Antibiotic use and the development of inflammatory bowel disease: a national case-control study in Sweden

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Summary

Background Use of antibiotics in early life has been linked with childhood inflammatory bowel disease (IBD), but data for adults are mixed, and based on smaller investigations that did not compare risk among siblings with shared genetic or environmental risk factors. We aimed to investigate the association between antibiotic therapy and IBD in a large, population-based study.

Methods In this prospective case-control study, we identified people living in Sweden aged 16 years or older, with a diagnosis of IBD based on histology and at least one diagnosis code for IBD or its subtypes (ulcerative colitis and Crohn's disease). We identified consecutive patients with incident IBD from the ESPRESSO (Epidemiology Strengthened by histoPathology Reports in Sweden) study, cross-referenced with the Swedish Patient Register and the Prescribed Drug Register. We accrued data for cumulative antibiotic dispensations until 1 year before time of matching for patients and up to five general population controls per patient (matched on the basis of age, sex, county, and calendar year). We also included unaffected full siblings as a secondary control group. Conditional logistic regression was used to estimate multivariable-adjusted odds ratios (aORs) and 95% CIs for diagnosis of incident IBD.

Findings We identified 23 982 new patients with IBD (15 951 ulcerative colitis, 7898 Crohn's disease, 133 unclassified IBD) diagnosed between Jan 1, 2007, and Dec 31, 2016. 117 827 matched controls and 28 732 siblings were also identified. After adjusting for several risk factors, aOR in patients who had used antibiotics versus those who had never used antibiotics was 1·88 (95% CI 1·79–1·98) for diagnosis of incident IBD, 1·74 (1·64–1·85) for ulcerative colitis, and 2·27 (2·06–2·49) for Crohn's disease. aOR was higher in patients who had received one antibiotic dispensation (1·11, 1·07–1·15), two antibiotic dispensations (1·38, 1·32–1·44), and three or more antibiotic dispensations (1·55, 1·49–1·61) than patients who had none. Increased risk was noted for ulcerative colitis (aOR with three or more antibiotic dispensations 1·47, 95% CI 1·40–1·54) and Crohn's disease (1·64, 1·53–1·76) with higher estimates corresponding to broad-spectrum antibiotics. Similar but attenuated results were observed when siblings were used as the reference group, with an aOR of 1·35 (95% CI 1·28–1·43) for patients who had received three or more dispensations, compared with general population controls.

Interpretation Higher cumulative exposure to systemic antibiotic therapy, particularly treatments with greater spectrum of microbial coverage, may be associated with a greater risk of new-onset IBD and its subtypes. The association between antimicrobial treatment and IBD did not appear to differ when predisposed siblings were used as the reference controls. Our findings, if substantiated by longer-term prospective studies in humans or mechanistic preclinical investigations, suggest the need to further emphasise antibiotic stewardship to prevent the rise in dysbiosis-related chronic diseases, including IBD.

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Introduction

Host genetics, environmental factors, and the gut microbiome are known to contribute to the aetiopathogenesis of inflammatory bowel disease (IBD).[1] Whereas host genetics have been studied extensively, less clearly understood are the contributions of specific environmental determinants to an alarming rise in IBD disproportionately affecting Europe, the USA, and parts of the world undergoing rapid economic development.[2,3] Increased sanitation and widespread use of anti-infective agents[4] have been proposed as reasons for this emerging disparity in disease burden—the so-called hygiene hypothesis. Previous large-scale efforts have shown that individuals with IBD harbour more facultative anaerobes (including *Escherichia coli*) and fewer obligate anaerobic producers of short-chain fatty acids compared with people without IBD.[5] As appreciation for the richness and diversity of the gut microbiome and its role in maintaining human health has grown, so too has concern that antibiotics might perturb and permanently alter these microbial communities, increasing risk for IBD and other disorders similarly characterised by dysregulated host–microbial interactions.

Despite expanded reliance on antimicrobial therapy being a leading suspected contributor to this relationship,

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Research in context

Evidence before this study

Rates of inflammatory bowel disease (IBD) are increasing, particularly in Europe, the USA, and other parts of the world undergoing rapid economic development, increased sanitation, and more frequent use of antibiotics. With growing appreciation for the role of the gut microbiome in maintaining human health, concern has also grown that antibiotics could perturb and permanently alter these fragile microbial communities. We searched PubMed for articles published between Jan 1, 1990, and April 30, 2020, using the terms "inflammatory bowel disease" and "antibiotics". Efforts to address this question have been conflicting—particularly in ulcerative colitis—and have been characterised by smaller-scale investigations, including a meta-analysis on the topic that reported an increased risk of IBD and Crohn's disease, but was not significant for ulcerative colitis. Whether or not antibiotic therapy is linked to new-onset IBD remains controversial.

Added value of this study

Among 23 982 individuals with IBD, matched to 117 827 controls, number of antibiotic dispensations was significantly associated with frequency-dependent risk for both ulcerative colitis and Crohn's disease. Risk appeared greater with more frequent use of broad-spectrum antibiotics. The positive association between antibiotic therapy and IBD remained observable after patients were compared with their unaffected siblings with whom they most likely shared genetic susceptibilities and childhood exposures, offering further support for the potential independent role of antibiotics in IBD development.

Implications of all the available evidence

Our findings, if substantiated by longer-term prospective studies in humans or mechanistic preclinical investigations, suggest the need to further emphasise antibiotic stewardship to prevent the rise in dysbiosis-related chronic diseases, including IBD.

studies are limited by small sample size[6–12] and paucity of histopathological case ascertainment (ie, studies that rely on clinical diagnosis by coding without confirming histopathology),[6–8,10,12–17] and mainly address risk associated with paediatric IBD.[13–18] No studies have assessed whether risk related to antibiotics is modified within families who are already genetically predisposed to the development of IBD. Finally, careful assessment of pre-diagnostic antibiotic use with an appropriate exclusionary lead-in period (ie, a period of time for which antibiotic dispensations just before IBD diagnosis are not accrued) is necessary to limit the possibility of reverse causation, and exclude therapy prescribed for symptoms related to undiagnosed IBD. This is particularly important for IBD, since the time to diagnosis can be delayed by 4–9 months.[19] Population-scale investigations with careful case ascertainment and an appropriate lead-in period are urgently needed to help answer this controversial and unsettled question.

Methods

Study design and participants

In Sweden, universal access to care is tax-funded and includes prescription medication coverage.[20] The Swedish National Board of Health and Welfare has collected patient data on hospital discharges nationally since 1987 using the Swedish Patient Register. Each patient record includes sex, date of birth, dates of hospital admission, and procedural and discharge diagnoses, recorded by International Classification of Diseases (ICD) code (appendix p 2). In 2001, this registry was expanded to include outpatient specialty care, including visits to gastroenterology providers. The positive predictive value for most diagnoses in this register is 85–95%.[21] We further integrated this national registry data with the Epidemiology Strengthened by histoPathology Reports in Sweden (ESPRESSO) study.[22] The ESPRESSO study is an ongoing, comprehensive data harmonising effort involving 28 pathology laboratories in Sweden, which includes all computerised gastrointestinal pathology reports generated for clinical care or research purposes between 1965 and 2016, encompassing more than 2·1 million unique individuals with detailed information on topography (ie, the anatomical location of the obtained tissue), morphology, appearance, and the pathologist's diagnostic impression. Since July, 2005, the Swedish Prescribed Drug Register has collected information on all medications prescribed to the entire Swedish population, date of redemption, amount dispensed, and dose allotted.[20] Patient data from the ESPRESSO cohort and two national registries (Patient Register and the Prescribed Drug Register) were linked by a unique personal identity number assigned at birth or at the time when permanent residence was established. Thus, our study encompasses all consecutive eligible patients for the period of overlap during which the National Patient Register, the ESPRESSO study, and the Prescribed Drug Register were each actively enrolling (July 1, 2005, to Dec 31, 2016). This investigation was approved by the Stockholm Ethics Review Board (protocol 2014/1287-31/4). Due to the strictly registry-based nature of the study, informed consent was waived.

See Online for appendix

Using predefined anatomical and histological criteria, and the attending pathologist's diagnostic impression (appendix p 2), we identified people in the ESPRESSO study with gastrointestinal tract histopathology compatible with the diagnosis of new-onset IBD and its subtypes (ulcerative colitis and Crohn's disease) from 2005 to 2016. If a distinction between subtypes could not be made, patients were defined as (non-infectious)

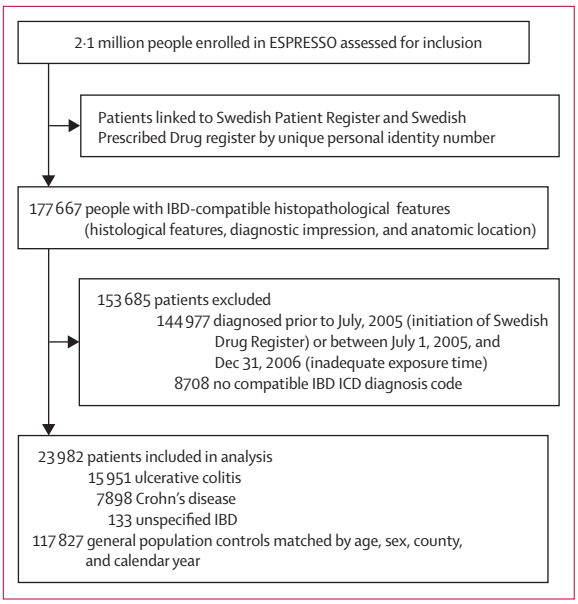

Figure 1: **Study profile**
ESPRESSO=Epidemiology strengthened by histopathology reports in Sweden. IBD=inflammatory bowel disease. ICD=International Classification of Diseases.

	Patients (n=23 982*)		Controls (n=117 827)
	Ulcerative colitis (n=15 951)	Crohn's disease (n=7898)	
Age, years	36 (24–56)	31 (19–51)	35 (22–54)
<18	1755 (11%)	1686 (21%)	17 699 (15%)
18–24	2552 (16%)	1422 (18%)	20 421 (17%)
25–34	3190 (20%)	1343 (17%)	22 785 (19%)
35–44	2233 (14%)	948 (12%)	15 843 (13%)
45–54	1914 (12%)	790 (10%)	13 451 (12%)
55–64	2072 (13%)	869 (11%)	13 487 (12%)
≥65	2235 (14%)	840 (11%)	14 141 (12%)
Sex			
Male	8543 (54%)	3997 (51%)	62 010 (52%)
Female	7408 (46%)	3901 (49%)	55 817 (48%)
Region of residence			
Northern Sweden	1417 (9%)	590 (7%)	9926 (8%)
Southeastern Sweden	1816 (11%)	923 (12%)	13 572 (12%)
Southern Sweden	3030 (19%)	1518 (19%)	22 390 (19%)
Stockholm-Gotland	3071 (19%)	2047 (26%)	25 229 (21%)
Uppsala-Örebro	3489 (22%)	1592 (20%)	25 216 (21%)
Western Sweden	3026 (19%)	1178 (15%)	20 922 (18%)
Unknown	102 (1%)	50 (1%)	572 (1%)
Education			
≤9 years	2829 (18%)	1540 (20%)	21 030 (18%)
10–12 years	7121 (45%)	3473 (44%)	50 542 (43%)
≥13 years	5563 (35%)	2430 (31%)	41 092 (35%)
Unknown	438 (3%)	455 (6%)	5163 (4%)
Number of encounters†			
Inpatient	2 (0–4)	2 (0–4)	1 (0–3)
Outpatient	5 (2–11)	6 (2–13)	3 (1–9)
Calendar year			
2007–09	4786 (30%)	2186 (28%)	34 449 (29%)
2010–13	7031 (44%)	3570 (45%)	52 373 (44%)
2014–16	4134 (26%)	2142 (27%)	31 005 (26%)

Data are n (%) or median (IQR). Polytomous variables might not sum to 100% due to rounding. *Includes ulcerative colitis, Crohn's disease, and unclassified inflammatory bowel disease (n=133). †Number of inpatient and outpatient encounters (continuous) for each participant during the study period up until the time of matching or case diagnosis.

Table 1: **Patient characteristics at the time of inflammatory bowel disease diagnosis and matched general population controls**

indeterminate colitis or unclassified IBD. We then cross-referenced potential patients and the entirety of their inpatient and outpatient records in search of at least one ICD code consistent with IBD.

We excluded patients with IBD-compatible pathology or ICD diagnostic coding before our study baseline. The date of IBD diagnosis was defined as the earliest between the date of relevant pathology findings and the first appearance of an IBD-related diagnosis code. In a random subset of individuals with both compatible histopathology and an ICD code for IBD, we were able to validate case status using manual chart review in 95 of 100 individuals,[22] yielding a positive predictive value of 95% (95% CI 89–99). To account for the possibility of reverse causation, or antibiotic therapy prescribed for symptoms related to undiagnosed IBD, we did not count antibiotic dispensations in the 1 year leading up to IBD diagnosis. To ensure adequate at-risk exposure time (since antibiotics were not counted in the preceding 12 months), we excluded patients diagnosed with IBD in the first 18 months from study baseline or initiation of the Swedish Prescribed Drug Register (appendix p 8).

At the time of ESPRESSO inclusion, individuals were paired with up to five reference controls from the general population, matched by age, sex, calendar year, and county. Controls with undiagnosed IBD at the time of matching were later considered to be patients if they met the prespecified diagnostic criteria, and they were then subsequently matched to five other reference controls of their own. To further assess the association between cumulative antibiotic use and IBD among genetically related individuals, we also identified and enrolled unaffected full siblings of our index patients who were still living at the time of their siblings' IBD diagnosis.[22]

Ascertainment of primary exposure and other covariates

Our primary exposure was cumulative antibiotic use up to 1 year before IBD diagnosis, defined as the cumulative sum of antibiotic dispensations. This was assessed using the Swedish Prescribed Drug Register and categorised using established WHO Anatomical Therapeutic Chemical (ATC) codes for the therapeutic subgroup of antibacterials approved for systemic use. To achieve adequate case

	None	1 dispensation	2 dispensations	≥3 dispensations	p value*
Inflammatory bowel disease†					
Patients (n=23 982)	9677 (40%)	4813 (20%)	3087 (13%)	6405 (27%)	..
Controls (n=117 827)	56 240 (48%)	24 864 (21%)	13 152 (11%)	23 571 (20%)	..
Unadjusted OR‡	1 (ref)	1·14 (1·10–1·19)	1·46 (1·40–1·53)	1·78 (1·71–1·84)	<0·0001
Multivariable aOR§	1 (ref)	1·11 (1·07–1·15)	1·38 (1·32–1·44)	1·55 (1·49–1·61)	<0·0001
Ulcerative colitis					
Patients (n=15 951)	6587 (41%)	3274 (21%)	2004 (13%)	4086 (26%)	..
Controls (n=78 349)	37 642 (48%)	16 570 (21%)	8656 (11%)	15 481 (20%)	..
Unadjusted OR‡	1 (ref)	1·16 (1·11–1·22)	1·39 (1·32–1·47)	1·63 (1·55–1·71)	<0·0001
Multivariable aOR§	1 (ref)	1·13 (1·08–1·19)	1·33 (1·25–1·41)	1·47 (1·40–1·54)	<0·0001
Crohn's disease					
Patients (n=7898)	3030 (38%)	1521 (19%)	1061 (13%)	2286 (29%)	..
Controls (n=38 818)	18 255 (47%)	8152 (21%)	4425 (11%)	7986 (21%)	..
Unadjusted OR‡	1 (ref)	1·18 (1·10–1·26)	1·56 (1·44–1·69)	1·94 (1·81–2·07)	<0·0001
Multivariable aOR§	1 (ref)	1·14 (1·06–1·22)	1·46 (1·35–1·58)	1·64 (1·53–1·76)	<0·0001

Data are n (%) or OR (95% CI). Cumulative dispensations accrued from study baseline to 1 year before diagnosis or match. OR=odds ratio. *Calculated using the median of each category as a continuous variable. †Includes ulcerative colitis, Crohn's disease, and unclassified inflammatory bowel disease. ‡Conditional logistic regression matched for age, sex, calendar year, and county. §Conditional logistic regression matched for age,sex, calendar year, and country, and further adjusted for number of inpatient and outpatient encounters and level of education.

Table 2: **Antibiotic use and inflammatory bowel disease in patients and matched general population controls**

balance, we collected information about the number of dispensations (categorised into zero, one, two, and three or more dispensations, corresponding with medians and IQRs for dispensations across the entire study population). We also collected information about the cumulative number of prescribed days and the cumulative defined daily dose. In secondary analyses to further define the relationship between antimicrobial coverage, dysbiosis, and risk of IBD, we assessed whether ATC class of antibiotic (penicillins, cephalosporins, macrolides, fluoroquinolones, tetracyclines, sulphonamides, and others) or spectrum of coverage (broad, narrow, or both)[23] influenced risk of disease (appendix pp 3–4).

When available, we obtained data about the level of education (≤9 years, 10–12 years, ≥13 years, and unknown) from Statistics Sweden and the longitudinal integrated database for health insurance and labour market studies, which, since 1990, has annually updated administrative information from the labour market and educational and social sectors for all individuals aged 16 years or older. This information is available in more than 98% of all individuals aged 25–64 years.[24] We also calculated the number of inpatient and outpatient encounters (continuous) for each participant during the study period up until the time of matching.

Statistical analysis

To evaluate the association between previous antibiotic therapy and IBD, we did conditional logistic regression between patients with IBD and reference controls to estimate crude and multivariable-adjusted odds ratios (aORs) and their 95% CIs conditioned on matching factors (age, sex, calendar year, and county of residence) and further adjusted for potential confounding factors (level of education and number of inpatient and outpatient encounters during follow-up [health-care use]). Tests for linear trend were calculated using the midpoint of each frequency category as a continuous variable. Two-sided p values of less than 0·05 were considered to be significant.

As a sensitivity analysis and to assess the robustness of our primary findings, we lengthened the lead-in period from 1 year in our primary analysis to a more conservative 2 years. We also did subgroup analyses according to the spectrum of antibiotic coverage and class of antibiotic therapy prescribed. We did a joint association analysis to establish whether the number of combined broad-spectrum or narrow-spectrum dispensations altered the association between antimicrobial therapy and IBD. Finally, to minimise the influence of genetic predisposition and shared childhood exposures, we compared patients with their (unmatched) full siblings using logistic regression with adjustment for age, sex, year of match, county, level of education, and health-care use. Statistical analyses were done using SAS version 9.4 (Cary, NC, USA) and R 3.5.1 (Vienna, Austria).

Role of the funding source

The funders had no role in study design; the collection, analysis, and interpretation of data; writing of the report; or the decision to submit for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

We identified 32 690 unique individuals aged 16 years or older in the ESPRESSO study with gastrointestinal tract

	None	1 dispensation	2 dispensations	≥3 dispensations	p value
Inflammatory bowel disease*					
Broad-spectrum antibiotics					
Patients (n=16 999)	9677 (57%)	3690 (22%)	1592 (9%)	2040 (12%)	..
Controls (n=84 965)	56 240 (66%)	16 302 (19%)	5803 (7%)	6620 (8%)	..
Multivariable aOR†	1 (ref)	1·31 (1·25–1·37)	1·58 (1·48–1·68)	1·69 (1·59–1·79)	<0·0001
Narrow-spectrum antibiotics					
Patients (n=21 795)	9677 (44%)	5212 (24%)	2797 (13%)	4109 (19%)	..
Controls (n=108 165)	56 240 (52%)	25 023 (23%)	11 768 (11%)	15 134 (14%)	..
Multivariable aOR†	1 (ref)	1·18 (1·13–1·22)	1·37 (1·30–1·43)	1·49 (1·43–1·56)	..
Ulcerative colitis					
Broad-spectrum antibiotics					
Patients (n=11 341)	6587 (58%)	2440 (22%)	1015 (9%)	1299 (11%)	..
Controls (n=56 914)	37 642 (66%)	10 934 (19%)	3873 (7%)	4465 (8%)	..
Multivariable aOR†	1 (ref)	1·29 (1·22–1·36)	1·50 (1·38–1·63)	1·57 (1·45–1·70)	<0·0001
Narrow-spectrum antibiotics					
Patients (n=14 480)	6587 (45%)	3524 (24%)	1779 (12%)	2590 (18%)	..
Controls (n=71 738)	37 642 (52%)	16 571 (23%)	7775 (11%)	9750 (14%)	..
Multivariable aOR†	1 (ref)	1·20 (1·15–1·26)	1·28 (1·21–1·36)	1·43 (1·35–1·52)	..
Crohn's disease					
Broad-spectrum antibiotics					
Patients (n=5582)	3030 (54%)	1236 (22%)	587 (11%)	729 (13%)	..
Controls (n=27 571)	18 255 (66%)	5277 (19%)	1909 (7%)	2130 (8%)	..
Multivariable aOR†	1 (ref)	1·40 (1·29–1·52)	1·79 (1·60–2·00)	1·78 (1·59–1·99)	<0·0001
Narrow-spectrum antibiotics					
Patients (n=7197)	3030 (42%)	1667 (23%)	1003 (14%)	1497 (21%)	..
Controls (n=35 815)	18 255 (51%)	8310 (23%)	3933 (11%)	5317 (15%)	..
Multivariable aOR†	1 (ref)	1·21 (1·13–1·30)	1·50 (1·37–1·63)	1·57 (1·44–1·70)	..

Data are n (%) or OR (95% CI). Patients might have received both narrow-spectrum and broad-spectrum antibiotics, and might therefore appear in more than one category. Cumulative dispensations accrued from study baseline up until 1 year before diagnosis or match. OR=odds ratio. *Includes ulcerative colitis, Crohn's disease, and unclassified inflammatory bowel disease. †Conditional logistic regression matched for age, sex, calendar year, and county, further adjusted for number of inpatient and outpatient encounters and level education.

***Table 3*: Antibiotic coverage in patients with inflammatory bowel disease and matched general population controls**

histopathology compatible with the diagnosis of new-onset IBD or its subtypes (ulcerative colitis and Crohn's disease) from July 1, 2005, to Dec 31, 2016. All potential study patients were identified in ESPRESSO and cross-referenced to the Patient Register to confirm diagnosis and to the Drug Register to assess antibiotic use. After excluding patients with a compatible IBD ICD diagnosis code at or before baseline and patients without adequate exposure time according to our prespecified lead-in period, we enrolled 23 982 patients with IBD, confirmed by biopsy and diagnosis code between Jan 1, 2007, and Dec 31, 2016 (15 951 ulcerative colitis, 7898 Crohn's disease, 133 unclassified IBD; figure 1). Patients with IBD were similar to the 117 827 matched controls by mean age, sex, level of education, and county, but tended to have more frequent engagement with inpatient and outpatient providers (table 1; appendix p 5). Only 952 (0·8%) of 117 827 general population individuals would become patients themselves and were matched to their own reference individuals. We found a strong correlation between the presence of an IBD-compatible ICD code recorded in the inpatient setting with one in the outpatient setting (Spearman's $\rho>0·62$ for IBD, Crohn's disease, and ulcerative colitis), which would suggest that either clinical setting (eg, inpatient or outpatient) is suitable for valid case identification.

The aOR in patients who had used antibiotics versus those who had never used antibiotics was 1·88 (95% CI 1·79–1·98) for diagnosis of incident IBD, 1·74 (1·64–1·85) for ulcerative colitis, and 2·27 (2·06–2·49) for Crohn's disease. Increased cumulative antibiotic use was associated with an increased risk for new-onset IBD and its two primary subtypes, ulcerative colitis and Crohn's disease (table 2). Inclusive of our matching criteria (age, sex, calendar year, and county), level of education, and number of previous inpatient and outpatient encounters, multivariable conditional logistic regression showed a frequency-dependent relationship between increased antibiotic prescriptions and risk of IBD (table 2; $p<0·0001$). Three or more antibiotic dispensations more than 1 year before IBD diagnosis was associated with an increased risk for IBD (multivariable aOR 1·55, 95% CI

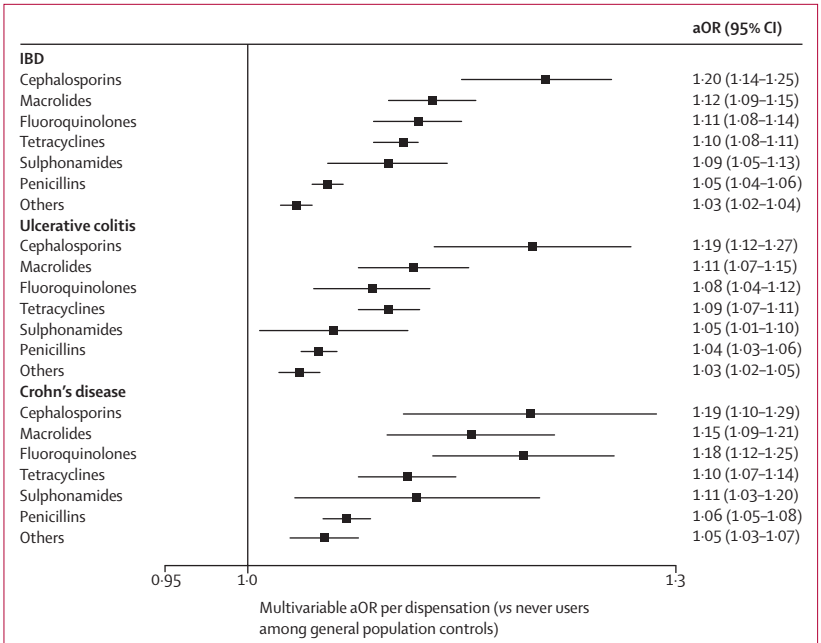

Figure 2: **Antibiotic use in patients with IBD and matched general population controls**
Conditional logistic regression matched for age, sex, calendar year, and county, and further adjusted for number of inpatient and outpatient encounters and level of education. The reference group had no previous exposure to antibiotics of any kind at the time of matching. Cumulative dispensations accrued from study baseline up until 1 year before diagnosis or match. IBD includes ulcerative colitis, Crohn's disease, and unclassified IBD. aOR=adjusted odds ratio. IBD=inflammatory bowel disease.

	None	1 dispensation	2 dispensations	≥3 dispensations	p value
Inflammatory bowel disease*					
Patients (n=16 353)	6502 (40%)	3407 (21%)	2170 (13%)	4274 (26%)	..
Siblings (n=28 732)	12 688 (44%)	6278 (22%)	3341 (12%)	6425 (22%)	..
Multivariable aOR†	1 (ref)	1·06 (1·01–1·12)	1·32 (1·24–1·41)	1·35 (1·28–1·43)	<0·0001
Ulcerative colitis					
Patients (n=10 838)	4428 (41%)	2279 (21%)	1392 (13%)	2739 (25%)	..
Siblings (n=19 032)	8459 (44%)	4209 (22%)	2204 (11%)	4160 (22%)	..
Multivariable aOR†	1 (ref)	1·06 (0·99–1·14)	1·23 (1·13–1·34)	1·29 (1·20–1·39)	<0·0001
Crohn's disease					
Patients (n=5429)	2036 (38%)	1117 (21%)	762 (14%)	1514 (28%)	..
Siblings (n=9435)	4073 (43%)	2030 (22%)	1111 (12%)	2221 (24%)	..
Multivariable aOR†	1 (ref)	1·13 (1·02–1·25)	1·41 (1·26–1·58)	1·46 (1·23–1·62)	<0·0001

Data are n (%) or OR (95% CI). aOR=adjusted odds ratio. *Includes ulcerative colitis, Crohn's disease, and unclassified inflammatory bowel disease. †Further adjusted for age, sex, county, calendar year, number of inpatient and outpatient encounters, and level of education. Cumulative dispensations accrued from study baseline up until 1 year before diagnosis.

Table 4: **Antibiotic use in patients with inflammatory bowel disease and unaffected siblings**

1·49–1·61) compared with no use. Due to collinearity in their measure, results were similar when cumulative antibiotic therapy was assessed by number of days prescribed or cumulative defined daily dose (data not shown). Risk estimates were slightly higher for Crohn's disease than with ulcerative colitis (all aORs by dispensation; table 2). Our estimates were similar when we used a 2-year, rather than 1-year lead-in (multivariable aOR for ≥3 dispensations compared with no use 1·47, 95% CI 1·41–1·53; p<0·0001). Results remained consistent when we removed controls who eventually became patients (data not shown).

Subgroup analyses by spectrum of antibiotic coverage showed an increased risk among patients reporting more frequent use of broad-spectrum antibiotics than with narrow spectrum (table 3). Heterogeneity in risk estimates appeared most pronounced for Crohn's disease, though formal tests for interaction were highly significant for IBD, ulcerative colitis, and Crohn's disease (all p<0·0001). There was no clear synergistic effect between increasing frequency of combined broad and narrow-spectrum antimicrobial therapy (data not shown). Each class of antibiotic assessed, categorised by WHO ATC subgroup, was associated with a significant increase in risk per dispensation compared with no use (multivariable aOR between 1·03 and 1·20; figure 2), and was highest for each dispensation of a cephalosporin class antibiotic (multivariable aOR 1·20, 95% CI 1·14–1·25). To further ensure our primary findings were not a consequence of infections related to undiagnosed IBD (ie, confounding by indication), we compared the use of a composite antibiotic, either ciprofloxacin or metronidazole—two commonly prescribed antibiotics for bacterial gastroenteritis—and found similar results to our overall estimate (multivariable aOR 1·15, 95% CI 1·11–1·20). In general, we found consistent results between ulcerative colitis and Crohn's disease (figure 2). A stratified analysis by age group (appendix p 6) appeared to show a greater association among older individuals.

Finally, among individuals with a genetic predisposition to disease development and to account in part for shared but unspecified childhood exposures, we compared antimicrobial therapy use among patients with IBD and their full siblings, when available. 16 353 (68%) of 23 982 patients had at least one living sibling identified in our cohort, all of whom were captured by linkage to the National Patient Register. Mean age, region of residence, and level of education were all similar between patients and 28 732 unaffected siblings (appendix p 7). Notably, number of inpatient and outpatient encounters were increased—like their siblings with IBD—compared with unrelated reference individuals, suggesting similar childhood exposures, infections, predisposition for chronic disease, and access to similar childhood care. When siblings were used as the reference group, IBD risk estimates were only slightly attenuated compared with general population controls, with a multivariable aOR of

1·35 (95% CI 1·28–1·43) when comparing three or greater antibiotic dispensations to none (p<0·0001; table 4). Estimates were similar for ulcerative colitis and Crohn's disease (table 4).

Discussion

In this population-based study of nearly 24 000 unique patients with IBD over 10 years, we report a potential frequency-dependent relationship between the number of antibiotic dispensations and the development of IBD. This potential association appeared to be robust to adjustment for age, sex, location, calendar year, level of education, and degree of health-care use. Furthermore, this risk association appeared greater with more frequent use of broad-spectrum antibiotics, which supports the hypothesis linking increased risk of chronic disease development with more drastic taxonomic changes to the normal gut ecological state due to antimicrobial therapy.

To our knowledge, this is the largest investigation exploring the link between previous antimicrobial therapy and subsequent risk of IBD, which not only allowed for subgroup analyses by WHO antibiotic class, but also suggested an association between more frequent antibiotic therapy and risk of ulcerative colitis, a disease subtype for which previous, smaller investigations have produced mixed results. Moreover, in a secondary analysis among patients and their siblings without IBD with whom they most likely shared childhood exposures and a genetic predilection for the development of IBD, we were able to show that risk associated with antibiotic use was only slightly attenuated compared with tests against general reference individuals. This comparison among similarly predisposed people provides further evidence to implicate cumulative antibiotic exposure as an independent risk factor in the aetiopathogenesis of IBD, regardless of underlying genetic susceptibility.

Disentangling the environmental exposures that could culminate in the diagnosis of IBD is crucial, particularly as some data suggest that risk of IBD due to antibiotics might be restricted to developed areas of the world.[3] Globally, rates of IBD are rising, particularly in areas undergoing rapid economic development. Since these trends are unlikely to be explained by drastic changes in underlying genetic architecture, the widespread adoption of increased sanitation and pervasive use of anti-infective agents is implicated.[25–28] Despite considerable progress in our understanding of IBD's genetic and familial underpinnings, concordance rates among monozygotic twins is just 20–50%,[29] highlighting the importance of considerable non-genetic determinants in new-onset IBD, such as antibiotic therapy.

Although antibiotics have been widely beneficial for the maintenance of human health, they greatly affect the fragile ecology of human gut microbial communities, resulting in individualised and sometimes incomplete recovery from such insults, potentially predisposing users to long-term chronic disease risk.[30–33] The consequences of altering the taxonomic makeup and collective activities of the gut microbiome can influence IBD risk by several, interconnected mechanisms, including a change in vital metabolic functions, vitamin and nutrient production, and energy extraction. Most importantly, gut microbial perturbations promote the onset of intestinal barrier dysfunction,[34,35] altered immune response,[36,37] defective autophagy,[38] and permissive pathogenic blooms[39–41] that are typically viewed as inciting events as early as several years before the development of IBD. Notably, our group has previously linked early life antibiotic exposures to a more severe, paediatric form of the disease, adding credibility to the findings.[15]

Evidence on the relationship between antimicrobial therapy and risk of IBD has been mounting. The largest meta-analysis to date,[42] encompassing 7208 patients with IBD from 11 separate investigations, noted similar findings with respect to antibiotic therapy and risk of Crohn's disease and also observed heterogeneous risk estimates related to class of antibiotic therapy and included several studies in which no clear association was noted. However, Ungaro and colleagues[42] did not find evidence of an association between antibiotic use and an increase in risk for ulcerative colitis, possibly because of sample size considerations (3207 patients with ulcerative colitis). A case-control study of 455 patients with IBD by Aniwan and colleagues[7] showed an association between antimicrobial therapy and risk of ulcerative colitis, albeit with much stronger risk estimates across IBD and its subtypes (aOR 2·93 for IBD, 95% CI 2·40–3·58; 2·94 for ulcerative colitis, 95% CI 2·23–3·88).[7] Our study was nationally representative in Sweden and population-based; by contrast, Aniwan and colleagues enrolled individuals from an ethnically and socioeconomically homogeneous region of the USA. Given our hypothesis linking hygiene, economic development, and increased use of antibiotics to IBD, it is unclear how generalisable their findings are. Additionally, Aniwan and colleagues[7] only excluded prescriptions in the 3 months before diagnosis, increasing the possibility of reverse causation, or therapy initiated for symptoms due to undiagnosed IBD, a disease for which the time to diagnosis can be delayed by months.[19] Our study did not allow dispensations to accrue in the 1 year before IBD diagnosis or time of matching, making it less likely that antibiotics were masking symptoms of IBD that had already developed, and our estimates were not significantly attenuated when we used an even stricter 2-year lead-in period. Finally, comparable observational studies have helped elucidate the role of antibiotics in other gastrointestinal conditions, such as colorectal cancer,[23] its precursor lesions,[43] and coeliac disease.[44]

Strengths of this investigation include the enrolment of all consecutive, eligible patients with new-onset IBD from a population-based register over 10 years, reducing selection bias. In Sweden, there is universal medication coverage with virtually complete information on all drug dispensations, including antibiotics, minimising

ascertainment bias (<0·3% of all prescriptions lack identifying information).[45] To complement the use of a large, nationally representative sample, we used stringent outcome ascertainment, requiring both compatible histopathological findings and confirmatory ICD coding for adjudicating cases. With a positive predictive value of 95%, this validated method of case identification allowed us to confidently leverage and retain the power to detect even modest risk increases among some antibiotic classes. Thus, we were able to show that all antimicrobial classes tested conferred additive—at times, modest—disease risk, strongly arguing for universal antibiotic stewardship and prescriber restraint.

We acknowledge several limitations. As with all large-scale pharmacoepidemiological studies, medication dispensation ascertained through the Swedish Prescribed Drug Register might not capture any given patient's actual use. However, due to the short-term nature of most antibiotic courses and the presumption that most dispensations were attributable to positive symptoms suggestive of an infection, adherence was not likely to be a major issue. Furthermore, such bias would have resulted in attenuation towards the null from non-differential misclassification. The Prescribed Drug Register does not contain information about drugs administered in hospital, though this could be accounted for in part in our multivariable models adjusting for number of hospital admissions over the follow-up period. Given the observational nature and epidemiological scale of this investigation, the possibility of unmeasured confounding remains, and our case-control design did not allow estimates of incidence or absolute risks. Our results will need to be validated in other populations given Sweden's elevated rates of IBD[2,46,47] and lower antibiotic dispensation patterns compared with other nations.[48,49] We did not have information about the type of infection or indication for antibiotics dispensed. We found a low rate of unclassified IBD during the study period, which might be a function of improved histopathological criteria,[50,51] our focus on adult-onset IBD,[52,53] or a protracted lead-in period that might not have allowed unclassified IBD to develop from patients with presumed ulcerative colitis or Crohn's disease.[50,53] Finally, we cannot fully eliminate the possibility of reverse causation (therapy initiated for undiagnosed IBD) or confounding by indication (therapy initiated for gastrointestinal infections related to IBD), though we attempted to minimise this in several ways. For instance, by only accounting for prescriptions at least 1 year before diagnosis, we can be more confident that antibiotic dispensations were not likely to be prescribed for undiagnosed IBD, a disease with a typical time to diagnosis between 4 and 9 months.[19] An even more stringent sensitivity analysis extended the lead-in period to 2 years yielded similar findings. We also showed a consistent frequency-response relationship in our primary analysis, and a secondary analysis showed an elevated risk, with broad-spectrum antibiotics more likely to adversely affect gut microbial communities. Lastly, we used an early, conservative date of diagnosis (at the time of either the earliest IBD-compatible histopathology or ICD coding) that minimises the time period for which antibiotic dispensations could be attributable to symptoms of IBD.

Higher cumulative exposure to systemic antibiotic therapy in the past 10 years, particularly those with greater spectrum of microbial coverage, was associated with an increase in risk for new-onset IBD and its two main subtypes, ulcerative colitis and Crohn's disease. The relationship between antimicrobial treatment and IBD was not materially altered when predisposed siblings were used as the referent controls. Further studies are needed to investigate how antibiotics might permanently alter gut microbial communities, potentially culminating in disease development, and whether that risk could be reduced by probiotics to prevent expansive blooms of pathogenic bacteria in place of beneficial microbes affected by antibiotic treatment.

Contributors

LHN and JFL were responsible for study concept and design, and for analysis and interpretation of data. JFL was responsible for the acquisition of data. LHN drafted the manuscript. AKO, YC, TGS, BR, ADJ, KS, MS, ATC, HK, OO, and JFL contributed to critical revision of the manuscript. LHN and YC did the statistical analysis. JFL was responsible for study supervision.

Declaration of interests

KS reports grants from AstraZeneca, Gelesis, and Takeda, and personal fees from Shire, Boston Pharmaceuticals, and Arena, all unrelated to the scope of the submitted work. HK received unrelated grant funding and consulting fees from Pfizer and Takeda. JFL coordinates a separate study on behalf of the Swedish IBD quality register (SWIBREG), which has received funding from Janssen.

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