Antibiotic Exposure and IBD Development Among Children: A Population-Based Cohort Study

WHAT'S KNOWN ON THIS SUBJECT: Inflammatory bowel disease pathogenesis is incompletely understood. Previous pediatric studies suggested associations between antibiotic use and inflammatory bowel disease development but were limited by recall bias, lack of controls, incomplete antibiotic capture, or included exposures between symptom onset and diagnosis.

WHAT THIS STUDY ADDS: Our population-based cohort study suggests that certain childhood antibiotic exposures are associated with an increased risk of developing inflammatory bowel disease. Our findings have implications for understanding the condition's pathogenesis and provide additional stimulus for reducing unnecessary childhood antibiotic use.

abstract

OBJECTIVE: To determine whether childhood antianaerobic antibiotic exposure is associated with the development of inflammatory bowel disease (IBD).

METHODS: This retrospective cohort study employed data from 464 UK ambulatory practices participating in The Health Improvement Network. All children with ≥2 years of follow-up from 1994 to 2009 were followed between practice enrollment and IBD development, practice deregistration, 19 years of age, or death; those with previous IBD were excluded. All antibiotic prescriptions were captured. Antianaerobic antibiotic agents were defined as penicillin, amoxicillin, ampicillin, penicillin/β-lactamase inhibitor combinations, tetracyclines, clindamycin, metronidazole, cefoxitin, carbapenems, and oral vancomycin.

RESULTS: A total of 1 072 426 subjects contributed 6.6 million person-years of follow-up; 748 developed IBD. IBD incidence rates among antianaerobic antibiotic unexposed and exposed subjects were 0.83 and 1.52/10 000 person-years, respectively, for an 84% relative risk increase. Exposure throughout childhood was associated with developing IBD, but this relationship decreased with increasing age at exposure. Exposure before 1 year of age had an adjusted hazard ratio of 5.51 (95% confidence interval [CI]: 1.66–18.28) but decreased to 2.62 (95% CI: 1.61–4.25) and 1.57 (95% CI: 1.35–1.84) by 5 and 15 years, respectively. Each antibiotic course increased the IBD hazard by 6% (4%–8%). A dose-response effect existed, with receipt of >2 antibiotic courses more highly associated with IBD development than receipt of 1 to 2 courses, with adjusted hazard ratios of 4.77 (95% CI: 2.13–10.68) versus 3.33 (95% CI: 1.69–6.58).

CONCLUSIONS: Childhood antianaerobic antibiotic exposure is associated with IBD development. *Pediatrics* 2012;130:e794–e803

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KEY WORDS

antimicrobials, epidemiology, inflammatory bowel disease, pediatric, population-based studies

ABBREVIATIONS

aHR—adjusted hazard ratio
CI—confidence interval
HR—hazard ratio
IBD—inflammatory bowel disease
IQR—interquartile range
THIN—The Health Improvement Network

Drs Kronman, Zaoutis, Haynes, Feng, and Coffin participated in study conception and design; Dr Haynes conducted acquisition of data; Drs Kronman, Zaoutis, Feng, and Coffin assisted with analysis and interpretation of the data; Dr Kronman drafted the manuscript; Drs Zaoutis, Haynes, Feng, and Coffin performed critical revision of the manuscript for important intellectual content; Drs Kronman and Feng conducted statistical analysis; Drs Kronman, Zaoutis, and Coffin obtained funding; Dr Haynes contributed administrative, technical, or material support; and Drs Zaoutis and Coffin provided supervision of study conduct and analysis.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the Agency for Healthcare Research and Quality or the National Institutes of Health. All authors had full access to all of the data in the study and the corresponding author (Dr Kronman) takes responsibility for the integrity of the data and the accuracy of the data analysis.

www.pediatrics.org/cgi/doi/10.1542/peds.2011-3886

doi:10.1542/peds.2011-3886

Accepted for publication May 17, 2012

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory condition without clear etiology. Although genome-wide association studies have identified novel genetic defects associated with IBD, these defects account for only one-half of IBD cases.[1,2] Childhood IBD incidence and prevalence seem to have doubled over the last decade.[3] A leading hypothesis of IBD pathogenesis is that gut bacterial community alterations, with either increases in pathogenic bacteria or decreases in protective bacteria, trigger inflammation.[4]

Consistent with this hypothesis, multiple studies have noted associations between intestinal infections and IBD onset, although these associations may be secondary to ascertainment bias and increased stool culture testing in those undergoing evaluation for intestinal symptoms.[5–7] In addition, patients with IBD have reduced diversity of intestinal microbiota (specifically, anaerobic bacteria) relative to healthy controls, although the causal relationship is unclear.[8] Moreover, murine IBD models demonstrate that the IBD phenotype can be induced or ameliorated through flora sharing alone.[9,10] Lastly, antibiotics alter the human gut microbiome, with transiently decreased bacterial diversity returning to its preantibiotic state within ~1 month.[11]

Previous studies have shown associations between antibiotic use and IBD development but were limited due to recall bias,[12–15] lack of controls,[16] incomplete antibiotic capture,[17,18] or potential selection bias due to case-control design[19]; examined predominantly adults[20,21] or only certain antibiotic classes[22]; or included antibiotic exposure between IBD symptom onset and eventual IBD diagnosis.[23]

We sought to examine the association between childhood antianaerobic antibiotic exposure and subsequent IBD development using a large population-based cohort, hypothesizing that exposure to antibiotics with anaerobic activity would be associated with the development of IBD.

METHODS

Study Design and Data Source

We performed a retrospective cohort study by using The Health Improvement Network (THIN) database. THIN is a UK collaborative effort collecting de-identified electronic medical record data from 464 participating general practices during the study period. THIN contains data on >9 million (>3 million active) patients, representing ~5.7% of UK patient visits.[24] The Vision general practice computing system (InPractice Systems, London, UK) is an electronic medical record capturing patient demographic characteristics, diagnoses, prescriptions, and some laboratory data, and was adopted by THIN practices from 1994 to 2008.

This study was approved by the institutional review board of the University of Pennsylvania and the THIN Scientific Review Committee.

Study Population

All children ≤17 years of age enrolled in THIN practices using the Vision electronic medical record between 1994 and 2009 and with ≥2 years' follow-up were included, because some adults experience symptoms for 2 years before diagnosis of IBD.[20] Subjects with an IBD diagnosis occurring before their practice's computerized medical record adoption were excluded. In addition, research in the UK's General Practice Research Database, which extracts data from the same medical practice software and overlaps some THIN practices, demonstrated that chronic conditions are overdiagnosed in the first 6 months after registration (as new providers record previous conditions), so we excluded subjects with first IBD diagnosis within 6 months of cohort entry to ensure capture of incident IBD cases only.[25] Subjects contributed person-time until first IBD diagnosis, practice deregistration, 19 years of age, or death. The last available data were from November 25, 2009.

Exposure Identification

Data on all systemic antibiotic prescriptions were captured; antibiotics are unavailable in the United Kingdom without prescription. Antianaerobic antibiotics were defined as penicillin, amoxicillin, ampicillin, penicillin/β-lactamase inhibitor combinations, tetracyclines, clindamycin, metronidazole, cefoxitin, carbapenems, and oral vancomycin. We first classified subjects as ever- or never-exposed. An antibiotic course was defined as continuous antibiotic exposure with <3 days' interruption. Antibiotic courses were measured in weeks.

THIN does not capture inpatient prescribing. To determine whether confounding by hospital admissions might exist (when subjects might receive unobserved antibiotics and which might represent treatment of early IBD symptoms), hospital admissions were defined as any diagnoses or procedures recorded on unique days with an inpatient location assigned. We did not assess probiotic exposure because probiotics are available without prescription.

Latency Period

Because IBD symptoms exist before IBD diagnosis, the potential for misclassification bias and reverse causality exists, when subjects might receive antibiotics as treatment of symptoms arising from as-yet undiagnosed IBD. This time between symptom onset and diagnosis was termed the "latency period." To address this potential for

reverse causality, we measured the latency period for subjects who developed IBD, defined as the time between the earliest potential IBD symptom diagnosis (eg, abdominal pain, diarrhea, abnormal weight loss) within 5 years before the first IBD diagnosis and the first IBD diagnosis. We then excluded all antibiotic exposures occurring from the median latency period until censorship for all subjects, regardless of outcome.

Outcome Identification

The primary outcome was IBD development, identified by using diagnosis codes (Read codes). Using the general practitioner survey report as the gold standard, IBD codes have been validated as having 92% positive predictive value for identifying patients with probable IBD in the General Practice Research Database.[26] THIN data elements have been previously validated against this database for diverse associations.[27]

Demographic Characteristics and Comorbidities

We obtained demographic information, including age at cohort entry, gender, and Townsend score (a measure of socioeconomic deprivation quintile provided by THIN). The Townsend score was dichotomized as those with most deprivation versus all others. Race and ethnicity data are unavailable in THIN. We identified subjects diagnosed with conditions known to be associated with IBD that might also be associated with antibiotic exposure (ie, chronic granulomatous disease, primary sclerosing cholangitis, chronic osteomyelitis, Behçet's syndrome, hyperimmunoglobulin E syndrome, common variable immunodeficiency).

Statistical Analysis

Demographic variables were described by using frequencies, mean, median, and interquartile range (IQR) as appropriate. The association between each covariate and IBD was examined by using χ^2 tests and logistic regression as appropriate, and all covariates associated with IBD development were examined, with $P < .2$ used for inclusion in the multivariable model. Confounders were defined as covariates whose adjustment produced an adjusted hazard ratio (aHR) >10% different than the unadjusted hazard ratio (HR).

A Cox proportional hazards model, which jointly considers IBD status and age of IBD onset, was used to examine the association between antibiotic exposure and IBD development. Each analysis was stratified according to primary care site, to allow separate hazard functions for each site because prescribing practices and patient populations may differ across sites. Because we were interested in estimating IBD risk at different ages, age was used as the time scale for cohort entry, exit, and exposure time points in multivariate analysis. Proportional hazards assumptions were checked statistically, and graphically if violated, and covariates were treated as time varying as appropriate.

Our primary a priori analysis examined the association between exposure to antianaerobic antibiotics and IBD development. Secondary analyses examined the association between antibiotic exposure and IBD development according to any antibiotic use and according to specific individual antibiotic classes. We additionally evaluated the primary association of interest for Crohn's disease and ulcerative colitis separately. Sensitivity analyses were performed by using a 1-year latency period (when the proportion of subjects with previous diagnoses for possible IBD symptoms tapered to an apparent baseline) and assigning missing Townsend score data first all in the highest deprivation category and then all in the lower deprivation category.

A P value $< .05$ was considered significant for our primary analysis, and precise P values are reported. Stata version 11 (Stata Corp, College Station, TX) was used for all analyses.

RESULTS

Cohort Description

There were 1 072 668 children eligible for inclusion. A total of 242 children were excluded: IBD was diagnosed before or within 6 months of cohort entry in 201 and 38, respectively; 3 had no IBD diagnosis date. The resulting cohort contained 1 072 426 subjects for analysis, followed up for 6.6 million person-years; 748 subjects (0.07%) developed IBD, for an overall incidence rate of 1.2/10 000 person-years (Tables 1 and 2). Only 30 of the 225 100 subjects followed up from birth developed IBD, precluding meaningful analyses in this subgroup.

Latency Period

Among the 748 subjects who developed IBD, the median latency period between first visit with a gastrointestinal diagnosis potentially consistent with IBD and first IBD diagnosis was 3.9 months (IQR: 0.5–17.9 months), and 68.2% had latencies ≤1 year (Fig 1). Fewer than 25% of other subjects had such diagnoses within 5 years before censorship.

Antibiotic Exposures

Antibiotic exposures were common, with 57.7% of subjects exposed to at least 1 antianaerobic antibiotic and 64.0% exposed to any antibiotic. Subjects received a median of 1 week of antianaerobic antibiotics (IQR: 0–2), with 42.3% receiving none, 31.9% receiving 1 to 2 weeks, and 25.8% receiving >2 weeks. Exposure distribution according

TABLE 1 Baseline Cohort Demographic Characteristics According to Antianaerobic Antibiotic Exposure Status

Overall Cohort (N = 1 072 426)	Unexposed (N = 453 763)	Exposed (N = 618 663)
Total person time, y	3 776 942	2 859 887
Male gender, *n* (%)	243 726 (53.7%)	311 796 (50.4)
Years in cohort, median (IQR)	4.6 (2.9–7.3)	6.5 (4.2–9.1)
Years of age at entry, median (IQR)	7.3 (2.2–12.1)	4.2 (0.2–9.8)
Country of residence, *n* (%)		
England	379 971 (83.7)	504 069 (81.5)
North Ireland	12 157 (2.7)	30 539 (4.9)
Scotland	40 370 (8.9)	46 996 (7.6)
Wales	21 265 (4.7)	37 059 (6.0)
Developed IBD, *n* (%)	312 (0.07)	436 (0.07)
Subjects with predisposition to IBD, *n* (%)		
Chronic osteomyelitis	16 (<0.01)	60 (0.01)
Behçet's syndrome	1 (<0.01)	19 (<0.01)
Chronic granulomatous disease	7 (<0.01)	12 (<0.01)
Primary sclerosing cholangitis	7 (<0.01)	10 (<0.01)
Common variable immunodeficiency	1 (<0.01)	16 (<0.01)
Hyperimmunoglobulin E syndrome	1 (<0.01)	1 (<0.01)
Family history of IBD, *n* (%)	121 (0.03)	235 (0.04)
Townsend quintile, *n* (%)		
1–4 (less deprivation)	363 747 (80.2)	503 162 (81.3)
5 (most deprivation)	59 604 (13.1)	81 165 (13.1)
Missing	30 412 (6.7)	34 336 (5.6)

to courses received was similar, with 42.3% receiving none, 34.9% receiving 1 to 2 courses, and 22.8% receiving >2 courses. Excluding antianaerobic antibiotic courses during the latency period excluded exposures for 12.8% of subjects who developed IBD and 6.9% of those who did not.

TABLE 2 Antibiotic Exposures According to Class

Subjects Ever Exposed to Antibiotic	Overall Cohort (N = 1 072 426)
Any antibiotic	686 730 (64.0)
Antianaerobic antibiotics[a]	618 663 (57.7)
Penicillins	585 695 (54.6)
Broad-spectrum penicillins[b]	86 629 (8.1)
Tetracyclines	28 930 (2.7)
Metronidazole	14 506 (1.4)
Lincosamides (eg, clindamycin)	59 (<0.01)
Glycopeptides (oral vancomycin)	7 (<0.01)
Carbapenems (eg, meropenem)	6 (<0.01)
Cefoxitin	0 (0)
Macrolides	131 576 (12.3)
Sulfonamides	103 384 (9.6)
Cephalosporins (other than cefoxitin)	87 762 (8.2)
Fluoroquinolones	9046 (0.84)
Other	198 (0.02)
Aminoglycosides	72 (<0.01)
Glycopeptides (intravenous vancomycin)	10 (<0.01)

Data are presented as *n* (%).

[a] Some subjects were exposed to >1 individual class of antianaerobic antibiotics.

[b] Broad-spectrum penicillins included amoxicillin-clavulanate, ampicillin-sulbactam, and pivmecillinam.

Demographic Characteristics and Covariates Associated With IBD

Female gender and extreme socioeconomic deprivation were negatively associated with developing IBD (Table 3). Age at cohort entry was associated with developing IBD (HR: 1.21 per year [95% confidence interval (CI): 1.19–1.24]). Family history of IBD, chronic granulomatous disease, and primary sclerosing cholangitis were all significantly associated with an increased hazard of developing IBD, whereas other immunodeficiencies were not ($P > .99$). Calendar year of study entry was not associated with IBD (HR: 0.99 per year [95% CI: 0.97–1.02]). Hospital admissions were associated with increased IBD risk (HR: 1.08 per admission [95% CI: 1.06–1.09]). In multivariable analysis, country and hospital admissions altered the association between antianaerobic antibiotics and IBD by <1% and were not included in final models.

Primary Outcome: Association Between Antianaerobic Antibiotics and IBD

Although 0.07% of subjects developed IBD in both unexposed and exposed groups, the IBD incidence rates (calculated by using observed person-time as denominator) in these groups were 0.83 and 1.52/10 000 person-years, respectively, an absolute risk increase of 0.69 case/10 000 person-years and an 84% relative risk increase. In univariate analysis, any antianaerobic antibiotic exposure was associated with developing IBD ($P < .001$, log-rank test; Fig 2A), a dose-response effect existed ($P < .001$, log-rank test; Fig 2B), and this relationship remained significant throughout childhood (Fig 3).

Because the survival and hazard curves suggested that the relationship between any exposure to antianaerobic antibiotics and IBD decreased with increasing age, we incorporated an interaction term of log(age) to reflect this time-varying association. In multivariate analysis, exposure before 1 year of age was associated with a 5.5-fold increased IBD risk (aHR: 5.51 [95% CI: 1.66–18.28]) compared with those unexposed at that age, with decreasing IBD risks by 5 years (aHR: 2.62 [95% CI: 1.61–4.25]) and 15 years (aHR: 1.57 [95% CI: 1.35–1.84]).

Each antianaerobic antibiotic course was associated with a 6% increase in IBD hazard (aHR: 1.06 [95% CI: 1.04–1.08]), and each week of exposure was associated with a 1% increased hazard (aHR: 1.01 [95% CI: 1.00–1.02]). Exposure to >2 antianaerobic antibiotic courses by 1 year of age was more highly associated with IBD development than exposure to 1 to 2 courses, with an aHR of 4.77 (95% CI: 2.13–10.68) versus 3.33 (95% CI: 1.69–6.58).

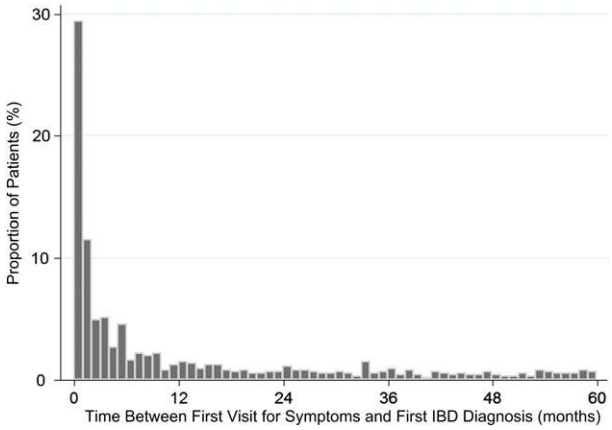

FIGURE 1
Latency period between first diagnosis for IBD-related symptoms (eg, abdominal pain) and first IBD diagnosis. Each bar represents a 1-month interval.

Secondary Associations With IBD and Sensitivity Analyses

Adjusted associations between individual antibiotic class exposure measures and IBD are summarized without the other model covariates in Tables 4 and 5. Exposure to any antibiotic, penicillins, broad-spectrum penicillins, and cephalosporins were associated with IBD development, whereas macrolide, sulfonamide, and tetracycline exposures were not. Antibiotic classes to which <0.05% of subjects were exposed were not included.

The 748 subjects who developed IBD received 1642 antibiotic prescriptions; prescription day diagnoses included 440 (26.8%) head and neck infections, 292 (17.8%) chest infections, 163 (9.9%) skin and soft tissue infections, 82 (5.0%) genitourinary infections, 27 (1.6%) gastrointestinal infections, and 638 (38.9%) without specific infectious diagnoses. Excluding from the analysis those subjects who received antianaerobic antibiotics for gastrointestinal illnesses and developed IBD did not alter the primary association of interest.

Metronidazole or fluoroquinolone exposure was associated with the development of IBD. To determine whether outcome timing misclassification caused these associations, 2 additional sensitivity analyses were performed. For those who developed IBD and were prescribed metronidazole or fluoroquinolones in the year before diagnosis, we reassigned IBD outcome at the initial date of metronidazole or fluoroquinolone prescription. Doing so decreased the magnitude, but did not eliminate, the initial associations: any fluoroquinolone exposure (aHR: 2.09 [95% CI: 1.10–3.98]) and any metronidazole exposure (aHR: 186.25 [95% CI: 10.86–3193.65]) by 1 year of age remained significant. Lastly, among all 14 506 subjects who received metronidazole, prescription day diagnoses were available for 11 936 (82.2%): 34.9% had vaginitis/vaginosis, 15.1% had oral infections (eg, dental abscess), 7.5% had gastrointestinal symptoms, 3.1% had specific infections treated with metronidazole (eg, giardiasis), and 39.5% were for unclassifiable reasons (eg, "telephone encounter").

Results among the 2 IBD subgroups, Crohn's disease and ulcerative colitis, were similar to the main results but did not vary with age (Table 6). Sensitivity analysis assigning all missing Townsend scores first in the highest, then the lower, deprivation category did not alter the primary outcome. Sensitivity analysis using a 1-year latency period altered the primary outcome's precision and magnitude but not its direction: any antianaerobic antibiotic exposure by 1 year of age (aHR: 3.73 [95% CI: 1.17–11.84]); each

TABLE 3 Multivariable Model for the Association Between Antianaerobic Antibiotic Exposure and IBD Development[a]

Variable	*N*	Developed IBD	Unadjusted HR (95% CI)	aHR (95% CI)
Antianaerobic antibiotics[b]				
Exposed	618 663	436	6.00 (1.93–18.66)	5.51 (1.66–18.28)
Unexposed	453 763	312	1 (Reference)	
Family history of IBD				
Present	356	12	42.05 (23.77–74.39)	35.57 (19.67–64.31)
Absent	1 072 070	736	1 (Reference)	
Gender				
Female	516 904	305	0.76 (0.66–0.88)	0.75 (0.64–0.87)
Male	555 522	443	1 (Reference)	
Chronic granulomatous disease				
Present	19	4	286.62 (107.29–765.74)	243.93 (76.89–773.78)
Absent	1 072 407	744	1 (Reference)	
Primary sclerosing cholangitis				
Present	17	9	734.29 (380.36–1417.57)	852.12 (328.43–2210.88)
Absent	1 072 409	739	1 (Reference)	
Socioeconomic deprivation[c]				
Most deprived	866 909	76	0.76 (0.60–0.96)	0.75 (0.57–0.97)
All others	140 769	645	1 (Reference)	

[a] Excludes exposures during the median latency period (3.9 months) before cohort censorship for all subjects.
[b] Represents the increased hazard of developing IBD with exposure before 1 year of age; hazard decreased with increasing age.
[c] Subject numbers do not sum to overall cohort totals due to missing values.

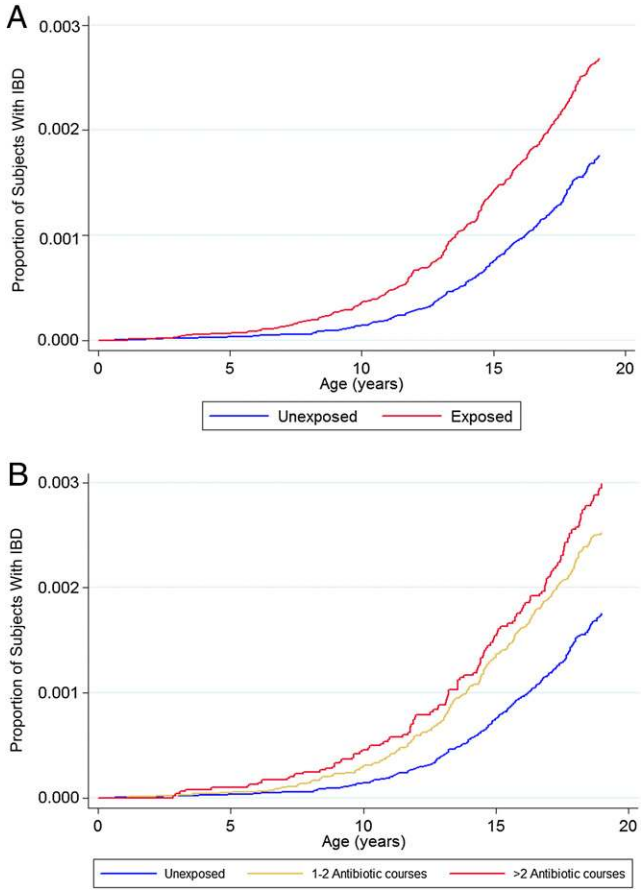

FIGURE 2

A, Proportion of subjects developing IBD according to age and antianaerobic antibiotic exposure status. $P < .001$ for the difference between groups by using the log-rank test. B, proportion of subjects developing IBD according to age and antianaerobic antibiotic exposure level. $P < .001$ for the difference among groups by using the log-rank test.

course (aHR: 1.03 [95% CI: 0.99–1.06]); exposure to 1 to 2 courses (aHR: 2.18 [95% CI: 1.07–4.50]); and exposure to >2 courses (aHR: 4.14 [95% CI: 1.74–9.87]).

DISCUSSION

Our large, population-based cohort study demonstrates that childhood antianaerobic antibiotic exposure was associated with IBD development; increasing early and cumulative exposure was directly associated with IBD risk. Our results suggest that for every 14 300 antianaerobic prescriptions given to children annually, 1 child will develop IBD. An estimated 49 million outpatient pediatric antibiotic prescriptions, approximately one-half for penicillins, occur in the United States annually; our data suggest those prescriptions would be associated with 1700 additional IBD cases yearly.[28]

Other findings in our multivariable model are consistent with previous epidemiologic IBD studies. Girls were 25% less likely to develop IBD as previously observed, and our overall IBD incidence rate (1.2/10 000 person-years) was similar to previous population-based incidence rate estimates among children.[29] Those with extreme social deprivation were less likely to develop IBD, consistent with the "hygiene hypothesis" (persons living in cleaner environments may be more likely to develop autoimmune disorders).[30] Chronic granulomatous disease and primary sclerosing cholangitis, both known to be associated with IBD, were highly but imprecisely associated with IBD, reflecting their rarity.[31,32]

Our findings are consistent with previous studies demonstrating increased IBD risk with earlier and cumulative antibiotic exposure.[18,19,23] The odds of 1.3 for the association between antibiotic use and IBD among adults is consistent with the hazard we noted in older adolescents.[20] Unlike Margolis et al,[22] however, we found no association between tetracycline exposure and IBD, although our subject population was younger and less exposed to tetracycline.

Our findings are consistent with the hypothesis that antianaerobic antibiotic exposure might alter gut flora and trigger increased inflammation in some individuals. Alternately, if specific

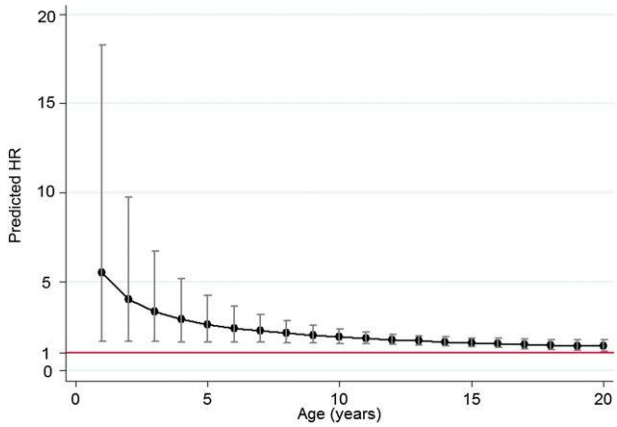

FIGURE 3

Hazard of developing IBD if ever previously exposed to antianaerobic antibiotics, according to age.

TABLE 4 Summary of Adjusted Multivariate Associations Between Antibiotic Exposure and IBD Development According to Antibiotic Class and Exposure Measure[a]

Exposure Measure	Any Antibiotic		Antianaerobic Antibiotics		Penicillins	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Any exposure[b]	5.00 (1.47–17.03)	*P* = .01	5.51 (1.66–18.28)	*P* = .005	5.26 (1.60–17.25)	*P* = .006
Per course	1.04 (1.03–1.05)	*P* < .001	1.06 (1.04–1.08)	*P* < .001	1.06 (1.03–1.08)	*P* < .001
No. of courses[b]						
0	1 (Reference)	—	1 (Reference)	—	1 (Reference)	—
1–2	3.13 (1.54-6.36)	*P* = .002	3.33 (1.69–6.58)	*P* = .001	2.83 (1.44–5.56)	*P* = .003
>2	5.15 (2.36–11.25)	*P* < .001	4.77 (2.13–10.68)	*P* < .001	5.83 (2.59–13.14)	*P* < .001
Per week	1.01 (1.00–1.02)	*P* = .001	1.01 (1.00–1.02)	*P* = .018	1.01 (1.00–1.02)	*P* = .017
No. of weeks[b]						
0	1 [Reference]	—	1 (Reference)	…	1 (Reference)	—
1–2	3.10 (1.50–6.41)	*P* = .002	3.04 (1.51–6.11)	*P* = .002	2.57 (1.28–5.16)	*P* = .008
>2	4.87 (2.30–10.34)	*P* < .001	5.23 (2.43–11.29)	*P* < .001	6.19 (2.84–13.49)	*P* < .001
Exposure Measure	Macrolides		Sulfonamides		Cephalosporins	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Any exposure[c]	1.21 (0.96–1.51)	*P* = .10	1.31 (0.98–1.74)	*P* = .068	1.58 (1.21–2.05)	*P* = .001
Per course	1.03 (0.95–1.11)	*P* = .52	1.03 (0.99–1.08)	*P* = .163	1.04 (1.00–1.07)	*P* = .026
No. of courses[c]						
0	1 (Reference)	—	1 (Reference)	—	1 (Reference)	—
1–2	1.22 (0.96–1.55)	*P* = .11	1.29 (0.95–1.74)	*P* = .10	1.56 (1.18–2.06)	*P* = .002
>2	1.16 (0.69–1.94)	*P* = .59	1.45 (0.67–3.14)	*P* = .34	1.71 (0.88–3.34)	*P* = .12
Per week	1.01 (0.99–1.03)	*P* = .42	1.01 (1.00–1.03)	*P* = .043	1.01 (0.99–1.03)	*P* = .17
No. of weeks[c]						
0	1 (Reference)	—	1 (Reference)	—	1 (Reference)	—
1–2	1.27 (0.98–1.63)	*P* = .06	1.31 (0.96–1.80)	*P* = .092	1.59 (1.20–2.12)	*P* = .001
>2	1.04 (0.68–1.60)	*P* = .86	1.28 (0.69–2.38)	*P* = .44	1.49 (0.81–2.74)	*P* = .20

—, not applicable.

[a] Excludes exposures during the median latency period (3.9 months) before cohort censorship for all subjects. The model included family history of IBD, gender, chronic granulomatous disease, primary sclerosing cholangitis, and socioeconomic deprivation for all listed associations.

[b] Represents the increased hazard of developing IBD with exposure before 1 year of age; hazard decreased with increasing age.

[c] Hazard did not vary according to age.

pathogens are directly associated with IBD, antibiotic exposure might simply be a surrogate for those infectious agents. One might expect such infections to be primarily gastrointestinal, yet only 1.6% of antibiotic prescriptions to our subjects who developed IBD were for gastrointestinal infections, and excluding these subjects from analysis did not change our results. Conversely, gut colonization with certain organisms might protect against IBD (as in the "hygiene hypothesis"), and antibiotic exposure could remove these protective organisms. If, for example, giardiasis protected against IBD, treatment with metronidazole would be associated with developing IBD. This outcome seems less likely, however, because of the multiple antibiotic classes associated with IBD and the diversity of diseases they treat.

A final explanation of our findings could be reverse causality; that is, misclassification of IBD diagnosis timing caused the association, with subjects receiving antianaerobic antibiotics as treatment of as-yet undiagnosed IBD. We addressed this outcome misclassification in multiple ways, however. First, we used validated IBD diagnosis codes.[26,27] Second, we excluded antibiotic exposures for all subjects during the median diagnosis latency period. Sensitivity analysis extending this period to 1 year altered the findings' precision and magnitude but not direction. Third, the strong association between metronidazole and fluoroquinolones (2 potential IBD treatments) and IBD development increased our concern about IBD diagnosis misclassification. However, these class-specific findings remained significant after re-setting the IBD outcome at the first metronidazole or fluoroquinolone prescription in the year before IBD diagnosis, suggesting that outcome misclassification was not significantly present. Moreover, metronidazole exclusively targets anaerobes and therefore may be more associated with developing IBD if altering anaerobes alone is more inflammatory than altering anaerobes and aerobes simultaneously. Lastly, penicillins and cephalosporins are not routine IBD treatments, yet were associated with IBD development. The associations between nonanaerobic antibiotic exposure and IBD may be because those antibiotics have activity against difficult-to-culture anaerobes or because IBD pathogenesis does not

TABLE 5 Summary of Adjusted Multivariate Associations Between Antibiotic Exposure and IBD Development According to Antibiotic Class and Exposure Measure[a]

Exposure Measure	Broad Penicillins		Fluoroquinolones		Tetracyclines	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Any exposure[b]	1.72 (1.32–2.24)	*P* < .001	3.70 (2.25–6.08)	*P* < .001	1.05 (0.65–1.69)	*P* = .85
Per course	1.06 (1.02–1.11)	*P* = .006	1.07 (0.99–1.16)	*P* = .064	0.97 (0.82–1.16)	*P* = .78
No. of courses[b]						
0	1 (Reference)	—	1 (Reference)	—	1 (Reference)	—
1–2	1.66 (1.26–2.19)	*P* < .001	3.71 (2.22–6.19)	*P* < .001	1.02 (0.58–1.78)	*P* = .95
>2	2.42 (1.13–5.18)	*P* = .023	3.57 (0.50–25.53)	*P* = .20	1.13 (0.47–2.75)	*P* = .78
Per week	1.02 (0.99–1.04)	*P* = .061	1.01 (0.98–1.05)	*P* = .51	1.00 (0.98–1.02)	*P* = .91
No. of weeks[b]						
0	1 (Reference)	—	1 (Reference)	—	1 (Reference)	—
1–2	1.62 (1.22–2.15)	*P* = .001	3.81 (2.24–6.48)	*P* < .001	0.75 (0.24–2.33)	*P* = .61
>2	2.52 (1.37–4.62)	*P* = .003	3.07 (0.76–12.36)	*P* = .11	1.14 (0.68–1.93)	*P* = .62

Exposure Measure	Metronidazole	
	HR (95% CI)	*P*
Any exposure[c]	337.78 (37.42–3048.96)	*P* < .001
Per course	1.15 (1.07–1.22)	*P* < .001
No. of courses[c]		
0	1 (Reference)	—
1–2	50.84 (12.71–203.39)	*P* < .001
>2	35.82 (0.02–60450.42)	*P* = .35
Per week	1.04 (1.02–1.07)	*P* < .001
No. of weeks[c]		
0	1 (Reference)	—
1–2	57.25 (13.79–237.67)	*P* < .001
>2	14.51 (0.14–1530.87)	*P* = .26

—, not applicable.

[a] Excludes exposures during the median latency period (3.9 months) before cohort censorship for all subjects. The model included family history of IBD, gender, chronic granulomatous disease, primary sclerosing cholangitis, and socioeconomic deprivation for all listed associations.

[b] Represents the increased hazard of developing IBD with exposure before 1 year of age; hazard decreased with increasing age.

[c] Hazard did not vary according to age.

TABLE 6 Adjusted Association Between Antianaerobic Antibiotic Exposure and IBD Development According to IBD Type and Exposure Measure[a]

Exposure Measure	Crohn's Disease (*n* = 449)[b]		Ulcerative Colitis (*n* = 272)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Any exposure[c]	1.71 (1.41–2.08)	*P* < .001	1.39 (1.08–1.79)	*P* = .011
Per course	1.06 (1.03–1.08)	*P* < .001	1.05 (1.01–1.09)	*P* = .017
No. of courses[c]				
0	1 (Reference)	—	1 (Reference)	—
1–2	1.61 (1.30–2.00)	*P* < .001	1.28 (0.97–1.70)	*P* = .084
>2	1.94 (1.49–2.52)	*P* < .001	1.65 (1.16–2.35)	*P* = .005
Per week	1.01 (0.99–1.02)	*P* = .072	1.01 (0.99–1.02)	*P* = .37
No. of weeks[c]				
0	1 (Reference)	—	1 (Reference)	—
1–2	1.66 (1.33–2.06)	*P* < .001	1.28 (0.96–1.71)	*P* = .091
>2	1.80 (1.40–2.33)	*P* < .001	1.59 (1.14–2.22)	*P* = .007

—, not applicable.

[a] Excludes exposures during the median latency period (3.9 months) before cohort censorship for all subjects. The model included family history of IBD, gender, chronic granulomatous disease, primary sclerosing cholangitis, and socioeconomic deprivation for all listed associations.

[b] Subjects with Crohn's disease and ulcerative colitis do not total 748 because 27 subjects developed IBD with unclassified subtype.

[c] Hazard did not vary according to age.

require perturbations in anaerobic flora alone.

Strengths of our study include its large, population-based nature; comprehensive exposure ascertainment; validated outcome ascertainment; and the results' robustness to multiple sensitivity analyses. Although this study would be neither feasible nor ethical in prospective or randomized fashions, administrative data have limitations, with misclassification most concerning. We addressed outcome misclassification as mentioned earlier. We also addressed exposure misclassification by accounting for potential unobserved inpatient antibiotic exposures, without altering the main findings. We cannot account for antibiotics received without prescription, but this potential exposure misclassification should be nondifferential across our exposed and unexposed groups, biasing the results to the null. Finally, we did not observe all subjects' entire childhood. The earliest subject enrollment was 1994, so subjects enrolled at birth could become as old as 15 years. Because most childhood antibiotics are prescribed in those <5 years of age,[33,34] and median childhood IBD diagnosis age is 12 years,[3] those enrolled from birth were younger, more likely to be antibiotic exposed, and less likely to develop IBD. Those enrolled later in childhood would be more likely to be classified as unexposed and to develop IBD. This possible exposure misclassification would therefore bias our results toward the null and may help explain the decreasing IBD risk we noted with increasing age.

CONCLUSIONS

Exposure to antianaerobic antibiotics during childhood was associated with development of the lifelong autoimmune condition IBD. Our study suggests that reduction in childhood antianaerobic

antibiotic use may have the potential to help curb the rising incidence of childhood IBD. Many unanswered questions remain, however, such as whether specific difficult-to-culture organisms could play roles in either IBD pathogenesis or protection against IBD, and whether alteration of flora through antibiotic exposure alters the immune system directly.

ACKNOWLEDGMENT

The authors thank James D. Lewis, MD, MSCE (Department of Medicine, Department of Biostatistics and Epidemiology, and Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA) for his thoughtful help with the conception and design of this work.

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FINANCIAL DISCLOSURE: *Dr Zaoutis has received research support from Merck and Company, Inc for other projects and has served as a consultant for Pfizer, Inc, HemoCue, Inc, Merck and Company, Inc, Cubist Pharmaceuticals, and Astellas Pharma US, Inc. He has also received a lecture honorarium from Merck and Company, Inc. Dr Coffin previously received research support from Sage Pharmaceuticals, Inc for other projects. Drs Kronman, Haynes, and Feng have indicated they have no financial relationships relevant to this article to disclose.*

FUNDING: Dr Kronman received support from a National Research Service Award Institutional Research Training grant T32-HD043021 at The Children's Hospital of Philadelphia as well as a University of Pennsylvania Center for Education and Research on Therapeutics grant U18HS016946 from the Agency for Healthcare Research and Quality. This work was also supported by a National Institutes of Health Clinical and Translational Science Award (grant UL1-RR024134 to Dr Haynes). No funding agency participated directly in the study design or conduct; data collection, management, analysis, or interpretation; or manuscript preparation, review, or approval. Funded by the National Institutes of Health (NIH).