American Journal of Epidemiology

Vol. 175, No. 8
DOI: 10.1093/aje/kwr400
Advance Access publication: February 24, 2012

Original Contribution

Association of Repeated Exposure to Antibiotics With the Development of Pediatric Crohn's Disease—A Nationwide, Register-based Finnish Case-Control Study

Lauri Virta, Anssi Auvinen, Hans Helenius, Pentti Huovinen, and Kaija-Leena Kolho*

* Correspondence to Dr. Kaija-Leena Kolho, Children's Hospital, University of Helsinki, Box 281, FIN-00029 Helsinki, Finland (e-mail: kaija-leena.kolho@helsinki.fi).

Initially submitted June 10, 2011; accepted for publication October 6, 2011.

To determine whether childhood exposure to antibiotics is associated with the risk of developing inflammatory bowel disease (IBD), the authors conducted a national, register-based study comprising all children born in 1994–2008 in Finland and diagnosed with IBD by October 2010. The authors identified 595 children with IBD (233 with Crohn's disease and 362 with ulcerative colitis) and 2,380 controls matched for age, gender, and place of residence. The risk of pediatric Crohn's disease increased with the number of antibiotic purchases from birth to the index date and persisted when the 6 months preceding the case's diagnosis were excluded (for 7–10 purchases vs. none, odds ratio = 3.48, 95% confidence interval: 1.57, 7.34; conditional logistic regression). The association between Crohn's disease and antibiotic use was stronger in boys than in girls ($P = 0.01$). Cephalosporins showed the strongest association with Crohn's disease (for 3 purchases vs. nonuse, odds ratio = 2.82, 95% confidence interval: 1.65, 4.81). Antibiotic exposure was not associated with the development of pediatric ulcerative colitis. Repeated use of antibiotics may reflect shared susceptibility to childhood infections and pediatric Crohn's disease or alternatively may trigger disease development.

anti-infective agents; case-control studies; child; colitis, ulcerative; Crohn disease; inflammatory bowel diseases

Abbreviations: ATC, Anatomic-Therapeutic-Chemical; CD, Crohn's disease; CI, confidence interval; IBD, inflammatory bowel disease; OR, odds ratio.

The incidence of inflammatory bowel disease (IBD), which encompasses Crohn's disease (CD), ulcerative colitis, and unclassified colitis, has rapidly increased in pediatric populations recently in most high-resource countries (1–7). In Canada, a population-based study showed a 5%–7% annual increase in the incidence of IBD in children under age 10 years from 1995 to 2005 (6). In Finland, the incidence of pediatric IBD increased 6%–8% per year in the early 2000s at ages up to 18 years (7).

The causes for the rapid increase in pediatric IBD remain unknown. Several social-environmental risk factors have been studied (e.g., household characteristics and dietary patterns) in addition to family history of IBD (8–11), but none have been firmly established. Intriguingly, the microbial environment may affect genetically controlled immune responses and thus contribute to the development of immune-mediated disease such as IBD (12, 13). It is agreed that intestinal microbiota play a crucial role in triggering IBD-related inflammation, but the specific role of different bacteria or alterations in bacterial diversity is unresolved (14, 15).

Use of antibiotics modifies the intestinal microbiota for a period of months (16–18), and there is some evidence that antibiotic use might be linked to the development of CD (19–21). Few studies have evaluated the association between exposure to antibiotics and subsequent risk of developing pediatric IBD (19, 20, 22, 23). In most studies, antibiotic exposure was mainly based on recall, not on objective data, or was indirect and related to the frequency of severe infections (24). Further, previous studies mainly assessed the risk of CD, not ulcerative colitis. In adults, antibiotic use prior to diagnosis of CD has been shown to be more frequent than in population-based controls (21). However, no clear

link between the type of antibiotic used and IBD has been established, and the type of antibiotic has not always been reported (19, 24). We used the opportunity provided by the availability of lifetime antibiotic-use data taken from a comprehensive nationwide database of antibiotic purchases to assess exposure to antibiotics and the risk of IBD in children up to 16 years of age. We hypothesized that alteration of intestinal flora by antibiotic use either 1) during the first year of life or 2) during the 2 years prior to IBD diagnosis, excluding an average prediagnostic phase of 6 months, would be associated with the development of pediatric IBD.

MATERIALS AND METHODS

Data sources

We utilized data from 3 Finnish national registers: the Special Reimbursement Register (for identifying cases) and the Drug Purchase Register (for exposure to antibiotics), both maintained by the Social Insurance Institution of Finland, and the Population Register Centre (for identifying controls).

Finland has a national health insurance system funded through tax revenue that covers all 5.3 million residents (25) and is governed by the Social Insurance Institution. Each beneficiary has a unique personal identifier including date of birth and gender, and registry linkage was based on this identifier. All medications prescribed by a physician and reimbursed by the national health insurance system are registered in the Drug Purchase Register (26), including information on dispensation dates for prescriptions and information on pharmaceuticals (27). Antibiotics are only available in pharmacies by prescription. The Drug Purchase Register does not include information on antibiotics administered in hospitals or indications for use (28). Patients with chronic diseases such as IBD are entitled to a higher refund (28), with entitlement decisions being entered in the Special Reimbursement Register. To be eligible for special reimbursement, the diagnosis has to be verified and must meet specific criteria for IBD, including endoscopy and usually histologic verification. The administrative process for decision-making by the Social Insurance Institution takes only a couple of weeks. During 2001–2009, 98% of the special reimbursement applications for IBD were accepted by the Social Insurance Institution; rejections are exceptional, particularly in children. Besides the subtypes of diagnoses for IBD (*International Classification of Diseases*, Tenth Revision, code K50 or K51), the register information includes the date of the special refund decision.

Study population

The case series was identified from the Special Reimbursement Register and comprised a total of 601 children born between January 1, 1994, and December 31, 2008, who had received approval for special reimbursement for IBD (had been diagnosed with IBD) by the end of September 2010. Six children with an unknown home municipality at birth were excluded. For each incident case, 4 eligible control children (children with no reimbursement for IBD) were randomly selected from the Population Register Centre and were individually matched to the cases with regard to date of birth (during the same quarter of the year), gender, and place of residence at birth. For 2 cases, a control child had to be selected from the neighboring municipality because of the small number of children in the locality. Finally, 595 cases and 2,380 controls were included in the analysis.

Antibiotic exposure

To evaluate exposure to antibiotics, we used the Drug Purchase Register to extract information on all antibiotics (Anatomic-Therapeutic-Chemical (ATC) code J01) purchased for the study children from birth to the index date, that is, the date of special reimbursement reflecting the date of diagnosis of IBD. For each control child, purchases of antibiotics were recorded from birth to the index date of the respective IBD case. The number of purchases was a proxy measure for repeated use.

Exposure to antibiotics was analyzed using 3 different time periods of purchases: from birth to the age of 12 months, from birth to the index date, and the 24-month time period preceding the index date. The amount of exposure to antibiotics was studied in 2 ways: first, as overall use of any antibiotics, and second, as number of purchases. Overall use dichotomously compares any purchases of antibiotics with no purchases. Repeated use of antibiotics during the total study period was categorized according to the number of purchases as none, 1–3, 4–6, 7–10, 11–16, or $\geq$17 purchases (with a median of 6 purchases). For the other time periods, the categories of purchases were none, 1, 2, and $\geq$3.

The types of antibiotics used were analyzed in specific groups comprising extended-spectrum penicillins (ATC codes J01CA and J01CR), phenoxymethylpenicillin (ATC code J01CE02), macrolides (ATC code J01F), cephalosporins (ATC code J01D), and combinations of sulfonamides and trimethoprim (ATC code J01E). Their use up to the index date was categorized as none, 1, 2, 3, or $\geq$4 purchases. Specific results are not shown for fluoroquinolones (ATC code J01MA) because of a small number of purchases (they were purchased by only 8.2% of CD cases and 4.1% of ulcerative colitis cases and by $<$1% of control children). Use of metronidazol (ATC codes J01XD01 and P01AB01) is not shown because purchases were infrequent and more than 86% occurred within the 3 months before the study endpoint.

In the subanalyses, purchases of antibiotics during the 6 months preceding the index date were excluded to avoid a possible bias related to prescription of antibiotics for potentially IBD-related symptoms (29, 30). Further, the associations between IBD and antibiotic exposure were controlled for the presence of additional chronic diseases (e.g., asthma, diabetes, epilepsy, juvenile idiopathic arthritis) with a special reimbursement entitlement before the index date of the study.

Ethics

The protocol was approved by the ethical committee of the Social Insurance Institution's Research Department. In accordance with Finnish regulations, no informed consent is required for registry-based studies.

Table 1. Characteristics of Children With Inflammatory Bowel Disease and Their Individually Matched (1:4) Population Controls, by Disease Subtype, Finland, 1994–2010

	Crohn's Disease (n = 233)			Ulcerative Colitis (n = 362)			Controls (n = 2,380)		
	%	Mean (SD)	Median	%	Mean (SD)	Median	%	Mean (SD)	Median
Male gender	63.1			52.2**			56.5		
Calendar year of birth									
1994–1995	38.6			37.8			38.1		
1996–1999	40.8			37.8			39.0		
2000–2008	20.6			24.3			22.9		
Age at study endpoint, years		9.7 (3.9)	11		8.5 (4.1)***	9		9.0 (4.1)	10
Age group, years									
1–5	17.2			27.6			23.7		
6–10	32.2			35.1			33.8		
11–16	50.6			37.3***			42.5		
Year of receiving IBD diagnosis									
1996–2004	12.0			24.0					
2005–2007	27.1			29.8					
2008–2010	60.9			46.2					
History of ≥1 drug-treated chronic diseases other than IBD prior to index date	19.7			17.7			10.6		
Presence of asthma, by age group (years)									
1–5	7.5			2.0			3.0		
6–10	17.3			7.9*			6.1		
11–16	9.3			8.2			6.8		

Abbreviations: IBD, inflammatory bowel disease; SD, standard deviation.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (chi-square test or t test for Crohn's disease vs. ulcerative colitis).

Statistical analyses

The data consisted of individually matched sets with 1 case and 4 controls. The associations between use of antibiotics and the risk of development of IBD were analyzed using conditional logistic regression analysis. The strengths of the associations were quantified using odds ratios with 95% confidence intervals. The analysis for the child's exposure to any antibiotics during the last 24 months was controlled for the presence of additional diagnoses of chronic disease (other than IBD). Further, the analyses of specific antibiotics included adjustment for the total number of antibiotic purchases (except the antibiotic in question). The test for departure from linear trend in the association was performed with the likelihood ratio test. In this test, the difference between the deviance (−2 log likelihood) and the degrees of freedom for the linear model and the deviance and the degrees of freedom for the general type association model were calculated. The interactions between gender and each risk factor were tested by fitting a conditional logistic model which included the main effect of the risk factor and the gender × risk factor interaction term but left the main effect of gender out of the model (31). Cross-tabulation with the chi-square test or ordinary logistic regression analysis was used when associations were separately studied in cases and controls. Statistical significance was set at the 5% level (2-sided P value). Statistical analyses were performed using SAS for Windows (version 9.2; SAS Institute Inc., Cary, North Carolina).

RESULTS

A total of 595 children with IBD, comprising 233 children with CD and 362 with ulcerative colitis, and 2,380 control children were included in the analyses (Table 1). Prior to the index date, every fifth child (19%) with IBD and every tenth (11%) control child had been diagnosed with 1 or more drug-treated chronic diseases (Table 1).

Exposure to all antibiotics

Almost all children in the study had at least 1 antibiotic purchased for them between birth and the index date (94.3% in the IBD group and 92.9% in the controls). During the first 12 months of life, antibiotics were purchased for 52.6% and 52.3% of the IBD case children and the control children, respectively. The respective figures were 60.3% and 51.1% during the 24 months before the index date. The children with a diagnosis of a chronic disease other than IBD (asthma being most frequent) had used antibiotics more often during the past 2 years than those with no such diagnosis.

Table 2. Risk of Pediatric Crohn's Disease and Ulcerative Colitis According to Exposure to Antibiotics During the 2 Years Before the Study Endpoint, Finland, 1994–2010

Outcome, Exposure Period, and Antibiotic Use	No. of Cases	No. of Controls	Crude OR	95% CI	Adjusted[a] OR	95% CI
Crohn's disease						
24 months prior to index date						
None	81	471	1	Referent	1	Referent
Overall	152	461	1.99	1.46, 2.71	1.87	1.37, 2.56
Male	97	296	2.03	1.36, 3.01	1.88	1.25, 2.81
Female	55	165	1.94	1.19, 3.17	1.91	1.16, 3.13
7–24 months prior to index date						
None	113	545	1	Referent	1	Referent
Overall	120	387	1.55	1.14, 2.09	1.46	1.08, 1.98
Male	77	252	1.52	1.04, 2.21	1.43	0.97, 2.10
Female	43	135	1.60	0.98, 2.61	1.56	0.95, 2.57
7–12 months prior to index date						
None	161	748	1	Referent	1	Referent
Overall	72	184	1.95	1.38, 2.74	1.84	1.30, 2.60
Male	50	114	2.40	1.55, 3.71	2.22	1.42, 3.47
Female	22	70	1.38	0.78, 2.45	1.35	0.76, 2.45
6 months prior to index date						
None	150	754	1	Referent	1	Referent
Overall	83	178	2.52	1.81, 3.52	2.47	1.77, 3.46
Male	57	116	2.90	1.90, 4.43	2.81	1.83, 4.32
Female	26	62	2.01	1.16, 3.47	1.99	1.16, 3.46
Ulcerative colitis						
24 months prior to index date						
None	155	692	1	Referent	1	Referent
Overall	207	756	1.25	0.98, 1.60	1.18	0.92, 1.52
Male	111	391	1.36	0.97, 1.90	1.31	0.93, 1.84
Female	96	365	1.14	0.79, 1.63	1.05	0.73, 1.52
7–24 months prior to index date						
None	191	819	1	Referent	1	Referent
Overall	171	629	1.19	0.93, 1.52	1.15	0.89, 1.47
Male	95	321	1.43	1.02, 2.01	1.39	0.98, 1.97
Female	76	308	0.97	0.68, 1.39	0.92	0.64, 1.33
7–12 months prior to index date						
None	286	1,143	1	Referent	1	Referent
Overall	76	305	0.99	0.74, 1.34	0.98	0.72, 1.32
Male	40	159	1.01	0.67, 1.52	1.01	0.66, 1.53
Female	36	146	0.98	0.64, 1.51	0.93	0.60, 1.45
6 months prior to index date						
None	263	1,088	1	Referent	1	Referent
Overall	99	360	1.15	0.88, 1.51	1.09	0.83, 1.43
Male	55	196	1.19	0.82, 1.71	1.14	0.79, 1.65
Female	44	164	1.11	0.74, 1.66	1.03	0.68, 1.55

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Results were controlled for the presence of additional chronic diseases prior to the index date.

Overall use of antibiotics was more common among children with CD than among their controls but did not significantly differ between the ulcerative colitis cases and their controls. In analyses by time period, overall use of antibiotics during the 2 years before the index date was significantly associated with CD (Table 2). Use of antibiotics from

Table 3. Risk of Pediatric Crohn's Disease and Ulcerative Colitis According to Exposure to Antibiotics From Birth Through the Study Period (Excluding the Last 6 Months), Finland, 1994–2010

Outcome, Gender, and Antibiotic Use	No. of Cases	No. of Controls	Crude OR	95% CI	Adjusted[a] OR	95% CI
Crohn's disease						
None	10	75	1	Referent	1	Referent
Overall	223	857	2.18	1.03, 4.61	2.06	0.97, 4.36
Male						
None	1	46	1	Referent	1	Referent
Overall	146	542	12.67	1.73, 92.82	11.86	1.61, 87.37
Female						
None	9	29	1	Referent	1	Referent
Overall	77	315	0.74	0.31, 1.78	0.73	0.30, 1.75
No. of antibiotic purchases						
0	10	75	1	Referent	1	Referent
1–3	40	204	1.62	0.73, 3.58	1.61	0.72, 3.56
4–6	37	196	1.71	0.76, 3.86	1.68	0.74, 3.79
7–10	63	171	3.48	1.57, 7.34	3.19	1.43, 7.13
11–16	46	154	2.93	1.28, 6.68	2.70	1.18, 6.19
≥17	37	132	2.81	1.21, 6.54	2.40	1.02, 5.64
P-trend			0.001		0.009	
Ulcerative colitis						
None	29	131	1	Referent	1	Referent
Overall	333	1,317	1.17	0.74, 1.84	1.11	0.71, 1.76
Male						
None	16	71	1	Referent	1	Referent
Overall	173	685	1.14	0.62, 2.10	1.09	0.59, 2.02
Female						
None	13	60	1	Referent	1	Referent
Overall	160	632	1.20	0.61, 2.37	1.14	0.58, 2.26
No. of antibiotic purchases						
0	29	131	1	Referent	1	Referent
1–3	78	334	1.08	0.66, 1.76	1.07	0.66, 1.74
4–6	72	273	1.24	0.74, 2.08	1.18	0.71, 1.98
7–10	88	307	1.36	0.82, 2.27	1.28	0.77, 2.15
11–16	50	239	0.99	0.57, 1.72	0.89	0.51, 1.55
≥17	45	164	1.31	0.74, 2.33	1.13	0.63, 2.02
P-trend			0.55		0.92	

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Results were controlled for the presence of additional chronic diseases prior to the index date.

birth to the index date or from birth to age 1 year was not significant (data not shown). However, when exposure to antibiotics during the 6 months preceding the index date was excluded, the association with overall use of antibiotics from birth to the index date was significant (Table 3). The association was notably higher in boys than in girls (Table 3), and this gender difference was statistically significant ($P = 0.011$). Overall use of antibiotics 24 months prior to the index date, excluding the last 6 months, somewhat lost strength with the association of CD (Table 2). The risk with overall use varied according to age (comparison related to the median age of the disease group): In children with CD diagnosed before the age of 11 years, the odds ratio was 1.90 (95% confidence interval (CI): 1.20, 2.99), and in older users it was 1.30 (95% CI: 0.86, 1.97). When the associations for overall use of antibiotics during the last 2 years were controlled for the presence of additional chronic diseases, the adjusted odds ratios were somewhat weaker than the crude odds ratios but remained significant in most analyses (Table 2). Overall use of antibiotics during the previous 2 years was not significantly associated with pediatric ulcerative colitis (Table 2).

Table 4. Risk of Pediatric Crohn's Disease and Ulcerative Colitis According to Frequency of Purchase of Antibiotics During the 7–24 Months Before the Study Endpoint, Finland, 1994–2010

Outcome and No. of Antibiotic Purchases	No. of Cases	No. of Controls	Crude OR	95% CI	Adjusted[a] OR	95% CI
Crohn's disease						
0	113	545	1	Referent	1	Referent
1	59	214	1.35	0.95, 1.92	1.32	0.92, 1.88
2	30	71	2.12	1.31, 3.41	1.98	1.22, 3.20
≥3	31	102	1.62	1.00, 2.66	1.42	0.87, 2.33
P-trend			0.004		0.023	
Ulcerative colitis						
0	191	819	1	Referent	1	Referent
1	69	296	1.02	0.75, 1.38	0.98	0.72, 1.34
2	42	127	1.47	0.99, 2.18	1.45	0.98, 2.16
≥3	60	206	1.34	0.93, 1.92	1.26	0.87, 1.82
P-trend			0.05		0.10	

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Controlled for the presence of additional chronic diseases prior to the index date.

Additionally, an increasing number of antibiotic purchases was associated with CD but not with ulcerative colitis. This association with CD was seen from birth to the index date (Table 3). The risk was highest in users with 7–10 purchases, but in users with 11–16 or more purchases, the odds ratio did not further increase ($P = 0.06$ for departure from linearity in the trend). In line with this, an increasing number of antibiotic purchases during the 24 months preceding the index date (excluding the last 6 months) was associated with an increased risk of CD, and the highest risk was seen in users with 2 purchases (Table 4).

Exposure to specific antibiotics

The most commonly used specific antibiotics were extended-spectrum penicillins (91% of CD cases, 86% of ulcerative colitis cases, and 84% of controls), followed by macrolides (70%, 64%, and 61%, respectively) and cephalosporins (64%, 52%, and 52%). Of the cephalosporins, the first-generation cephalosporins comprised the main group (cephalexin was used in 55% of CD cases). Phenoxymethylpenicillin (33%, 26%, and 29%) and combinations of sulfonamides and trimethoprim (33%, 30%, and 29%) were used less frequently.

Exposure to cephalosporins was associated with an increased risk of CD (Figure 1), for both overall use (odds ratio (OR) = 1.89, 95% CI: 1.37, 2.62) and an increasing number of purchases (e.g., for users with 3 purchases compared with nonusers, OR = 2.82, 95% CI: 1.65, 4.81). In addition, during the past 2 years but not during the first year of life, the number of purchases was associated with the risk of CD (Table 5). All children with purchases of cephalosporins also had purchases of other antibiotics.

Borderline statistical significance for trend was seen for exposure to macrolides ($P = 0.048$) and exposure to extended-spectrum penicillins ($P = 0.055$) (Figure 1). From birth to age 1 year, the overall use of phenoxymethylpenicillin was associated with increased risk of CD (Table 5). No other specific antibiotic groups were significantly associated with the risk of IBD.

DISCUSSION

This national population-based case-control study provides evidence that use of antibiotics, especially use of cephalosporins in childhood, predisposes people to CD but not to ulcerative colitis. This risk persisted after exclusion of the 6 months preceding diagnosis (of the case) but was not associated with overall use during the first year of life. The risk was more marked in boys. More frequent purchases of antibiotics were associated with an increased risk of CD, but without a linear exposure-effect relation. Having 7 or more lifetime antibiotic purchases was associated with a doubling of the risk in comparison with having 6 or fewer lifetime antibiotic purchases. Frequent use of antibiotics may interfere with the normal intestinal microbiota and thus predispose people to the development of CD, or it may reflect the fact that CD patients are more prone to infections treated with antibiotics before the manifestation of intestinal disease.

The main strength of this study was the use of comprehensive nationwide data from several registers combined using deterministic record linkage. For the identification of IBD cases, the coverage of the Special Reimbursement Register has been shown to be very high (94%) (7). Furthermore, 98% of the IBD cases met modern diagnostic criteria, indicating excellent specificity (7). Lifetime antibiotic exposure was assessed using the Drug Purchase Register, a nationwide register on medication reimbursements that extensively covers prescription antibiotic purchases—a proxy indicator for actual use. Although compliance could not be assessed, these data suggest better correspondence with actual antibiotic use than would data retrieved from registries based solely on

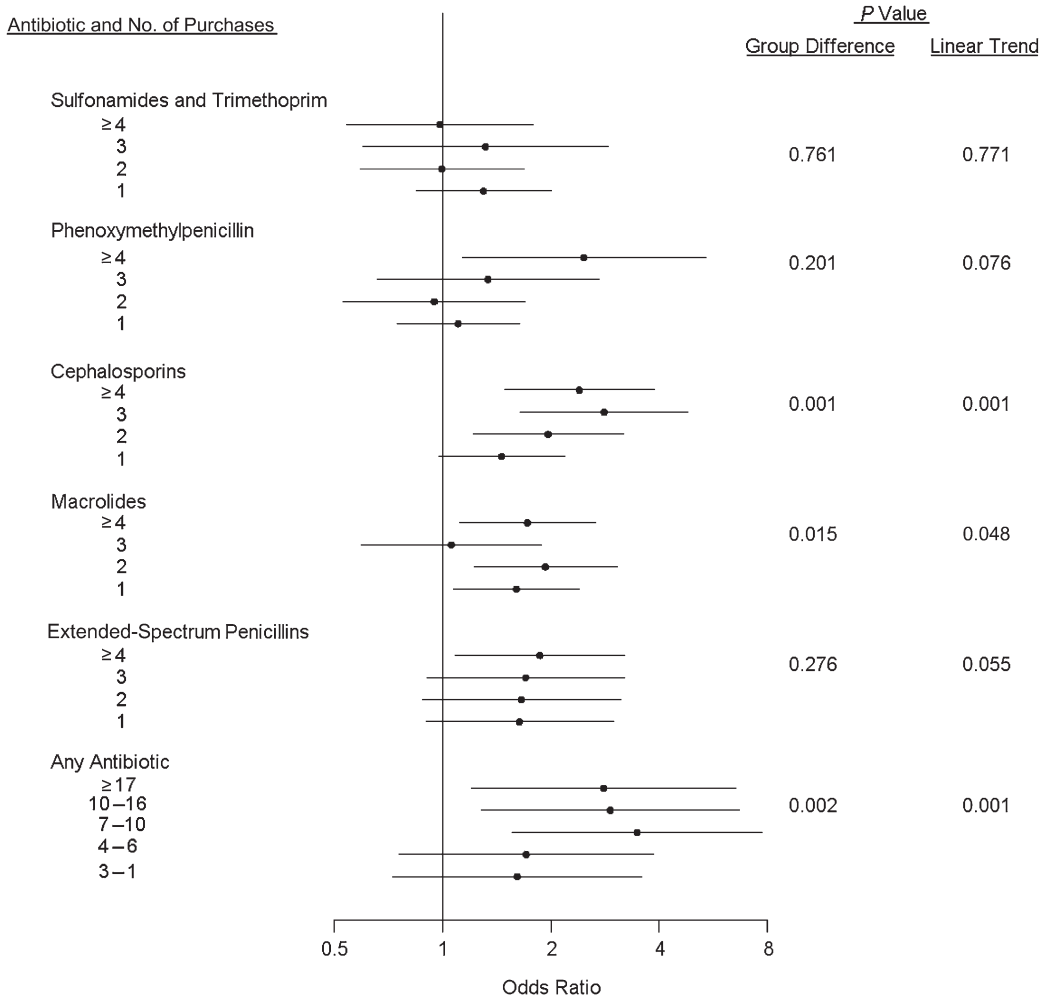

Figure 1. Odds ratios for pediatric Crohn's disease according to exposure to commonly used antibiotics from birth to the index date of the study (excluding the past 6 months), Finland, 1994–2010. No purchase of antibiotics was the reference category (conditional logistic regression analysis). Bars, 95% confidence interval.

prescriptions (32). This register covered 83% of total antibiotic consumption Finnish outpatients during 2006–2009 (28). In 2000–2005 and in 1997–1999, the respective figures were somewhat lower (75% and 70%). Prior to 2006, the most frequent reason for not being covered by the database was the low price of medication (the threshold of €10 was abandoned in 2006). Although the coverage of the Drug Purchase Register was suboptimal during the early years, the missing data would not have caused information bias between the cases and controls. The fact that hospital use was not covered may have caused nondifferential misclassification, diluting the real effect; however, the impact is likely to have been small.

As a limitation, we had no information on the indications for antibiotic use (diagnosis). However, we analyzed the data after exclusion of the 6-month period before diagnosis to minimize the possibility that antibiotics were used for IBD-related symptoms (reverse causality due to protopathic bias (29)). This exclusion did not remove the observed association with antibiotic use and the development of CD. Nevertheless, the latency period for IBD is not well established and is likely to vary between subjects. The controls were matched not only for age and gender but also for place of residence to avoid the effect of regional differences in antibiotic prescriptions. It is also noteworthy that in Finland, eligibility for the basic and special reimbursements is not dependent on a family's socioeconomic situation or place of residence.

The very first study to link antibiotic use to a risk of IBD was a questionnaire survey involving patients with IBD diagnosed by the age of 20 years (19). Another questionnaire-based case-control study in adults showed increased antibiotic use during childhood in persons with CD (20). However, these results were prone to recall bias because of the self-reported exposure information. A study based on a national prescription registry showed an increased risk of CD (adjusted

Table 5. Risk of Pediatric Crohn's Disease According to Exposure to Commonly Used Antibiotics During the 2 Years Before the Study Endpoint and During the First Year of Life, Finland, 1994–2010

Type of Antibiotic, Exposure Period, and No. of Antibiotic Purchases	No. of Cases	No. of Controls	Crude OR	95% CI	Adjusted OR	95% CI
Cephalosporins						
6 months prior to index date						
0	197	893	1	Referent	1[a]	Referent
1	22	30	3.40	1.90, 6.07	3.21	1.77, 5.83
≥2	14	9	8.13	3.25, 20.34	6.11	2.37, 15.75
P-trend			<0.001		<0.001	
7–24 months prior to index date						
0	183	832	1	Referent	1[a]	Referent
1	34	72	2.24	1.43, 3.52	2.10	1.33, 3.33
≥2	16	28	2.59	1.38, 4.83	2.42	1.28, 4.57
P-trend			<0.001		<0.001	
Phenoxymethylpenicillin						
Birth to age 1 year						
0	218	907	1	Referent	1[b]	Referent
≥1	15	25	2.56	1.30, 5.00	2.54	1.30, 4.98

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Results were controlled for the total number of antibiotic purchases (any antibiotic) made for the child during the period and for the presence of additional chronic diseases before the study endpoint.

[b] Results were controlled for the total number of antibiotic purchases (any antibiotic) made for the child during the period.

OR = 1.32) in adults with antibiotic use, but patients with ulcerative colitis were excluded. The finding was not specific, since a similar association was found with use of cardiovascular drugs (21).

There have been only 2 published pediatric registry-based studies on antibiotic use suggesting that early exposure to antibiotics might predispose people to IBD, especially CD. A nationwide Danish cohort study by Hviid et al. (22) comprised 117 cases with IBD and showed a risk ratio of 3.4 for CD in antibiotic users. The children were younger than 11 years, the median age of patients with CD in our study. However, as the authors discussed, confounding by indication may arise if antibiotics are prescribed for intestinal symptoms of yet-undiagnosed IBD, since the highest risk was related to the 3-month period prior to diagnosis (22). Compared with our study, the number of CD cases was small (less than 20 cases in most analyses), the patients were younger, no uniform diagnostic criteria for IBD could be applied, and the exclusion period before diagnosis was only 3 months. In another small case-control study, Shaw et al. (23) investigated antibiotic use in 36 children with IBD and found a significant association between IBD and childhood use of antibiotics. Accordingly, the use of antibiotics for acne has been linked to an increased risk of IBD, particularly CD (33).

Use of antibiotics always interferes with the normal intestinal microbiota and thus may predispose people to the development of CD. However, more frequent antibiotic use may reflect the fact that CD patients are prone to infections treated with antibiotics before the manifestation of intestinal disease. More frequent "physician-diagnosed infections" (34), pneumonia, and otitis media have been reported in patients with subsequent IBD (24). Previously, it was shown that recurrent respiratory infections were more frequent in IBD patients prior to diagnosis than in controls, but only CD was associated with repetitive antibiotic use (19). In line with this, a study showed that hospitalization for pneumonia by the age of 5 years resulted in odds ratios of 2.7 and 4.9 for pediatric and adult-onset CD, respectively (24). Here the odds ratio associating antibiotic use with CD reached 1.9 in children younger than age 11 years as compared with 1.3 in children diagnosed at an older age. So far, there is no explanation for the high odds ratio (exceeding 12) for boys. Whether this association reflects an increased predisposition to infectious diseases and to subsequent antibiotic use in CD is currently not known. Notably, antibiotic use itself may increase risk of further bacterial infections, most likely because of disturbed microbiota (35).

There has been no definite link in any of the previous studies between the type of antibiotic used and IBD. In the early studies, investigators did not report the types of antibiotics used, and the recent small study suggested a role for penicillins and macrolides (23). In our study, repeated use of cephalosporins showed the strongest association with CD, with some indication of excess risk also related to phenoxymethylpenicillin. Although the indications for use could not be traced, the latter most likely reflects therapy for suspected pneumococcal infections, since the risk was present only for use during the first year of life, and use for gastrointestinal infections is unlikely. In Finland, use of cephalosporins is higher than in many other European countries (36), the first-generation cephalosporins comprising the major group. It

is possible that cephalosporins have a greater tendency to modify the intestinal microbiota towards CD development; that is to say, they may alter the relative proportion of disease-associated bacteria such as *Faecalibacterium prausnitzii* in the intestine (37, 38), but so far there has been no such evidence. Recent reports have shown an association between asthma and CD in adults (11, 39, 40), suggesting shared genetic-environmental triggers for these diseases. Here 20% of the IBD patients had an additional chronic disease, most often asthma, diagnosed prior to IBD diagnosis and relating to more frequent use of antibiotics. Notably, antibiotic use has also been linked to the development of asthma (41, 42) and other chronic conditions with possible involvement of autoimmune mechanisms, such as type I diabetes (43). In an animal model, intestinal microbiota have been linked to the development of type I diabetes (44), supporting the possibility that modification of intestinal microbiota following the use of antibiotics may alter immune response and trigger the development of autoimmune diseases. Interestingly, the use of antibiotics prior to 12 months of age did not carry a clearer risk of CD than later use, suggesting that early alteration in microbiota is not a key factor for development of CD.

In summary, repeated use of antibiotics was associated with an increased risk of pediatric CD but not ulcerative colitis. The strength of the association with CD was related to the number of purchases and exposure periods but was not completely linear. Antibiotic use may reflect a shared susceptibility to childhood infections and CD or, alternatively, may trigger disease development.

ACKNOWLEDGMENTS

Author affiliations: Research Department, Social Insurance Institution, Turku, Finland (Lauri Virta); School of Public Health, University of Tampere, Tampere, Finland (Anssi Auvinen); Pediatric Research Centre, University of Tampere and Tampere University Hospital, Tampere, Finland (Anssi Auvinen); Department of Biostatistics, Faculty of Medicine, University of Turku, Turku, Finland (Hans Helenius); Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Turku, Turku, Finland (Pentti Huovinen); Division of Health Protection, National Institute of Health and Welfare, Turku, Finland (Pentti Huovinen); and Children's Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland (Kaija-Leena Kolho).

This work was supported by grants from the Finnish Pediatric Research Foundation, the Päivikki and Sakari Sohlberg Foundation, and the Helsinki University Central Hospital Research Fund.

The authors thank Kristiina Tyrkkö of the Social Insurance Institution for management of the register sample.

The study sponsors played no role in the study design, in the collection, analysis, and interpretation of the data, or in the writing of the article or the decision to submit it for publication. The researchers are independent from the funders and have access to all of the data.

Conflict of interest: none declared.

REFERENCES

1. Benchimol EI, Fortinsky KJ, Gozdyra P, et al. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis*. 2011;17(1):423–439.
2. Armitage E, Drummond HE, Wilson DC, et al. Increasing incidence of both juvenile-onset Crohn's disease and ulcerative colitis in Scotland. *Eur J Gastroenterol Hepatol*. 2001;13(12):1439–1447.
3. Auvin S, Molinié F, Gower-Rousseau C, et al. Incidence, clinical presentation and location at diagnosis of pediatric inflammatory bowel disease: a prospective population-based study in northern France (1988–1999). *J Pediatr Gastroenterol Nutr*. 2005;41(1):49–55.
4. Kappelman MD, Rifas-Shiman SL, Kleinman K, et al. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin Gastroenterol Hepatol*. 2007;5(12):1424–1429.
5. Perminow G, Brackmann S, Lyckander LG, et al. A characterization in childhood inflammatory bowel disease, a new population-based inception cohort from South-Eastern Norway, 2005–07, showing increased incidence in Crohn's disease. *Scand J Gastroenterol*. 2009;44(4):446–456.
6. Benchimol EI, Guttmann A, Griffiths AM, et al. Increasing incidence of paediatric inflammatory bowel disease in Ontario, Canada: evidence from health administrative data. *Gut*. 2009;58(11):1490–1497.
7. Lehtinen P, Ashorn M, Iltanen S, et al. Incidence trends of pediatric inflammatory bowel disease in Finland, 1987–2003, a nationwide study. *Inflamm Bowel Dis*. 2011;17(8):1778–1783.
8. Baron S, Turck D, Leplat C, et al. Environmental risk factors in paediatric inflammatory bowel diseases: a population based case control study. *Gut*. 2005;54(3):357–363.
9. Aspberg S, Dahlquist G, Kahan T, et al. Fetal and perinatal risk factors for inflammatory bowel disease. *Acta Paediatr*. 2006;95(8):1001–1004.
10. Innis SM, Jacobson K. Dietary lipids in early development and intestinal inflammatory disease. *Nutr Rev*. 2007;65(12):S188–S193.
11. Gearry RB, Richardson AK, Frampton CM, et al. Population-based cases control study of inflammatory bowel disease risk factors. *J Gastroenterol Hepatol*. 2010;25(2):325–333.
12. Bernstein CN. Epidemiologic clues to inflammatory bowel disease. *Curr Gastroenterol Rep*. 2010;12(6):495–501.
13. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science*. 2010;330(6012):1768–1773.
14. Tannock GW. The bowel microbiota and inflammatory bowel diseases. *Int J Inflam*. 2010;2010:954051. (doi:10.4061/2010/954051).
15. Mondot S, Kang S, Furet JP, et al. Highlighting new phylogenetic specificities of Crohn's disease microbiota. *Inflamm Bowel Dis*. 2011;17(1):185–192.
16. Edlund C, Beyer G, Hiemer-Bau M, et al. Comparative effects of moxifloxacin and clarithromycin on the normal intestinal microflora. *Scand J Infect Dis*. 2000;32(1):81–85.
17. Jernberg C, Löfmark S, Edlund C, et al. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J*. 2007;1(1):56–66.
18. Lindgren M, Löfmark S, Edlund C, et al. Prolonged impact of a one-week course of clindamycin on Enterococcus spp. in human normal microbiota. *Scand J Infect Dis*. 2009;41(3):215–219.
19. Gilat T, Hacohen D, Lilos P, et al. Childhood factors in ulcerative colitis and Crohn's disease. An international cooperative study. *Scand J Gastroenterol*. 1987;22(8):1009–1024.

20. Wurzelmann JI, Lyles CM, Sandler RS. Childhood infections and the risk of inflammatory bowel disease. *Dig Dis Sci*. 1994; 39(3):555–560.
21. Card T, Logan RF, Rodrigues LC, et al. Antibiotic use and the development of Crohn's disease. *Gut*. 2004;53(2): 246–250.
22. Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. *Gut*. 2011;60(1):49–54.
23. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. *Am J Gastroenterol*. 2010;105(12): 2687–2692.
24. Hildebrand H, Malmborg P, Askling J, et al. Early-life exposures associated with antibiotic use and risk of subsequent Crohn's disease. *Scand J Gastroenterol*. 2008;43(8): 961–966.
25. Niemelä H, Salminen K. *Social Security in Finland*. Helsinki, Finland: Social Insurance Institution (KELA), Finnish Centre for Pensions (ETK), Finnish Pension Alliance (TELA), and Finnish Ministry of Social Affairs and Health; 2006.
26. Furu K, Wettermark B, Andersen M, et al. The Nordic countries as a cohort for pharmacoepidemiological research. *Basic Clin Pharmacol Toxicol*. 2010;106(2):86–94.
27. WHO Collaborating Centre for Drug Statistics Methodology, Norwegian Institute of Public Health. *ATC/DDD Index 2012* [database]. Oslo, Norway: Norwegian Institute of Public Health; 2011. (http://www.whocc.no/atcddd/). (Accessed June 10, 2011).
28. Finnish Medicines Agency and Social Insurance Institution. *Finnish Statistics on Medicines 2009* [in Finnish]. Helsinki, Finland: Finnish Medicines Agency and Social Insurance Institution; 2010.
29. Horwitz RI, Feinstein AR. The problem of ''protopathic bias'' in case-control studies. *Am J Med*. 1980;68(2):255–258.
30. Salas M, Hofman A, Stricker BH. Confounding by indication: an example of variation in the use of epidemiologic terminology. *Am J Epidemiol*. 1999;149(11):981–983.
31. Collett D. *Modelling Binary Data*. 2nd ed. London, United Kingdom: Chapman & Hall Ltd; 2002.
32. Beardon PH, McGilchrist MM, McKendrick AD, et al. Primary non-compliance with prescribed medication in primary care. *BMJ*. 1993;307(6908):846–848.
33. Margolis DJ, Fanelli M, Hoffstad O, et al. Potential association between the oral tetracycline class of antimicrobials used to treat acne and inflammatory bowel disease. *Am J Gastroenterol*. 2010;105(12):2610–2616.
34. Amre DK, Lambrette P, Law L, et al. Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case-control study. *Am J Gastroenterol*. 2006;101(5): 1005–1011.
35. Huovinen P. Bacteriotherapy: the time has come. *BMJ*. 2001; 323(7309):353–354.
36. Goossens H, Ferech M, Vander Stichele R, et al. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet*. 2005;365(9459): 579–587.
37. Sokol H, Pigneur B, Watterlot L, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A*. 2008;105(43): 16731–16736.
38. Jia W, Whitehead RN, Griffiths L, et al. Is the abundance of *Faecalibacterium prausnitzii* relevant to Crohn's disease? *FEMS Microbiol Lett*. 2010;310(2):138–144.
39. Bernstein CN, Wajda A, Blanchard JF. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. *Gastroenterology.* 2005; 129(3):827–836.
40. Hemminki K, Li X, Sundquist J, et al. Subsequent autoimmune or related disease in asthma patients: clustering of diseases or medical care? *Ann Epidemiol*. 2010;20(3):217–222.
41. Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. *Chest*. 2007;131(6): 1753–1759.
42. Risnes KR, Belanger K, Murk W, et al. Antibiotic exposure by 6 months and asthma and allergy at 6 years: findings in a cohort of 1,401 US children. *Am J Epidemiol*. 2011;173(3): 310–318.
43. Kilkkinen A, Virtanen SM, Klaukka T, et al. Use of antimicrobials and risk of type 1 diabetes in a population-based mother-child cohort. *Diabetologia*. 2006;49(1):66–70.
44. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature*. 2008;455(7216):1109–1113.