Antibiotic Exposures and the Likelihood of Developing Pediatric Autoimmune Diseases: a Register-based Matched Case-control Study

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Abstract

Incidences of pediatric autoimmune diseases (ADs) have been increasing without clearly identified environmental risk factors. Using national registers (special reimbursement and drug purchase register), this matched case-control study aims to discover whether antibiotic exposures are related to development of ADs in general. From a cohort of 11,407 children (born 2000-2005, followed-up until December 2018), 242 children with ADs (type 1 diabetes (DM), autoimmune thyroiditis (AIT), juvenile idiopathic arthritis (JIA), and inflammatory bowel diseases (IBD)) generated the case group. Each case was matched with one to four child(ren) with similar age, sex, residential area, gestational age, and delivery mode, generating 708 matched controls. Number of antibiotic purchases throughout childhood was unrelated to the development of the studied ADs as one group, nor with DM, AIT, or IBD individually. While antibiotic exposures in infancy did not increase the likelihood of ADs, exposures to macrolides within two years before diagnosis showed minor association (OR 1.24, 95% CI 1.01-1.51). Exposures to cephalosporins, macrolides, and amoxicillin-clavulanic acid throughout childhood may increase the likelihood of JIA (OR 1.25, 95% CI 1.03-1.52; OR 1.21, 95% CI 1.06-1.38; OR 1.19, 95% CI 1.02-1.39, respectively). Regardless of frequent use in childhood (40% of all antibiotics), penicillins were not associated with the onset of any ADs.

Conclusion: Occasional use of antibiotics are relatively safe regarding overall development of ADs. Penicillins are unlikely to be associated with any ADs, while broad-spectrum antibiotics (including macrolides) should be used considerately as they may associate with an increased likelihood of ADs, especially JIA.

What Is Known

- Role of antibiotics and/or infections in triggering autoimmune diseases have been speculated
- Antibiotic exposures in early childhood have been associated with the development of juvenile idiopathic arthritis (JIA) and inflammatory bowel diseases (IBD), while in type 1 diabetes (DM) the association is controversial
- The role of antibiotic exposures as a potential mutual risk factor for several autoimmune diseases is unclear

What Is New

- Number of antibiotic courses in the first year of life are unlikely to be associated with overall development of pediatric autoimmune diseases
- Exposures to broad-spectrum antibiotics, particularly in early childhood, may increase the likelihood of developing JIA
- Exposures to macrolides may be associated with the development of autoimmune diseases in general, while exposures to penicillins did not seem to be

Introduction

Autoimmune diseases (ADs) are disorders in which the immune system attacks normal tissues. Some ADs such as type 1 diabetes (DM), autoimmune thyroiditis (AIT), juvenile idiopathic arthritis (JIA), and inflammatory bowel diseases (IBD) may have overlapping genetic pathways [1, 2] and similarities in their pathogenesis [3–7]. For unknown reasons, the incidences of these four chronic ADs in pediatric population in Finland have been particularly high, presuming a presence of mutual environmental risk factors [8]. Since Finnish children have used more antibiotics in the first and second year of life compared with Estonian or Russian Karelian children, and since the incidence of ADs in Finland are higher than in eastern Europe, this study focuses on the potential relationship between antibiotics and ADs [9–13].

Previous studies have connected antibiotic exposures (especially in early childhood) with the onset of JIA and Crohn's disease, while findings regarding DM were controversial [14–19]. AIT has been related to tetracycline use in adolescence, but the mechanism remained unspecified [20]. Most of previous studies addressed each disease individually in different settings, making it challenging to estimate whether antibiotic exposures could be associated with the development of pediatric autoimmunity in general, yet manifesting as different diseases. The aim of this register-based matched case-control study is to investigate whether ADs (represented by DM, AIT, JIA, and IBD) might be associated with: (1) number of antibiotic exposures during different periods in childhood; (2) exposures to particular types of. antibiotics.

Methods

Data sources for the study population

The study population was derived from Finnish Health in Teens (Fin-HIT) cohort – a nationwide prospective school-based cohort to address health behaviors of Finnish children and adolescents, comprising over 11,000 children (born 2000-2005) without specific exclusion criteria. More details on recruitment process and characteristics of the cohort have been described elsewhere [21]. The cohort represents children from densely populated areas across Finland, with relatively high maternal socioeconomic status [8]. Using a unique personal identity code of every Finnish resident, children in the Fin-HIT cohort were linked to three well established national registers: (1) the Special Reimbursement Register (SRR) – containing records on patients with chronic diseases requiring medication (including entry dates and physician verified diagnosis), who are entitled to drug refunds (i.e. special reimbursements) regardless of their socioeconomic status; (2) the Drug Purchase Register (DPR) – containing data on all purchased drugs by prescriptions in Finland (including dispensation dates and pharmaceutical information); and (3) the Medical Birth Register (MBR) – containing information on gestational age, delivery modes, and postnatal antibiotic treatment before discharge. These registers are maintained by the Finnish Social Insurance Institution (SRR and DPR) and Finnish Institute for Health and Welfare (MBR).

Identifying children eligible for the matched case-control study

The outcome of this study was the diagnosis of at least one AD at the end of the follow-up (31 December 2018). DM, JIA, and IBD (including Crohn's disease, ulcerative colitis, and IBD unclassified) diagnoses were obtained from the SRR using ICD-10 codes (International Classification of Diseases (ICD), 10th revision): E10 for DM; M08 for JIA; K50 (CD) and K51 (UC/IBDU) for IBD. AIT diagnoses were obtained from the DPR using ATC (Anatomical Therapeutic Chemical) code H03AA01 for thyroxin – the prescription-only drug used for AIT. The excellent coverage of these registers has been described previously [22, 23]. DPR was chosen for identifying AIT because thyroxin is inexpensive and not everyone using this medication is registered in the SRR.

Of over 11,000 children in the Fin-HIT cohort, 242 developed a primary AD after first year of life and generated the case group. Depending on the availability of potential controls, each child in the case group was matched with one to four children from the same cohort with similar age (0-4 days of age difference), sex, and residential area. Since preterm birth has been identified as a potential risk factor for ADs, gestational age (preterm/term), and delivery mode (cesarean section/vaginal delivery) were also considered in the matching [8]. Due to the limited number of potential controls, most children born preterm and/or with cesarean section had only one matching control.

Number and types of antibiotic purchases

Data on perinatal antibiotic treatment in birth hospital were collected from the MBR and outpatient antibiotic purchases from the DPR with ATC codes starting with J01. The data was collected from birth until two months prior to index date (date of diagnosis for children with ADs/ compatible date for their matched controls). The two-month period was chosen to reduce the possibility of including antibiotic purchases during exacerbation phase of ADs.

Antibiotic exposures were analyzed in several observation periods based on age distribution of antibiotic purchases (Figure 1 a): (1) throughout childhood – from birth to the date of diagnosis / the index date; (2) infancy – during the first year of life; (3) toddler phase – from the age 1 up to 3rd birthday/index date (in those who were diagnosed <3 years old); (4) preschool to adolescence – from the age of 3 years to the index date; and (5) within two years before the index date. Due to observed nonlinear associations between antibiotic exposures and ADs, the numbers of antibiotic purchases in each observation periods were first categorized into quartiles and then modified accordingly: no purchases or 0 to Q1 (when the number of children without antibiotic purchases in that specific observation period is low), Q2, and combined Q3-Q4. The odds of developing an AD in relation to the number of antibiotic exposures in each observation period (while exposures in earlier periods were disregarded) were analyzed individually.

Different types of antibiotics were categorized based on level 4 ATC codes (Supplementary Table 1) into 5 groups: (A) penicillins; (B) macrolides; (C) cephalosporines; (D) amoxicillin-clavulanic acid; and (E) sulphonamides and trimethoprim. Clindamycin, tetracyclines, fluoroquinolones and other antibiotics such as nitrofurantoin and metronidazole were considered in the analysis regarding the total number of antibiotic purchases but dismissed from the subgroup analysis due to low frequency of usage. Purchases of different antibiotic subgroups were also analyzed in the same observation periods as above (1-5).

Statistical methods

The background data of cases and controls are presented as mean and standard deviation (SD) or number/proportion (%). The likelihood of developing an AD was obtained by comparing each case and matched control(s) using conditional logistic regression. Results were presented with Odds Ratio (OR) and 95% confidence interval (CI). The software used was IBM SPSS Statistics 26.0 and a 5% statistical significance level was adopted.

Results

The background characteristics of the 242 children who developed ADs (cases) and 708 children who did not (matched controls) are presented in Table 1. Throughout childhood, only 14 (5.8%) children in the case group and 34 (4.8%) in the matched control group had no record of antibiotic purchases (p=0.596). Age at and the type of the first antibiotic exposure did not differ between cases and matched controls (Supplementary Table 2). Penicillins were the most common purchased antibiotics (40% of all antibiotics, of which over 80% were amoxicillin), followed by macrolides (20% of all antibiotics, of which over 80% were azithromycin) (Supplementary Table 1).

Table 1
Background characteristics of children in the study population.

	DM (N=102)	AIT (N=68)	JIA (N=54)	IBD (N=27)	Cases[a] (N=242)	Matched controls[b] (N=708)
Age at the end of follow-up (years), mean±SD	16.5±1.6	17.1±1.1	16.6±1.3	16.8±1.2	16.7±1.4	16.8±1.4
Sex, N (%)						
- Girl	41 (40.2)	47 (69.1)	43 (79.6)	13 (48.1)	140 (57.9)	407 (57.5)
- Boy	61 (59.8)	21 (30.9)	11 (20.4)	14 (51.9)	102 (42.1)	301 (42.5)
Residential area, N (%)						
- Capital (South)	34 (33.3)	20 (29.4)	10 (18.5)	11 (40.7)	72 (29.8)	248 (35.0)
- Inner South	6 (5.9)	7 (10.3)	13 (24.1)	4 (14.8)	30 (12.4)	93 (13.1)
- West	9 (8.8)	18 (26.5)	10 (18.5)	2 (7.4)	36 (14.9)	92 (13.0)
- East	33 (32.4)	18 (26.5)	9 (16.7)	5 (18.5)	63 (26.0)	161 (22.7)
- North	20 (19.6)	5 (7.4)	12 (22.2)	5 (18.5)	41 (16.9)	114 (16.1)
Age of diagnosis/ age at the index date[e], N (%)						
- <6 years	36 (35.3)	3 (4.4)	19 (35.2)	2 (7.4)	59 (24.4)	154 (21.8)
- 6-12 years	42 (41.2)	21 (30.9)	18 (33.3)	11 (40.7)	88 (36.4)	273 (38.6)
- >12 years	24 (23.5)	44 (64.7)	17 (31.5)	14 (51.9)	95 (39.3)	281 (39.7)
Gestational age, N (%)						

[a] Cases=children with autoimmune diseases (represented with DM (type 1 diabetes mellitus), AIT (autoimmune thyroiditis), JIA (juvenile idiopathic arthritis), and IBD (inflammatory bowel diseases)). Nine chidren have two diagnoses. [b] Each child in the case group were matched with one to four children with similar age, sex, residential area, gestational age (preterm/term), delivery mode (cesarean section/vaginal). Due to limited potential controls, most children born preterm/with cesarean section have only one control instead of four.

	DM (N=102)	AIT (N=68)	JIA (N=54)	IBD (N=27)	Cases[a] (N=242)	Matched controls[b] (N=708)
- Term (≥ 37 weeks)	90 (88.2)	63 (92.6)	49 (90.7)	24 (88.9)	217 (89.7)	682 (96.3)
- Preterm (<37 weeks)	9 (8.9)	4 (5.9)	4 (7.4)	3 (11.1)	20 (8.3)	26 (3.7)
- Missing	3 (2.9)	1 (1.5)	1 (1.9)	0	5 (2.0)	0
Delivery mode, N(%)						
- Vaginal	80 (78.4)	58 (85.3)	47 (87.0)	21 (77.8)	198 (81.8)	644 (91.0)
- Cesarean section	19 (18.6)	9 (13.2)	7 (13.0)	6 (22.2)	40 (16.5)	64 (9.0)
- Missing	3 (2.9)	1 (1.5)	0	0	4 (1.7)	0
Maternal antibiotic purchase during pregnancy, N(%)						
- None	79 (77.5)	58 (85.3)	45 (83.3)	23 (85.2)	198 (81.8)	776 (80.9)
- Up to 60 days before delivery	5 (4.9)	1 (1.5)	0	0	6 (2.5)	36 (3.8)
- > 60 days before delivery	18 (17.6)	9 (13.2)	9 (16.7)	4 (14.8)	38 (15.7)	147 (15.3)

[a] Cases=children with autoimmune diseases (represented with DM (type 1 diabetes mellitus), AIT (autoimmune thyroiditis), JIA (juvenile idiopathic arthritis), and IBD (inflammatory bowel diseases)). Nine chidren have two diagnoses. [b] Each child in the case group were matched with one to four children with similar age, sex, residential area, gestational age (preterm/term), delivery mode (cesarean section/vaginal). Due to limited potential controls, most children born preterm/with cesarean section have only one control instead of four.

Table 2

Association between numbers of antibiotic purchases in different periods and the development of an autoimmune disease (DM, AIT, JIA, or IBD)[a].

Antibiotic purchases at different ages, N(%)		Cases[a] (N=242)	Matched controls[b] (N=708)	Odds Ratio (95%CI)[c]
Throughout childhood	**AD**			
(From birth to the index date[d])	<4 courses	53 (21.9)	174 (24.6)	1.00 (Ref)
	4-8 courses	82 (33.9)	223 (31.5)	1.32 (0.87-2.01)
	>8 courses	107 (44.2)	311 (43.9)	1.22 (0.79-1.88)
	DM			
	<4 courses	28 (27.5)	81 (28.9)	1.00 (Ref)
	4-8 courses	36 (35.3)	101 (36.1)	1.07 (0.60-1.93)
	>8 courses	38 (37.3)	98 (35.0)	1.27 (0.69-2.36)
	AIT			
	<4 courses	11 (16.2)	25 (13.2)	1.00 (Ref)
	4-8 courses	24 (35.3)	52 (27.4)	1.08 (0.45-2.58)
	>8 courses	33 (48.5)	113 (59.5)	0.68 (0.29-1.60)
	JIA			
	<4 courses	10 (18.5)	56 (35.9)	1.00 (Ref)
	4-8 courses	15 (27.8)	45 (28.8)	*2.91 (1.05-8.05)*
	>8 courses	29 (53.7)	55 (35.3)	*6.60 (2.12-20.5)*
	IBD			
	<4 courses	5 (18.5)	11 (15.1)	1.00 (Ref)

	4-8 courses	9 (33.3)	21 (28.8)	0.90 (0.21-3.86)
	>8 courses	13 (48.1)	41 (56.2)	0.69 (0.17-2.79)
Infancy (<age of 1 year)	**AD**			
	None	128 (52.9)	356 (50.3)	1.00 (0.72-1.38)
	1-2 courses	85 (35.1)	262 (37.0)	1.00 (Ref)
	≥3 courses	29 (12.0)	90 (12.7)	0.88 (0.54-1.44)
	DM			
	None	59 (57.8)	147 (52.5)	1.09 (0.65-1.83)
	1-2 courses	34 (33.3)	102 (36.4)	1.00 (Ref)
	≥3 courses	9 (8.8)	31 (11.1)	0.66 (0.26-1.65)
	AIT			
	None	37 (54.4)	89 (46.8)	1.37 (0.71-2.62)
	1-2 courses	22 (32.4)	73 (38.4)	1.00 (Ref)
	≥3 courses	9 (13.2)	28 (14.7)	1.00 (0.41-2.44)
	JIA			
	None	24 (44.4)	82 (52.6)	0.76 (0.37-1.54)
	1-2 courses	19 (35.2)	56 (35.9)	1.00 (Ref)
	≥3 courses	11 (20.4)	18 (11.5)	1.71 (0.72-4.11)
	IBD			
	None	14 (51.9)	31 (42.5)	1.01 (0.42-2.45)
	1-2 courses	12 (44.4)	29 (39.7)	1.00 (Ref)
	≥3 courses	1	13 (17.8)	0.21 (0.03-

		(3.7)		1.77)
Toddler phase	**AD**			
(From age of 1 up to third birthday/ the index date[e])	None	45 (23.3)	146 (20.6)	0.98 (0.65-1.50)
	1-2 courses	73 (30.2)	216 (30.5)	1.00 (Ref)
	≥3 courses	124 (51.2)	346 (48.9)	*1.63 (1.14-2.34)*
	DM			
	None	21 (20.6)	56 (20.0)	1.25 (0.66-2.34)
	1-2 courses	27 (26.5)	96 (34.3)	1.00 (Ref)
	≥3 courses	54 (52.9)	128 (45.7)	*1.74 (1.01-3.00)*
	AIT			
	None	11 (16.2)	40 (21.1)	0.60 (0.26-1.37)
	1-2 courses	25 (36.8)	44 (23.2)	1.00 (Ref)
	≥3 courses	32 (26.4)	106 (55.8)	0.81 (0.41-1.60)
	JIA			
	None	13 (24.1)	35 (22.4)	1.47 (0.61-3.47)
	1-2 courses	12 (22.2)	57 (36.5)	1.00 (Ref)
	≥3 courses	29 (53.7)	64 (41.0)	*4.80 (1.90-12.1)*
	IBD			
	None	2 (7.4)	10 (13.7)	0.24 (0.03-1.96)
	1-2 courses	9 (33.3)	17 (23.3)	1.00 (Ref)
	≥3 courses	16 (59.3)	46 (63.0)	1.67 (0.65-4.30)
Preschool to adolsescence	**AD**			

(From age of 3 years to index date)	None	33 (13.6)	96 (13.6)	1.23 (0.68-2.23)
	1-2 courses	38 (15.7)	147 (20.8)	1.00 (Ref)
	≥3 courses	151 (62.3)	40 (57.3)	*1.56 (1.01-2.43)*
	Index date <3 years	20 (8.3)	59 (8.3)	
	DM			
	None	20 (19.6)	54 (19.3)	1.75 (0.69-4.39)
	1-2 courses	13 (12.7)	71 (25.4)	1.00 (Ref)
	≥3 courses	57 (55.9)	126 (45.0)	*2.63 (1.29-5.57)*
	Index date <3 years	12 (11.8)	29 (10.4)	
	AIT			
	None	7 (10.3)	13 (6.8)	1.81 (0.54-6.05)
	1-2 courses	10 (14.7))	29 (15.3)	1.00 (Ref)
	≥3 courses	51 (75.0)	148 (77.9)	1.15 (0.49-2.70)
	Index date <3 years	0	0	
	JIA			
	None	6 (11.1)	27 (17.3)	0.53 (0.15-1.91)
	1-2 courses	9 (16.7)	35 (22.4)	1.00 (Ref)
	≥3 courses	33 (61.1)	72 (46.2)	2.09 (0.82-5.31)
	Index date <3 years	6 (11.1)	22 (14.1)	
	IBD			
	None	1 (3.7)	2 (2.7)	0.86 (0.06-13.2)
	1-2 courses	6 (16.2)	12 (16.4)	1.00 (Ref)

	≥3 courses	18 (66.7)	52 (71.2)	0.69 (0.21-2.22)
	Index date <3 years	2 (7.4)	7 (9.6)	
Purchases within 2 years before index date	**Autoimmune diseases**			
	None	120 (49.6)	371 (52.4)	1.02 (0.71-1.45)
	1-2 courses	70 (28.9)	235 (33.2)	1.00 (Ref)
	≥3 courses	52 (21.5)	102 (14.4)	*1.81 (1.14-2.86)*
	DM			
	None	54 (52.9)	153 (54.6)	1.07 (0.61-1.88)
	1-2 courses	27 (26.5)	83 (29.6)	1.00 (Ref)
	≥3 courses	21 (20.6)	44 (15.7)	1.43 (0.66-3.11)
	AIT			
	None	39 (57.4)	101 (53.2)	1.06 (0.39-2.84)
	1-2 courses	21 (30.9)	64 (33.7)	1.00 (Ref)
	≥3 courses	8 (11.8)	25 (13.2)	1.10 (0.58-2.09)
	JIA			
	None	24 (44.4)	76 (48.7)	1.14 (0.49-2.64)
	1-2 courses	14 (25.9)	56 (35.9)	1.00 (Ref)
	≥3 courses	16 (29.6)	24 (15.4)	*2.67 (1.16-6.16)*
	IBD			
	None	10 (37.0)	37 (50.7)	0.69 (0.25-1.90)
	1-2 courses	9 (33.3)	27 (37.0)	1.00 (Ref)

	≥3 courses	8 (29.6)	9 (12.3)	2.87 (0.72-11.5)

[a] AD=autoimmune diseases. Cases=children with ADs, N=242 (represented with DM (type 1 diabetes mellitus), N=102; AIT (autoimmune thyroiditis), N=68; JIA (juvenile idiopathic arthritis), N=54; and IBD (inflammatory bowel diseases), N=27). Nine children had two diagnoses.

[b] Each child in the case group were matched with one to four children with similar age, sex, residential area, gestational age (preterm/term), and delivery mode (cesarean section/vaginal). Due to limited potential controls, most children born preterm/with cesarean section have only one control instead of four.

[c] Odds ratio and CI (95% Confidence Interval) was obtained using conditional logistic regression.

[d] Throughout childhood, only 14 children in the case group and 34 in the control group had no records of antibiotic purchases.

Index date= date of diagnosis for children who developed autoimmune diseases and compatible date for their matching controls.

[e] When the age of diagnosis were between the age of 1 and 3 years.

Total number of antibiotic purchases throughout childhood (from birth to the date of diagnosis/index date) was not associated with the development of the studied ADs as one group, nor with DM, AIT, and IBD individually. Of all purchased antibiotics, 44.3% (n=3,825) were bought before the age of 3 years. Concerning antibiotic purchases in each age, the only significant difference between cases and matched controls was seen during the second year of life: children who later developed ADs purchased more antibiotics than those who did not develop ADs (OR 1.11, 95%CI 1.03-1.20) (Figure 1). As for antibiotic types, penicillins were not associated with the development of any ADs, while purchases of macrolides within two years before the index date were associated with the onset of ADs in general. Moreover, purchases of broader-spectrum antibiotics (macrolides, cephalosporins, or amoxicillin-clavulanic acid) throughout childhood (but particularly during infancy and toddler phase) increased the likelihood of developing JIA.

Number of antibiotic purchases

The relationship between the total number of antibiotic purchases before the index date and the proportion of ADs was non-linear and required categorized analyses (Figure 2). The highest odds for developing ADs in general (not associated with any specific diagnosis) were observed in children with frequent antibiotic purchases (≥ 3 courses) when compared with those without antibiotic purchases (OR 1.72, 95%CI 1.08-2.74) within the two years before the index date (Supplementary Table 3).

Regarding particular diagnosis, children receiving 4-8 courses and >8 courses had an increased likelihood of developing JIA (OR 2.91, 95% CI 1.05-8.05 and OR 6.60, 95% CI 2.12-20.5 – respectively) compared

with those receiving <4 antibiotic courses throughout childhood. The likelihood of developing JIA was also increased after frequent antibiotic purchases during toddler phase (OR 4.80, 95% CI 1.90-12.1) and within 2 years before the index date (OR 2.67 95% CI 1.16-6.16) when compared with infrequent antibiotic purchases (1-2 courses) in both of these periods. Furthermore, JIA was more common in children with frequent antibiotic purchases (OR 3.94, 95% CI 1.16-13.4) than in those without antibiotic purchases during preschool to adolescence (Supplementary Table 3). As for other ADs, frequent antibiotic purchases (≥ 3 courses) in toddler phase (OR 1.74, 95% CI 1.01-3.00) and from preschool to adolescence (OR 2.63, 95% CI 1.29-5.57) increased the odds of developing DM when compared with infrequent antibiotic purchases during these periods.

Types of antibiotic purchases

When purchases of the five most common antibiotic types in the study population (penicillins, macrolides, cephalosporins, amoxicillin-clavulanic acid, and sulphonamides and trimethoprim) throughout childhood were analyzed individually, none of them was associated with the onset of the four ADs as one group (Supplementary Table 4). The likelihood of JIA was increasing with the number broader-spectrum antibiotics (cephalosporins, macrolides, and amoxicillin-clavulanic acid) purchases, particularly in infancy (OR 2.54, 95%CI 1.01-6.38; OR 1.80, 95%CI 1.08-3.01; and OR 1.03, 95%CI 1.12-3.32 – respectively) and in toddler phase (OR 1.69, 95%CI 1.15-2.48; OR 1.40, 95% CI 1.09-1.79; and OR 1.43, 95%CI 1.03-2.00 – respectively). However, this finding was inconspicuous regarding DM, AIT, and IBD – as their odds were low after purchases of broader-spectrum antibiotics in infancy. Furthermore, purchases of sulphonamides and trimethoprim from preschool to adolescence was associated with DM (OR 1.35, 95%CI 1.03-1.77). Finally, purchases of macrolides within two years before the index date was associated with the development of ADs in general (OR 1.24, 95%CI 1.01-1.51) (Figure 3, Supplementary Table 4), while purchases of penicillin were scarcely ever associated with any types of ADs in this study.

Discussion

Our study is the first to investigate the association between number and types of antibiotic exposures in different stages of childhood and the onset of four common pediatric ADs (DM, AIT, JIA, or IBD) in a mutual setting. We showed that the relationship between the number of antibiotic exposures and the onset of ADs seems to be non-linear. In addition, the likelihood of developing ADs after antibiotic exposures in infancy remained low, especially regarding DM, AIT, and IBD. Despite of being the most common antibiotic used in childhood, penicillins (predominantly amoxicillin) would be relatively safe to use at any age in relation with the development of ADs, while exposures to macrolides within the two years prior to diagnosis may present minor association. Regarding specific diagnosis, the number of antibiotic exposures throughout childhood was mainly associated with JIA. Even then, exposures to penicillin group antibiotics did not increase the likelihood of JIA, while exposures to cephalosporin, macrolide, and amoxicillin-clavulanic (especially before the age of three) might moderately do so.

In our study, antibiotic exposures in infancy did not increase the likelihood of developing any ADs. Furthermore, we showed that frequent antibiotic exposures after first year of life may be related with ADs when compared with occasional exposures, but not when compared with those having no records of antibiotic purchases in each observation period. However, this finding seems to be predominantly related to JIA and DM, but not with AIT and IBD. How do these findings align with previous studies? A Swedish register-based study presented a connection between prescribed antibiotics during infancy and the onset of DM [18], while studies from which countries have not reported significant association between early childhood antibiotic exposures and DM [19, 24]. Studies from United Kingdom and Finland have associated both lifetime and early life antibiotic exposures and the onset of JIA [14, 15]. As for IBD, early antibiotic exposures have been related with Crohn's disease but not with ulcerative colitis [17, 25]. Most of these studies assumed a linear association between antibiotic exposures and the onset of a particular AD, thus assuming that the lowest risk for ADs is retained by those without previous antibiotic exposures. Our study challenges this presumption. Since infancy is the most susceptible period for common infectious diseases and is the period of most frequent antibiotic use [26], it is reassuring that antibiotics used in early childhood hardly increased the likelihood of developing pediatric ADs. Beyond first year of life, occasional antibiotic courses (approximately 1-2 courses per year) can generally be seen as a low-risk approach in relation to the development of ADs.

We showed that exposures to macrolides within the two years before obtaining diagnosis may be related with the overall development of ADs. In addition, exposures to antibiotics with broader spectrum (cephalosporine, macrolide, and amoxicillin-clavulanic acid), particularly before the age of three years, were moderately associated with JIA. On the contrary, penicillins (as the most common antibiotic type used to treat infections throughout childhood) were not related to the development of any ADs at any age. Hence, the relationship between antibiotics and ADs could not solely be explained by number of infections, as often speculated.

Several studies have reported an association between gut dysbiosis and autoimmune diseases [27–30]. Since antibiotics have been shown to have an influence on gut microbial homeostasis [31], antibiotic exposures could as well be related to ADs through facilitating gut dysbiosis. Variations in magnitude and specificity of gut microbiota modification by different antibiotics have been reported, hence requiring variable recovery time after exposures [32–34]. For example, macrolides have both a broad spectrum and long-term influence on gut microbiota that may persist even for several years [35, 36]. In our study, azithromycin was the most often used antibiotic among macrolides. Azithromycin has a broad bacteriostatic spectrum, a marked tissue penetration, a high stability and a low clearance rate due to its long half-life, which enable it to reach a higher cellular concentration compared to penicillin [37]. These characteristics may explain the long-term influences of azithromycin (as a macrolide) on gut microbiota compared with penicillin. Furthermore, previous Fin-HIT study showed that azithromycin presented the strongest inverse association with salivary microbiota diversity [38]. Since dysbiosis of gut and salivary microbiota have also been associated with ADs [39], we suggest that macrolides might catalyze longer-term dysbiosis, explaining their association with the development of ADs, particularly up to two years prior to diagnosis, while penicillin was not associated with any type of ADs at any ages. Further studies to

examine the potential link between the use of broad-spectrum antibiotics, the time of their influence on gut microbiota, gut dysbiosis, and the onset of ADs is warranted.

The strength of our study lies in the comprehensive longitudinal data from national registers. For example, we were able to trace purchased antibiotics as outpatients rather than just prescribed. In addition, we studied several ADs in a mutual setting, using rather homogenous Fin-HIT cohort with small variations in socioeconomic status as the source of study population[8]. The control population was matched with details based on age (with only up to 4 days of age difference), sex, residential area, gestational age, and delivery method to limit the number of potential confounding factors. Therefore, our study setting made it possible to examine the association between childhood antibiotic exposures and onsets of the four pediatric ADs together, and to reliably compare one disease to another in this subject.

As for limitations, we lack the information on the children's genetic susceptibility to infections or to ADs. We also did not know why the antibiotics were purchased – for treating infections (and if so, for what kind of infection) or for prophylactic purposes – and on whether secondary antibiotic courses for the same infection were needed. In addition, we had no access on the length of purchased antibiotic courses nor on the antibiotics given during inpatient care. Yet, antibiotic treatments during hospitalization are often continued orally after discharge, and our data cover these post-discharge antibiotic purchases.

Conclusion

Use of antibiotics in the first year of life and occasional use throughout childhood can be considered relatively safe in relation with the development of pediatric autoimmunity. Antibiotics in the penicillin group are unlikely to be associated with the development of any ADs. In contrast, broad-spectrum antibiotics (including macrolides) should be used considerately as they may associate with an increased likelihood of ADs, especially JIA.

Abbreviations

AD Autoimmune disease

AIT Autoimmune Thyroiditis

ATC Anatomical Therapeutic Chemical

DM Type 1 Diabetes

DPR Drug Purchase Register

Fin-HIT Finnish Health in Teens

IBD Inflammatory Bowel Diseases

JIA Juvenile Idiopathic Arthritis

MBR Maternal Birth Register

SRR Special Reimbursement Register

Declarations

Funding

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Conflict of interest

The authors have no conflicts of interest to declare, nor have any affiliations with financial or non-financial interest in subjects relevant to the content of this manuscript.

Ethics approval

The Fin-HIT study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (decision number 169/13/03/00/10).

Consent to participate

The children and a guardian provided a written informed consent for the register linkage.

Consent for publication

All authors approved the final manuscript as submitted, and agreed to be accountable for all aspects of the work.

Availability of data and material

Data available upon request.

Code availability

N/A

Contributions

LR, EE, HV, KLK conceptualized the study. HV participated in data collection. LR carried out the analyses and interpreted the results with the help of RS and SK, and wrote the initial manuscript, and made the figures and tables. LR and SK searched the literatures. HV and KLK supervised the study. All authors critically reviewed and revised the manuscript for important intellectual content.

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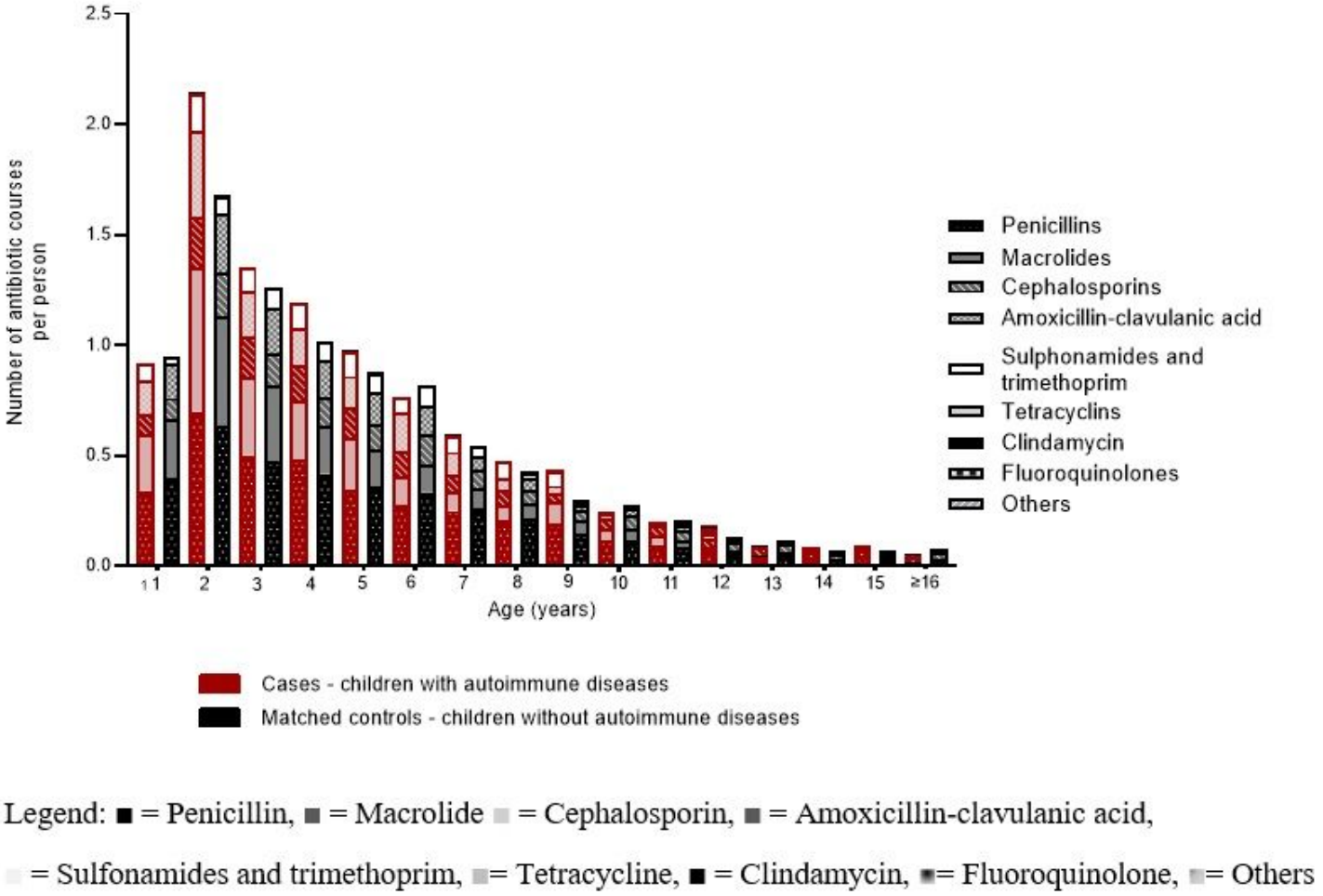
Figures



Legend: ■ = Penicillin, ■ = Macrolide ■ = Cephalosporin, ■ = Amoxicillin-clavulanic acid, ■ = Sulfonamides and trimethoprim, ■= Tetracycline, ■ = Clindamycin, ■= Fluoroquinolone, ■= Others

Figure 1

Purchased antibiotic courses before index date[a] in the matched case-control[b] study. Number of tetracyclin, clindamycin, fluoroquinolone, and other antibiotics courses were relatively low.

[a] Index date= Date of diagnosis for children who developed autoimmune diseases (type 1 diabetes, autoimmune thyroiditis, juvenile idiopathic arthritis, and inflammatory bowel diseases) and compatible date for their matching controls

[b] Cases= 242 children who developed autoimmune diseases by the end of follow-up (31 December 2018). Each child in the case group was matched with one to four children with similar age, sex, residential area, gestational age (preterm/term), and delivery mode (cesarean section/vaginal), comprising control group of 708 children.

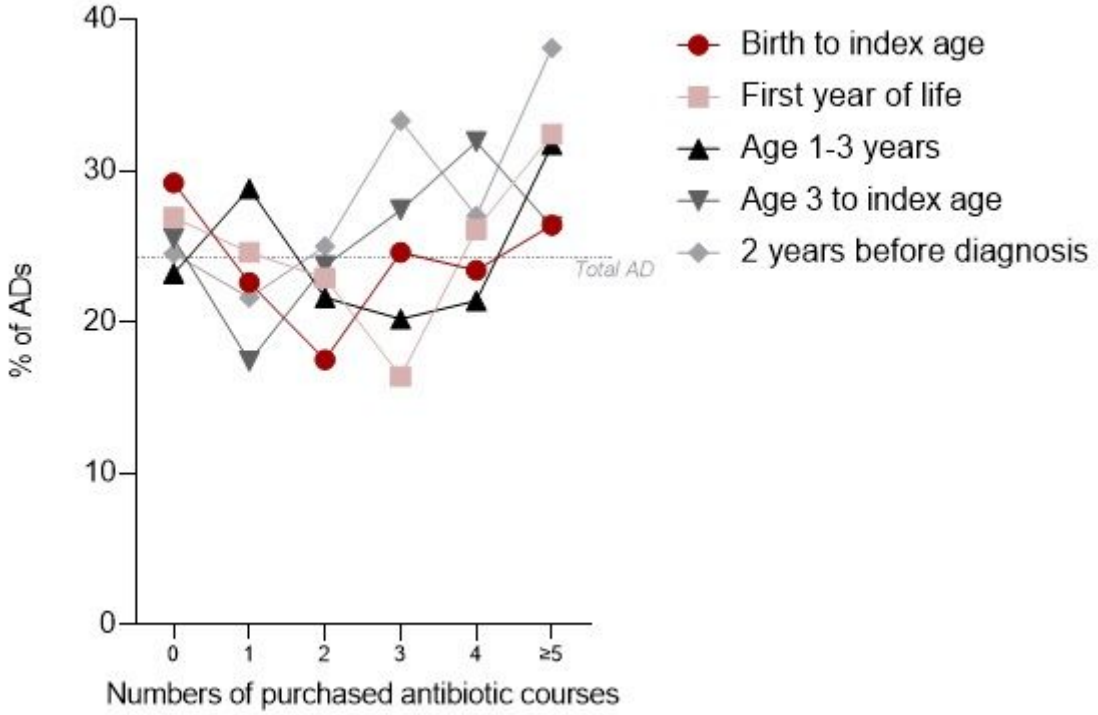


Figure 2

Proportion of autoimmune diseases[a] in groups of children with different number of antibiotic purchases in different periods of childhood.

[a] Autoimmune diseases in this study were type 1 diabetes, autoimmune thyroiditis, juvenile idiopathic arthritis, and inflammatory bowel diseases. Index date = Date of diagnosis for children who developed autoimmune diseases and compatible date for their age, sex, residential areas, gestational age (preterm/term), and delivery method (Cesarean section/vaginal delivery) matched controls.

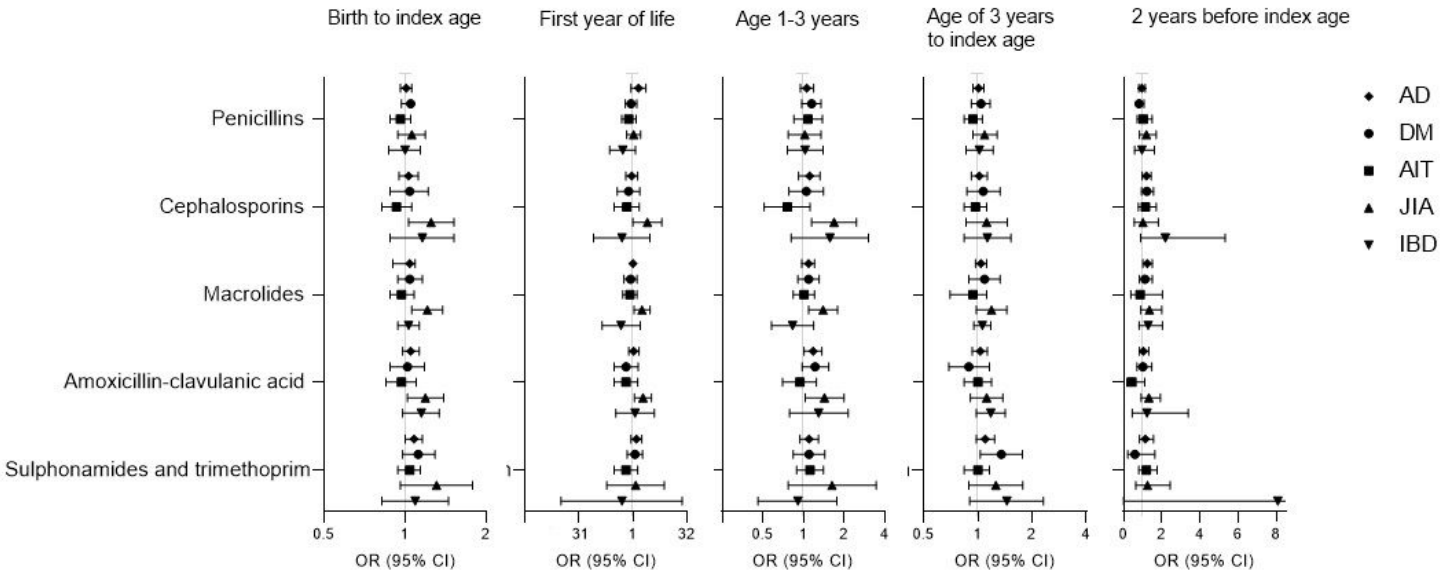


Figure 3

Association between types of antibiotic purchases in different periods and the development of an autoimmune disease (DM, AIT, JIA, or IBD)[a].

[a] AD=autoimmune diseases. Cases=children with ADs (represented with DM (type 1 diabetes mellitus), AIT (autoimmune thyroiditis), JIA (juvenile idiopathic arthritis), and IBD (inflammatory bowel diseases)). Nine children have two diagnoses. OR=Odds ratio, CI=Confidence Interval. Analyses was performed using conditional logistic regression. Index date= age of diagnosis for children who developed autoimmune diseases and compatible date for their matching controls.

Supplementary Files

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