Repeated Exposure to Antibiotics in Infancy: A Predisposing Factor for Juvenile Idiopathic Arthritis or a Sign of This Group's Greater Susceptibility to Infections?

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ABSTRACT. ***Objective.*** Previous exposure to antibiotics has been associated with the pathogenesis of several autoimmune diseases. Our objective was to explore whether childhood exposure to antibiotics would be associated with the risk of developing juvenile idiopathic arthritis (JIA).

Methods. The material was collected from national registers containing all children born in 2000–2010 in Finland and diagnosed with JIA by the end of December 2012 (n = 1298) and appropriate controls (n = 5179) matched for age, sex, and place of birth. All purchases of antibiotics were collected from birth until the index date (i.e., the date of special reimbursement for JIA medications). A conditional logistic regression was performed to evaluate the association between the exposure to antibiotics and the risk of JIA.

Results. The risk of JIA increased with the number of antibiotic purchases from birth to the index date: for ≥ 1 purchases versus none, OR 1.6, 95% CI 1.3–1.9 with an upward trend in OR ($p < 0.001$). Antibiotic groups lincosamides and cephalosporins showed the strongest association with JIA (OR 6.6, 95% CI 3.7–11.7, and OR 1.6, 95% CI 1.4–1.8, respectively). Overall exposure to antibiotics before 2 years of age was associated with an increased risk of JIA (OR 1.4, 95% CI 1.2–1.6), with the trend test of OR ($p < 0.001$).

Conclusion. Previous early and repeated exposure to antibiotics may predispose individuals to develop JIA. Alternatively, the apparent association may reflect shared susceptibility to infections and JIA. (First Release Oct 15 2014; J Rheumatol 2015;42:521–6; doi:10.3899/jrheum.140348)

Key Indexing Terms:
ANTIBACTERIAL AGENTS MATCHED CASE-CONTROL STUDIES CHILD EPIDEMIOLOGY ENVIRONMENTAL EXPOSURE JUVENILE IDIOPATHIC ARTHRITIS

The etiology of juvenile idiopathic arthritis (JIA) is largely unknown. It is believed that the onset of the disease is influenced by genetic, epigenetic, and environmental factors. In most of the JIA subtypes, there is a remarkable linkage of genetic factors with HLA, which are involved in antigen-driven immunity[1]. Studies on monozygotic and dizygotic twins have emphasized the contribution of environmental antigens and HLA-linked immunity in the pathogenesis of JIA[2]. The recognition of potential environmental risk factors could provide an opportunity to prevent the development of JIA.

It is believed that when environmental microbial antigens encounter mucosal membranes, they promote the programming of the immune system[3]. The child's postnatal period is a time for the programming of the immune system, allowing it to tolerate microbial and nutritional antigens[3]. During this period, the colonization of the newborn's mucosal tissue with commensal bacteria promotes intestinal tolerance[3]. However, this process can be impaired by agents like antibiotics, which affect mucosal homeostasis by causing longterm modification of commensal microbial flora[4,5]. Alteration in fecal flora has been associated with the appearance of polyarticular JIA[6] and rheumatoid arthritis (RA)[7,8].

Relatively little is known about the links between

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mucosal flora and mucosal immunity in JIA. Some mucosal alterations in JIA[9,10] resemble the changes seen in type 1 diabetes mellitus and Crohn disease (i.e., HLA-DR expression in intestinal crypts and increased intestinal permeability[11,12,13,14]). The risks for Crohn disease and type 1 diabetes mellitus have been associated with previous exposure to antibacterial agents[15,16,17]. To the best of our knowledge, the potential risk of JIA associated with exposure to antibacterial agents has not been studied previously. We aimed to investigate whether the repeated use of antibiotics would be associated with the development of JIA.

MATERIALS AND METHODS

Data sources. Data were obtained from 3 national registers and linked by the unique personal identity codes (including the date of birth and sex) that are assigned to all Finnish citizens shortly after birth or immigration. The cases were identified through the Special Reimbursement Register for drug costs maintained by the Social Insurance Institution (SII) of Finland with the controls being sampled from the Population Register Centre. Information on purchased antibiotics was obtained from the Drug Prescription Register also maintained by SII. All drugs prescribed by physicians and reimbursed by the National Sickness Insurance Scheme are registered in the Drug Prescription Register, which has been in operation since 1994[18]. In Finland, antibiotics for outpatients are available only by prescription and are sold only in pharmacies. The Drug Prescription Register includes information on drug class [Anatomical Therapeutic Chemical (ATC) Classification system][19] and the dispensing date of the prescription.

Study population. We identified all children (n = 1298) who were born between January 1, 2000, and December 31, 2010, in Finland, and who received a special reimbursement for the cost of antirheumatic drugs based on diagnosed JIA (International Classification of Diseases code M08) by the end of December 2012. In Finland, patients with JIA are entitled to a higher refund[20], which covers disease-modifying antirheumatic drugs (e.g., methotrexate), glucocorticoids for systemic use (e.g., prednisolone), and the immunosuppressant drugs (e.g., azathioprine). To be eligible for special reimbursement, a patient's condition and the diagnosis have to be verified by the pediatric rheumatologist. The drug certificates are checked by a medical examiner, physician, or pharmacist at the SII before entitlement to the special refund can be granted. The administrative process for decision making by the SII takes a couple of weeks. During 2000–2010, 99% of the special reimbursement applications for JIA were approved by the SII. In addition to the diagnosis of JIA, the register information includes the date of the special refund decision and the presence of other chronic diseases (e.g., asthma). For each incident case, 4 eligible control children (with no special reimbursement for JIA) were randomly selected and individually matched according to date of birth (during the same quarter of the year), sex, and municipality of residence at birth. In 7 cases, fewer than 4 controls could be found. Ultimately, our study identified 1298 cases and 5179 matched controls.

Antibiotic exposure. To evaluate a child's exposure to antibiotics, we extracted information on all antibiotics (ATC code J01, antibacterials for systemic use) purchased for the study children from birth to the index date, with the starting date of special reimbursement reflecting the date of diagnosis of JIA. For the control children, exposure to antibiotics was recorded from birth to the index date of the respective JIA case. All in all, the data comprised 8559 purchases of antibiotics for cases and 27,374 for controls before the index date. The mean of the number of purchases of antibiotics was 6.6 (SD = 7.0) for cases and 5.3 (SD = 5.7) for controls.

Exposure to antibiotics was analyzed using 4 different time periods of purchases: from birth to the ages of 6, 12, and 24 months, and to the index date of the study. In addition, the hypothesis in all subjects was reevaluated according to age in very early onset arthritis at age of < 3 years and 3 years or later. The reevaluation was done because the phylogenetic composition of the bacterial communities evolves toward an adult-like configuration within 3 years of age[21] and because the incidence peak of JIA is before the age of 3 years[22]. The amount of exposure to antibiotics was also studied in 2 ways: first, as an overall exposure to any antibiotic, and second, as a repeated exposure to antibiotics. The overall exposure dichotomously compared no purchases versus ≥ 1 purchase of any antibiotic. In the repeated exposure, the total number of antibiotics during the study period was categorized according to the number of purchases as none, 1, 2–3, and ≥ 4 purchases. The type of antibiotics purchased was subdivided into specific groups of antibiotics comprising β-lactam penicillins (ATC code J01C), cephalosporins (J01D), macrolides (J01FA), sulfonamides and trimethoprim (J01E), and lincosamides (J01FF). Within the β-lactam penicillin group, we also tested the hypothesis separately for phenoxymethylpenicillin (ATC code J01CE02), amoxicillin (J01CA04), and amoxicillin with enzyme inhibitor (J01CR02).

Statistical analyses. We analyzed the associations between exposure to antibiotics and the risk of development of JIA, and examined the upward trend in OR using conditional logistic regression analysis. The strengths of associations were quantified using OR with 95% CI. Because asthma may be associated with both the exposure to antibiotics and the outcome, its effect on the OR was examined, but we determined that it had only a minor effect; thus, only the unadjusted OR are presented. Group differences between independent continuous variables were tested by the Student t test, and the differences in proportions were tested by the standard normal deviate test. Statistical significance was set at the 5% level (2-sided). Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp.) and StatsDirect statistical software, Version 2.7.9.

Ethical considerations. According to the Finnish legislation, ethical approval was not necessary because we used only encrypted register data and did not contact the unidentifiable study subjects (Personal Data Act (523/1999).

RESULTS

A total of 1298 children with JIA and 5179 matched control children were included in the analyses. The median age at the diagnosis of JIA was 3.8 years (range 0.8 to 12.9 yrs), and 63% of the individuals in the cases were girls (Table 1). Before the index date, asthma was more common among cases than controls (p = 0.003). In addition, children with a diagnosis of asthma had more purchases of antibiotics than those without asthma (mean 13.4 vs 5.3 purchases, difference 8.1, 95% CI 7.3–8.9, p < 0.001).

Exposure to antibiotics during the first 6 months of life was not significantly associated with a risk of JIA; at least 1 antibiotic regimen was purchased for 15% of the JIA cases compared to 14% of controls. The purchase of ≥ 1 antibiotic regimen during the first 12 months of life was associated with an increased risk of JIA (OR 1.2, 95% CI 1.1–1.4; Figure 1). The strongest associations were observed for cephalosporins (OR 1.3, 95% CI 1.1–1.7) and β-lactam penicillins (OR 1.2, 95% CI 1.1–1.4).

Antibiotic exposure (0 vs any) at < 24 months was associated with an increased risk of JIA (OR 1.4, 95% CI 1.2–1.6), and the risk increased according to the number of purchases (for the purchase of any antibiotics, p for the linear trend < 0.001; Figure 1). The number of purchases of specific antibiotics revealed that the strongest association

Table 1. Characteristics of children with JIA and their individually matched (1:4) population controls.

Characteristics	JIA, n = 1298	Controls, n = 5179
Female sex, n (%)	822 (63.3)	3276 (63.3)
Age groups, yrs at index date		
0.7–2.9	476	1903
3.0–12.9	822	3276
Age at index date, yrs		
Mean (SD)	4.7 (2.8)	4.7 (2.8)
Median (range)	3.8 (0.8–12.9)	3.8 (0.8–12.9)
Presence of asthma, n (%)	63 (4.9)	162 (3.1)

JIA: juvenile idiopathic arthritis.

for lincosamides (0 vs any, OR 3.5, 95% CI 1.3–9.7) and the risk of 0 versus ≥ 2 purchases was 8.0 (95% CI 0.7–88.2; Figure 1). The association did not change substantially in the further analysis according to age at diagnosis of JIA: OR was 1.5 (95% CI 1.2–1.9) for children diagnosed before age 3 years and 1.4 (95% CI 1.1–1.6) for children diagnosed at the age of 3 years or later.

Overall purchases of any antibiotics from birth to diagnosis was associated with an increased risk of JIA (OR 1.6, 95% CI 1.3–1.9). The strongest associations were observed for lincosamides (0 vs any, OR 6.6, 95% CI 3.7–11.7) and cephalosporins (0 vs any, OR 1.6, 95% CI 1.4–1.8). The subanalysis within the β-lactam penicillin group revealed that exposure to phenoxymethylpenicillin (OR 1.2, 95% CI 1.0–1.5) displayed a slightly smaller association for the development of JIA than exposure to amoxicillin (OR 1.4, 95% CI 1.2–1.6) or amoxicillin with enzyme inhibitor (OR 1.3, 95% CI 1.1–1.5). There was an association of increased risk of JIA and the number of purchases analyzed for any antibiotics and separately for specific antibiotics (p for linear trend at least 0.001; Figure 1).

DISCUSSION

To the best of our knowledge, ours is the first register-based, case-control study to examine the antibiotic exposure in relation to the development of JIA. Exposure to antibiotics was associated with an increased risk of JIA, and there was an upward trend in OR in more frequent purchases of antibiotics. This association was also separately observed for specific antibacterial agents. It is possible that the frequent exposure to antibiotics may interfere with the normal intestinal microbiota and increase mucosal permeability and consequently, antigen leakage[10], ultimately contributing to the pathogenesis of arthritis[23].

There was a link between the type of antibiotic purchased and the risk of developing JIA. The exposure risk increased in the following order: macrolides, sulphonamides and/or trimethoprim, penicillins, cephalosporins, and lincosamides. One can suggest that this order reflects either the indication of antibiotics previously reported to be associated with the onset of JIA (e.g., *Streptococcus pyogenes*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*)[24,25], the anti-inflammatory properties of certain antibiotics[26,27], or the different effects of specific antibiotics on mucosal bacterial flora. Certain antibacterial agents, such as lincosamides (clindamycine), may cause long-lasting dysbiosis[28], giving way to a different bacterial community structure[29]. Certain intestinal bacterial species have been shown to be able to trigger arthritis in animal models[30]. Moreover, in a recent human study, fecal prevotella copri was associated with new-onset RA[8]. Still, there is only limited evidence of mucosal dysbiosis in JIA[6,31] or restoration of the immune balance in JIA by the provision of probiotics[32].

In addition to our putative hypothesis that antibiotics can be a risk factor for JIA, it could be postulated that infections themselves may trigger arthritis[25] or repetitive purchase of antibiotics may reflect the susceptibility of patients with JIA to infections. In a large nationwide, register-based, case-control study in Sweden[33], the prevalence of hospitalization for any infection in the first year of life was almost 2-fold in patients who later developed JIA. In particular, gastrointestinal infections appeared to be associated with JIA. In the same study, a tendency was noted for cesarean birth to be a risk factor for JIA. These authors also speculated that early infection could be a risk factor for JIA[33]. In addition, it should be noted that cesarean birth and the concordant use of antibiotics for infection interfere with normal mucosal commensal flora, and this in turn could increase the risk of subsequent bacterial infections[34,35]. In contrast, the rapid recolonization of mucosal commensal flora after a course of antibiotics has been shown to reduce the risk of repeated bacterial infections[35,36].

One limitation of our current study is the absence of information about the JIA categories. Another limitation is the absence of data about antibiotics during hospitalization; hence, there is a lack of information of any parenteral antibiotics. This, in turn, minimizes the input of erroneous data of antibiotics often used before the diagnosis of systemic arthritis is made. The antibiotic purchase and JIA association cannot be explained by the possible antibiotic treatment in cases of systemic arthritis, which also has low incidence in northern countries[22]. However, data of the antibiotic purchase as an indicator of infection is inaccurately low in children under 1 month of age because infections in neonates are, in some instances, treated only with parenteral antibiotics. In older hospitalized children with verified infection, the parenteral medications are continued orally after discharge, and these oral medications are compiled appropriately in the prescription register[20]. Another limitation in this register-based study is the lack of information of any genetic factors or other environmental risk factors that may contribute to the study results.

Subgroup or Antibiotic	Purchases	Cases No.	Controls No.	Odds ratio (95% CI)	Linear trend test
From birth to 12 months					
Penicillins	≥ 1	534	1911	1.2 (1.1 to 1.4)	NA
	0	764	3268	1.0	
Cephalosporins	≥ 1	119	364	1.3 (1.1 to 1.7)	NA
	0	1179	4815	1.0	
Macrolides	≥ 1	262	906	1.2 (1.02 to 1.4)	NA
	0	1036	4273	1.0	
Sulfonamides	≥ 1	80	274	1.2 (0.9 to 1.5)	NA
	0	1218	4905	1.0	
Lincosamides	≥ 1	1	4	1.0 (0.1 to 8.9)	NA
	0	1297	5175	1.0	
Any antibiotics	≥ 1	644	2342	1.2 (1.1 to 1.4)	NA
	0	654	2837	1.0	
From birth to 24 months					
Penicillins	≥ 2	563	1894	1.5 (1.3 to 1.7)	< 0.001
	1	347	1361	1.3 (1.1 to 1.5)	
	0	388	1924	1.0	
Cephalosporins	≥ 2	84	280	1.3 (0.99 to 1.6)	0.001
	1	202	627	1.4 (1.1 to 1.6)	
	0	1012	4272	1.0	
Macrolides	≥ 2	245	831	1.3 (1.1 to 1.5)	0.006
	1	282	1088	1.1 (0.9 to 1.3)	
	0	771	3260	1.0	
Sulfonamides	≥ 2	66	218	1.3 (0.9 to 1.7)	0.039
	1	134	467	1.2 (0.97 to 1.5)	
	0	1098	4494	1.0	
Lincosamides	≥ 2	2	1	8.0 (0.7 to 88.2)	0.014
	1	5	7	2.9 (0.9 to 9.0)	
	0	1291	5171	1.0	
Any antibiotics	≥ 2	782	2725	1.5 (1.3 to 1.7)	< 0.001
	1	233	997	1.2 (0.99 to 1.5)	
	0	283	1457	1.0	
From birth to index date					
Penicillins	≥ 4	471	1586	1.8 (1.5 to 2.2)	< 0.001
	2–3	377	1415	1.5 (1.2 to 1.8)	
	1	235	1030	1.2 (1.01 to 1.5)	
	0	215	1148	1.0	
Cephalosporins	≥ 4	94	222	2.2 (1.7 to 2.9)	< 0.001
	2–3	205	603	1.7 (1.4 to 2.0)	
	1	241	852	1.4 (1.2 to 1.6)	
	0	758	3502	1.0	
Macrolides	≥ 4	173	516	1.5 (1.2 to 1.9)	< 0.001
	2–3	246	900	1.2 (1.03 to 1.5)	
	1	267	1128	1.04 (0.9 to 1.2)	
	0	612	2635	1.0	
Sulfonamides	≥ 4	57	154	1.6 (1.2 to 2.2)	0.001
	2–3	112	415	1.2 (0.9 to 1.5)	
	1	189	640	1.3 (1.1 to 1.5)	
	0	940	3970	1.0	
Lincosamides	≥ 4	0	1	0 (0 to ∞)	< 0.001
	2–3	12	3	16.0 (4.5 to 56.7)	
	1	20	15	5.3 (2.7 to 10.4)	
	0	1266	5160	1.0	
Any antibiotics	≥ 4	763	2623	1.9 (1.5 to 2.3)	< 0.001
	2–3	254	1064	1.5 (1.2 to 1.8)	
	1	141	684	1.2 (0.9 to 1.6)	
	0	140	808	1.0	

NA = not applicable

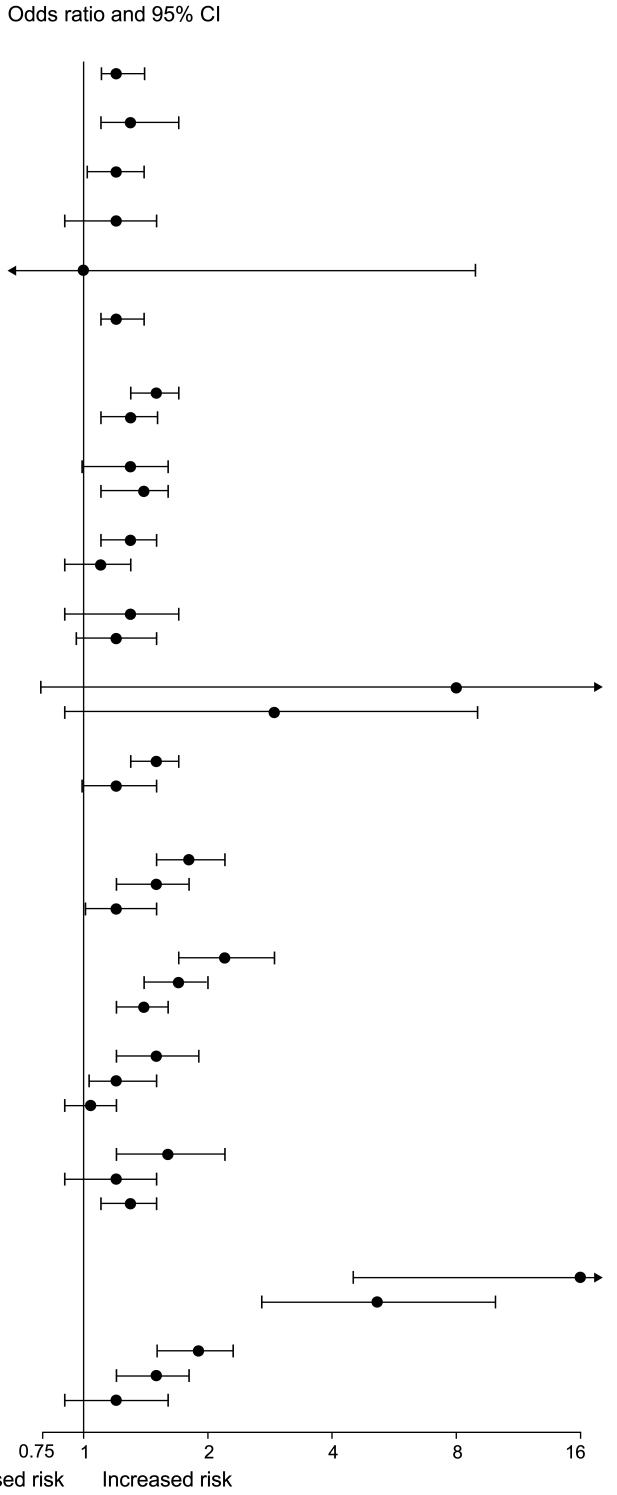

Figure 1. OR for JIA according to exposure to commonly used antibiotics from birth to 12 and 24 months of age, and to the date of special reimbursement of the medications for JIA, Finland, 2000–2012. No purchase of antibiotics was the reference category (conditional logistic regression analysis). JIA: juvenile idiopathic arthritis.

Therefore, more comprehensive data should be collected to confirm the association of antibiotic exposure and development of JIA observed in our study.

If the early use of antibiotics is causally associated with JIA, then antibiotics could be among the first players in a pathological cascade in the development of JIA. It is

important to clarify the potential pathological cascade of mucosal dysbiosis and immune failure by combining epigenetic, microbial, and immunological data. One important factor in this cascade could be the Toll-like receptors (TLR), which are innate immune receptors recognizing conserved structures of the bacteria. We studied mRNA levels of TLR in small intestinal mucosal samples of patients with JIA and found that the expression levels of intestinal TLR 2 and 4 were inversely correlated with JIA activity[9]. Simultaneously, the expression levels of antiinflammatory mediators of intestinal mucosa, FoxP3, interleukin 10, and transforming growth factor-β were downregulated. An optimal TLR stimulus by commensal bacterial antigens[37] is known to be important for regulatory T cell function[38] and for the homeostasis of the intestinal barrier[39,40]. It can be speculated that excess antigen leakage through the intestinal barrier may lead to bystander activation of autoreactive T cells and the disrupted guidance of mucosal T cells by dendritic cells, consequently predisposing the individual to arthritis[41,42,43].

To the best of our knowledge, ours is the first report to suggest that there may be an association between the use of antibiotics in early childhood and later development of JIA. The strength of the association varied according to the type of antibiotic used and it also seems that the risk of JIA increased as the number of purchases of antibiotics increased. The use of antibiotics may reflect a shared susceptibility to infections and JIA, or antibiotics may trigger the development of the disease by intervening with mucosal commensal flora and homeostasis.

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