RESEARCH ARTICLE

Otitis Media, Antibiotics, and Risk of Autism Spectrum Disorder

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Otitis media infections and antibiotic treatment have been linked to the risk of developing autism spectrum disorder. Broad-spectrum antibiotics may alter the composition of the gut flora microbiota, which is hypothesized to be involved in the regulation of the immune system. This study examines the interplay among otitis media, antibiotics, and the subsequent risk of developing autism. Based on the entire Danish population, 780,547 children were followed from birth (January 1, 1997 to December 31, 2008) until December 31, 2012. We calculated adjusted hazard ratios and absolute risks of autism with 95% confidence intervals (CIs) related to previous otitis media diagnoses and antibiotic prescriptions redeemed at Danish pharmacies. The absolute risk of autism before age 10 was increased among children with otitis media (1.2% for females and 3.3% for males) and in children who had redeemed an antibiotic prescription (0.6% and 2.7% for females and males) compared to children without a history of otitis media and antibiotics usage (0.4% for females and 1.9% for males). Similarly, we found an increased hazard ratio of autism associated with otitis media (1.83 95% CI 1.71–1.95) and antibiotics usage (1.29 95% CI 1.17–1.43). A history of both otitis media and antibiotic treatment did not further increase the risk of autism. Although the risk of autism was associated with otitis media and treatment with antibiotics, we found little evidence of a synergistic effect between otitis media infections and treatment with antibiotics. ***Autism Research*** *2018, 11: 1432–1440.* © 2018 International Society for Autism Research, Wiley Periodicals, Inc.

Lay Summary:: We investigated whether otitis media ear infections and antibiotic treatment were associated with autism spectrum disorder. Autism was more common in children who had had an otitis media infection or who had been treated with antibiotics. Given the observational nature of our data, our study cannot be used to conclude that otitis media or use of antibiotics cause autism, as our findings may be subject to unobserved confounding.

Keywords: autism spectrum disorder; otitis media; antibiotics; epidemiology

Introduction

Autism spectrum disorder (autism) is a neurodevelopmental disorder with early childhood onset, affecting approximately 1%–2% of the general population [Hansen, Schendel, & Parner, 2015]. The prevalence is higher among males and the incidence of the diagnosis has increased remarkably over the past three decades [Hansen et al., 2015]. Previous studies have indicated a bidirectional association between otitis media and autism [Adams et al., 2016; Atladottir et al., 2010]. A Danish population-based study showed increased rates of autism in children previously hospitalized for infections, particularly after otitis media [Atladottir et al., 2010], and a recent US study observed a significantly increased rate of otitis media and otitis-related complications in children with autism [Adams et al., 2016].

The antibiotic prescribing rate among children in Denmark has been shown to be relatively stable at a high level from 2000 to 2012, with a gradual shift from narrow-spectrum penicillin V to the broader-spectrum amoxicillin among children younger than 5 years [Pottegard et al., 2015]. Antibiotics affect the microbiome of the intestinal tract and other mucosal sites, and particularly the shift towards higher use of broad-spectrum antibiotics may have long-term consequences [Biedermann & Rogler, 2015]. Alterations and disruptions in the microbiome have been hypothesized to be correlated with

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autism [Mangiola et al., 2016; Mayer, Padua, & Tillisch, 2014; Rosenfeld, 2015; Sharon, Sampson, Geschwind, & Mazmanian, 2016], and it has been suggested that autism may represent a disorder, that may partly be viewed as "a disorder of the brain–gut axis" because up to 70% of patients with autism report symptoms from the gastrointestinal tract [Dinan & Cryan, 2017].

A recent study found an excess risk of severe adult mental disorders among individuals with infections treated with antibiotics, with the most pronounced effects observed for broad-spectrum and moderate-spectrum antibiotics [Kohler et al., 2017]. Another population-based cohort study indicated an association between maternal use of antibiotics during pregnancy and development of autism in the child [Atladottir, Henriksen, Schendel, & Parner, 2012; Sharon et al., 2016]. However, it remains unknown whether it is the infection per se or the antibiotic treatment that increases the risk of autism. The importance of broad-spectrum antibiotics as a potential independent risk factor for autism and/or a potential mediator of the association between otitis media and autism, has not yet been investigated.

We aimed to examine the risk of autism in relation to otitis media and broad-spectrum antibiotics usage. More precisely, we aimed to: (a) explore the association between otitis media and the risk of autism; (b) explore whether broad-spectrum antibiotics usage is associated with the risk of autism; and (c) investigate whether children exposed to otitis media and broad-spectrum antibiotics were associated with an even larger risk increase for autism.

Methods

Data Sources and Study Population

The study was based on linking nationwide Danish registers, using the unique personal identification number assigned to all individuals who were alive and resident in Denmark. Since 1969, the Danish Civil Registration System contains dates of birth, deaths, emigrations, and links to parents [Pedersen, 2011]. Data on all hospital admissions were available from the Danish Psychiatric Central Research Register, computerized in 1969 [Mors, Perto, & Mortensen, 2011], and the Danish National Patient Register, established in 1977 [Lynge, Sandegaard, & Rebolj, 2011]. Since 1995, both registers include information on outpatient visits. Information on birth-related factors such as gestational age and parity was obtained from the Danish Medical Birth Registry [Knudsen & Olsen, 1998]. Information on all redeemed prescriptions were extracted from the Danish National Prescription Registry which covers all pharmacies in Denmark since January 1, 1995 [Pottegard et al., 2016]. Until January 1, 1997, prescriptions redeemed for a child were registered under the personal identification number of the parent. Thus, the study period started January 1, 1997. The study cohort included a total of 780,547 children, covering all births in Denmark between January 1, 1997 and December 31, 2008.

Otitis Media and Other Infections

Otitis media was identified as the first registered diagnosis of otitis media from the Danish National Patient Register, that is, at the date of the first outpatient or emergency room contact, or the date of discharge after inpatient hospitalization with a diagnosis of otitis media (International Classification of Diseases, 10th edition [ICD-10]: H65: nonsuppurative otitis media, H66: suppurative and unspecified otitis media, H67: otitis media in diseases classified elsewhere). Other infections were classified according to ICD-10 codes used in a previous study [Atladottir et al., 2010; Supporting Information Table S1]. Throughout this paper, the term "hospital contacts" refers to all contacts in secondary care, registered in the Danish National Patient Register, including emergency room contacts, outpatient, and inpatient contacts.

Antibiotics

Broad-spectrum antibiotic prescription redemptions were identified using the following anatomical therapeutical chemical codes: J01A, J01D, J01E, J01G, and J01M. In sensitivity analyses, moderate-spectrum and narrow-spectrum antibiotics, including macrolides, were also studied (Supporting Information Table S2). We only considered the first redeemed antibiotic of each category in the analyses, and the individual was then considered exposed from the day of redemption until the end of follow-up.

Autism Spectrum Disorder

Using the Danish Psychiatric Central Research Register, cases of autism were identified by the date of their first diagnosis of autism (ICD-10 codes 84.0, F84.1, F84.5, F84.8, and F84.9).

Statistical Analyses

The individuals included in the study population were followed from the date of birth until first registered diagnosis of autism, death, emigration, or end of follow-up on December 31, 2012. The associations are between: (a) otitis media and autism; and (b) broad-spectrum antibiotics and autism, were investigated in independent models. Furthermore, a model including a combined exposure of otitis media and broad-spectrum antibiotics was analyzed. All exposures were included as time-dependent variables in a Cox regression model, such that

an individual could change status from unexposed to exposed during follow-up. We estimated adjusted hazard rate ratios (aHRs) and 95% confidence intervals (CIs) in partly and fully adjusted models, with age as the underlying time scale. The following potential confounders were considered at birth (baseline): sex, maternal and paternal age (<25, 25–35, ≥35 years), gestational age (<28, 28–37, ≥37 weeks), parity (first-born child, second-born or later-born child), parental history of any psychiatric disorder (any psychiatric contacts (ICD-8: 290-315, ICD-10: F00-99) of mother and/or father since 1969, until the birth of the offspring). Furthermore, we included somatic hospitalizations of the child in the previous year, identified time-dependently as inpatient hospitalization with a nonpsychiatric condition. These potential confounders were chosen a priori according to data availability and known associations with the exposure and outcome [Atladottir et al., 2010; Korvel-Hanquist et al., 2016]. A few variables had missing values: parity, 0.7%; gestational age, 0.6%; paternal age, 0.5%; maternal age, <0.001%. Thus, in 768,379 (98.4%) individuals, we had complete information.

Proportionality of hazards was checked for time-fixed covariates by diagnostic plots, and no major violations were detected. In adjusted analyses, changes in the incidence of autism over calendar time and time-dependent effect of parity were taken into account by allowing separate baseline hazards for different calendar time periods of birth (1997–1999, 2000–2002, 2003–2005, and 2006–2008) and parity (first-born child, second-born or later-born child). A robust variance estimator was applied to control for dependencies between maternal siblings.

To visualize temporal relationships of diagnosis of otitis media and treatment with broad-spectrum antibiotics on the risk of autism, we estimated absolute risks using multistate models. A multistate model deals with data obtained by observing individuals over time, focusing on events or transitions occurring for the individuals by describing transitions between states [Meira-Machado, de Una-Alvarez, Cadarso-Suarez, & Andersen, 2009; Putter, van der Hage, de Bock, Elgalta, & van de Velde, 2006]. In the current study, the followed five states are considered: (a) healthy; (b) otitis media (first diagnosis); (c) broad-spectrum antibiotics (first redeemed prescription); (d) otitis media + broad-spectrum antibiotics (first time both events have occurred); and (e) autism. Similar to the Cox-regression model, individuals were followed from time of birth until first diagnosis of autism or end of follow-up, whichever came first. The process of possible transitions (events) and possible states (exposure/outcome status) is illustrated in Figure 1. In order to avoid the Simpsons paradox [Hernan, Clayton, & Keiding, 2011] because of autism being more prevalent among boys and antibiotics usage being more common among girls (especially the broad-spectrum antibiotics trimethoprim and sulfamethizole because of urinary tract infections [Geerlings, 2016]), all analyses were stratified by sex. To consider the sensitivity of putative associations with otitis media and broad-spectrum antibiotics, we repeated the analyses while including other infections and narrow-spectrum or moderate-spectrum antibiotics into separate categories, respectively.

Secondary Analyses

We repeated the analyses using time-dependent cumulative measures of otitis media and prescriptions of broad-spectrum antibiotics, defined categorically as 0, 1, and 2 or more events. If several hospital contacts with otitis media or several redemptions of prescriptions of antibiotics were registered within 7 days, only the first event was counted. In order to mitigate confounding by geographic patterns in the distribution of the disorders, we repeated the analysis by additionally adjusting for the degree of urbanicity at birth: (a) capital area; (b) suburb to the capital; (c) provincial cities; (d) provincial towns; and (e) rural areas [Pedersen & Mortensen, 2001]. To assess potential differences in the associations by calendar periods, separate analyses were conducted for two birth cohorts (birth years 1997–2001 and 2002–2008). The supplementary file contains hazard rate ratios based on analyses with restrictions on length of follow-up (Supporting Information Table S4), age (Supporting Information Table S5), and timing of exposure (Supporting Information Table S6), as well as hazard rate ratios associated with (Supporting Information Table S7) and adjusted for

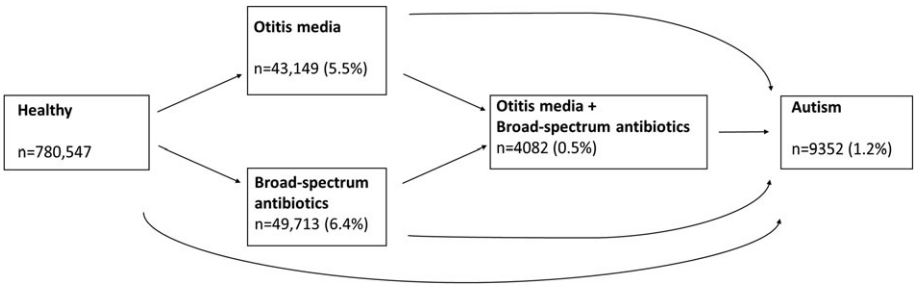

Figure 1. The five-state model showing the eight possible transitions including four possible transitions into autism.

maternal antibiotics usage during pregnancy (Supporting Information Table S8). Lastly, the confounding impact of other infections was examined (Supporting Information Table S9). Supporting Information Tables S1–S9 are shown as supplementary data in the supporting information file. Hazard rate ratios were estimated using Stata version 13. The multistate models were estimated using the mstate package in R version 3.2.2.

Results

Among the 780,547 children who were born in Denmark between January 1, 1997 and December 31, 2008, a total of 9,352 developed autism during the 7,631,510 person-years of follow-up until December 31, 2012, corresponding to an autism incidence of 12 per 10,000 person-years. Analogously, 43,149 individuals were diagnosed with otitis media, corresponding to an incidence rate of 59 per 10,000 person-years at risk. Diagnoses of otitis media were given during admissions to hospital as inpatients (49.4%) and outpatients (42.9%), whereas emergency room contacts constituted 7.8% of all otitis media diagnoses. Among individuals with autism, 14.5% had a diagnosis of intellectual disability. A total of 49,713 individuals redeemed a broad-spectrum antibiotic prescription, corresponding to an incidence rate of 67 per 10,000 person-years at risk. The median ages were 1.5 years (inter-quartile range: 1.0–3.1) for first otitis media diagnosis, 5.4 years (3.3–8.6) for first redeemed broad-spectrum antibiotics, and 7.1 years (4.9–10.0) for first autism diagnosis. Males had higher rates of both otitis media and autism, but used broad-spectrum antibiotics less frequently. The rates of autism, otitis media as well as antibiotic usage were statistically associated with the following baseline factors: low parental age, low gestational age, first-born child, and a parental history of a psychiatric disorder (Table 1).

A diagnosis of otitis media increased the risk of autism (aHR = 1.83, 95% CI 1.71–1.95; Table 2). This association was only slightly attenuated after further adjustment for broad-spectrum antibiotics usage (aHR = 1.81 [1.70–1.94]). Similarly, an increased risk of autism was observed in children who had been exposed to broad-spectrum antibiotics compared to children who had not (aHR = 1.29 [1.17–1.43]). When examining mutually exclusive groups defined by exposure history of otitis media (yes/no) and broad-spectrum antibiotics usage (yes/no), the numerically largest association was found for otitis media (only; aHR = 1.85 [1.73–1.98]). Additional exposure to broad-spectrum antibiotics did not increase the association with autism, aHR = 1.77 (1.36–2.30), indicating no evidence of any synergistic effect between otitis media and broad-spectrum antibiotics usage (Table 2). Analyses conducted separately for males and females similarly indicated effects of otitis media and broad-spectrum antibiotics, both in crude and adjusted models. For otitis media, the association with autism was stronger among females (aHR = 2.41 [2.08–2.80]) than among males (aHR = 1.72 [1.59–1.85]).

Crude autism risks as a function of age are shown in Figure 2. Table 2 shows absolute 10-year risks for males and females. The pattern was similar for males and females, suggesting independent effects of otitis media (twofold increased 10-year risk for males and threefold increased 10-year risk for females) and broad-spectrum antibiotics (1.5-fold increased 10-year risk), but no further autism risk increase was observed among individuals who had both had otitis media and redeemed prescriptions for broad-spectrum antibiotics (Table 2 and Fig. 2).

Individuals who had been exposed to other infections than otitis media were at an increased risk of being diagnosed with autism, aHR = 1.23 (1.17–1.29), but at a statistically significantly lower incidence than individuals with otitis media (aHR = 1.96 [1.83–2.10]). When compared to individuals who had never used antibiotics, broad-spectrum antibiotics usage as well as moderate-spectrum antibiotics usage significantly increased the incidence of autism: aHR = 1.51 (1.33–1.72) and 1.22 (1.13–1.32), respectively, whereas treatment with narrow-spectrum antibiotics only showed limited association with autism (aHR = 1.08 [0.99–1.18]; Table 3). Analogous patterns were seen for males and females, separately (results not shown).

Secondary Analyses

When investigating potential dose–response relationships, we found that, the higher the number of hospital contacts due to otitis media, the higher the rate of autism. However, no clear trend was observed for the association between number of redeemed prescriptions of broad-spectrum antibiotics and autism (Supporting Information Table S3). When additionally adjusting the effect of main model with the otitis media exposure (Table 2), the adjusted hazard ratio estimate did not change markedly, that is, from 1.83 (1.71–1.95) to 1.79 (1.67–1.92). Repeating the main analysis for two different calendar periods of birth (1997–2001 and 2002–2008), showed that the effects of both otitis media and broad-spectrum antibiotics remained statistically significant, with a higher influence of otitis media in later birth years (aHR = 1.70 [1.56–1.86] for years 1997–2001; aHR = 2.04 [1.83–2.26] for years 2002–2008).

Discussion

In this prospective study, we included longitudinal information on diagnosis of otitis media and use of antibiotics

Table 1. Baseline characteristics of the 780,547 persons born in Denmark 1997–2008 who were followed for otitis media (*n* = 43,149), broad-spectrum antibiotics (*n* = 49,713), and autism (*n* = 9,352)

	Total	Otitis media[1] rate/1,000 person-years (95% CI)		Broad-spectrum antibiotics[1] rate/1,000 person-years (95% CI)		Autism[1] rate/1,000 person-years (95% CI)	
Baseline variables	*N* (%)	Females	Males	Females	Males	Females	Males
Total	780,547 (100.0)	4.9 (4.9–5.0)	6.7 (6.8–6.9)	10.7 (10.6–10.8)	3.1 (3.1–3.2)	0.5 (0.5–0.5)	1.9 (1.9–1.10)
Maternal age, years							
<25	103,949 (13.3)	6.0 (5.8–6.2)	8.3 (8.0–8.5)	11.8 (11.5–12.1)	3.3 (3.2–3.5)	0.6 (0.5–0.6)	2.3 (2.1–2.4)
25–34	547,338 (70.1)	4.8 (4.7–4.9)	6.7 (6.6–6.8)	10.5 (10.4–10.7)	3.1 (3.0–3.2)	0.5 (0.4–0.5)	1.8 (1.8–1.9)
≥35	129,255 (16.6)	4.5 (4.4–4.7)	6.4 (6.2–6.6)	10.3 (10.0–10.5)	3.0 (2.9–3.2)	0.6 (0.5–0.6)	2.0 (1.9–2.2)
Paternal age, years							
<25	47,882 (6.2)	5.9 (5.6–6.3)	8.8 (8.4–9.2)	12.2 (11.8–12.7)	3.3 (3.1–3.6)	0.5 (0.5–0.6)	2.3 (2.1–2.5)
25–34	480,071 (61.8)	4.9 (4.8–5.0)	6.8 (6.7–6.9)	10.7 (10.6–10.8)	3.1 (3.1–3.2)	0.5 (0.4–0.5)	1.8 (1.8–1.9)
≥35	248,585 (32.0)	4.5 (4.3–4.6)	6.5 (6.4–6.7)	10.3 (10.0–10.5)	3.0 (2.9–3.1)	0.5 (0.5–0.6)	2.0 (1.9–2.1)
Gestational age, weeks							
<28	8,103 (1.0)	13.2 (11.9–14.6)	15.6 (14.3–17.1)	10.6 (9.5–11.8)	4.5 (3.8–5.3)	0.8 (0.6–1.2)	2.8 (2.3–3.4)
28–36	43,090 (5.6)	7.4 (7.0–7.8)	9.8 (9.4–10.2)	11.5 (11.0–12.0)	3.6 (3.3–3.8)	0.6 (0.5–0.8)	2.1 (1.9–2.3)
≥37	724,828 (93.4)	4.7 (4.7–4.8)	6.6 (6.5–6.7)	10.6 (10.5–10.8)	3.1 (3.0–3.1)	0.5 (0.5–0.5)	1.9 (1.9–2.0)
Parity							
First-born child	339,047 (43.7)	5.2 (5.1–5.3)	7.0 (6.9–7.2)	11.4 (11.3–11.6)	3.4 (3.3–3.5)	0.6 (0.5–0.6)	2.3 (2.2–2.4)
Second-born or later-born	436,076 (56.3)	4.7 (4.6–4.8)	6.7 (6.6–6.8)	10.1 (10.0–10.2)	2.9 (2.8–2.9)	0.4 (0.4–0.5)	1.6 (1.6–1.7)
Parental history of psychiatric disorder							
Yes	59,463 (7.6)	7.3 (7.0–7.6)	10.2 (9.8–10.6)	11.9 (11.5–12.4)	3.2 (3.0–3.5)	0.8 (0.7–0.9)	3.3 (3.1–3.5)
No	721,084 (92.4)	6.7 (6.6–6.8)	6.6 (6.5–6.7)	10.6 (10.5–10.7)	3.1 (3.0–3.2)	0.5 (0.5–0.5)	1.8 (1.8–1.9)

[1] Data were censored at the first autism diagnosis, emigration, death, or December 31, 2012.

Table 2. Adjusted hazard ratios (aHRs) and 10-year risks for autism by otitis media, broad-spectrum antibiotics and mutually exclusive combinations (n = 780,547)

Exposures (time-dependent)	Total person-years	N (autism)	aHR[1]	95% CI	aHR[2]	95% CI	Females absolute 10-year risk[3] (%)	95% CI (%)	Males absolute 10-year risk[3] (%)	95% CI (%)
No otitis media (ref)	7,292,452	8,351	1.00		1.00		0.42%	(0.39–0.44)	1.89%	(1.84–1.94)
Otitis media	339,059	1,001	1.94	(1.82–2.08)	1.83	(1.71–1.95)	1.18%	(0.99–1.37)	3.32%	(3.06–3.58)
No broad-spectrum antibiotics (ref)	7,372,895	8,948	1.00		1.00		0.41%	(0.39–0.44)	1.89%	(1.84–1.95)
Broad-spectrum antibiotics	258,616	404	1.37	(1.24–1.52)	1.29	(1.17–1.43)	0.60%	(0.42–0.78)	2.66%	(2.21–3.10)
Combined exposure										
No otitis media or broad-spectrum antibiotics (ref)	7,056,027	8,005	1.00		1.00		0.41%	(0.39–0.44)	1.89%	(1.84–1.94)
Broad-spectrum antibiotics (only)	236,425	346	1.38	(1.24–1.54)	1.31	(1.17–1.46)	0.64%	(0.45–0.83)	2.71%	(2.25–3.18)
Otitis media (only)	316,868	943	1.96	(1.83–2.10)	1.85	(1.73–1.98)	1.18%	(0.98–1.36)	3.32%	(3.06–3.58)
Otitis media + broad-spectrum antibiotics	22,191	58	1.99	(1.53–2.58)	1.77[4]	(1.36–2.30)	1.06%	(0.48–1.63)	3.63%	(2.24–5.01)

[1] Adjusted for sex, age, and allowing different baseline hazards for different periods of calendar year at birth.
[2] Adjusted for sex, age, maternal, and paternal age at birth, gestational age, parental psychiatric disorders prior to birth, somatic hospitalization during the previous year (included time-dependently), allowing different baseline hazards for each calendat period and parity.
[3] Absolute risk of autism at age 10 based on multistate models. For the combined exposure, the estimates correspond to those illustrated in Figure 2.
[4] This model including a four-level categorical exposure variable corresponds to a model with otitis media, broad-spectrum antibiotics) as well as the interaction (P = 0.033).

to assess their separate and combined association with later autism. We found that children who were hospitalized with otitis media experienced an increased risk of autism, and in keeping with previous research, this association appeared to be more pronounced among girls [Atladottir et al., 2010]. Analogously, children who had been exposed to broad-spectrum antibiotics were at increased risk of autism. However, the effect of otitis media was not altered by additional exposure to broad-spectrum antibiotics, thus indicating no synergistic effect between exposure to both otitis media and broad-spectrum antibiotics. The association between otitis media and autism, independent of antibiotics, is still largely unexplained. It may be related to congenital malformations such as abnormalities of the orofacial architecture, difficulties in providing preventative medical care to individuals with behavioral abnormalities, deficiencies in the innate immune system related to the clearance of infectious organisms, aberrant inflammatory responses or other neuroimmonulogical factors [Atladottir et al., 2009; Jyonouchi, Geng, Cushing-Ruby, & Quraishi, 2008; Rosenhall, Nordin, Sandstrom, Ahlsen, & Gillberg, 1999]. Although the seminal paper by Sharon and colleagues clearly explains the rational and background [Sharon et al., 2016], further studies are required to determine which of these factors are the most important contributors to this association.

Strengths and Limitations

One strength of the present study is the large nationwide cohort of 780,547 children followed from birth and until a maximum age of 17 years. For the entire period, we used information from all prescriptions that were redeemed from all pharmacies in Denmark and any hospital contacts with infections. Both otitis media and antibiotics usage were considered as time-dependent exposures. We estimated both absolute and relative risks of autism as a function of age in relation to otitis media and broad-spectrum antibiotics usage. Moreover, several confunders were taken into account.

Our study had several limitations. First, we only had access to hospital and emergency room contacts with otitis media, and as a consequence, we could not include children with mild cases of otitis media treated by general practitioners, otologists, or pediatricians in private practices, which constitutes the majority of children with otitis media; and hence, not estimate their risk of autism. On the other hand, the validity of the otitis media diagnoses based on hospital contacts is presumably higher. Similarly, in the Danish registers, diagnoses of autism are based on clinical assessments following national guidelines, including a hearing test [The Danish Child and Adolescent Psychiatric Association [Børne- og Ungdomspsykiatrisk Selskab i Danmark], 2014]; and the validity of

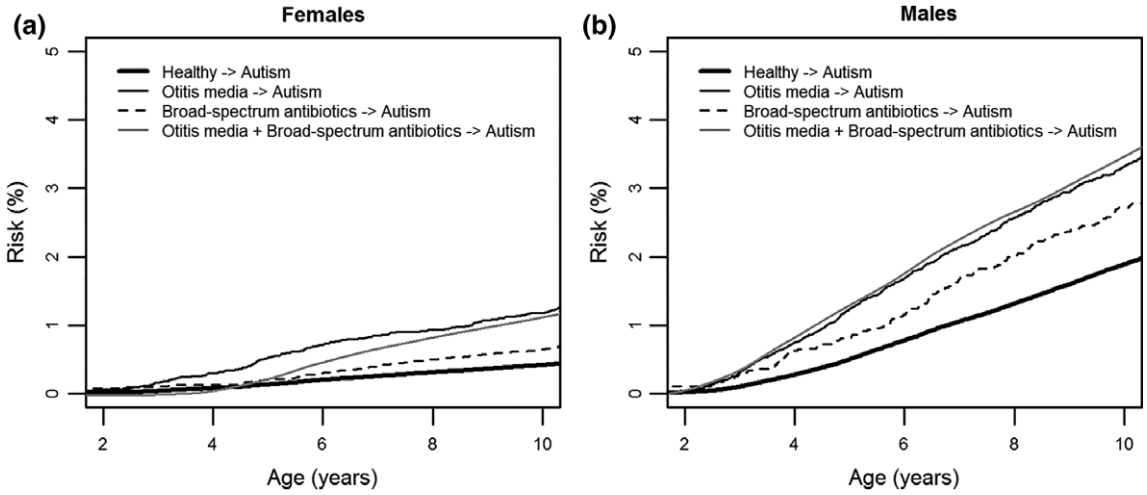

Figure 2. Absolute risk of autism for females (**A**) and males (**B**). The risk of transitioning from [otitis media + broad-spectrum antibiotics] → [autism] was smoothened to avoid potential identification of individuals.

Table 3. Adjusted hazard ratios (aHRs) for autism by exposure categories of otitis media and other infections, broad-spectrum antibiotics and other antibiotics (*n* = 780,547)

Exposures (time-dependent)	Total person-years	*N* (autism)	aHR[1]	95% CI	aHR[2]	95% CI
Infection						
No infection (ref)	5,549,054	5,458	1.00		1.00	
Other nonotitis media infection (only)	1,743,397	2,893	1.29	(1.23–1.35)	1.23	(1.17–1.29)
Otitis media	339,059	1,001	2.11	(1.97–2.26)	1.96	(1.83–2.10)
Antibiotics						
No antibiotitcs (ref)	1,683,234	748	1.00		1.00	
Narrow-spectrum antibiotics (only)	1,565,907	1,915	1.08	(0.99–1.18)	1.08	(0.99–1.18)
Moderate-spectrum antibiotics (no broad)	4,123,754	6,285	1.26	(1.17–1.37)	1.22	(1.13–1.32)
Broad-spectrum antibiotics	258,616	404	1.64	(1.45–1.86)	1.51	(1.33–1.72)

[1] Adjusted for sex and age and allowing different baseline hazards for different periods of calendar year at birth.
[2] Adjusted for sex, age, maternal, and paternal age at birth, gestational age, parental psychiatric disorders prior to birth, somatic hospitalization in the previous year modeled as time-dependent covariates, allowing different baseline hazards for different periods of calendar year at birth and for parity (i.e., stratified by parity and calendar year).

autism diagnoses has shown to be good [Atladottir et al., 2007; Lauritsen et al., 2010; Madsen et al., 2002]. Secondly, we could not assess whether the antibiotic was actually ingested. However, the Danish National Prescription Register covers all Danish pharmacies and all redeemed medication, and furthermore, antibiotics are not sold over the counter in Denmark [Muscat et al., 2006]. Moreover, in our primary analyses, we only considered the first redeemed antibiotic of each type and did not take into account the amount of antibiotics redeemed. Because we do not know the biological mechanisms of otitis media or exposure to broad-spectrum antibiotics, our models considered any history of otitis media or broad-spectrum antibiotics as risk factors for autism, regardless of the time since the events or the number of events. However, when investigating the association between the number of broad-spectrum antibiotics and autism, we found little evidence to suggest that subsequent redemptions of broad-spectrum antibiotics further increased the incidence of autism. Our estimates were based on observational data, and the associations should thus not be interpreted as causal. While examining whether associations are related to severity of otitis media could be important; we do not believe that type of hospital contact is a valid proxy for severity of otitis media. To minimize the potential confounding by severity of infection, we adjusted for recent inpatient admissions to somatic hospitals, but residual confounding may still be present. Although otitis media may result in hearing impairments, a differential diagnosis to autism, we did not include data on this, as a recent systematic review concluded that there is no conclusive evidence, that autism is associated with hearing impairments [Beers, McBoyle, Kakande, Dar Santos, & Kozak, 2014]. Finally, it should be noted that the present study only looked at the associations between otitis media and broad-spectrum antibiotics and subsequent diagnosis of autism. A previous study [Adams et al., 2016] examined the occurrence

of otitis media after autism, and found otitis media to be more frequent in children with autism, supporting a bidirectional temporal association between otitis media and autism.

Conclusion

Our findings are in keeping with previous studies which have found associations between otitis media and autism. Otitis media was more strongly associated with the risk of autism than other infections. Furthermore, we found an association between antibiotic usage and autism. However, we found little evidence of any synergistic effect between otitis media and broad-spectrum antibiotics usage. Thus, we found no evidence to suggest that the association between otitis media and autism was modified by broad-spectrum antibiotics usage, which is sometimes perceived as a proxy marker for alteration of the gut flora microbiota.

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Conflict of Interest

The authors declare no conflicts to declare.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: ICD-10 Diagnostic Codes of Infectious Disease Categories.
Table S2: ATC-codes and classification of antibiotics based on the spectrum of antibacterial effects.
Table S3: Adjusted hazard ratios (aHRs) for autism by cumulative exposure categories of otitis media contacts and broad-spectrum antibiotics (n = 780,547).
Table S4: Analogous hazard ratios to the hazard ratios shown in Table with the exception being that follow-up was followed until their 10 year birthday (n = 780,547).
Table S5: Analogous hazard ratios to the hazard ratios shown in Table with the exception being that individuals were divided into those who were exposed before and after their 5th birthday.
Table S6: Analogous hazard ratios to the hazard ratios shown in Table with the exception being that the exposure to otitis media infection and antibiotics treatment were time-dependently split in before and after 1 year of exposure.
Table S7: Adjusted hazard ratios (aHRs) of offspring autism associated with maternal antibiotics usage during pregnancy (n = 780,547).
Table S8: Analogous hazard ratios to the hazard ratios shown in Table adjusted for maternal antibiotics usage during pregnancy
Table S9: Analogous hazard ratios to the hazard ratios shown in Table adjusted for other infections