Respiratory infections in early life predict the development of islet autoimmunity in children at increased type 1 diabetes risk: Evidence from the BABYDIET study

Andreas Beyerlein[1], PhD; Fabienne Wehweck[1]; Anette-Gabriele Ziegler[1,2], MD; Maren Pflueger[1], PhD

[1]Institute of Diabetes Research, Helmholtz Zentrum München, Munich, Germany, and Forschergruppe Diabetes der Technischen Universität München, Munich, Germany

[2]Forschergruppe Diabetes e.V. am Helmholtz Zentrum München, Munich, Germany

Corresponding author:

Prof. Dr. Anette-G. Ziegler, MD

Institute of Diabetes Research

Helmholtz Zentrum München

Ingolstädter Landstraße 1

85764 Neuherberg, Germany

Phone +49(0)89 3187-2836

Fax +49(0)89 3081-733

E-mail: anette-g.ziegler@helmholtz-muenchen.de

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Abstract

Importance: There is evidence for a role of infections within the pathogenesis of islet autoimmunity and type 1 diabetes (T1D), but previous studies did not allow assessing potential critical time windows in this context.

Objective: To examine whether early, short term or cumulative exposures to episodes of infection and fever during the first three years of life were associated with the initiation of persistent islet autoimmunity in children at increased T1D risk.

Design: Prospective cohort study with daily infection records and regular assessment of islet autoimmunity.

Setting: Diabetes Research Institute, Munich, Germany.

Participants: 148 high T1D risk children with documentation of 1,245 infectious events in 90,750 person days during their first three years of life.

Main outcome measures: Hazard ratios (HR) for seroconversion to persistent islet autoantibodies were assessed in Cox regression models with numbers of respiratory, gastrointestinal and other infections, adjusting for sex, delivery mode, intervention group, season of birth and antibiotic use.

Results: An increased HR of islet autoantibody seroconversion was associated with respiratory infections during the first six months of life (HR, 2.27; 95% CI, 1.32-3.91) and in the age of 6.0-11.9 months (1.32; 95% CI, 1.08-1.61). During the second year of life, no meaningful effects were detected for any infectious category. A higher number of respiratory infections in the six months prior to islet autoantibody seroconversion was also associated with an increased HR (1.42; 95% CI, 1.12-1.80).

Conclusion: Our study identified respiratory infections in early childhood as a potential risk factor for the development of T1D.

Introduction

The incidence of type 1 diabetes (T1D) increases worldwide [1], but its etiology is still not well understood. Infections have been discussed as important environmental determinants in the pathogenesis of T1D [2-4]. Retrospective case-control studies showed that patients with newly diagnosed T1D had higher titres of enterovirus antibodies [5] and were more likely to be positive for enterovirus RNA [6, 7] compared to healthy controls. Prospective studies from Finland confirmed the potential association between enterovirus infections and islet autoimmunity [8, 9], while others from Germany and the USA did not [10-12].

These studies were based on a nested case-control design, which has only a limited ability to control for potential confounding factors. Furthermore, the approaches used did not allow detailed analyses on whether there are critical time windows in which infections might have a particularly strong influence on the development of islet autoimmunity. Moreover, infectious agents other than enteroviruses, e. g. rotaviruses, have been reported to be related to T1D [13-15].

The occurrence of fever is also of interest in this context, as fever indicates a strong immune response and therefore a potentially sufficient and fast removal of the possibly autoimmunity triggering pathogen [16, 17]. However, fever might also indicate a severe infection and excessive immune response leading into tissue damage and activation of autoreactive cells [18, 19]. Therefore it is not yet known whether fever is a favorable or unfavorable prognostic factor in the pathogenesis of autoimmunity diseases.

The aim of this study was to examine whether infections and fever episodes during the first three years of life were associated with the risk to develop islet autoimmunity. We hypothesized three scenarios to explain how infectious diseases might be involved in this context. First, early exposure, e.g. in the first months of life, may be instrumental in inducing a state of susceptibility for future seroconversion. Alternatively, infections may have a short-term impact, causing islet autoantibody seroconversion in the following few months. Third,

infections might have a cumulative impact such that the frequency of infectious events would increase the likelihood of islet autoimmunity irrespectively of when they occur. To study this, we took advantage of data from children with high risk for T1D who had been followed from three months of age within the BABYDIET study.

Materials and Methods

Data collection

The BABYDIET study is an intensively monitored German dietary intervention study testing the potential effect of delayed gluten exposure on the development of islet autoimmunity in children at increased risk for diabetes. The study cohort is still followed and has been described in detail elsewhere [20, 21]. In brief, 150 children younger than three months with at least one first-degree relative with T1D and one of three specific T1D-associated HLA genotypes were recruited between 2000 and 2006 (participation rate: 88.8 %) and randomised to exposure to dietary gluten in the first year of life. After inclusion, children were followed in three-monthly intervals until the age of three years and yearly thereafter for efficacy (persistent islet autoantibodies) and safety assessment, including intensive monitoring with three-monthly sample collection of venous blood, urine and stool.

Assessment of infectious episodes

At the three-months visit, parents completed a detailed questionnaire on their children's history of infections, fever and medication. Specifically, they were asked about fever, infectious symptoms (such as diarrhea, vomiting, constipation and allergies) and the name of administered pharmaceutical agents or their active ingredient with starting date and duration of infections and medication. The questionnaire additionally addressed the occurrence of diabetes and other autoimmune diseases in the child or its family and lifestyle habits such as

smoking during pregnancy. All families then received a BABYDIET book in which they were asked to record all infections and fever events (>38° C) together with medication and health visits of their children on a daily basis until the age of three years. Parents were instructed to report disease-free days in the BABYDIET book as well. The diseases were classified using the ICD-10 code from 2011 based on the physicians' diagnosis (if medical care had been sought) or on parental reports of symptoms.

Infectious disease was defined as an acute event according to the ICD-10 Code or by a symptom indicating an infectious genesis. Infectious events were assigned to a specific time interval by their date of onset. We defined three categories of infectious diseases: a) infections of the respiratory tract, ear, nose, throat and the eye (if inflammatory symptoms of the respiratory tract were reported), b) gastrointestinal infections (if the main symptoms were diarrhoea and/or vomiting), c) other infections (e. g. with symptoms of skin or mucosa lesions). Other disease events such as allergies or accidents were not considered as infectious diseases. Separate infectious diseases of one category were defined as one infectious event if there were less than six days of potential remission between the respective infections, as these seemed likely to be caused by the same infectious agent. Additionally, we defined a category called "any infections", in which infectious diseases of different categories were considered as one infectious event if their time frames were overlapping.

Assessment of islet autoantibody seroconversion

Seroconversion was defined as the development of persistent autoantibodies to one or more of the antigens insulin, GAD65, IA-2 or Zn-T8. Only the first event of seroconversion in each subject was taken into account. Measurement of autoantibodies has been described elsewhere [21]. The upper limits of normal were determined using Q–Q plots, corresponded to the 99th percentile of control children, and were 1.5 local units/ml for IAA, 35 WHO units/ml for

GADA, 5 WHO units/ml for IA-2A, 16 units/ml for ZnT8RA, and 30 units/ml for ZnT8WA. Using these thresholds for positivity, the assays had sensitivities and specificities of 70% and 99% (IAA), 86% and 93% (GADA), 72% and 100% (IA-2A) and 84% and 100% for multiple islet autoantibodies in the Diabetes Autoantibody Standardization Program Proficiency Workshop [22]. Persistence was defined as being positive in at least two consecutive samples and in the last available sample. Islet autoantibodies were measured in venous blood samples from all scheduled visits and therefore every three months. Diabetes development was monitored and diagnosed according to the American Diabetes Association Expert Committee criteria [23].

Statistical analyses

Due to the daily records in the BABYDIET books which covered also disease-free days, it was possible to distinguish between documented days (with or without disease) and undocumented days. To avoid potential bias due to variation in the number of documented days between subjects and time periods, we calculated the numbers of infectious disease and fever events per 100 documented days for each child as a measure of incidence. Due to the detailed questionnaire at the three-months visit, we considered all days from birth to this visit as being documented. The analyses were restricted to observations based on at least 20 documented days in the respective time interval.

In order to address the early exposure hypothesis, we calculated hazard ratios (HRs) of time until seroconversion to islet autoantibodies with respective 95% confidence intervals (CIs) by the number of infectious events per 100 documented days in the intervals of 0-5.9 months, 6-11.9 months and 12-23.9 months of age using Cox's proportional hazard regression model. For any time point, we calculated four models, using total number of infectious events as predictor in the first model, numbers of respiratory, gastrointestinal and other infections as

separate predictors in the second model, number of any fever events (recorded with or without an infectious episode) as predictor in the third model, and number of fever events without infection in the fourth model. In total, the dataset included 26 events of islet autoantibody seroconversion, which occurred at the age of 0.59 to 7.90 years. There were no observations with reported islet autoantibody seroconversion in the first six months of life; those with seroconversion at the age of 6-11.9 months were excluded from the 12-23.9 months analyses.

To examine the short term impact hypothesis, we calculated Cox regression models with number of infectious and fever events per 100 documented days in the last six months before autoantibody seroconversion within the first three years of life as time-varying predictors [24].

In order to disentangle potential early exposure and short term effects, we performed two sensitivity analyses. In the first one, we restricted the models with infectious / fever events during the first half year of life to those seroconversion events which occurred after the first year of life. In the second one, we restricted the models with time-varying predictors to those seroconversion events which happened between 1.5 and three years of age.

All Cox regression models were adjusted for sex, delivery mode (caesarean section / vaginal), intervention group, season of birth (March-May / June-August / September-November / December-February) and frequency of antibiotics use, and tested for interaction with the virus-sensing interferon induced with helicase C domain 1 (*IFIH1*) gene polymorphism [25].

With respect to the cumulative exposure hypothesis, we compared the distributions of the recorded numbers of infectious events in children with and without autoantibody seroconversion during their first two years of life in three-monthly intervals.

One subject dropped out of the study after the first visit, and another one had less than 20 documented days in every age interval examined. Both children were therefore excluded from the sample, leaving a final sample size of n=148.

The level of significance was set to 0.05 in all analyses. All calculations were carried out with SAS 9.2 (SAS Institute Inc, Cary, NC). Plotting was done using R 2.14.1 (http://cran.r-project.org).

The BABYDIET study was conducted at the Diabetes Research Institute (Munich, Germany) and approved by the ethics committee of the Ludwig-Maximilian University, Munich, Germany.

Results

Descriptive statistics

In total, our data comprised 90,750 documented days (table 1). Overall, 1,245 events (8.41 per child) and 431 events (2.91 per child) had been reported with respect to infections and fever, respectively. Children with islet autoantibody seroconversion during follow-up were significantly more exposed to respiratory and gastrointestinal infections as well as to fever, both during the first year of life and overall (table 2). The median number of documented days decreased from 350.5 in the first year of life to 177.5 in the second and 12.5 in the third year, respectively. The median number of documented days and median follow-up time per child were similar in both groups, respectively.

While there were no islet autoantibody seroconversion events observed in the first six months of life, the incidence rates of seroconversion per 100 person years were 8.51 in the second half year, 4.07 in the second year and 3.67 in the third year of life. The mean incidences of the three infection categories also increased considerably after the first six months of life and remained relatively constant thereafter, with a slight decline in the third year of life (figure 1). The same tendency was observed for the incidence of fever. Respiratory infections constituted the majority of the infections recorded.

Addressing the early exposure hypothesis

Despite the overall lower incidence of infections in the first 6 months of life, the number of infections during this age period per 100 documented days was associated with an increase in the HR for islet autoantibody seroconversion in both crude and adjusted analyses (table 3). The effect size was particularly high for respiratory infections (adjusted HR=2.27; 95% CI: 1.32-3.91), while there were no significant associations with gastrointestinal and other infections in this early period. The results were similar when we excluded all seroconversion events which happened during the first year of life (data not shown).

In the following six months of life, any infections and respiratory infections were again significantly associated with islet autoantibody seroconversion. However, the effect size was much lower than in the preceding period (e. g. respiratory infections: adjusted HR=1.32; 1.08-1.61). There were no significant associations with fever events in any of the two periods. During the second year of life, no meaningful effects were detected for infections or fever (data not shown). We did not detect significant interactions with *IFIH1* genotype in any model (data not shown).

When we explored the associations observed in the Cox regression analyses in further detail (figure 2), we found that the rate of islet autoantibody seroconversion was highest in children with >5 recorded events of respiratory infections in the first year of life. For all seroconverters, at least two infections had been reported in this period, including respiratory infections for all except one case.

Addressing the short term impact hypothesis

Calculations with time-dependent covariates indicated that the HR for seroconversion was significantly increased by a higher number of respiratory infections per 100 documented days within the preceding six months (adjusted HR=1.42 (1.12-1.80), table 4). Again,

gastrointestinal and other infections did not have a positive association with the seroconversion rate. However, we detected significant associations with any fever events and fever events without infection. When we restricted these analyses to seroconversion events after 1.5 years of life in order to disentangle potential effects of the first year of life and the preceding six months, the results were similar, but not significant, with the exception of any fever events. Again, we found no interactions with *IFIH1* genotype (data not shown). For all seroconverters, at least one infection had been reported within six months prior to the seroconversion event, including respiratory infections for all except two cases (data not shown).

Addressing the cumulative exposure hypothesis

Between nine and 18 months of life, subjects with later islet autoantibody seroconversion had on average a higher total number of infectious events recorded than their peers without seroconversion as determined by Mann-Whitney U tests (data not shown). The respective interquartile ranges of their cumulative infections numbers differed accordingly, although the highest exposures were observed in children without seroconversion (figure 3). The upper quartiles (75^{th} percentiles) of the distributions of the cumulative numbers of respiratory infections increased considerably faster in seroconverters compared to healthy peers during the first year of life, but this difference remained relatively constant thereafter (figure 4). There were no meaningful results with respect to gastrointestinal and other infections. Therefore, the on average higher cumulative infection number in children with later seroconversion (as concluded from higher interquartile ranges) reflected mainly a higher exposure to respiratory infections during the first year of life. All these findings were similar when we compared numbers of events per 100 documented days (data not shown).

Additional analyses

In an attempt to identify specific infectious diseases which might be mainly responsible for the increased rate of seroconversion by respiratory infections, we compared infection rates between children with and without seroconversion. We observed significantly higher rates of infections of the upper respiratory tract (Mann-Whitney-U test: p=0.005) and among these of acute rhinopharyngitis (ICD-10 code J00, p=0.001). When we used infections of the upper respiratory tract as predictor variable in the Cox regression models, we found significant associations at 0-5.9 months, 6-11.9 months and for six months before seroconversion (table 5). We observed no significant associations with infections of the lower respiratory tract (table 5), of ear, nose and throat and of the eye (data not shown).

Discussion

Through the daily documentation of illnesses and fever in children with a family history of type 1 diabetes during the first three years of life we could demonstrate that exposure to infectious diseases, and in particular to respiratory infections, in early life predicted the development of islet autoimmunity. Primarily, we found strong associations with infections occurring in the first year of life, confirming the early exposure hypothesis.

There was also some evidence for the short-term impact hypothesis, as infections and fever events within six months prior to islet autoantibody seroconversion were also associated with an increased islet autoimmunity risk. Notably, all children who developed islet autoimmunity had at least two infections in the first year of life and at least one infection within six months before islet autoantibody seroconversion. However, cumulative exposure alone seemed not to be instrumental, as potential differences in the cumulative disease numbers between children with and without islet autoantibody seroconversion mainly reflected different exposure to infections in the first year of life only. Fever seemed to be associated with a detrimental rather

than a protective effect, particularly with respect to short-term effects, but these findings were not conclusive.

These results are novel, as this study for the first time prospectively assessed infectious diseases together with their starting date and duration in children at risk for T1D. Overall, only few data exist about the incidence of infections and fever in infancy. Our finding that the infection rates increased largely after the first six months of life and remained relatively stable thereafter is comparable to results from another study [26] and appears likely to reflect protection by maternal passive immunity which is known to decline during the first year of life [27-29]. Extrapolated to yearly rates, our incidences of respiratory infections were comparable to those observed in another German population [30]. Other strengths of our study lie in the frequent 3-monthly measurement of all four relevant islet autoantibodies and in the fact that parents reported disease-free days as well, so that we were able to estimate disease frequencies without reporting bias. Accompanied collection of medication data even enabled us to rule out that our results were confounded by antibiotics use.

A potential limitation is the sample size, but such frequent follow-up of study subjects, detailed phenotyping and daily documentation of infectious events constitutes a challenge for both investigators and participants, so that it appears difficult to obtain a larger population size with such a study design. As a consequence, our data did not allow us finally excluding that other than upper respiratory tract infections might also have a potential impact on development of islet autoimmunity, as the statistical power might simply have been too low to detect such associations. Apparently for the same reason, we were not able to finally exclude that potential short term associations observed in our data might be due to early exposure effects. Although we performed a number of sensitivity analyses, it appears unlikely that this has resulted in a multiple testing problem. The results of these analyses were only used to

assess the validity of the main analyses (which had been performed beforehand) and were not interpreted otherwise.

Various mechanisms have been discussed to explain how infectious diseases might induce autoreactivity in T1D. Results from animal studies indicate that different viruses can affect the development of islet autoimmunity via various mechanisms such as direct beta-cell lysis, bystander activation of autoreactive T cells, loss of regulatory T cells and molecular mimicry [31, 32], which might therefore also be relevant in humans [33-36].

The age of six months to three years has been identified as peak incidence period of islet autoimmunity in the BABYDIET data as well as in other studies [37-39], implying that most of the relevant causative events occur at or before this age. Our results indicate that early exposure to infections increases the susceptibility for developing islet autoantibodies. Although it might appear plausible that such a state of susceptibility would soon lead to islet autoantibody seroconversion, we found that exposure to infections in the first six months of life was also associated with seroconversion after the first year of life, thus indicating also longer-term effects by early exposure.

Potential prevention strategies against T1D derived from studies like this might address early vaccination against specific infectious agents. Unfortunately, we were not able to identify a single infectious agent which might be instrumental in the development of T1D. Our results point to a potential role of infections in the upper respiratory tract and specifically of acute rhinopharyngitis. These results are partly supported by the MIDIA study [40], which also indicated that respiratory tract infections are associated with the development of islet autoimmunity. In general, our findings are in accordance with other previous studies which showed associations with enterovirus infections [5, 6, 8, 9], as rhinoviruses – an enterovirus species – are known to be the major causative agents of respiratory infections [41-43].

In conclusion, our study identified respiratory infections in early childhood, especially in the first year of life, as a risk factor for the development of T1D. We also found some evidence for short term effects of infectious events on development of autoimmunity, while cumulative exposure alone seemed not to be causative.

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The authors' responsibilities were as follows: AGZ is the principal investigator of the BABYDIET study, and together with MP developed the study hypothesis. AB conducted data management and statistical analyses together with FW and wrote the first and subsequent drafts of the paper together with AGZ and MP.

AB had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors had no conflicts of interest.

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Table 1. Total documented days and numbers of infectious and fever events in the BABYDIET study (n=148).

Events of islet autoantibody seroconversion	26
Total documented days	90,750
Days with infections	12,910
Days with fever	1,137
Total number of any infections [a]	843
Total number of respiratory infections	669
Total number of gastrointestinal infections	257
Total number of other infections	319
Total number of fever events	431

[a] In the category "any infections", respiratory, gastrointestinal and other infections were considered as one infectious event if their time frames were overlapping. Therefore, the total number of infections recorded in this category was lower than the total number of infections summed up over the three categories respiratory, gastrointestinal and other infections.

Table 2. Description of the BABYDIET study population. Data are presented as n (%) for categorical or median (range) for continuous variables in children with and without islet autoantibody seroconversion. Infections and fever were documented up to the age of 3 years. P-values for differences between groups were determined by chi-square test and Mann-Whitney U test (as appropriate).

	Seroconversion	**No seroconversion**	**p-value**
Male sex	12 (46 %)	54 (44 %)	0.86
Mother smoking during pregnancy	0 (0 %)[a]	13 (9.2 %)[a]	0.13
Follow-up time [years]	5.76 (0.75, 9.18)	5.09 (0.26, 10.01)	0.38
Documented days per child	484 (145, 1098)	535 (95, 1110)	0.89
First year of life	309.5 (123, 366)	357.5 (94, 366)	0.86
Any infections [per 100 dd]	2.20 (1.30, 5.15)	1.70 (0.00, 5.42)	0.02
First year of life [per 100 dd]	1.94 (0.82, 4.88)	1.37 (0.00, 3.84)	0.002
Respiratory infections [per 100 dd]	1.55 (0.64, 4.64)	1.30 (0.00, 4.90)	0.02
First year of life [per 100 dd]	1.66 (0.00, 4.88)	1.04 (0.00, 3.62)	0.002
Gastrointestinal infections [per 100 dd]	0.38 (0.00, 1.03)	0.13 (0.00, 2.29)	0.01
First year of life [per 100 dd]	0.30 (0.00, 1.63)	0.00 (0.00, 1.79)	0.006
Other infections [per 100 dd]	0.33 (0.00, 1.64)	0.27 (0.00, 1.84)	0.22
First year of life [per 100 dd]	0.00 (0.00, 1.31)	0.27 (0.00, 1.84)	1.00
Fever events [per 100 dd]	1.00 (0.27, 3.51)	0.60 (0.00, 2.53)	0.001
First year of life [per 100 dd]	0.83 (0.00, 3.51)	0.55 (0.00, 2.47)	0.04

dd: documented days

[a] 3 missing values (in each group)

Table 3. Hazard ratios [95% confidence intervals] for islet autoantibody seroconversion at any time during follow-up by number of infectious diseases and fever events per 100 documented days during the age of 0-5.9 months and 6-11.9 months. Adjustment was made for sex, delivery mode, intervention group, season of birth and antibiotics use.

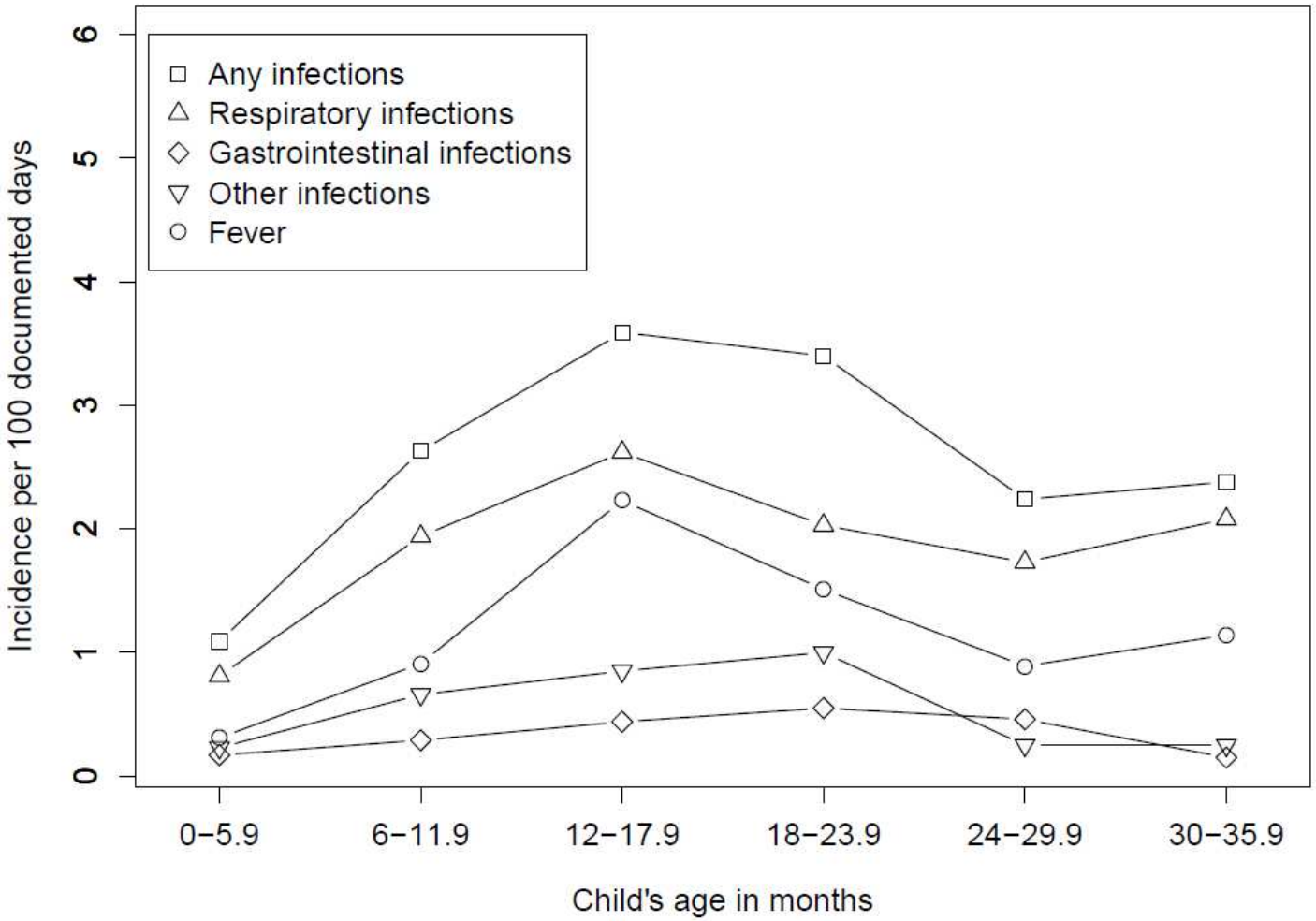
	0-5.9 months		**6-11.9 months**	
	Crude	**Adjusted**	**Crude**	**Adjusted**
Any infections	1.60 [1.06-2.42]	1.68 [1.04-2.72]	1.11 [0.998-1.24]	1.25 [1.06-1.47]
Respiratory infections	1.95 [1.18-3.22]	2.27 [1.32-3.91]	1.10 [0.97-1.25]	1.32 [1.08-1.61]
Gastrointestinal infections	1.38 [0.53-3.60]	1.29 [0.48-3.46]	1.46 [0.97-2.18]	1.37 [0.90-2.09]
Other infections	0.52 [0.15-1.73]	0.41 [0.12-1.47]	1.06 [0.77-1.45]	1.07 [0.75-1.52]
Any fever events	1.83 [0.84-3.96]	1.81 [0.77-4.26]	1.38 [0.93-2.05]	1.50 [0.99-2.28]
Fever events without infection	1.56 [0.34-7.05]	2.04 [0.43-9.72]	1.30 [0.57-3.00]	1.44 [0.53-3.95]

Table 4. Hazard ratios [95% confidence intervals] for islet autoantibody seroconversion at the age of 0-3 years and 1.5-3 years, respectively, by number of infectious diseases and fever events per 100 documented days in the 6 months prior to seroconversion. Adjustment was made for sex, delivery mode, intervention group, season of birth and antibiotics use.

	Seroconversion at 0-3 years		**Seroconversion at 1.5-3 years**	
	Crude	**Adjusted**	**Crude**	**Adjusted**
Any infections	1.17 [1.03-1.34]	1.17 [1.03-1.32]	1.21 [0.91-1.61]	1.29 [0.93-1.78]
Respiratory infections	1.25 [1.04-1.51]	1.42 [1.12-1.80]	1.25 [0.93-1.67]	1.32 [0.95-1.82]
Gastrointestinal infections	0.94 [0.51-1.74]	0.98 [0.53-1.81]	1.05 [0.63-1.75]	1.08 [0.64-1.82]
Other infections	1.08 [0.79-1.47]	0.94 [0.68-1.30]	0.33 [0.03-3.75]	0.31 [0.03-3.70]
Any fever events	1.15 [1.07-1.24]	1.15 [1.07-1.23]	1.33 [0.99-1.78]	1.35 [1.01-1.82]
Fever events without infection	1.29 [1.05-1.58]	1.27 [1.03-1.57]	1.53 [0.56-4.17]	1.48 [0.53-4.14]

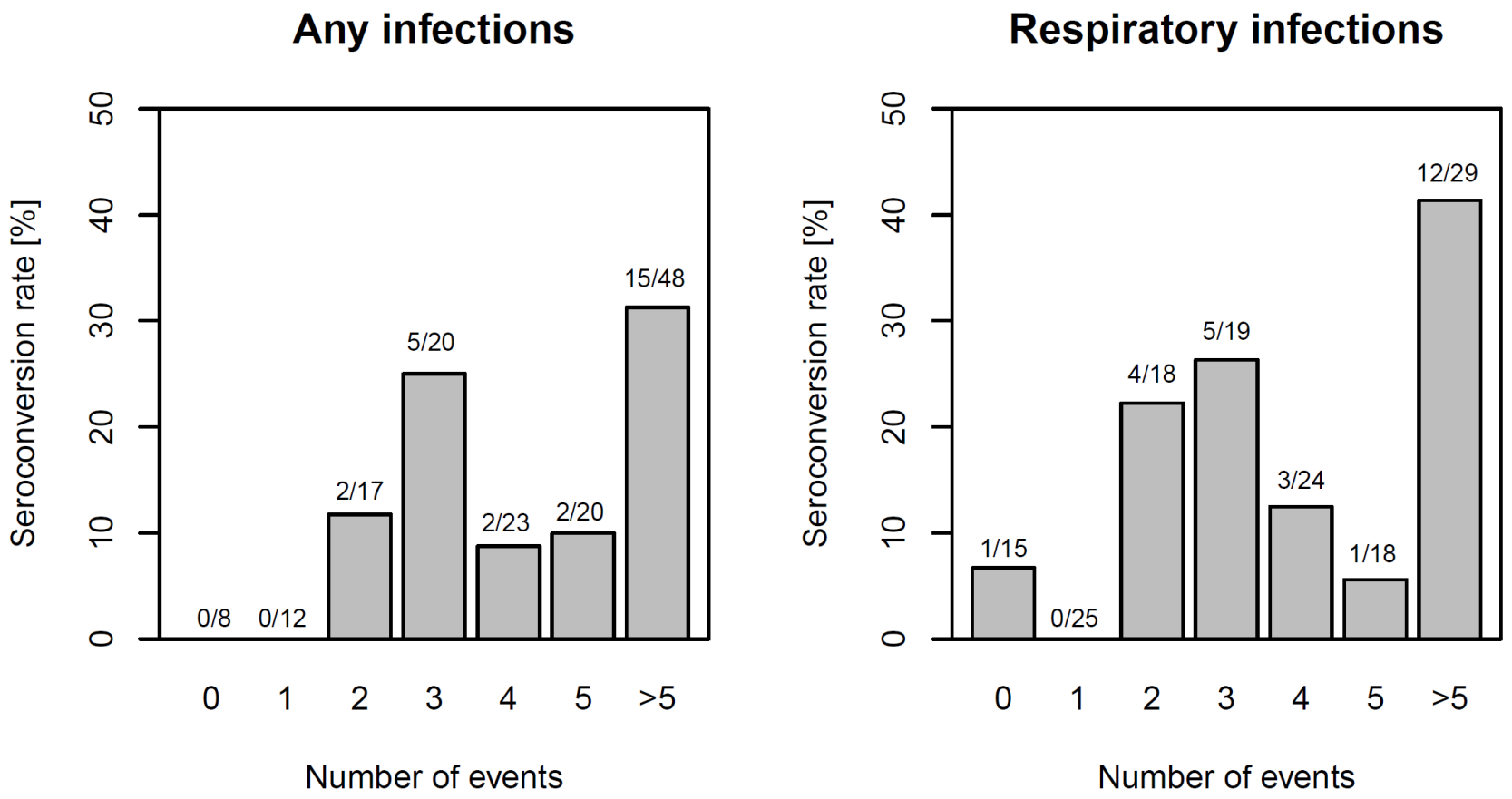
Table 5. Hazard ratios [95% confidence intervals] for islet autoantibody seroconversion by number of infections in the upper and lower respiratory tract (URT, LRT) and of acute rhinopharyngitis per 100 documented days. All analyses were adjusted for sex, delivery mode, intervention group, season of birth and antibiotics use as well as for other respiratory, gastrointestinal and other infections.

	0-5.9 months	**6-11.9 months**	**6 months before seroconversion**
URT infections	2.02 [1.10-3.72]	1.41 [1.09-1.82]	1.57 [1.26-1.95]
LRT infections	0.88 [0.17-4.64]	2.12 [0.96-4.69]	1.28 [0.51-3.17]
Acute rhinopharyngitis	1.77 [0.88-3.53]	1.34 [1.02-1.75]	1.31 [0.90-1.90]



13

14 **Figure 1.** Mean incidences of infectious and fever events per 100 documented days in the first three years of life in the BABYDIET data.



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16 **Figure 2.** Rates of islet autoantibody seroconversion by total numbers of infectious events in the first year of life as recorded in the BABYDIET

17 data.

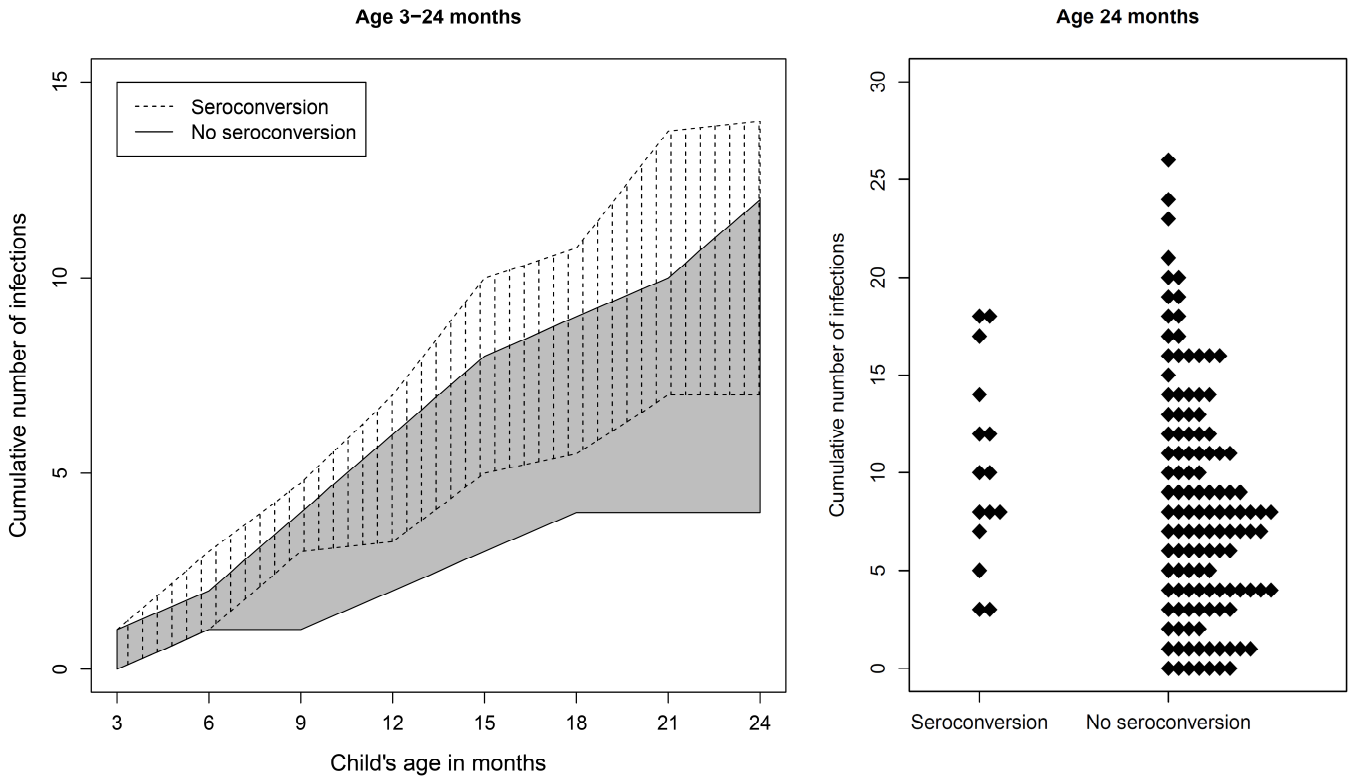

Figure 3. Total cumulative numbers of any infections in children with and without islet autoantibody seroconversion. In the left plot, interquartile ranges of cumulative infection numbers are depicted in 3-monthly intervals up to the age of 24 months. For each time point, subjects with prior seroconversion were excluded from the respective calculations. In the right plot, cumulative infection numbers at 24 months are shown in detail.

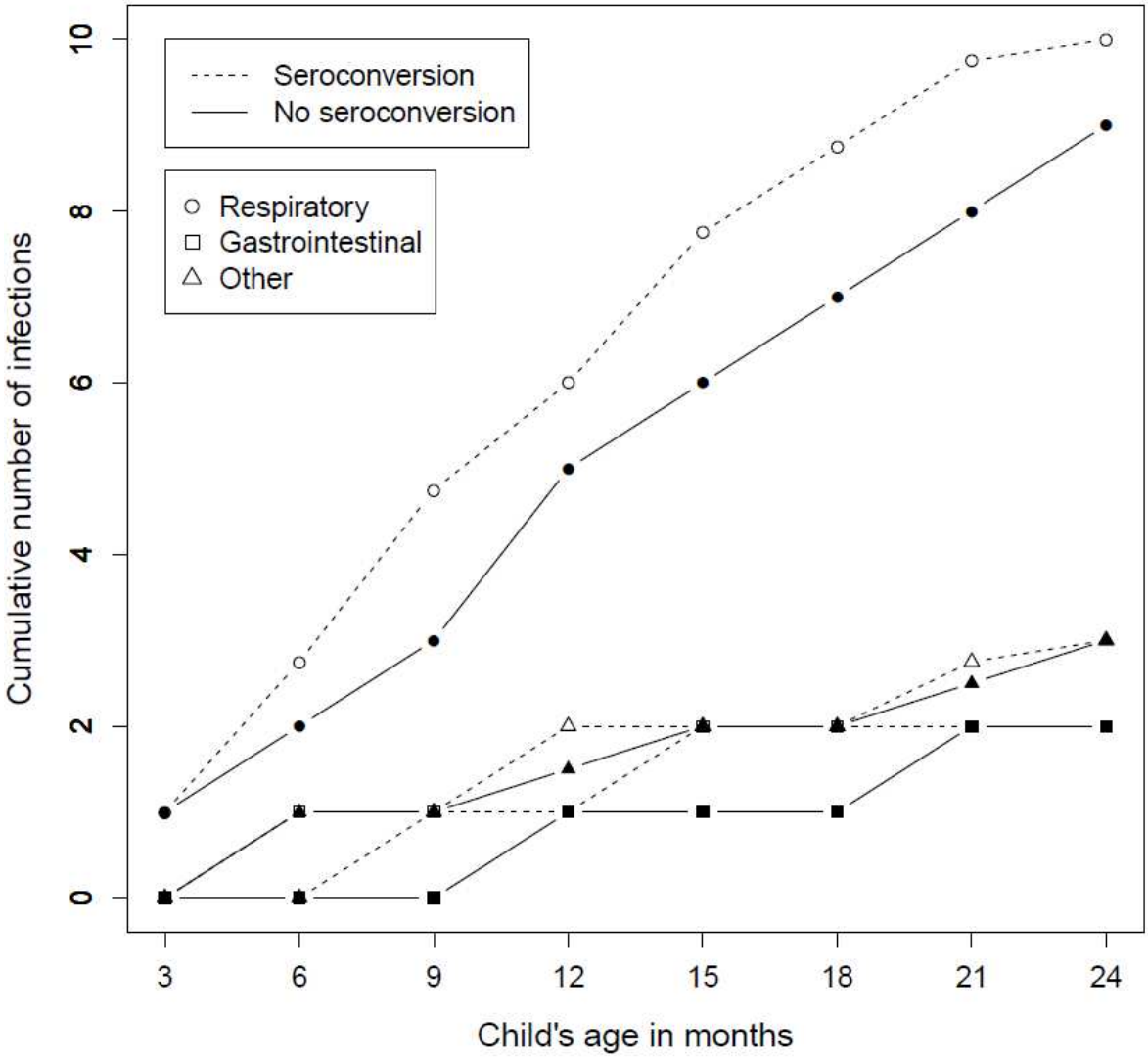

Figure 4. 75th percentiles of the distributions of total cumulative numbers of respiratory, gastrointestinal and other infections in children with and without islet autoantibody seroconversion in 3-monthly intervals up to the age of 24 months. For each time point, subjects with prior seroconversion were excluded from the respective calculations.