Gastrointestinal infections modulate the risk for insulin autoantibodies as the first-appearing autoantibody in the TEDDY Study

Short Running Title: GI infections modulate autoantibody-risk

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Gastrointestinal infections modulate the risk for insulin autoantibodies as the first-appearing autoantibody. This was found in the TEDDY study. #Autoimmunediabetes #TEDDYstudy

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Abstract

Objective: To investigate gastrointestinal infection episodes (GIE) in relation to the appearance of islet autoantibodies in The Environmental Determinants of Diabetes in the Young (TEDDY) cohort.

Research Design and Methods: GIE on risk of autoantibodies against either insulin (IAA) or glutamic acid decarboxylase (GADA) as the first-appearing autoantibody were assessed in a 10-year follow-up of 7867 children. Stool virome was characterized in a nested case-control study.

Results GIE reports (OR 2.17 [1.39 – 3.39]) as well as Norwalk viruses found in stool (OR 5.69 [1.36 – 23.7]) at <1 year of age associated with an increased IAA risk at 2-4 years of age. GIE reported at age 1 to <2 years correlated with a lower risk of IAA up to 10 years of age (OR 0.48 [95%CI 0.35 – 0.68]). GIE reports at any other age were associated with an increase in IAA-risk (OR 2.04 for IAA when GIE was observed 12 to 23 months prior [1.41 – 2.96]). Impacts on GADA-risk were limited to GIE <6 months prior to autoantibody development in <4 year old children (OR 2.16 [1.54 – 3.02]).

Conclusions: Bidirectional associations were observed. GIE associated with increased IAA-risk when reported before one year of age or 12-23 months prior to IAA. Norwalk virus was identified as one possible candidate factor. GIE reported during the second year of life associated with a decreased IAA-risk.

Article highlights

- Associations between gastrointestinal (GI) infections and autoantibodies were evaluated in a 10-year follow-up of 7867 children taking part in the TEDDY study.
- GI infection report and Norwalk virus in stool at < 1 year of age associated with an increased insulin autoantibody (IAA) -risk at 2-4 years of age.
- GI infection report at 1-2 years of age associated with a decreased IAA-risk up to 10 years of age.
- GI infections caused by Norwalk and other viruses modulate the risk of autoimmunity starting with IAA. Direction of their effect depends on the age and timing of infection.

The Environmental Determinants of Diabetes in the Young (TEDDY) Study is a large multinational prospective birth cohort aiming at identification and characterization of environmental factors at the onset of islet autoimmunity (IA) and its progression to clinical type 1 diabetes (T1D) in subjects at increased genetic risk for T1D (1-3). Respiratory infections - the most common infection type reported in TEDDY subjects (4) - were associated with the onset of IA, as well as for male subjects' progression to clinical T1D (5,6). Other studies have also found respiratory infections to be associated with IA (7-9). Furthermore, gestational respiratory infections modified the association between CTLA-4 gene polymorphism and the risk of insulin autoantibody (IAA) seroconversion in the offspring (10). In line with this, certain viruses causing respiratory symptoms, particularly *Enteroviruses*, have been linked to the risk of T1D (11-13). In addition, viruses that replicate both in the respiratory and gastrointestinal tract, such as *Enterovirus*, or in the gastrointestinal tract, such as *Rotavirus*, have been linked to IA and T1D (14-16). The mechanisms of these associations are not known. It is possible that enteral viruses can spread from the intestinal mucosa and gut-associated immune system to the closely located pancreas via common lymphatic networks and infect islet cells. Transmission to the pancreas may also occur via blood since enteral viruses have been detected in the blood during acute infection. The frequent detection of enterovirus protein and RNA in the pancreas of patients with T1D supports the possible role of virus in infecting pancreatic islet cells (17-19).

The present study sought to identify associations between prospective reports of gastrointestinal infections - the second most common type of infections in TEDDY subjects (4) – and onset of IA defined by the first-appearing islet autoantibody during the first ten years of life. The prospective study design's frequent monitoring of autoantibodies,

comprehensive collection of questionnaire data on infections, and use of modern sequencing technologies detecting viruses in longitudinal stool sample series (13) allowed us to identify time-dependent associations at different ages and correlate these associations with genetic and other host factors in relation to which islet autoantibody was first detected.

Research Design and Methods

Participants

Details of the study design can be found in previous publications (1-3). Six clinical research centers: three in the USA (Colorado, Georgia/Florida, and Washington state) and three in Europe (Finland, Germany, and Sweden) participated in a population-based HLA screening of newborns between 2004-2010. After screening, 8676 children with HLA haplotypes conferring an increased risk of T1D were enrolled between the ages 3-4.5 months (20). The children are followed until age 15 years and the study is on-going. Study clinic visits include a blood draw every three months until the age of four years, and every six months thereafter. Stool samples are collected monthly between 3-48 months of age and quarterly thereafter until 10 years of age (21). Written informed consent was obtained from primary caregiver separately for genetic screening and participation in the follow-up. TEDDY has been approved by local institutional ethics boards and is monitored by an external evaluation committee. The current study with data frozen as of month and year included 7867 children who were prospectively followed for the development of IA until their 10-year birthday. Participants missing four or more consecutive visits were considered withdrawn after the date of their last visit. Virome was analyzed in a subset of the cohort: a nested case control (NCC) study included 383 case children who presented with IA by May 31st, 2012, median age and interquartile range = 21 (13-33) months, along

with one matching control for each case selected from risk sets that did not develop IA by at least 6 months after date of case event (13).

Islet autoantibodies

Islet autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA) and insulinoma antigen-2 (IA-2A) were analyzed from blood samples (1,2). Persistent islet autoimmunity (IA) was defined by the presence of an islet autoantibody (GADA, IA-2A, or IAA) at each of the two TEDDY reference laboratories on two or more consecutive visits. The US reference laboratory was the Barbara Davis Center for Childhood Diabetes at the University of Colorado Denver, and for European samples the University of Bristol, Bristol, UK. All autoantibody-positive samples, as well as 5% of negative samples, were re-analyzed by the other reference laboratory. Both laboratories showed high sensitivity, specificity, and concordance (22). Onset of IA was the age at which a confirmed and persistent autoantibody was first detected (23).

Reported gastrointestinal infections

Illnesses were recorded by the parents at home in a diary. At each clinic visit, illnesses reported since the previous visit were translated into ICD-10 diagnosis codes (5) by study nurses (5). Infectious disease data processing and the infection episode approach have been previously described (4). In this study, gastrointestinal infection episodes (GIE) were identified as having a record of an ICD-10 code for an infective gastroenteritis.

Stool virome

The virome of stool samples collected monthly from the age of three months until IA development was characterized using Illumina mass sequencing that can identify widely different kinds of human viruses as described (24). Virome analyses were originally carried

out as part of the NCC study with 383 IA case children and risk set controls that were additionally matched by study site, sex, and family history with T1D. Stool samples from 751 children (379 case-control pairs) were eligible and available for the present study. Altogether 7202 stool samples up to 24 months of age collected monthly prior to IA onset were analyzed for association of common viruses (present in >2.5% of samples) with GIE reports within 2-weeks of stool sample collection.

Other factors

TEDDY has previously identified other predictors of islet autoimmunity:gestational respiratory infections (10), upper respiratory infections before age 3 months (6), respiratory infectious episodes (5), and the use of probiotics before 28 days of age (25). Therefore, HLA and other genetic risk factors as described elsewhere were considered as potential confounders or effect modifiers as some of these factors were related to age of seroconversion, particularly with type of first-appearing islet autoantibody (IAA or GADA) (5,9). SNP genotyping was performed by the Center for Public Health Genomics at the University of Virginia using the Illumina Immunochip, a custom array for genotyping selective SNPs from regions of the human genome associated with autoimmune diseases (26,27).

Statistical Methods

GIE and IA were examined across discrete time intervals at scheduled visits when both a blood draw sample and a diary of reported infections since the last visit were obtained. Time-varying correlations of GIE with first-appearing islet autoantibodies were assessed using discrete cause-specific hazards models. The models were specified by multinomial logistic regression models with four categories (IAA-first, GADA-first, other IA and a

category for survival beyond the discrete event time) (28, 29). The effect measures are considered odds ratio with 95% confidence intervals for IAA or GADA as the first-appearing islet autoantibody. The effect of GIE was modeled prospectively by several time varying predictors on two different time scales: subject's age (year) at infectious exposure and year lag from infection to IA. The age of GIE was modeled as a step function and GIEs prior to IA were modeled as a lag function. Prior infections were defined as the time prior to the last scheduled visit before the child developed IA. Secondary analysis further examined associations by age of seroconversion, by season of GIE report and autoantibody development and for clustering over shorter time periods. P-values were Bonferroni-adjusted to correct for family-wise error rate. All discrete time survival models were adjusted for sex, HLA-DR-DQ genotype, family history of T1D, country and age of child, with age modelled as a quadratic polynomial. The time varying GIE predictors were examined separately in relation to either IAA or GADA as the first-appearing autoantibody, but also later with single IA overall (IAA-first, GADA-first, IA2A-first) and multiple IA at seroconversion which represented two or more IA appearing for first time since the last blood draw.

A generalized linear mixed model with logit link was fitted to examine how common viruses (>2.5%) in stool related to a child's specific odds of having a GIE reported within two weeks of stool sample collection. The model included all viruses and adjusted for site, sex, family history with T1D and age of child when sample was taken. The aim of this analysis was to identify which viruses independently related to the likelihood of reporting a GIE. Based on results in full cohort, it was intended for specific viruses associated with reported GIE to be examined at important times of interest in relation to IA development using the NCC design. All viruses were individually examined in relation to IA by conditional logistic regression models with models adjusted for matching factors (site, sex, FDR) and HLA-

DR-DQ genotype. Results from conditional logistic regression models and generalized mixed models using the nested case control study were described as odds ratio (OR) with 95%CI. To examine potential confounding the final models were adjusted for factors that have previously shown association with GIE or appearance of autoantibodies in TEDDY. Unless otherwise stated, p-values less than 0.05 were considered significant. All statistical analyses were performed using SAS, Version 9.4 (SAS Institute Inc. Cary, NC, USA), R and GraphPad PRISM 5.03 (GraphPad Software Inc., Sand Diego, CA) for figures.

Data availability statement

The datasets generated and analyzed during the current study will be made available in the NIDDK Central Repository at https://repository.niddk.nih.gov/studies/teddy.

Results

Incidence of first-appearing islet autoantibodies and GIE

Islet autoantibodies developed in 763 (9.7%) children. IAA was the first-appearing autoantibody in 283 (37.1%) children, 333 (43.6%) had GADA-first, 20 (2.6%) had IA2A-first, 92 (12.1%) had both IAA and GADA with no determination of which was first, and 35 (4.6%) had other autoantibody combinations. The low number of subjects with IA2A-first did not allow meaningful statistical analyses in this subgroup. The peak incidence of IAA-first was between 6 and 9 months (20/1000 person-years) with a rapid decline in incidence between 9 and 24 months and a median (IQR) age of seroconversion at 2.0 (1 – 3.9) years (Fig. 1A). Incidence of GADA-first rose to 12/1000 person-years at age 27 months and then stabilized to 5-7/1000 person-years from 2 to 9 years of age, median (IQR)

seroconversion age of 4.5 (2.3 – 7.4) years**.** While children were still observed at risk for IA, incidence of GIE peaked at 15 months of age (63.3/100 person-years) (Fig. 1B**)**.

Age-specific GIE reports on IAA- and GADA-risk

A GIE reported at 3-12 months of age associated with higher odds of IAA (p = 0.03), especially at the age of 25 – 60 (OR= 2.17, 95%CI = 1.39 – 3.39, p=0.0006) (Fig. 2A). A GIE reported at 13 - 24 months of age was associated with a lower risk of IAA-first (OR = 0.48, 95%CI = 0.35 – 0.68, p <0.0001) (Fig. 2B). This inverse association was strongest when IAA-first appeared between 13 and 24 months of age (OR = 0.30, 95%CI = 0.14 – 0.63, p=0.002) but was still observed when IAA appeared after 24 months of age (OR= 0.57, 95%CI = 0.39 – 0.84, p=0.0004). No seasonal correlation between the age-specific GIE reports and IAA-risk was observed. No significant associations were observed between age-specific reports of GIE and GADA (Supplemental Fig. S1A-C).

Lag correlations between GIE reports and IAA- and GADA-risk

GIE was associated with an immediate lower risk of IAA-first (OR= 0.63 if GIE was reported 0-11 months prior, 95%CI = 0.45 – 0.87, p=0.005), followed by an increased risk of IAA-first (OR= 1.67 if GIE was reported 12-23 months prior, 95%CI = 1.18 – 2.38, p=0.004) (Fig 2D-E). When these lag correlations were examined by age of IAA appearance (cross signs in Fig 2D-F), strong inverse associations were observed at around 2, 3 and 4 years of age in figures 2D, 2E and 2F, respectively. These inverse associations linked with the strong age-specific inverse effect of GIE at 13-24 months of age on subsequent IAA-risk and explained the immediate (0-11 months prior) inverse lag effect (Fig 2D) and reduced the 12-23 months prior lag effect (Fig 2E). No significant lag correlations between GIE and GADA were observed (Supplemental Fig. S1D-F), although lag 0-11 month was close to significant (p = 0.05). When this lag was examined further, a

significant lag influence was found for GIE six months prior to GADA development in children under 48 months of age (OR = 2.16, 95%CI = 1.54 – 3.02, p<0.0001) (Supplemental Fig S2). The lag correlations between GIE reports and IAA- or GADA -risk were not seasonal.

Stool viruses and GIE

Viruses from stool samples collected monthly until 24 months of age while at risk for IA were examined in relation to the likelihood of a GIE being reported within +/- 2 weeks of a stool sample collection (Fig. 3). The number of stool samples included 4450 between 3 - 12 months and 2752 between 13-24 months of age. Figure 3 shows common viruses found in stool (prevalence > 2.5 % of samples) including viruses causing frequent and/or intense gastrointestinal symptoms (lower part of y-axis: *Adenovirus F, Bocaparvovirus, Mamastrovirus, Norwalk* virus*, Sapporo* virus) and viruses causing infrequent and/or mild gastrointestinal symptoms (upper part of y-axis: *Enterovirus A and B, Rhinovirus A-C, Parechovirus 1-6, Adenovirus A* and *F*). *Norwalk* virus (OR = 3.71, 95%CI = 2.65 – 5.19, p<0.0001) and *Sapporo* virus (OR = 2.23, 95%CI = 1.47 – 3.37, p= 0.0002) were associated with higher odds of a GIE report.*Adenovirus F, Bocaparvovirus* and *Astrovirus* correlated with an elevated odds of a GIE report**.** In contrast, *Enterovirus B* (OR = 0.35, 95%CI – 0.17 – 0.73, p = 0.005) was associated with lower odds of a GIE report. There were no significant interactions between viruses and age of stool sample.

Stool viruses and first-appearing islet autoantibodies.

Next, viruses in stools were examined in relation to the subsequent risk of IAA-first (Fig. 4 A – C). *Norwalk* virus during the first year of life (≤12 months) correlated with significant

age dependent association with IAA-first (interaction, p<0.001) - *Norwalk* virus detected during the first year of life was associated with a reduced risk of IAA appearing until 24 months of age (OR = 0.40, 95%CI = 0.20 – 0.78) and an increased risk of IAA-first appearing between 25 and 60 months (OR = 5.69, 95%CI = 1.36 – 23.7). Species B *Enterovirus* up to age 24 months was strongly associated with risk of IAA-first between 25 and 60 months (OR=9.34, 95%CI 1.88 – 46.5, p=0.006), regardless of whether the virus was detected during the first (OR = 4.00, 95%CI = 1.02 – 15.7) or second year of life (OR = 5.57, 95%CI = 1.00 – 31.0). *Norwalk* virus between 13 and 24 months of age was not associated with IA.

***FUT2* variant**

SNP-rs601338G>A in *FUT2* gene with the non-secretor status (genotype A/A) confers resistance against *Norwalk* virus. This was validated in the present study. Children with A/A genotype reported fewer GIE before 2 years of age (Supplemental Fig. S3A), and their stools were less likely to be positive for *Norwalk* virus (Supplemental Fig. S3B). Correlations with first-appearing islet autoantibodies were re-examined for dependence on the *FUT2-A/A* genotype (Supplemental Table S1**)**. An inverse association between GIE reports between 13 and 24 months of age and risk of IA overall was dependent on the FUT2-A/A genotype. A GIE report during the second year of life was associated with lower risk of IA only among children carrying the non-secretor *FUT2-A/A* genotype (OR = 0.33, 95%CI = 0.19 – 0.57, p=0.0001), regardless of the first islet autoantibody to appear (IAA-first, OR = 0.26, 95%CI = 0.09 – 0.73; GADA-first 0.47, 95%CI = 0.22 – 1.03; IAA and GADA at first appearance of IA, OR=0.19, 95%CI = 0.04 – 0.80).

Stratification by country and adjusting for other factors

The associations between the major time dependent GIE variables and IAA-first were found relatively consistent in direction and magnitude across countries (Supplemental Fig. S4). Associations between Norwalk virus in the first year of life and IAA-risk at 2-4 years of age was stronger in Europe (OR = 9.02, 95%Ci = 1.31 – 62.3) than in the US (OR = 1.38, 95%CI = 0.11 – 17.4) matching what was observed with GIE during the first year and IAA-first (Supplemental Fig S4A).

The major observed GIE associations also remained after adjusting for previously reported factors with association to GIE or IA in the TEDDY cohort (Supplemental Tables S2 and S3). A GIE reported between 13 and 24 months of age was associated with a decreased risk of IAA from 2 years of age onwards (OR = 0.49, 95%CI 0.33 – 0.71, p=0.0002). A GIE before 1 year of age correlated with an increased risk of IAA from 2 years of age onwards (OR = 1.70, 95%CI 1.17 – 2.46, p=0.005).

Conclusions

Gastrointestinal infections showed a clear association with the appearance of IA. The impact depended on the type of the first-appearing islet autoantibody (IAA-first or GADA-first) and the age of infection. GIE before 1 year of age was associated with increased IAA-risk with a relatively long delay, and *Norwalk* virus seemed like a possible factor for this phenomenon. The observed time-lag between infection and increased IAA-risk suggest slowly operating mechanisms. It can also be a sign of other non-viral factors operating. The observed associations remained after adjusting for potential confounders, but this does not exclude the possibility of such additional factors. Virus infection may induce a sustained shift in the infant’s immune response pattern and make the child susceptible for onset of autoimmunity by additional factors. Furthermore, acute GI infection may

sometimes be followed by persistent low-level virus replication. For example, signs of a slow persistent enterovirus infection (*Coxsackievirus B*) have been found in beta-cells, and this may lead to chronic inflammation promoting onset of beta-cell autoimmunity (30).

GIE during the second year of life showed a clear correlation with a reduced risk of IAA as the first-appearing autoantibody. This inverse association was significant up to 10 years of age, but it was strongest up to 12 months after the infection. The reason why infections at this particular age could be important risk modifiers is not known. The fact that gastrointestinal infections peak at this age can be one factor since frequent exposure increases statistical power to detect such association. In addition, children at this age are susceptible to the virus since breast-feeding or maternally acquired virus antibodies are no longer protecting them. This leads to efficient virus replication in gut mucosa which, in turn, may stimulate immune regulatory pathways in the gut immune system. For example, both *Rotavirus* and *Norwalk* virus (*Norovirus*) infections have been shown to induce strong IL-10 responses in man (31, 32) and *Rotavirus* infection leads to strong Treg activation in gnotobiotic pigs (33). Murine *Norovirus* infection protects NOD mice from the development of diabetes and is associated with an expansion of Tregs and reduced proinflammatory T cells (34). Such effects could downregulate autoimmune reactions and IAA.

Detection of viruses that replicate in the gut and typically cause gastrointestinal symptoms were associated with GIE reports (Fig. 3). However, while GIE between 13 and 24 months of age was associated with a decreased IAA-risk, *Norwalk* virus found in stool at same age showed no association with IAA-risk. Additionally, while the inverse association between GIE and IAA-risk was most pronounced among children with *FUT2* genetic resistance against *Norwalk* virus, the association between GIE at the same age and GADA-risk was similarly impacted by this *FUT2* genetic resistant group. These findings suggest viruses other than *Norwalk* virus may account for the inverse association between GIE and IAA-

risk in such a way as to have an impact on risk of islet autoimmunity in general. Other viruses that were detected in stool and are typically causing gastrointestinal infections were not associated with this phenomenon. One should note, however, that the low detection rate of *Rotavirus* (N=42) made it impossible to study its possible contribution. Further virus serology studies are in progress to evaluate possible role of *Rotavirus* and other enteral viruses.

Associations between GIE and onset of IA with GADA-first were limited. The primary analyses showed no significant associations (Supplemental Fig S1). Only after secondary analysis of shorter lags we found that GADA risk was increased by GIE reported 0-6 months prior to GADA seroconversion in children under 4 years of age. Also, respiratory infections have been reported to increase IA risk (both GADA and IAA) when reported shortly prior (0-9 months) to IA seroconversion (5). Infections a few months prior to onset of IA may act as triggering or precipitating factors for IA, but they can also be due to reverse causation, i.e. immune dysregulation or low level autoimmunity may increase susceptibility to infections. We tried to minimize the possibility of reverse causation by not considering infections all the way up to the age of first detection of autoantibodies, but only up to the previous scheduled blood draw. Blood draw interval was 3 months in <4-year old children and 6 months in older children. Accordingly, infections were considered only up to 3 or 6 months before first detection of autoantibodies. .

Virus interference (type 1 interferons induced by ongoing virus infection protect against other viruses) may explain some of the findings in the present study (35). The decreased frequency of GIE reports at the time when *Enterovirus B* was detected in stools may reflect viral interference – ongoing replication of *Enteroviruses* could protect against other viruses. Furthermore, the inverse association between GIE at 13 to 24 months of age and risk for IAA-first may also be due to virus interference: GIEs may provide protection

against onset of IA simply by blocking concomitant infections that could promote autoimmunity, such as *Enterovirus B* infections (24).

This study has major advantages. Prospective study setting, large cohort size, multinational subjects, and recording of gastrointestinal infections in a diary on daily basis allow powerful statistical analyses and general applicability of results. Additional strength was gained by using stool virome data to validate and characterize the GIE reports. Stool was collected in monthly intervals, which allows diligent observation of viral exposures over time and helps in combining stool virome results with diary data. Furthermore, serum samples were drawn every three months, which allowed recognition of subjects with either IAA- or GADA-type onset of autoimmune process and analysis of GIE association separately for these two pathogenic pathways.

Limitation of the study is the fact that despite the extensive data and sample collection, the study still does not capture all gastrointestinal infections. This is because part of these infections may be symptomless and don't get recorded by the parents. Also, there may be viruses which replicate in the gut only briefly and are not captured on the day of stool sampling (e.g. *Rotavirus* detection rate was low). The role of *Rotaviruses* as well as mild symptomless infections could be evaluated by measuring virus antibody levels in serum. Finally, we cannot rule out the possibility of a bystander effect of some other environmental factor linking with GIE cannot be ruled out.

To our knowledge this is the first study to show that overt gastrointestinal infections may modulate the risk of IA when the autoimmune process starts with IAA as the first-appearing autoantibody. It is possible that viral influence on the risk of developing islet autoimmunity is strongest in IAA first cases, as also *Enterovirus B* exposure is linked with increased risk of particularly IAA-first (36). We observed that gastrointestinal infections

associated with either increased or decreased IAA-risk depending on the age and timing of infections. *Norwalk* virus was identified as one potential factor increasing the IAA-risk, whereas other viruses seem to account for the decreased IAA-risk. These results open new opportunities and directions to identify risk-modifying viruses and the mechanisms mediating their effect.

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Guarantor Statement M.L. and K.F.L. are the guarantors of this work, and as such, had full access to all the data in the study and take responsibility for the integrity of data and the accuracy of data analysis.

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Figure legends

Figure 1

Age specific incidences of IAA, GADA and IA2A as the first-appearing autoantibody (A) and GIE (B.

Figure 2

IAA-risk irrespective of age (horizontal line = OR ± 95%CI)) and by age (x = OR) when RIE was reported at age <1 year (A), 1 to <2 years (B) and 2 to <3 years (C). Time lag correlation at 0 to 11 months (D) 12 to 23 months (E) and 24 to 35 months (F) between GIE and subsequent risk of IAA-first development irrespective of age (horizontal line, OR ± 95%CI) and at the age when children developed IAA (x = OR). Period of autoantibody development is defined as time after a scheduled blood draw visit up until the next blood draw visit when autoantibodies can be first detected.

Figure 3

Common viruses in stools (n stool = 7202) collected monthly from 751 children aged 3 to 24 months and association with the odds of GIE report within +/- 2 weeks from stool sample collection date (A). Panel B shows the percentage of stool samples (separate bars for virus positive and virus negative samples) with a reported GIE within +/- 2 weeks from stool sample collection date.

Figure 4

Common viruses (>2.5% of samples) in stool of children between the ages of 3 to 12 months (A & B) and 13 to 24 months (C) in relation to risk of IAA-first appearance between the age of 6 and 24 months (A, n=127 IAA-first cases and match control pairs) and between 25 and 60 months (B & C, n=52 IAA-first cases and match control pairs). Viruses in relation to IAA-first were examined separately adjusting for HLA-DR genotype and

matching factors (gender, site and family history with type 1 diabetes). Associations with $p<0.05$ are shown in color (red = positive association, blue = inverse association) and # = significant interaction between virus and age of seroconversion.

Online-Only Supplemental Material - Table of Contents

Supplemental Table S1. Reporting of GIE during first year of life, between 13 and 24 months of age and during yearly lag periods prior to development of islet autoantibodies on the risk of first appearing IA among children reporting by non-secretor FUT2 genotype of SNP rs601338G>A.

FUT2 Genotype	Report of GIE by age (year) of child or lag period prior to IA development	Non-secretor AA genotype of rs601338G>A in FUT2 on risk of Islet autoantibodies overall and by first appearing islet autoantibodies			
		IA overall	IAA-first	GADA-first	Multiple IA
		OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
AG, GG	age ≤12 months (yes vs. no)	1.04 (0.87 – 1.24)	1.34 (1.00 – 1.79)	1.03 (0.79 – 1.35)	0.64 (0.40 – 1.02)
AA *	age ≤ 12months (yes vs. no)	1.17 (0.78 – 1.75)	1.59 (0.87 – 2.94)	1.32 (0.72 – 2.41)	0.31 (0.07 – 1.3))
FUT2-AA - GIE interaction		p-value = 0.71	p-value = 0.71	p-value = 0.54	p-value = 0.37
AG, GG	age 13 to 24 months (yes vs. no)	0.86 (0.71 – 1.03) §	0.53 (0.37 – 0.76) ‡	1.18 (0.91 – 1.53)	0.76 (0.49 – 1.18)
AA *	age 13 to 24 months (yes vs. no)	0.41 (0.25 – 0.67) ‡§	0.25 (0.09 – 0.70) ‡	0.58 (0.30 – 1.11)	0.30 (0.09 – 1.03)
FUT2-AA - GIE interaction		p-value = 0.005	p-value = 0.16	p-value = 0.03	p-value = 0.21
AG, GG	lag 0 to 11 months (yes vs. no)	0.94 (0.77 – 1.14)	0.68 (0.48 – 0.97)	1.34 (1.02 – 1.78)	0.73 (0.45 – 1.18)
AA *	lag 0 to 11 months (yes vs. no)	0.63 (0.82 – 1.03)	0.43 (0.18 – 1.01)	1.04 (0.53 – 2.04)	0.34 (0.08 – 1.48)
FUT2-AA - GIE interaction		p-value = 0.11	p-value = 0.34	p-value = 0.38	p-value = 0.35
AG, GG	lag 12 to 23 months (yes vs. no)	1.22 (0.98 – 1.53)	1.76 (1.17 – 2.63) †	1.12 (0.82 – 1.53)	0.92 (0.54 – 1.58)
AA *	lag 12 to 23 months (yes vs. no)	1.33 (0.82 – 2.13)	1.49 (0.70 – 3.17)	1.53 (0.78 – 3.00)	0.56 (0.12 – 2.52)
FUT2-AA - GIE interaction		p-value = 0.90	p-value = 0.89	p-value = 0.61	p-value = 0.59

* AA genotype is resistant to *Norwalk* **virus; Associated with lower rates of reported infective gastroenteritis and stools positive with Norwalk virus in TEDDY** (Supplemental Figure S3).

† Report of GIE is associated with signi**fi**cantly higher odds of outcome.

‡ Report of GIE is associated with signi**fi**cantly lower odds of outcome.

§ Interaction between report of GIE and FUT2 (AA vs. AG/GG) genotypes.

Supplemental Table S2. Logistic regression model of factors associated with propensity for a GIE to be reported between visits during the first and second year of life as estimated by Generalized Estimating Equations (GEE).

Factor	GIE between visits during first year of life		GIE between visits during second year of life	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age (/3-month visit)	1.18 (1.16 – 1.21)	<0.001	0.97 (0.96 – 0.99)	<0.001
Month visit report				
January	Reference		Reference	
February	1.25 (1.02 – 1.55)	0.04	1.29 (1.10 – 1.53)	0.002
March	1.63 (1.34 – 1.98)	<0.001	1.54 (1.32 – 1.80)	<0.001
April	1.84 (1.51 – 2.47)	<0.001	1.41 (1.20 – 1.66)	<0.001
May	1.27 (1.03 – 1.56)	0.03	1.25 (1.06 – 1.47)	0.008
June	0.82 (0.66 (1.02)	0.07	0.95 (0.80 – 1.12)	0.54
July	0.68 (0.52 – 0.89)	0.005	0.58 (0.46 – 0.72)	<0.001
August	0.70 (0.55 – 0.88)	0.003	0.54 (0.45 – 0.65)	<0.001
September	0.50 (0.39 – 0.64)	<0.001	0.47 (0.38 – 0.57)	<0.001
October	0.53 (0.41 – 0.69)	<0.001	0.43 (0.38 – 0.57)	<0.001
November	0.62 (0.49 – 0.79)	<0.001	0.46 (0.35 – 0.53)	<0.001
December	0.75 (0.59 – 0.96)	0.02	0.76 (0.64 – 0.92)	0.004
Sex				
Boy vs Girl (reference)	1.18 (1.16 – 1.21)	0.07	1.09 (1.01 – 1.17)	0.04
Country of residence				
US	Reference		Reference	
Finland	1.21 (1.05 – 1.39)	0.01	1.15 (1.03 – 1.29)	0.02
Germany	1.86 (1.52 – 2.88)	<0.001	1.66 (1.39 – 1.98)	<0.001
Sweden	1.68 (1.49 – 1.90)	<0.001	1.65 (1.50 – 1.83)	<0.001
Breastfeeding stopped by age 6 months				
Yes vs. No (reference)	1.28 (1.16 – 1.41)	<0.001	0.89 (0.82 – 0.97)	0.008
Daycare started by age 6 months				
Yes vs. No (reference)	1.28 (1.16 – 1.42)	<0.001	1.03 (0.96 (1.12)	0.40
Mom working when child aged 9 months				
Yes vs. No (reference)	1.01 (0.90 – 1.12)	0.92	0.92 (0.84 – 1.00)	0.06
URI reported before enrollment				
Yes vs. No (reference)	1.12 (1.021 – 1.25)	0.04	1.01 (0.93 – 1.11)	0.77
Respiratory febrile infection between visit				
Yes vs. No (reference)	1.33 (1.20 – 1.46)	<0.001	1.25 (1.15 – 1.35)	<0.001
Child diarrhea before enrollment				
Yes vs. No (reference)	1.53 (1.30 – 1.80)	<0.001	1.12 (0.97 – 1.29)	0.03

OR >1 = higher propensity for a GIE to be reported between visits during the year
OR<1 = lower propensity for a GIE to be reported between visits during the year

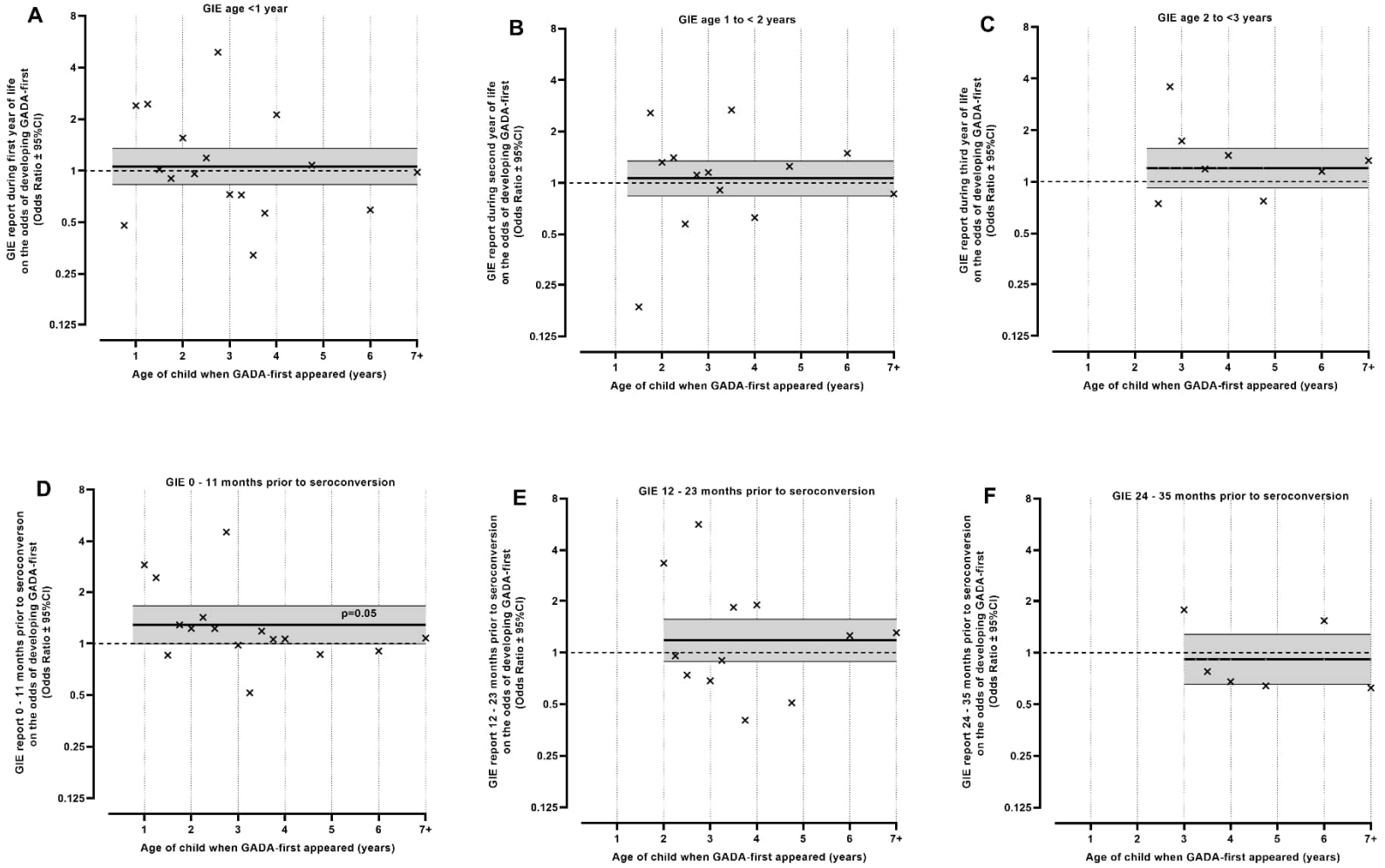
Supplemental Table S3. Multivariate multinomial model* of factors associated with IAA or GADA as first appearing autoantibodies from age 2 years onwards adjusting for age, country of residence, sex, and average propensity for child to have a GIE between visits during the first of life and separately during the second year of life.

Factor	IAA-first		GADA-first	
	OR (95%CI)	p-value	OR (95%CI)	p-value
HLA-DR-DQ genotype				
DR4-DQ8/DR4-DQ8	reference		Reference	
DR3-DQ2/DR4-DQ8	0.84 (0.54 – 1.32)	0.46	1.68 (1.17 – 2.41)	0.005
DR4-DQ8/DQ8-DR4	1.00 (0.59 – 1.70)	0.99	0.99 (0.61 – 1.60)	0.97
DR4-DQ8/ family T1D history	0.59 (0.22 – 1.54)	0.28	0.16 (0.04 – 0.68)	0.01
DR3-DQ2/DR3-DQ2	0.47 (0.25 – 0.87)	0.02	1.18 (0.77 – 1.79)	0.45
Family history with T1D				
Yes vs. No	2.11 (1.26 – 3.52)	0.004	2.19 (1.54 – 3.11)	<0.0001
Single nucleotide polymorphisms†				
rs2476601G>A in *PTPN22*	1.58 (1.08 – 2.32)	0.02	1.35 (1.01 – 1.79)	0.04
rs1004446G>A in *INS*	0.78 (0.55 – 1.10)	0.15	0.94 (0.73 – 1.21)	0.61
rs2292239G>T in *ERBB3*	1.04 (0.74 – 1.48)	0.82	1.40 (1.08 – 1.81)	0.01
rs3184504 C>T in *SH2B3*	1.90 (1.23 – 2.96)	0.004	1.37 (1.03 – 1.84)	0.04
rs3757247C>T in *BACH-2*	0.61 (0.43 – 0.86)	0.005	1.48 (1.11 – 1.98)	0.007
rs231775A>G in *CTLA-4*	1.05 (0.72 – 1.55)	0.79	1.49 (1.11 – 2.00)	0.008
rs601338G>A in *FUT-2*	1.35 (0.88 – 2.05)	0.17	1.04 (0.75 – 1.44)	0.80
Probiotics before age 90 days				
Yes vs. no	0.46 (0.25 – 0.83	0.01	0.86 (0.59 – 1.27)	0.45
Weight of child at age months				
(/1 SD)	1.21 (1.02 – 1.42)	0.03	1.19 (1.08 – 1.33)	0.0008
GIE during first year of life				
Yes vs. no	1.89 (1.31 – 2.73)	0.0007	0.97 (0.73 – 1.29)	0.84
GIE during second year of life				
Yes vs. No	0.51 (0.35 – 0.75)	0.0005	1.06 (0.82 – 1.36)	0.68

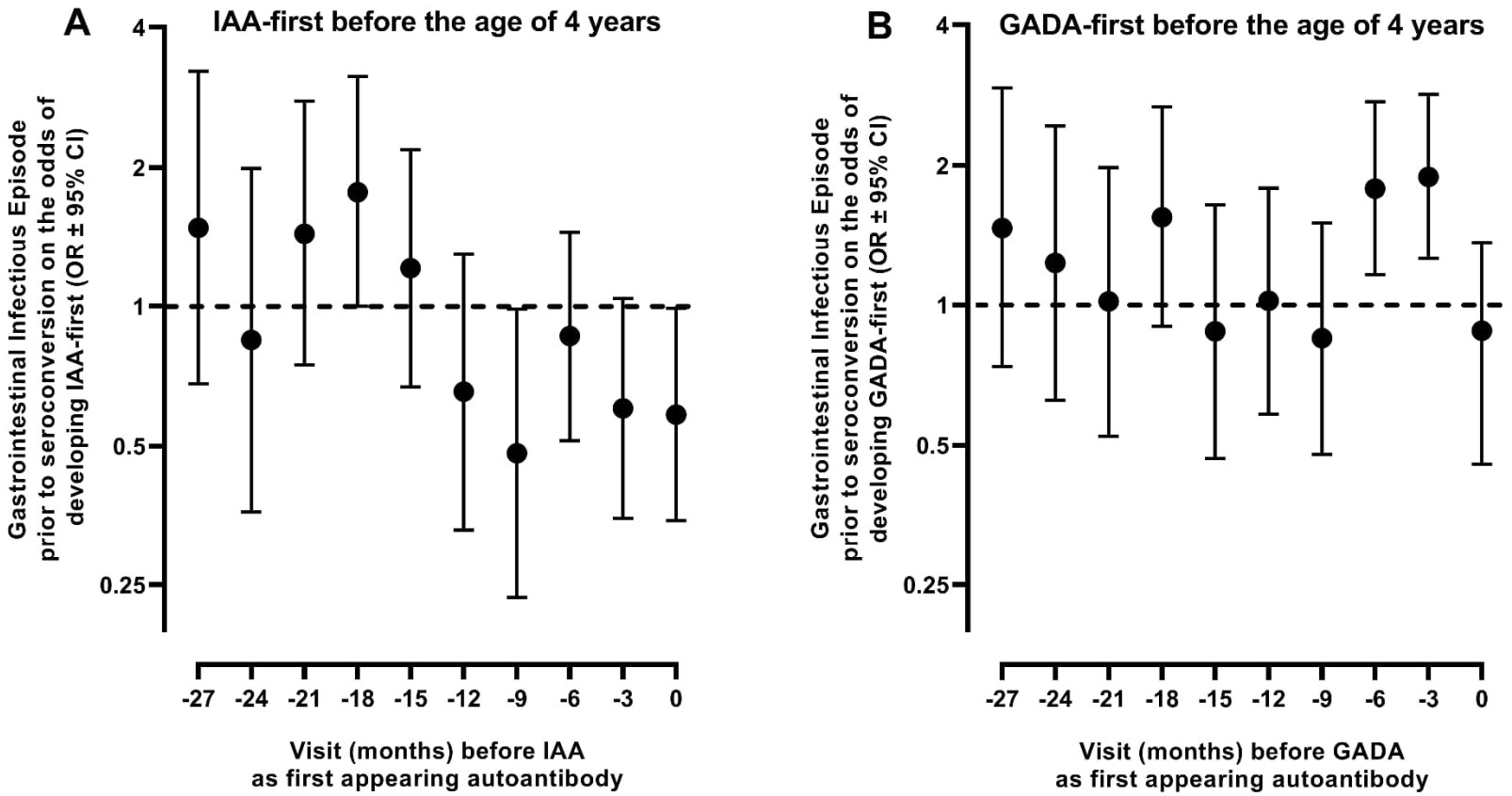
* = All factors were included in one model; propensity scores for GIE during **fi**rst and second years were **estimated using model**s in supplement table 2 and averaged for children across visits during the years; scores were included in models to account for factors strongly associated with GIE that may be unbalanced by cases and non-cases.

† = AA non-secretor genotype of SNP-rs601338G>A in FUT2 gene is resistant to *Norwalk* virus and is compared to remaining genotypes. Remaining SNPS are examined by minor allele (yes vs. no)

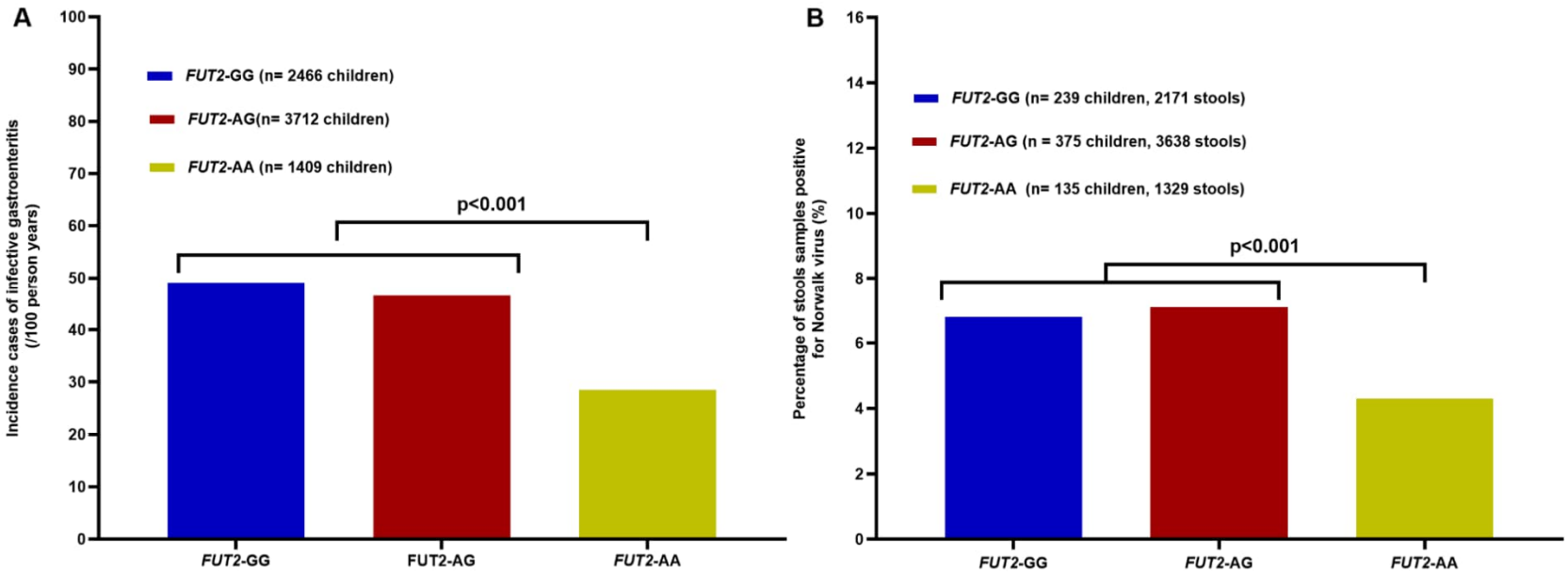
Supplemental Figure S1. GADA-risk irrespective of age (horizontal line = OR ± 95%CI)) and by age (x = OR) when GIE was reported at age <1 year (A), 1 to <2 years (B) and 2 to <3 years (C). Time lag correlation at 0 to 11 months (D) 12 to 23 months (E) and 24 to 35 months (F) between GIE and subsequent risk of GADA-first development irrespective of age (horizontal line, OR ± 95%CI) and at the age when children developed GADA (x = OR). The period of autoantibody development is defined as time after a scheduled blood draw visit up until the next blood draw visit when autoantibodies can be first detected.



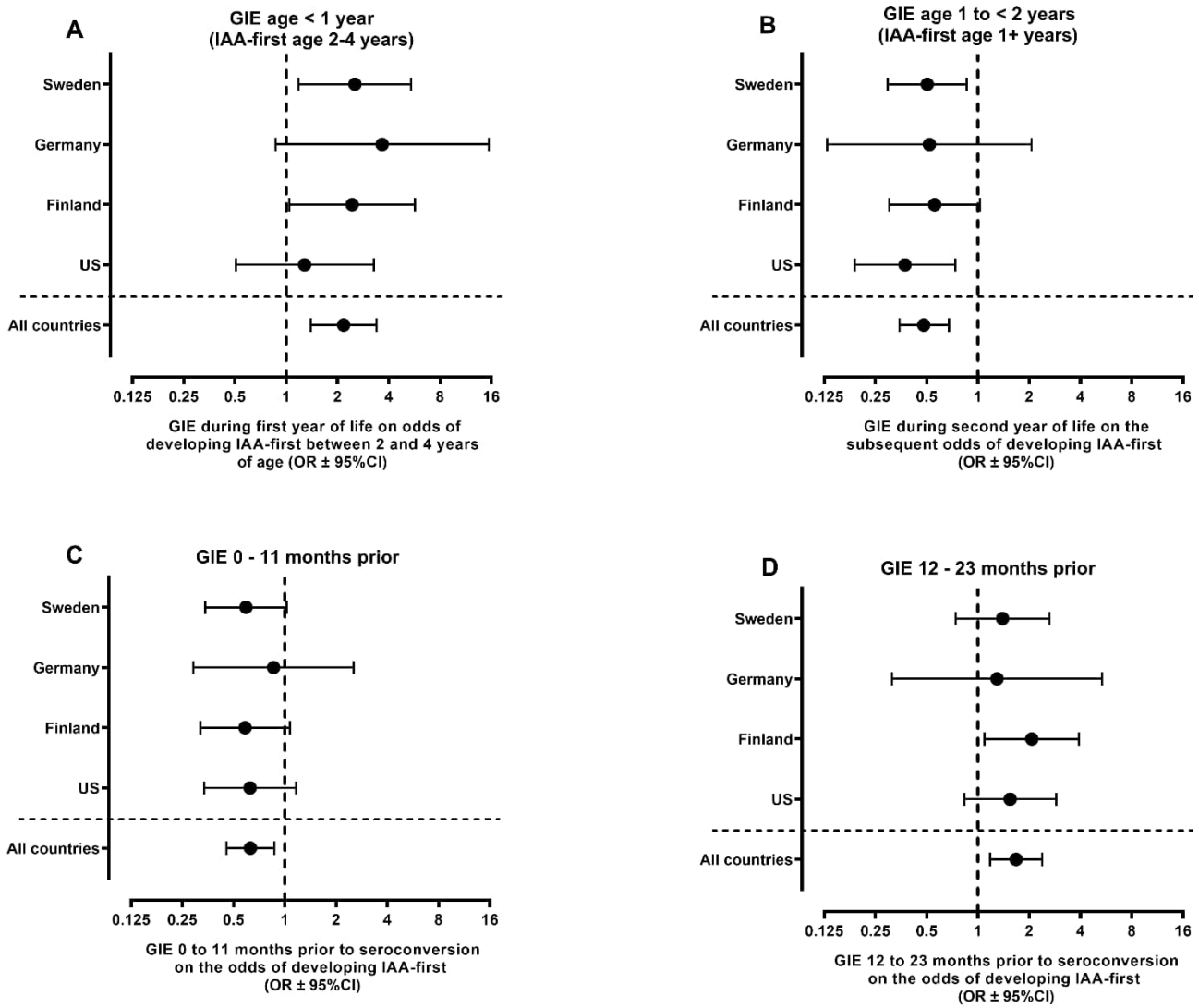
Supplemental Figure S2. Three-month lag correlations between GIE and the subsequent odds of (A) IAA-first and (B) GADA-first autoantibody development for children who seroconverted before 4 years of age while children were on a 3-month visit schedule.



Supplemental Figure S3. The incidence of GIE is shown in panel A by child's genotype for SNP-rs601337G>A in FUT2 gene. Similarly, percentage of stool samples positive for Norwalk virus are shown by child's FUT2 genotypes in Panel B.



Supplemental Figure S4. GIE reported before 1 year of age (A), during second year of life (B), 0 to 11 months prior to seroconversion (C) and 12 to 23 months prior to seroconversion (D) on the odds of a child developing IAA as a first appearing autoantibody.



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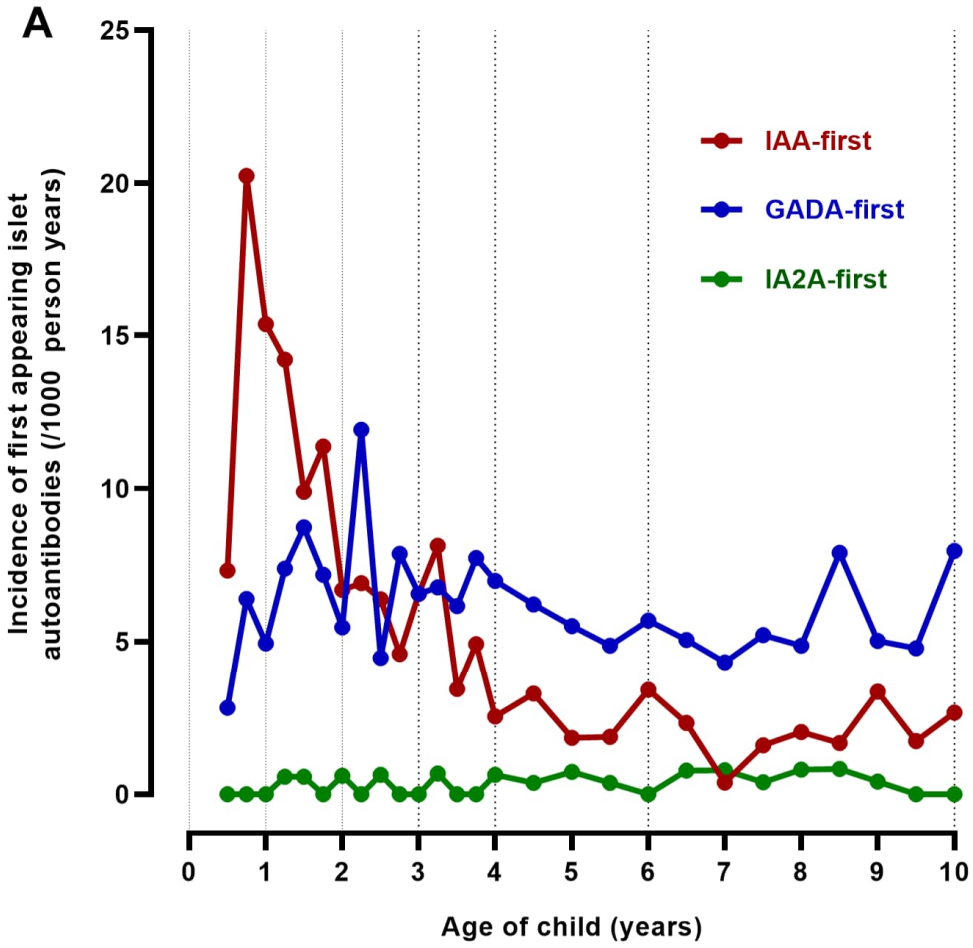
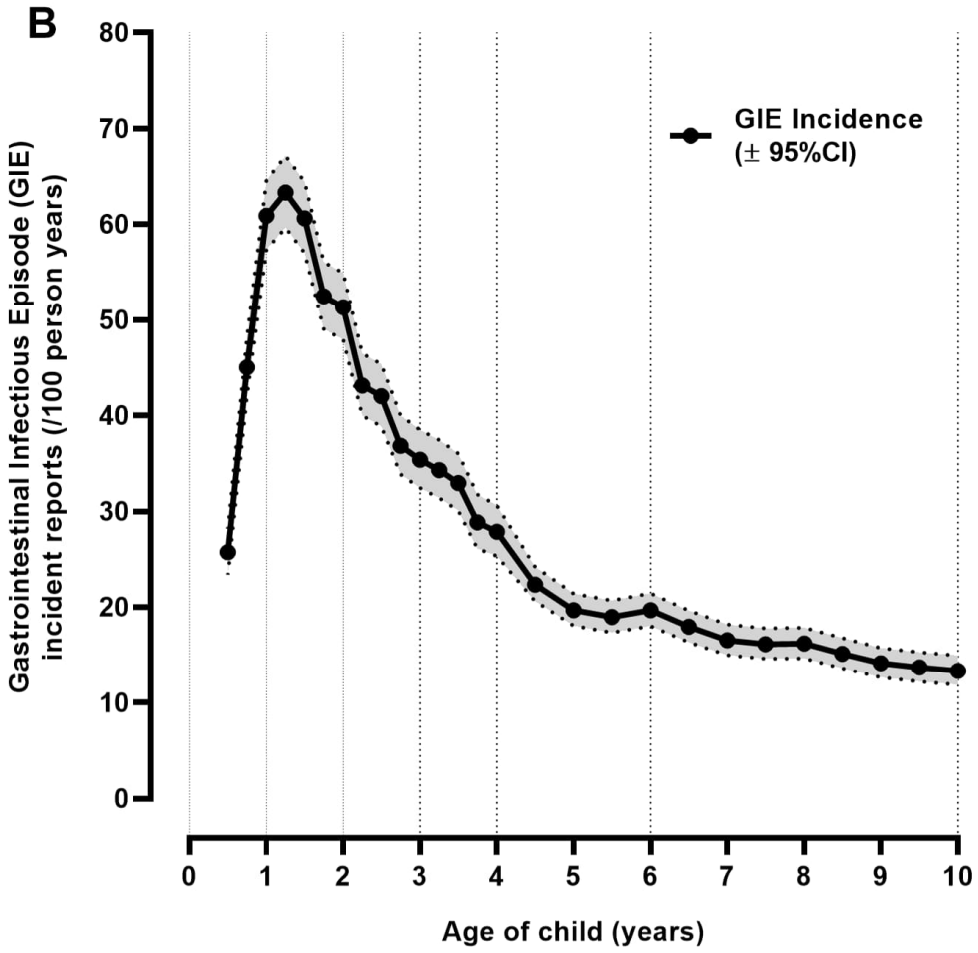
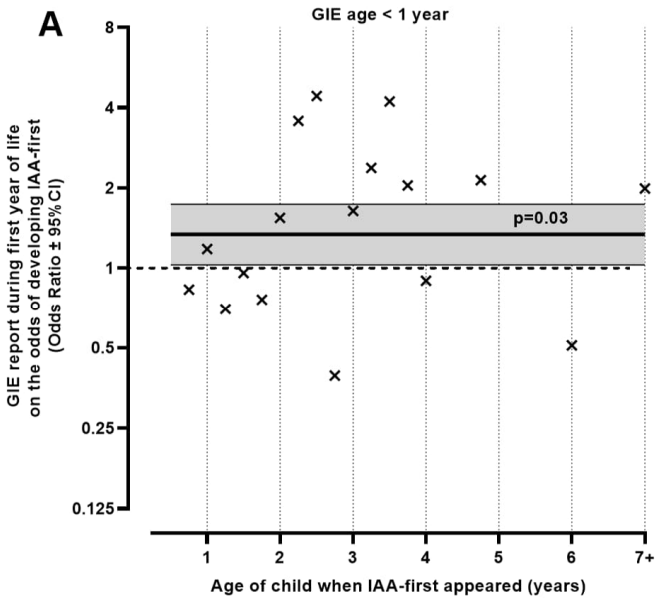
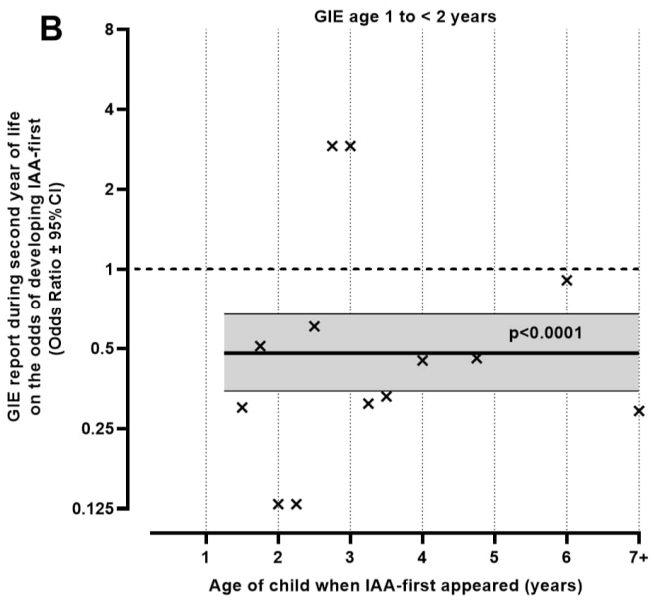
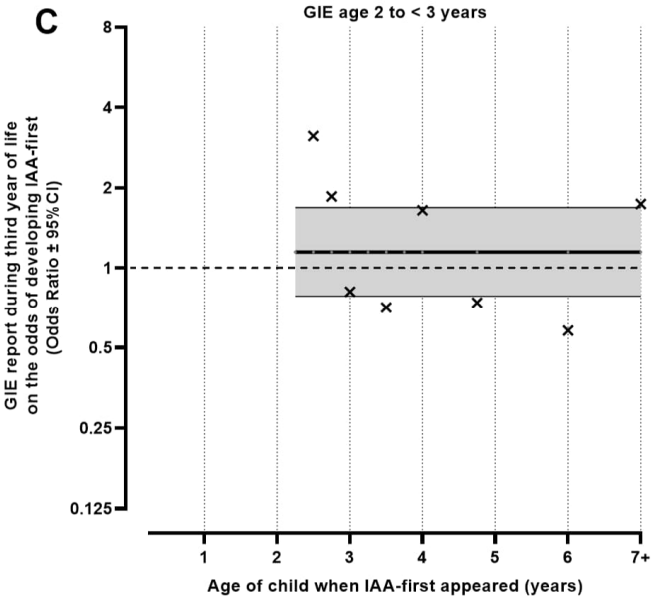
Committees:
[1]Ancillary Studies, [2]Diet, [3]Genetics, [4]Human Subjects/Publicity/Publications, [5]Immune Markers, [6]Infectious Agents, [7]Laboratory Implementation, [8]Psychosocial, [9]Quality Assurance, [10]Steering, [11]Study Coordinators, [12]Celiac Disease, [13]Clinical Implementation.

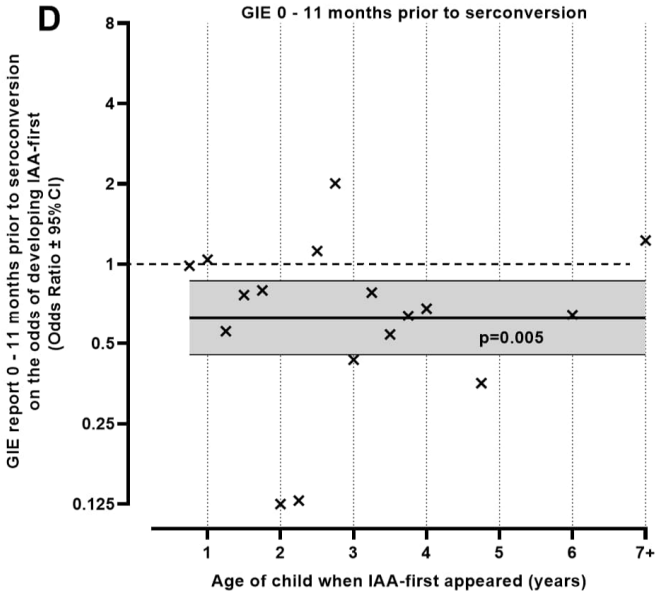

A
Incidence of first appearing islet autoantibodies (/1000 person years)
25
20
15
10
5
0
IAA-first
GADA-first
IA2A-first
0
1
2
3
4
5
6
7
8
9
10
Age of child (years)

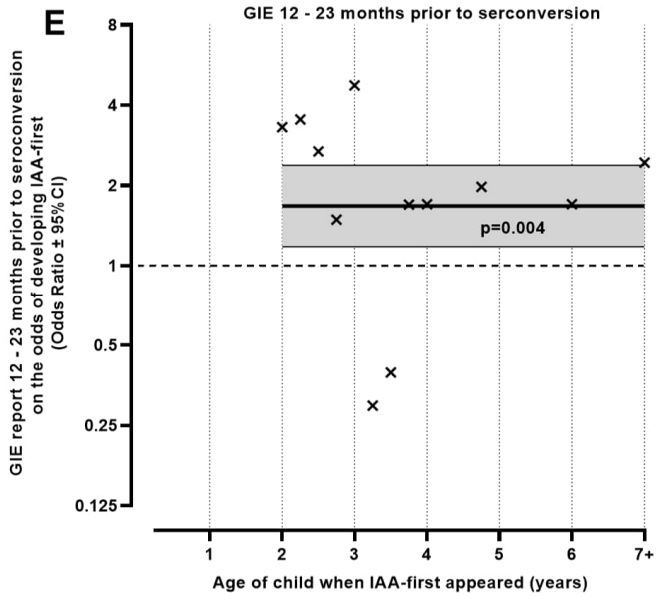

B
Gastrointestinal Infectious Episode (GIE) incident reports (/100 person years)
80
70
60
50
40
30
20
10
0
GIE Incidence
(± 95%CI)
0
1
2
3
4
5
6
7
8
9
10
Age of child (years)

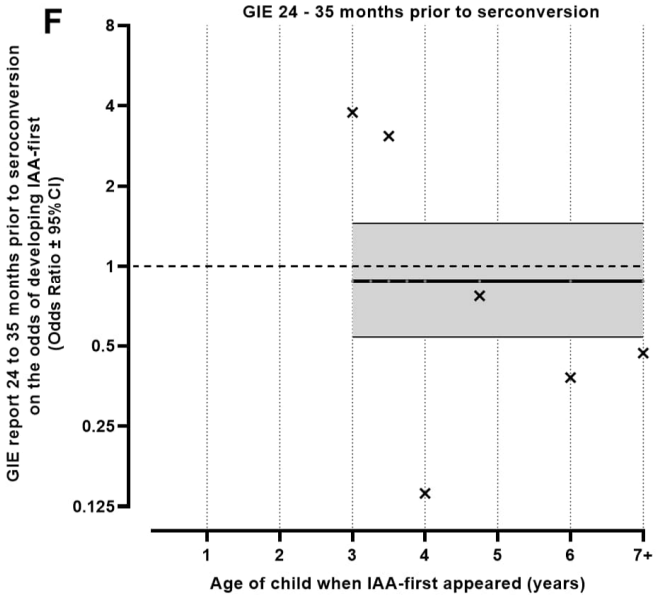

A
GIE age < 1 year
p=0.03
GIE report during first year of life on the odds of developing IAA-first (Odds Ratio ± 95% CI)
Age of child when IAA-first appeared (years)

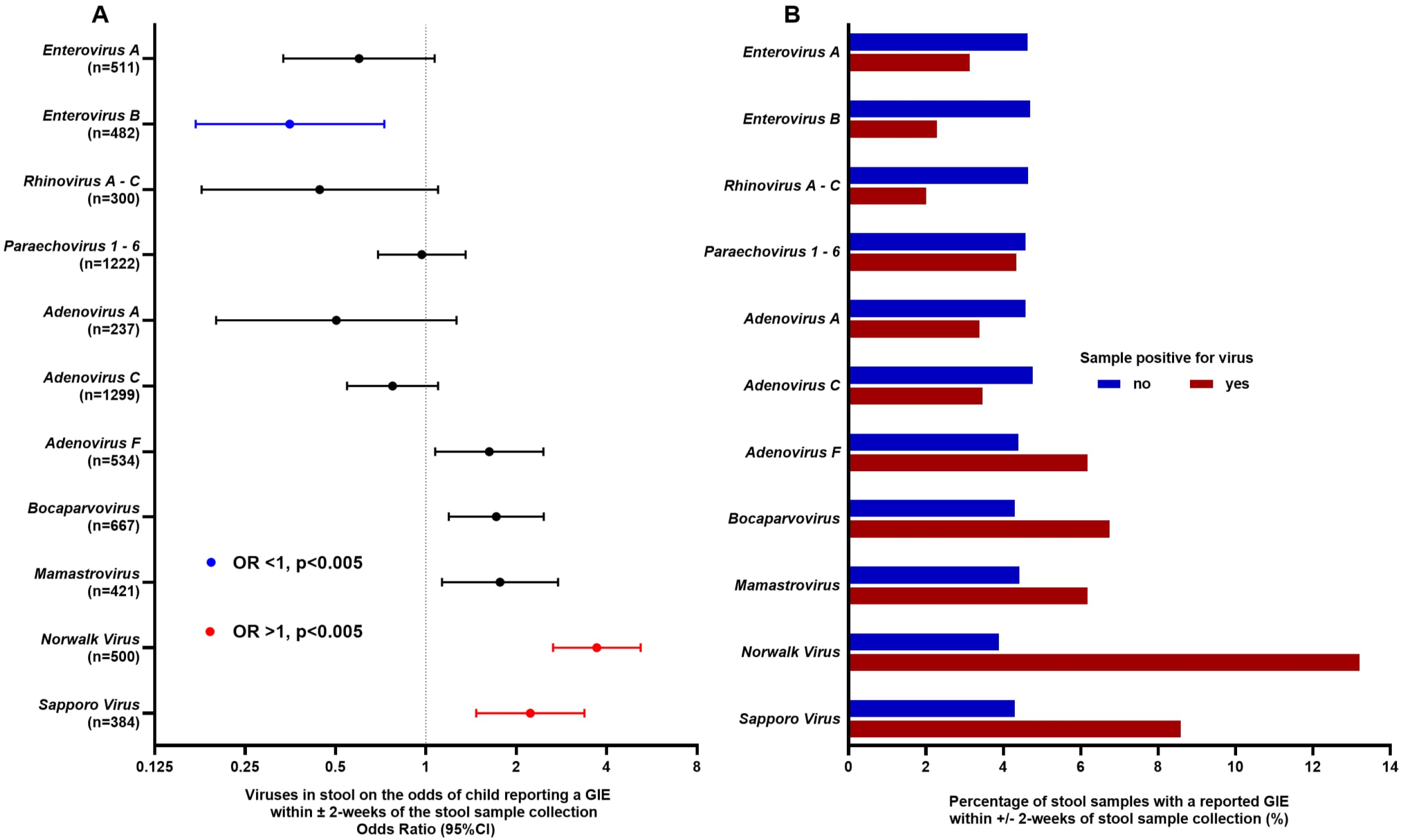

B
GIE age 1 to < 2 years
p<0.0001
GIE report during second year of life on the odds of developing IAA-first (Odds Ratio ± 95% CI)
Age of child when IAA-first appeared (years)

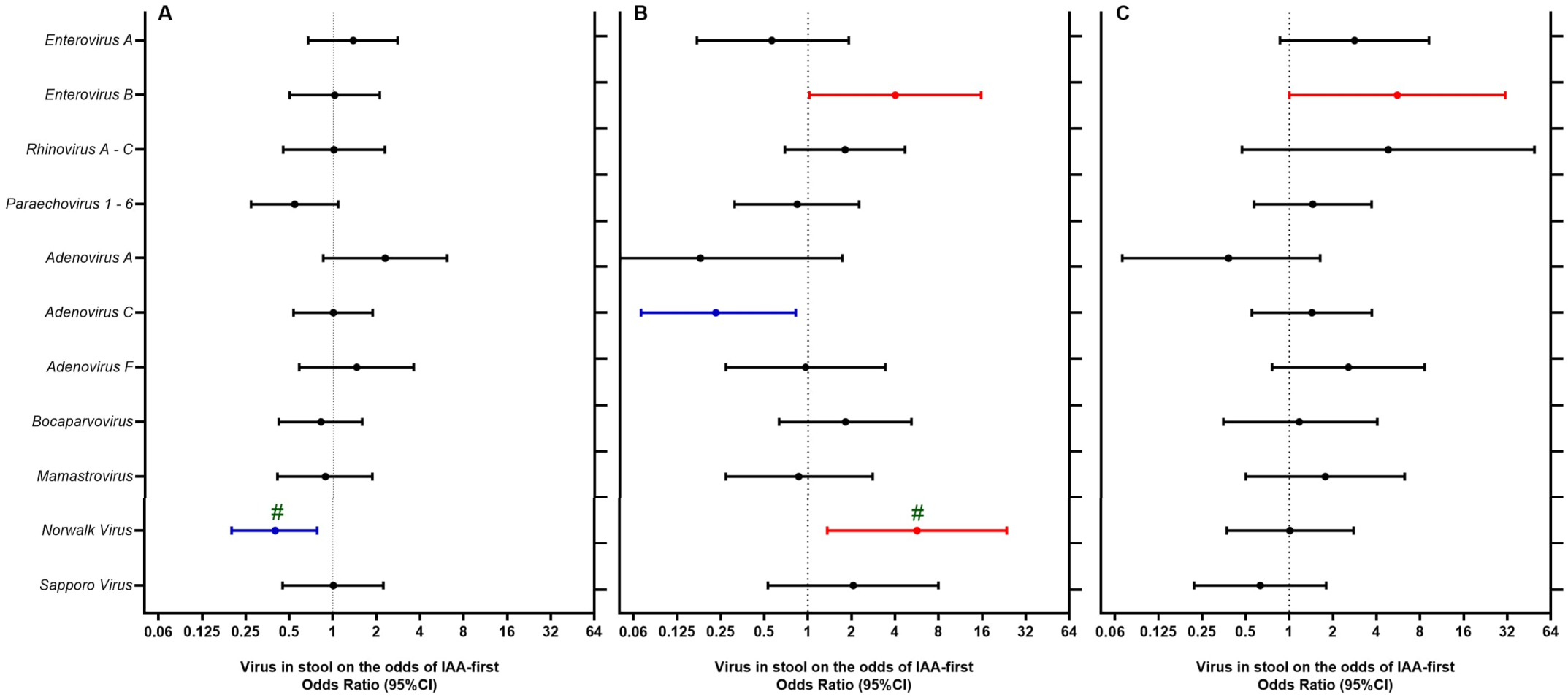

C
GIE age 2 to < 3 years
GIE report during third year of life on the odds of developing IAA-first (Odds Ratio ± 95% CI)
Age of child when IAA-first appeared (years)


D
GIE 0 - 11 months prior to serconversion
p=0.005
GIE report 0 - 11 months prior to seroconversion on the odds of developing IAA-first (Odds Ratio ± 95% CI)
Age of child when IAA-first appeared (years)


E
GIE 12 - 23 months prior to serconversion
p=0.004
GIE report 12 - 23 months prior to seroconversion on the odds of developing IAA-first (Odds Ratio ± 95% CI)
Age of child when IAA-first appeared (years)


F
GIE 24 - 35 months prior to serconversion
GIE report 24 to 35 months prior to seroconversion on the odds of developing IAA-first (Odds Ratio ± 95% CI)
Age of child when IAA-first appeared (years)


A
Enterovirus A (n=511)
Enterovirus B (n=482)
Rhinovirus A - C (n=300)
Paraechovirus 1 - 6 (n=1222)
Adenovirus A (n=237)
Adenovirus C (n=1299)
Adenovirus F (n=534)
Bocaparvovirus (n=667)
Mamastrovirus (n=421)
Norwalk Virus (n=500)
Sapporo Virus (n=384)
OR <1, p<0.005
OR >1, p<0.005
0.125
0.25
0.5
1
2
4
8
Viruses in stool on the odds of child reporting a GIE within ± 2-weeks of the stool sample collection Odds Ratio (95%CI)
B
Enterovirus A
Enterovirus B
Rhinovirus A - C
Paraechovirus 1 - 6
Adenovirus A
Adenovirus C
Adenovirus F
Bocaparvovirus
Mamastrovirus
Norwalk Virus
Sapporo Virus
Sample positive for virus
no
yes
0
2
4
6
8
10
12
14
Percentage of stool samples with a reported GIE within +/- 2-weeks of stool sample collection (%)


A
B
C
Enterovirus A
Enterovirus B
Rhinovirus A - C
Paraechovirus 1 - 6
Adenovirus A
Adenovirus C
Adenovirus F
Bocaparvovirus
Mamastrovirus
Norwalk Virus
Sapporo Virus
#
#
0.06 0.125 0.25 0.5 1 2 4 8 16 32 64
Virus in stool on the odds of IAA-first
Odds Ratio (95%CI)