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ARTICLE

First-appearing islet autoantibodies for type 1 diabetes in young children: maternal life events during pregnancy and the child's genetic risk

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Abstract

Aims/hypothesis Psychological stress has long been considered a possible trigger of type 1 diabetes, although prospective studies examining the link between psychological stress or life events during pregnancy and the child's type 1 diabetes risk are rare. The objective of this study was to examine the association between life events during pregnancy and first-appearing islet autoantibodies (IA) in young children, conditioned by the child's type 1 diabetes-related genetic risk.

Methods The IA status of 7317 genetically at-risk The Environmental Determinants of Diabetes in the Young (TEDDY) participants was assessed every 3 months from 3 months to 4 years, and bi-annually thereafter. Reports of major life events during pregnancy were collected at study inception when the child was 3 months of age and placed into one of six categories. Life events during pregnancy were examined for association with first-appearing insulin (IAA) ($N = 222$) or GAD (GADA) ($N = 209$) autoantibodies in the child until 6 years of age using proportional hazard models. Relative excess risk due to interaction (RERI) by the child's HLA-DR and SNP profile was estimated.

Results Overall, 65% of mothers reported a life event during pregnancy; disease/injury (25%), serious interpersonal (28%) and job-related (25%) life events were most common. The association of life events during pregnancy differed between IAA and GADA as the first-appearing autoantibody. Serious interpersonal life events correlated with increased risk of GADA-first only in HLA-DR3 children with the *BACH2*-T allele (HR 2.28, $p < 0.0001$), an additive interaction (RERI 1.87, $p = 0.0004$). Job-related life events were also associated with increased risk of GADA-first among HLA-DR3/4 children (HR 1.53, $p = 0.04$) independent of serious interpersonal life events (HR 1.90, $p = 0.002$), an additive interaction (RERI 1.19, $p = 0.004$). Job-related life events correlated with reduced risk of IAA-first (HR 0.55, $p = 0.004$), particularly in children with the *BTNL2*-GG allele (HR 0.48; 95% CI 0.31, 0.76).

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Research in context

What is already known about this subject?

- Prospective studies have suggested a link between psychological stress and type 1 diabetes
- Two prospective studies have documented an association between psychological stress or life events during pregnancy and risk of type 1 diabetes in the child

What is the key question?

- Are maternal life events experienced during pregnancy associated with first-appearing insulin autoantibodies (IAA) or GAD autoantibodies (GADA) in the young child and is any association conditioned by the child's type 1 diabetes genetic risk?

What are the new findings?

- The association of maternal life events during pregnancy differed between IAA and GADA as the first-appearing autoantibody and was often conditioned by the child's type 1 diabetes genetic risk
- A job-related life event during pregnancy was associated with reduced risk of IAA as the first-appearing autoantibody in all children except HLA-DR3/4 children with the *BTNL2*-A allele
- In contrast, a serious interpersonal life event during pregnancy was associated with increased risk for GADA as the first-appearing autoantibody in HLA-DR3 children with the *BACH2*-T allele; a job-related life event during pregnancy was also associated with an increased risk of GADA in HLA-DR3/4 children

How might this impact on clinical practice in the foreseeable future?

- The findings add to our understanding of the different pathways by which a child may develop type 1 diabetes and support the need for additional investigation of the role of life events during pregnancy in the child's development of type 1 diabetes-related autoimmunity

Conclusions/interpretation Specific life events during pregnancy are differentially related to IAA vs GADA as first-appearing IA and interact with different HLA and non-HLA genetic factors, supporting the concept of different endotypes underlying type 1 diabetes. However, the mechanisms underlying these associations remain to be discovered. Life events may be markers for other yet-to-be-identified factors important to the development of first-appearing IA.

Keywords *BACH2* single nucleotide polymorphism · *BTNL2* single nucleotide polymorphism · GAD autoantibodies · HLA-DR-DQ haplogenotype · Insulin autoantibodies · Islet autoimmunity · Prenatal life events · Psychosocial stress · Type 1 diabetes

Abbreviations

BACH2	rs3757247 SNP in *BACH2*
BTNL2	rs3763305 SNP in *BTNL2*
CTLA4	rs231775 SNP in *CTLA-4*
ERBB3	rs2292239 SNP in *ERBB3*
GADA	GAD autoantibodies
IA	Islet autoantibodies
IAA	Insulin autoantibodies
IA-2A	Insulinoma-associated protein 2 autoantibodies
INS	rs1004446 SNP in *INS*
LE	Life event
PTPN22	rs2476601 SNP in *PTPN22*
RERI	Relative excess risk due to interaction
SH2B3	rs3184504 SNP in *SH2B3*
TEDDY	The Environmental Determinants of Diabetes in the Young

Introduction

The Environmental Determinants of Diabetes in the Young (TEDDY) study seeks to identify environmental triggers of type 1 diabetes in genetically at-risk children followed from birth to age 15 at three centres in the USA (Colorado, Georgia/Florida and Washington) and three centres in Europe (Germany, Finland and Sweden). We previously confirmed

that diabetes-related insulin autoantibodies (IAA) first appear at an earlier age than GAD autoantibodies (GADA) and that the order of appearance is related to the child's HLA-DR-DQ haplogenotype [1], suggesting different pathways to the endotypes of type 1 diabetes [2].

Psychological stress has long been considered a possible trigger of type 1 diabetes; literature reviews and prospective studies provide evidence for such a linkage [3–8]. However, the mechanism by which psychological stress might lead to type 1 diabetes is unknown. Psychological stress could have a direct effect on the development of diabetes-related autoimmunity or it could have an indirect effect by increasing the likelihood of some other exposures associated with the aetiology of the disease [9]. Any link between stress and diabetes-related autoimmunity may also depend on the child's HLA and non-HLA genetic risk. No study has examined the association between psychological stress and first-appearing IAA and GADA separately nor has the impact of the child's HLA and non-HLA genetic risk on the possible relationship between stress and IA been explored. Although there is prospective literature documenting a link between life events (LEs) during pregnancy and subsequent infections or illnesses in the child [10–13], only two prospective studies examined the relationship between psychological stress or LEs during pregnancy and the child's subsequent risk of type 1 diabetes: death of the child's father or sibling during pregnancy was associated with increased risk [7] and interpersonal events during pregnancy (e.g. divorce, family conflict) were associated with increased risk in HLA-DR3/4 children [8]. Neither study assessed the possible link between LEs during pregnancy and first-appearing IA in the child.

In TEDDY, all children were recruited based on their high-risk HLA-DR-DQ haplogenotype. We have reported that non-HLA SNPs are strongly associated with the development of islet autoantibodies (IA) up to 6 years of age: rs2476601 (*PTPN22*), rs2292239 (*ERBB3*), rs1004446 (*INS*), rs3184504 (*SH2B3*) and rs3763305 (*BTNL2*). Several SNPs were found to be related differently with IAA compared with GADA: rs231775 (*CTLA4*), rs689 (*INS*) and rs3757247 (*BACH2*) [1, 14–16]. No study has examined whether the association between psychological stress and first-appearing IAA and GADA is dependent on these SNPs.

Methods

Participants The TEDDY study design and methods are published elsewhere [17], as well as the characteristics of those who enrolled and those who declined [18, 19]. Written, informed consents were obtained from parents of all participants and the study was approved by each site's institutional review or ethics board. All participants joined the TEDDY study before 4.5 months of age.

The current analysis focused on the TEDDY cohort as of March 2019. Of the 8676 children who entered TEDDY, the following were excluded from the analysis: twin or triplet (n = 252); child determined not to be HLA eligible (n = 120); someone other than the mother was interviewed at 3 months about the mother's LEs during the pregnancy (n = 168) or no one was interviewed (n = 5); mother had gestational, type 1 or type 2 diabetes (n = 791); or the child's antibody status was indeterminant (n = 23). Hence, we included 7317 TEDDY children followed for the development of IA until 6 years (<84 months) of age. During this interval, 532 (7.3%) of these children developed autoantibodies (222 IAA-first; 209 GADA-first; ten insulinoma antigen-2 (IA-2A)-first; 91 multiple IA at first detection). This paper focuses on the IAA-first and GADA-first samples since the first-appearing IA could not be determined in the multiple-IA cases and the IA-2A sample was too small.

Genotyping More than 400,000 newborns were HLA genotyped and those with DR3/4, DR4/4, DR4/8 and DR3/3 HLA haplogenotypes were eligible for TEDDY participation. For first-degree relatives of someone with type 1 diabetes, several additional haplogenotypes were eligible for inclusion (electronic supplementary material [ESM] Table 1). Of the 21,589 HLA eligible children, 8676 joined the TEDDY study. When the TEDDY participant was 9–12 months of age, the child's HLA status was confirmed and diabetes-related SNPs from the Illumina Immuno BeadChip (manifest file: Immuno_BeadChip_11419691.bpm from Illumina, San Diego, CA, USA) were assessed. The methods for genotyping have been published previously [1]. For the current analysis, we focused on the high-risk HLA groups and SNPs previously found to be associated with IAA, GADA or both in TEDDY (rs2476601 in *PTPN22*; rs2292239 in *ERBB3*; rs1004446 in *INS*; rs3757247 in *BACH2*; rs3184504 in *SH2B3*; rs231775 in *CTLA4*; and rs3763305 in *BTNL2*) [1, 11, 12].

Islet autoantibodies Blood draws for IA assay were done every 3 months for the first 4 years of participation and then bi-annually unless the child was positive for IA, in which case quarterly visits were maintained. A child was considered to have developed IA if the child had persistent confirmed autoimmunity defined as the presence of confirmed IAA, GADA or IA-2A at each of the two TEDDY reference laboratories on two or more consecutive visits. The assay methods have been published elsewhere [1].

LEs during pregnancy The most common method for assessing LEs is a self-reported checklist (e.g. Social Readjustment and Rating Scale, Life Experiences Survey) [20, 21]. To improve the quality of the data obtained, we modified this approach based on expert recommendations from the literature [22]. Instead of using an LE checklist, we

interviewed the mother at the time of the child's enrolment in TEDDY (during the child's first 3–4 months of life) about any major LE that occurred during her pregnancy. As part of the interview process, the mother was provided with a list of 20 LEs commonly reported [23] and was invited to report LEs not listed (ESM Table 2). To enhance accurate recall, the focus was on a specific, relatively short time-frame: her pregnancy. Our approach considered both overall LEs and type of LE grouped into six categories: disease/injury (self or others); significant loss (death of a family member or friend); serious interpersonal (marriage, separation, divorce, conflicts with spouse/relative/friend, moved or had a change in family composition); job-related (self or spouse quit/lost a job or started work/school); financial difficulties (self or spouse/partner); and other. To avoid concerns about quantifying the total number of LEs, and given the short time-frame of interest (pregnancy), we treated LEs as a dichotomous (yes/no) variable.

Statistical methods LEs during pregnancy and their association with IA overall as well as with IAA or GADA as the first-appearing autoantibodies were evaluated by proportional hazard models. Children negative for autoantibodies were right-censored on the day of the last negative autoantibody test result or on the day before the child's seventh birthday. The strength of associations was described by HRs and 95% CIs. Factors known to be associated with the development of autoimmunity (country, sex, having a father or sibling with type 1 diabetes, HLA haplogenotype and diabetes-related SNPs) were statistically controlled. Further testing of whether risk factors for IAA-first and GADA-first differed was performed by multivariate logistic regression, modelling factors significantly associated with the ratio of IAA-first to GADA-first. All factors included in the proportional hazard models were also included in the logistic models in addition to age of seroconversion. To account for correlation among the non-mutually exclusive LE categories, all categories were included in the multivariate models and a p value less than 0.05 was considered statistically significant. LEs significantly associated with IA overall or IAA-first vs GADA-first were further examined. Confounding and selection biases were considered by adjusting parsimoniously for maternal factors associated with maternal reports of LEs during pregnancy using an LE propensity score. An inverse probability of treatment (LE) weighting analysis, as described elsewhere [24], was performed to reduce selection bias by weighting children in the proportional hazard models by a stabilised weight created from the LE propensity score [25]. Since the propensity score was first an estimate and then a known quantity, standard errors were calculated from 1000 bootstrap samples. Maternal factors were also included in the models if they showed a significant association with outcome. Finally, maternal LEs showing an association with development of IA in the offspring were tested for effect modification by the child's genetic risk factors. Interactions were examined on the ratio scale (multiplicative interaction), by including a cross-product term in the proportional hazard model, or on the difference scale (additive interaction) by estimating the relative excess risk due to interaction (RERI). The RERI was estimated by including in the model a four-category variable describing the presence (1) and absence (0) of LEs and genetic factors and estimating the RERI as $HR_{11} - HR_{10} - HR_{01} + 1$ [26]. The RERI and 95% CI estimate the additional risk due to interaction, with RERI >0 suggesting synergistic interaction. The strongest interactions are considered to exist on both the additive and multiplicative scales, with an RERI >1 indicating possible sufficient cause interaction between LE and gene. To account for multiple genetic × LE comparisons, a false discovery rate was calculated to account for the number (HLA-DR haplogenotypes and SNPs) and the type (additive or multiplicative) of genetic interaction tests ($n = 22$), and a false discovery rate <0.05 was considered statistically significant. A sensitivity analysis was performed to determine whether results may have been influenced by knowing the first autoantibody for children who developed both IAA and GADA between visits ($n = 72$ children). These children were censored at the time of seroconversion in a competing risk analysis along with 29 children who had developed IA-2A. The model discriminating IAA-first from GADA-first was fitted to children with both IAA and GADA to predict which autoantibody might have come first. Associations examining LEs with first-appearing autoantibodies were repeated to include any additional first-appearing autoantibody cases that had a predicted ratio of 2:1 to have developed one autoantibody over the other (51/72). The sensitivity analysis was extended to examine the influence of attrition bias by including any first-time dropout or loss to follow-up (>1 year since last visit) as a competing risk.

Results

Nearly two-thirds (65%) of all participating mothers reported at least one LE during pregnancy (US mothers: 69%; European mothers: 62%). The most common categories of events reported were: disease/injury (25%); serious interpersonal (28%); and job-related (25%). Financial difficulties were reported by 19% of US mothers but only 5% of European mothers (Table 1).

Controlling for all demographic and genetic factors associated with any IA, IAA-first or GADA-first (ESM Table 3), having one or more LE of any kind during pregnancy was not associated with IA overall (ESM Table 4). However, it was associated differently for children developing IAA-first as compared with GADA-first ($p = 0.04$, ESM Table 4). The associations between specific LEs and any IA, IAA-first and

Table 1 LEs during pregnancy reported by US and European TEDDY mothers

LE	All mothers (*N*=7317), *n* (%) reporting the event	US mothers (*N*=3103), *n* (%) reporting the event	European mothers (*N*=4214), *n* (%) reporting the event
Any LE	4742 (65)	2132 (69)	2610 (62)
Disease/injury (self or others)	1842 (25)	847 (27)	996 (24)
Significant loss (death of family member or friend)	767 (11)	380 (12)	387 (9)
Serious interpersonal (marriage, separation, divorce, conflicts with spouse/relative/friend, move or change in family composition)	2010 (28)	982 (32)	1028 (24)
Job-related (self or spouse quit/lost job, started work/school)	1828 (25)	853 (28)	975 (23)
Financial difficulties (self or spouse)	807 (11)	588 (19)	219 (5)
Other	995 (14)	412 (13)	583 (14)

GADA-first are provided in Table 2. Having a serious interpersonal LE, independent of other LE categories, was associated with an increased risk of IA overall (HR 1.25; 95% CI 1.03, 1.52; $p = 0.02$). This was largely explained by an increased risk of GADA-first (HR 1.58; 95% CI 1.18, 2.12; $p = 0.002$). In contrast, a job-related LE correlated with a lower risk of IAA-first (HR 0.55; 95% CI 0.40, 0.84; $p = 0.004$). The association of job-related LE with IAA-first differed significantly compared with GADA-first ($p = 0.005$) and there was no association with IA overall. No other LE category showed a correlation with IA, IAA-first or GADA-first.

The serious interpersonal LE association with GADA and the job-related LE association with IAA remained significant (p values ≤0.01) when sensitivity analysis was performed to address possible bias due to other factors associated with maternal reports of LE (ESM Table 5). Attrition or interval censoring due to lack of determination of the first-appearing IA did not affect the associations. Since we previously reported that respiratory infections during pregnancy exhibited a protective association with IAA for certain genetic subgroups [15] and stressful LEs are known to increase risk of illness [9], we reasoned that maternal illness during pregnancy might explain our job-related LE–IAA association. However, multivariate modelling, controlling for respiratory illness during pregnancy, did not reduce the protective association between job-related LE during pregnancy and IAA-first in the child (ESM Table 6).

We next examined whether the associations of job-related LE with IAA-first and serious interpersonal LE with GADA-first were dependent on the child's type 1 diabetes genetic risk. Additive and multiplicative interactions were tested between maternal job-related LE and the ten genetic components of the child (HLA-DR3, HLA-DR4, HLA-DR8 and SNPs in *PTPN22*, *INS*, *ERRB3*, *SH2B3*, *BACH2*, *CTLA4* and *BTNL2*) (ESM Table 7). Adjusting for multiple comparisons, there were no statistically significant interactions between job-related LE and the genetic components. However, an interaction was observed between job-related LE and the *BTNL2* SNP on both the risk difference (additive interaction, RERI 0.56; 95% CI 0.05, 1.08; $p = 0.03$) and ratio scales (multiplicative interaction, HR 2.19; 95% CI 1.01, 4.77; $p = 0.048$). Job-related LE was associated with a reduced risk of IAA among children with the *BTNL2*-GG genotype (HR 0.48; 95% CI 0.31, 0.76), but no correlation was seen among children with the *BTLN2*-A allele (HR 1.03; 95% CI 0.54, 1.95). An examination of the overall absolute incidence of IAA-first at age 6 years by job-related LE and the child's HLA-DR and *BTNL2* genotypes showed that job-related LE reduced incidence of IAA-first consistently across HLA-DR haplogenotypes, although only in children with the *BTNL2*-GG genotype (Fig. 1a). The association was not consistent for children with the *BTNL2*-A allele (Fig. 1b). In this cohort, 99.4% of HLA-DR3/3 children have the *BTNL2*-GG genotype and the incidence of IAA-first among HLA-DR3/3 children was low. Thus, we examined the children with at least one HLA-DR4 haplogenotype. The reduced risk of IAA-first by a job-related LE during pregnancy was primarily observed before 3 years of age and only in children with the *BTNL2*-GG genotype (Fig. 1c,d).

The additive and multiplicative interactions between a serious interpersonal LE during pregnancy and the ten genetic components of the child on risk of GADA-first are summarised in ESM Table 8. An additive interaction was discovered between the *BACH2* SNP and a serious interpersonal LE (RERI 1.25; 95% CI 0.50, 2.00; $p = 0.001$; false discovery rate = 0.02). Taking *BACH2*-CC genotype and no serious interpersonal LE as a reference group, only children with a *BACH2*-CT or TT genotype and a serious interpersonal LE showed an increased risk of developing GADA-first (HR 2.22; 95% CI 1.48, 3.33), with no increase seen for children with a *BACH2*-CC genotype and a serious interpersonal LE (HR 0.77; 95% CI 0.40, 1.47) or for *BACH2*-CT and TT genotypes and no serious interpersonal LE (HR 1.21; 95% CI 0.83, 1.77). The child's HLA haplogenotype also showed evidence of modifying the serious interpersonal LE effect

Table 2 Maternal LEs reported during pregnancy in relation to the risk of IA in the child until 6 years of age

Specific LE	Outcome=IA multivariate and adjusted			Outcome=IAA-first multivariate and adjusted			Outcome=GADA-first multivariate and adjusted		
	LE among cases (% of *n*=532)	HR[a] (95% CI)	p value	LE among cases (% of *n*=222)	HR[a] (95% CI)	p value	LE among cases (% of *n*=209)	HR[a] (95% CI)	p value
Disease/injury (self or others)	24%	0.94 (0.77, 1.15)	0.56	28%	1.27 (0.94, 1.70)	0.12	22%	0.82 (0.59, 1.15)	0.25
Significant loss (death of family member or friend)	9%	0.89 (0.66, 1.20)	0.44	9%	0.84 (0.52, 1.35)	0.48	10%	0.97 (0.61, 1.54)	0.90
Serious interpersonal (marriage, separation, divorce, conflicts with spouse/relative/friend, move or change in family composition)	30%	1.25 (1.03, 1.52)	0.02	26%	1.02 (0.75, 1.39)	0.90	35%	1.58 (1.18, 2.12)	0.002
Job-related (self or spouse quit/lost job, started work/school)	23%	0.89 (0.72, 1.10)	0.26	16%	0.55 (0.40, 0.84)[b]	0.004	28%	1.07 (0.78, 1.47)[b]	0.68
Financial difficulties (self or spouse)	8%	0.95 (0.68, 1.32)	0.76	9%	1.24 (0.76, 2.04)	0.39	8%	0.78 (0.46, 1.32)	0.35
Other	9%	0.91 (0.68, 1.22)	0.53	10%	1.03 (0.66, 1.61)	0.85	10%	0.88 (0.55, 1.39)	0.57

[a] Multivariable proportional hazard model of specific reported maternal LEs examined together in relation to IA overall as well to IAA-first and GADA-first among 6364 children followed until 6 years of age

Models adjusted for sex, country of residence, family history of type 1 diabetes and genetic risk factors for IA as shown in ESM Table 3.

[b] LE is associated differently with hazard of IAA-first as compared with GADA-first ($p<0.05$)

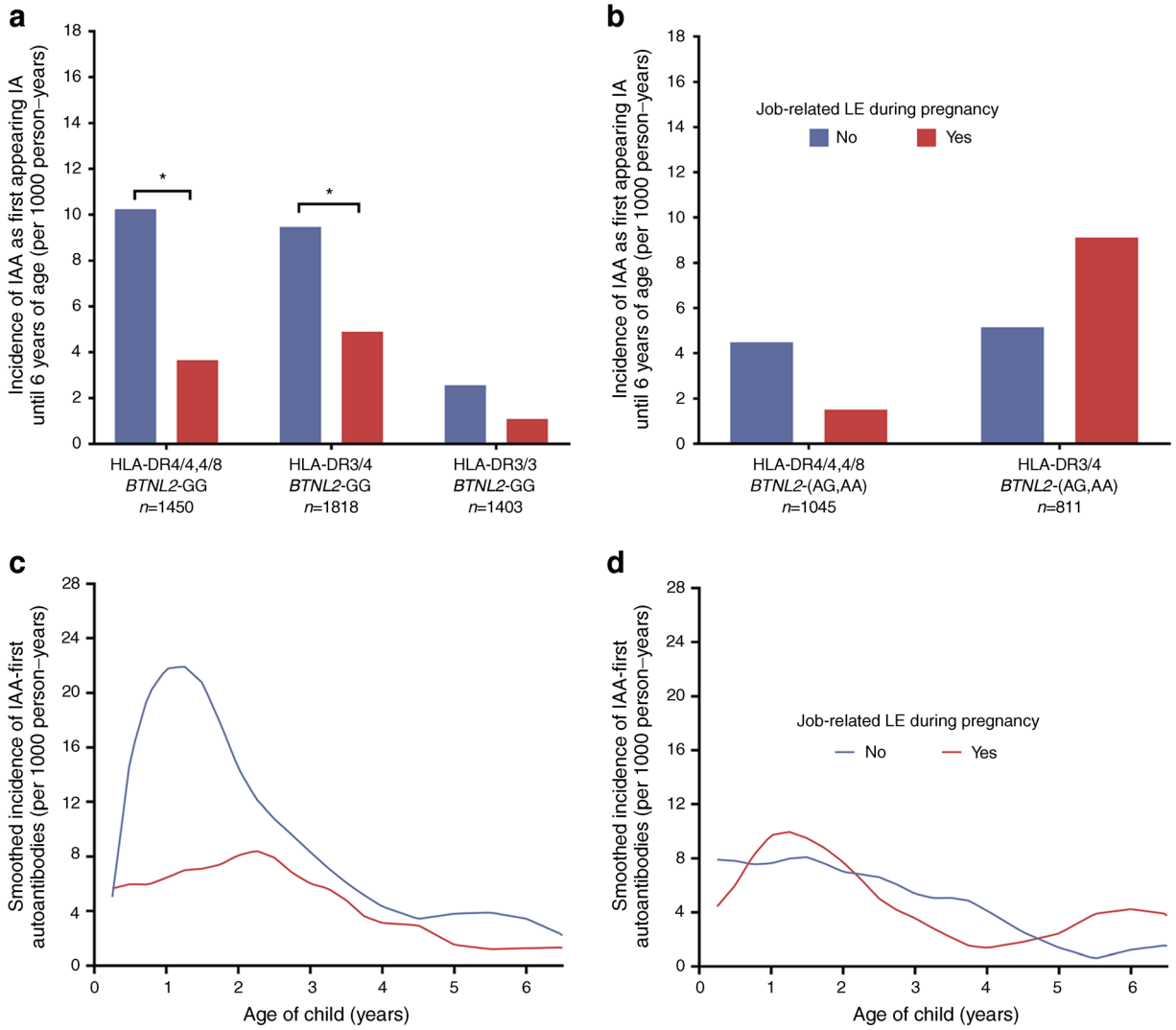

Fig. 1 (**a**) HLA-DR4/4 or 4/8 children and HLA-DR3/4 children with the *BTNL2*-GG genotype were less likely to develop IAA as the first-appearing IA if the mother experienced a job-related LE during pregnancy (*p<0.05). A job-related LE during pregnancy was unrelated to IAA as the first-appearing IA in HLA-DR3/3 children (p=0.26) with the *BTNL2*-GG genotype. (**b**) There was no association between job-related LE in pregnancy and IAA as the first-appearing antibody in HLA-DR4/4 or 4/8 children (p=0.14) or DR3/4 children (p=0.16) with the *BTNL2*-AA/AG genotype. (**c**) Age-specific incidence of IAA as the first-appearing IA by job-related LE in pregnancy for HLA-DR4 children with the *BTNL2*-GG genotype and (**d**) for HLA-DR4 children with the *BTNL2*-AA/AG genotype

(ESM Table 8). The absolute risk of GADA-first at 6 years of age stratifying on serious interpersonal LE during pregnancy as well as both HLA-DR and *BACH2* genotypes showed that the increased risk of GADA-first by serious interpersonal LE in pregnancy and *BACH2*-T allele only occurred if children had the HLA-DR3 haplogenotype (HR 2.28, $p < 0.0001$), an additive interaction (RERI 1.87, $p = 0.0004$). (Fig. 2a,b). Among HLA-DR3 children, the impact of the *BACH2*-T allele on the serious interpersonal LE correlation with GADA-first increased with the age of the child (Fig. 2c,d).

Since GADA-first generally occurs later than IAA-first, we also examined whether job-related LE interacted with the child's HLA-DR haplogenotype on risk of GADA-first. Job-related LE showed strong multiplicative (HR 2.44; 95% CI 1.29, 4.61; $p = 0.006$) and additive interactions (RERI 1.19; 95% CI 0.38, 2.00; $p = 0.004$) with the HLA-DR3/4 haplogenotype. Job-related LE correlated with increased risk of GADA-first (HR 1.75; 95% CI 1.17, 2.61; $p = 0.006$) among children with HLA-DR3/4, while no increase was seen in children without both the HLA-DR3 and the HLA-DR4 haplogenotypes (HR 0.68; 95% CI 0.41, 1.12; $p = 0.12$). The interaction was not dependent on the *BTNL2* genotype or age (ESM Fig. 1a–d). For children with HLA-DR3/4, serious interpersonal LE (HR 1.90; 95% CI 1.26, 2.84; $p = 0.002$) and job-related LE (HR 1.53; 95% CI 1.01, 2.30; $p = 0.04$) independently correlated with an increased risk of GADA-first after adjusting for all other factors. The incidence of GADA-first by 6 years of age among HLA-DR3/4 children

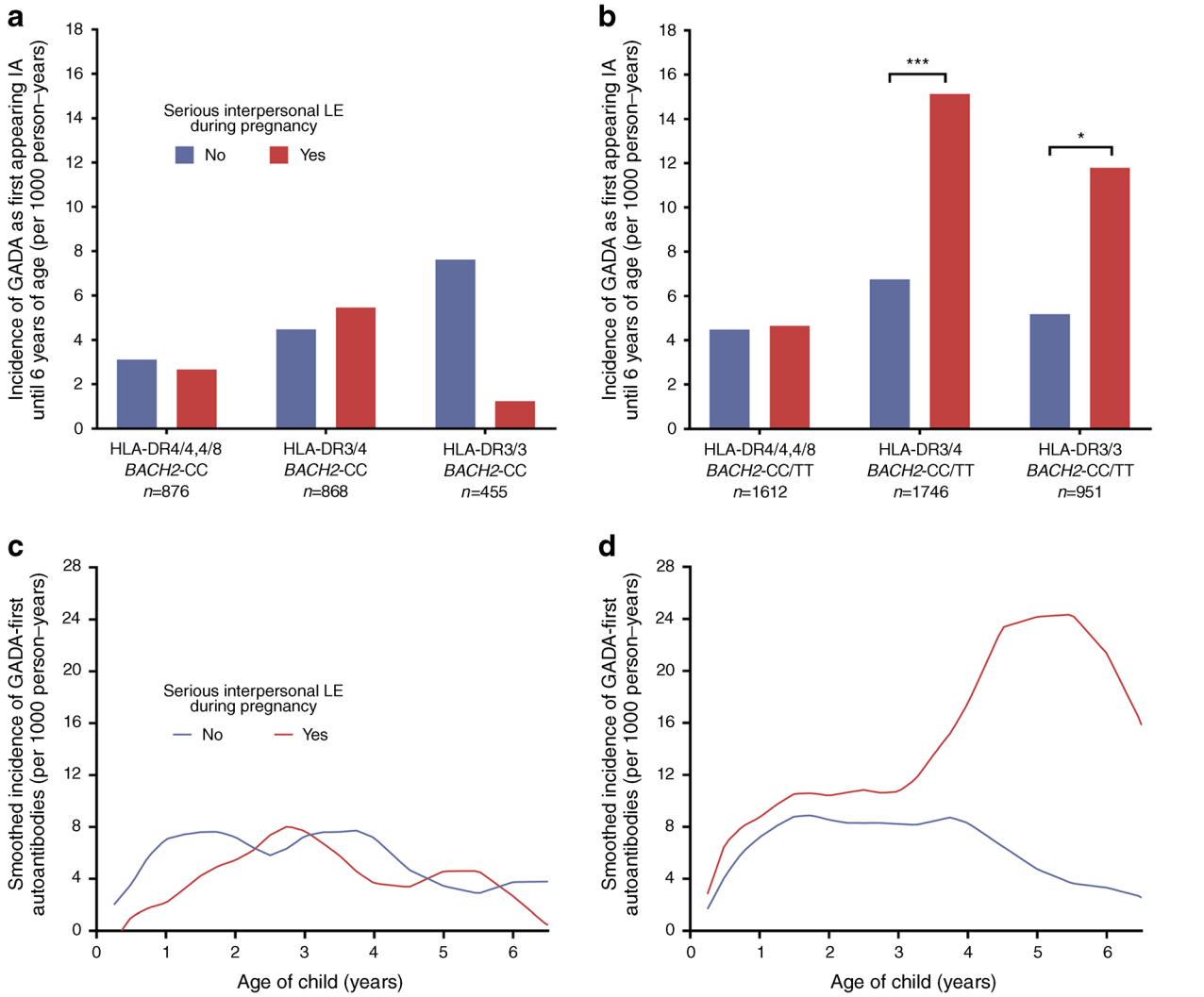

Fig. 2 (**a**) There was no association between a serious interpersonal LE in pregnancy and GADA as the first-appearing autoantibody for HLA-DR4/4 or 4/8 (p=0.79), HLA-DR3/4 (p=0.65) and HLA-DR3/3 (p=0.08) children with the *BACH2*-CC genotype. (**b**) A serious interpersonal LE was associated with increased risk of GADA as the first-appearing antibody in HLA-DR3/4 children with the *BACH2*-CT/TT genotype (***p<0.0005) and HLA-DR3/3 children with the *BACH2*-CT/TT genotype (*p<0.05), but not in HLA-DR4/4 or 4/8 children with the *BACH2*-CT/TT genotype (p=0.91). (**c**) Age-specific incidence of GADA as the first-appearing autoantibody by serious interpersonal LE during pregnancy for HLA-DR3 children with the *BACH2*-CC genotype and for (**d**) HLA-DR3 children with the *BACH2*-CT/TT genotype

was 13.7/1000 person-years if mothers reported both serious interpersonal LE and job-related LE (n = 308), 9.2/1000 person-years if mothers reported only one of these events (n = 979) and 5.1/1000 person-years if mothers reporting neither during pregnancy (n = 1602).

Discussion

Type 1 diabetes is a complex autoimmune disease, with age and HLA and non-HLA SNPs associated with the first-appearing IA [1]. Although the incidence of type 1 diabetes increases until early adolescence, the appearance of IAA as the first IA peaks in the first year of life, predominately among HLA-DR4 children, while GADA-first appears consistently throughout early childhood. Our findings add further clarification of these relationships by identifying specific LEs during pregnancy that correlated differently with IAA-first and GADA-first in genetically at-risk young children. For IAA-first, we found that a job-related LE reported during pregnancy was associated with lower risk, specifically among children having the *BTNL2*-GG genotype. For GADA-first, a serious interpersonal LE during pregnancy, interacting with HLA-DR3 and *BACH2*-G alleles, correlated with a significant excess risk. Taken together, these findings show that children born with the highest HLA risk for type 1 diabetes (HLA-DR3/4) had a greater risk of developing GADA-first over IAA-first, and thus IA at a later age, if the mother reported a job-related LE or serious interpersonal LE during pregnancy.

Although these findings are intriguing, they raise many questions about the mechanisms underlying these associations. For example, a job-related LE could be a proxy for other events that occur during pregnancy or after the birth of the child (e.g. exclusivity/duration of breast feeding, age placed in daycare) that could prove to be associated with IAA at a young age. Of interest is our finding that among HLA-DR4 children, the *BTNL2*-A allele had a protective association with IAA; a job-related LE during pregnancy was not associated with IAA in these children. In contrast, the protective association of a job-related LE occurred in those HLA-DR4 children with the higher-risk *BTNL2*-GG genotype. Although this interaction was no longer significant when controlling for multiple comparisons, this preliminary finding may warrant further exploration as the *BTNL2-G* allele, among HLA-DR3/4 children in the TEDDY cohort, was in nearly complete linkage disequilibrium with *HLA-DRB1*04* subtypes (*04:01, *04:02, *04:05), while the *BTNL2-A* allele was associated with *HLA-DRB1*04:04* and *04:07 [15]. Previously, the *BTNL2*-GG genotype was associated with increased risk for IA and type 1 diabetes, although its specific association with IAA and GADA as the first-appearing autoantibody was not explored [15]. *BTNL2* is a butyrophilin family member and mutations in this gene have been associated with several autoimmune diseases [15, 27]. There are few studies examining the association of *BTNL2* with type 1 diabetes [28, 29], although *BTNL2* is thought to have a regulatory function on T cell generation and function [15, 30–32] (ESM Table 9).

Whatever the mechanism, evidence of protection against IAA is important because IAA is seen in very young children who tend to go on to develop type 1 diabetes very rapidly [33]; reducing risk of IAA would likely delay type 1 diabetes onset even in those who later develop GADA. While HLA-DR4 is associated with IAA-first, HLA-DR3 is associated with GADA-first. We found that a serious interpersonal LE during pregnancy further increased the risk of GADA in HLA-DR3 children but not in HLA-DR4 children. Further, serious interpersonal LEs were associated with increased risk of GADA in children with the *BACH2*-T allele. A number of studies have documented an association between the *BACH2* SNP and increased risk for type 1 diabetes [34–38], and one study reported *BACH2* to be associated with increased risk for GADA as the first-appearing antibody [1]. Several studies have suggested that the *BACH2* SNP plays a key role in B cell differentiation [39] as well as T cell regulation [40–42]. While only 10% of the participants had all three risk factors (DR3, *BACH2*-T allele, serious interpersonal LE during pregnancy), 24% of the GADA participants did.

However, the mechanism by which a serious interpersonal LE during pregnancy might lead to GADA in DR3, *BACH2*-T allele children is unknown. Serious interpersonal LEs may be rather chronic in nature, resulting in a maternal stress response affecting the mother's own immune system as well as the development of the immune system in the child. It is also possible that a serious interpersonal LE during pregnancy is a proxy for other exposures or maternal behaviours (e.g. diet, sleep, prenatal care) that could affect the developing fetus, or mothers with serious interpersonal LEs during their pregnancy may continue to experience such events after the child's birth, influencing mother–child bonding, breastfeeding behaviour [43] and other parenting practices or environmental exposures that could affect the child.

The HLA-DR3/4 haplogenotype is the most common high-risk subgroup in TEDDY. Children in this subgroup are equally likely to get IAA as GADA as the first-appearing autoantibody. Like children with other HLA-DR haplogenotypes, a job-related LE during pregnancy was protective of IAA, particularly in those with the *BTNL2*-GG genotype. However, the same job-related LE during pregnancy actually increased risk of GADA in these children, suggesting that in these children the risk for IA was not reduced but simply shifted in time. In fact, these children were at exceedingly high risk for GADA if their mothers experienced both a job-related LE and a serious interpersonal LE during pregnancy and they had the *BACH2*-T allele.

Although gene–environment interactions are often cited as a causal influence for type 1 diabetes, there are few clear examples in the published literature. Here we report two gene–environment interactions relevant to GADA as the first-appearing antibody: a serious interpersonal LE in pregnancy increased a child's risk for GADA in HLA-DR3 but not HLA-DR4 children, and increased risk for GADA in children with the *BACH2*-T allele but not in children with the *BACH2*-CC genotype. We also report some preliminary evidence that a job-related LE during pregnancy may have a protective association with IAA in those HLA-DR4 children with the *BTNL2*-GG genotype, but may increase risk for GADA in HLA-DR3/4 children.

By focusing separately on IAA and GADA as the first-appearing autoantibody, we add to our understanding of the different pathways by which a child may develop this disease. By including diabetes-related genetic information in our analyses we have advanced our knowledge of the complex interplay of genes and environment in its early development. Although our study findings highlight important associations between different types of LEs during pregnancy and the child's subsequent likelihood of developing type 1 diabetes-related autoimmunity, we have yet to understand the mechanisms underlying these associations. Nevertheless, our study findings clearly support the concept of different endotypes underlying type 1 diabetes [2].

The study limitations include a rather crude measure of environmental stress exposure during pregnancy. Unfortunately, there is no gold standard for measuring human environmental stress exposure. We took a number of steps to promote accurate recall of LE data; our prospective study design eliminated the possibility that associations were a

product of recall bias. Further, our findings are consistent with a previously published report linking interpersonal events during pregnancy with type 1 diabetes in HLA-DR3/4 children [8]. However, certain types of LEs (significant loss and financial difficulties) were infrequently reported, limiting our ability to detect any association between these types of events and the development of IA in the child. Given the previously published study documenting a link between death of a father or a sibling during the prenatal period and the development of type 1 diabetes [7], we conducted an exploratory post hoc analysis examining onset of any IA by HLA-DR haplogenotype and specific LEs. This analysis suggested that a significant loss LE during pregnancy may indeed impact onset of IA in the child, but the effect is dependent on the child's HLA-DR haplogenotype (ESM Table 10).

Our focus on environmental stress exposure during pregnancy and not the mother's reaction to that exposure is an additional study limitation. Our purpose was to first test the possible association of environmental stress exposure during pregnancy with the development of diabetes-related autoimmunity in the child. Resilience, coping or adaptation in response to LEs are certainly additional factors important to consider in future studies [44].

Additional study limitations include its narrow focus on IAA-first and GADA-first in children of non-diabetic mothers until 6 years of age. We conducted post hoc analyses of 774 TEDDY children with diabetic mothers of whom 65 developed IA (29 IAA-first, 24 GADA-first). In this small sample, children of mothers reporting a job-related LE during pregnancy were more likely to develop GADA-first than IAA-first (HR 3.16; 95% CI 1.25, 9.02; $p = 0.02$), consistent with the findings we report here. However, we were unable to document an association between serious interpersonal LEs and any IA, possibly due to the small sample size. Future research will need to expand this work to the unique situation experienced by pregnant women with diabetes. Also important will be studies of prenatal LEs and IA in older children, as well as their possible role in a child's progression to multiple autoantibody status or type 1 diabetes. Because TEDDY study visits occur every 3 to 6 months, we were unable to determine the first IA for 20% of children who exhibited multiple autoantibodies in their first positive test results; this was another study limitation. However, sensitivity analysis showed little evidence that determination of first-appearing IA for these multiple-IA children would change the findings.

In an effort to address some of these limitations, TEDDY investigators plan to explore the role of LEs after the child's birth in both the development of IA and progression toward type 1 diabetes. Plans also include exploring possible associations with stress response-related genotypes in the child using genome-wide SNP coverage, epigenetic and transcriptome profiling, child gut microbiota, metabolomics and proteomics, and the child's immunological response. Although stress has long been considered a possible trigger of type 1 diabetes, this work will advance the field beyond the simple association studies that have characterised the literature to date. Elucidating the gene–environment interactions underlying the pathogenesis of this disease is critical to our ultimate goal of type 1 diabetes prevention.

Supplementary Information The online version contains peer-reviewed but unedited supplementary material available at https://doi.org/10.1007/s00125-020-05344-9.

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Data availability The datasets generated and analysed during the current study will be made available in the NIDDK Central Repository at https://www.niddkrepository.org/studies/teddy. The TEDDY ImmunoChip (SNP) data that support the findings of this study have been deposited in NCBI's database of Genotypes and Phenotypes (dbGaP) with the primary accession code phs001037.v2.p1.

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Authors' relationships and activities The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

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ESM Table 1: TEDDY HLA Eligibility Criteria for a First-degree Relative (FDR) and the General population (GP)

Code	Haplotype genotypes	Abbreviation	FDR	GP	N (%)
A	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*03-DQA1*05:01-DQB1*02:01*	HLA-DR3/4	Yes	Yes	2889 (39.5%)
B	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*04-DQA1*03-DQB1*03:02*	HLA-DR4/4	Yes	Yes	1435 (19.6%)
C	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*08-DQA1*04:01-DQB1*04:02*	HLA-DR4/8	Yes	Yes	1284 (17.5%)
D	*DRB1*03-DQA1*05:01-DQB1*02:01 / DRB1*03-DQA1*05:01-DQB1*02:01*	HLA-DR3/3	Yes	Yes	1553 (21.2%)
E	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*04-DQA1*03-DQB1*02:02*	HLA-DR4/4b	Yes	No	3 (0.04%)
F	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*01-DQA1*01:01-DQB1*05:01*	HLA-DR4/1	Yes	No	98 (1.3%)
G	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*13-DQA1*01:02-DQB1*06:04*	HLA-DR4/13	Yes	No	28 (0.4%)
H	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*04-DQA1*03-DQB1*03:04*	HLA-DR4/4c	Yes	No	3 (0.04%)
I	*DRB1*04-DQA1*03-DQB1*03:02 / DRB1*09-DQA1*03-DQB1*03:03*	HLA-DR4/9	Yes	No	12 (0.2%)
J	*DRB1*03-DQA1*05:01-DQB1*02:01 / DRB1*09-DQA1*03-DQB1*03:03*	HLA-DR3/9	Yes	No	12 (0.2%)

Genotypes A, B, C, D confer general population eligibility but exclude *DRB1*04:03*. Genotypes A through J confer eligible to a first degree relative with type 1 diabetes.

ESM Table 2. Experiences of the Parent/Primary Caretaker

	Life Experience	Life Event Category
1.	You became seriously ill or injured	Disease/injury
2.	A family member became seriously ill or injured	Disease/injury
3.	You were hospitalized	Disease/injury
4.	A family member was hospitalized	Disease/injury
5.	A family member died	Significant loss
6.	A close friend died	Significant loss
7.	You separated from your spouse of significant other	Serious interpersonal
8.	You got a divorce	Serious interpersonal
9.	You got married	Serious interpersonal
10.	You experienced violence	Other*
11.	A family member experienced violence	Other*
12.	You quit or lost your job	Job-related
13.	Your spouse/significant other quite or lost his/her job	Job-related
14.	You returned to work, started a new job or started school	Job-related
15.	Your spouse/significant other returned to work, started a new job or started school	Job-related
16.	You moved	Serious interpersonal
17.	You had serious arguments/conflict with your spouse/significant other	Serious interpersonal
18.	You had serious arguments/conflict with other relatives/friends	Serious interpersonal
19.	You had financial difficulties or money problems	Financial difficulties
20.	Your spouse/significant other had financial difficulties or money problems	Financial difficulties
21.	Other life event reported by the parent/primary caretaker	Placed into one of the categories listed above or Other

* = Placed into “Other” category due to very infrequent reports.

ESM Table 3. Study Sample Characteristics: Sex, Country, Family History of Type 1 Diabetes, Type 1 Diabetes Related HLA and SNPs and Association with Risk of First-appearing Islet Autoantibodies until 6 years of age

Total = (N)	**IA Negative**	**First Appearing Islet Autoantibody (IA) until 6 years of age**					
Age of seroconversion = Median (IQR)	**(N = 6785*)**	**Any IA⁺ (N=532*) Age = 27 (15 – 48) months**		**IAA as first appearing (N = 222*) Age = 21 (12 – 36) months**		**GADA as first appearing (N = 209*) Age = 38 (21 – 58) months**	
Characteristic	**N (%)**	**N (%)**	**Multivariate HR* (95%CI)**	**N (%)**	**Multivariate HR* (95%CI)**	**N (%)**	**Multivariate HR* (95%CI)**
Child sex							
Female	3355 (50%)	241 (45%)	1.00 (reference)	98 (44%)	1.00 (reference)	98 (47%)	1.00 (reference)
Male	3430 (51%)	291 (55%)	**1.22 (1.02 – 1.45)‡**	124 (56%)	1.27 (0.98 – 1.66)	111 (53%)	1.10 (0.84 – 1.45)
Country							
U.S.	2923 (43%)	180 (34%)	1.00 (reference)	64 (29%)	1.00 (reference)	84 (40%)	1.00 (reference)
Finland	1361 (20%)	122 (23%)	1.10 (0.87 – 1.41)	64 (29%)	**1.47 (1.02 – 2.14) §**	37 (18%)	**0.86 (0.57 – 1.28) §**
Germany	392 (6%)	38 (7%)	1.13 (0.76 – 1.64)	16 (7%)	1.35 (0.76 – 2.38)	8 (4%)	0.46 (0.20 – 1.06)
Sweden	2109 (31%)	192 (36%)	1.20 (0.98 – 1.48)	78 (35%)	1.38 (0.99 – 1.93)	80 (38%)	1.10 (0.80 – 1.50)
Family history of T1D							
None	6354 (94%)	449 (84%)	1.00 (reference)	183 (82%)	1.00 (reference)	181 (87%)	1.00 (reference)
First degree relative							
Father	340 (5%)	58 (11%)	**2.39 (1.75 – 3.26)‡**	25 (11%)	**2.51 (1.56 – 4.06)**	23 (11%)	**3.09 (1.95 – 4.90)**
Sibling	91 (1%)	25 (5%)	**3.48 (2.29 – 5.29)‡**	14 (6%)	**4.97 (2.80 – 8.82)**	5 (2%)	1.98 (0.81 – 4.86)
HLA high risk							
DR-4/4	1346 (20%)	89 (17%)	1.00 (reference)	37 (17%)	1.00 (reference)	30 (14%)	1.00 (reference)
DR-4/8	1200 (18%)	84 (16%)	1.05 (0.77 – 1.42)	51 (23%)	1.47 (0.96 – 2.27)	23 (11%)	0.91 (0.52 – 1.58)
DR-4/9,X‖	126 (2%)	18 (3%)	0.70 (0.40 – 1.25)	9 (4%)	0.81 (0.36 – 1.82) §	2 (1.0%)	**0.13 (0.02 – 0.96) §**
DR-3/4	2620 (39%)	269 (51%)	1.43 (1.12 – 1.82) ‡	108 (49%)	1.35 (0.92 – 1.97)	104 (50%)	1.72 (1.14 – 2.60)
DR-3/9‖	12 (0.2%)	0 (0%)	-	0 (0%)	-	0 (0%)	-
DR-3/3	1481 (22%)	72 (13%)	**0.63 (0.46 – 0.87)c**	17 (8%)	**0.36 (0.20 – 0.65) §**	50 (24%)	1.36 (0.84 – 2.20) §
Single Nucleotide Polymorphisms (SNPs)-Minor Allele							
***PTPN22*-A allele**							
No	4957 (81%)	367 (70%)	1.00 (reference)	155 (70%)	1.00 (reference)	148 (71%)	1.00 (reference)
Yes	1195 (19%)	161 (30%)	**1.74 (1.44 – 2.10) ‡**	66 (30%)	**1.64 (1.23 – 2.20)**	60 (29%)	**1.66 (1.23 – 2.25)**
***INS*-T allele**							
No	3165 (53%)	344 (66%)	1.00 (reference)	162 (74%)	1.00 (reference)	108 (52%)	1.00 (reference)

Yes	2759 (47%)	180 (34%)	**0.62 (0.52 – 0.75)** ‡	57 (26%)	**0.43 (0.32 – 0.58)** §	98 (48%)	1.06 (0.81 – 1.40) §
***ERBB3*-T allele**							
No	2871 (47%)	207 (39%)	1.00 (reference)	84 (38%)	1.00 (reference)	85 (41%)	1.00 (reference)
Yes	3280 (53%)	321 (61%)	**1.38 (1.16 – 1.64)** ‡	137 (62%)	**1.47 (1.12 – 1.94)**	123 (59%)	1.26 (0.96 – 1.67)
***SH2B3*-T allele**							
No	1961 (32%)	127 (24%)	1.00 (reference)	60 (27%)	1.00 (reference)	44 (21%)	1.00 (reference)
Yes	4191 (68%)	401 (76%)	**1.36 (1.11 – 1.66)** ‡	161 (3%)	1.19 (0.88 – 1.61)	164 (79%)	**1.58 (1.13 – 2.21)**
***BACH2*-T allele**							
No	2067 (34%)	167 (32%)	1.00 (reference)	85 (39%)	1.00 (reference)	50 (24%)	1.00 (reference)
Yes	4059 (66%)	360 (68%)	1.12 (0.93 – 1.34)	135 (61%)	0.87 (0.66 – 1.14) §	158 (76%)	**1.57 (1.14 – 2.16)** §
***CTLA4*-G allele**							
No	1951 (32%)	160 (30%)	1.00 (reference)	73 (33%)	1.00 (reference)	54 (26%)	1.00 (reference)
Yes	4200 (68%)	368 (70%)	1.02 (0.84 – 1.23)	148 (67%)	0.86 (0.65 – 1.15) §	154 (74%)	1.28 (0.94 – 1.76) §
***BTNL2*-A allele**							
No	4389 (71%)	406 (77%)	1.00 (reference)	172 (78%)	1.00 (reference)	157 (76%)	1.00 (reference)
Yes	1763 (29%)	122 (23%)	**0.73 (0.58 – 0.90)** ‡	49 (22%)	**0.66 (0.47 – 0.92)**	51 (25%)	0.91 (0.65 – 1.27)

* = multivariate model included gender, country, family history with T1D and HLA available on all children, and SNPs available on 5841/6785 negative and on 523/532 IA positive including 219/222 IAA-first and 206/209 GADA-first.

+ = an additional 10 children developed IA2A and 91 multiple as their first IA (GADA-IA2A n=1; GADA-IAA, n=72; IA2A-IAA, n=4; GADA-IAA-IA2A, n=14). They were censored in multivariate models at age of seroconversion (median (IQR) = 55 (38 – 67) for IA2A, 27 (15 – 42) for multiple IA)

‡ = an association exists with IA overall (p-value <0.05)

§ = the association with IAA-first and GADA-first is different (p-value<0.05)

‖ = children with a family history of T1D were included as HLA high risk if they had an additional haplotype, see ESM Table 1

ESM Table 4. Maternal Life Events Reported during Pregnancy in Relation to the Risk of Islet Autoantibodies in the Child until 6 years of age

Life Event categories mother experienced during pregnancy		Maternal LE on risk of IA		Maternal LE on risk of IAA-first		Maternal LE on risk of GADA-first	
	N (%)[$]	HR*(95%CI)	p-value	HR*(95%CI)	p-value	HR*(95%CI)	p-value
None	2667 (35.6%)	1.00 (reference)		1.00 (reference)		1.00 (reference)	
At least one	4097 (64.4%)	0.99 (0.82 – 1.18)	0.86	0.78 (0.60 – 1.03)[+]	0.08	1.18 (0.88 – 1.58)[+]	0.28
One	2086 (32.8%)	0.96 (0.78 – 1.18)		0.69 (0.50 – 0.96)		1.06 (0.75 – 1.49)	
Two	1218 (19.1%)	1.07 (0.85 – 1.37)		0.88 (0.61 – 1.27)		1.42 (0.98 – 2.06)	
Three	493 (7.7%)	0.78 (0.51 – 1.12)		0.76 (0.44 – 1.34)		1.06 (0.59 – 1.89)	
Four or more	300 (4.7%)	1.27 (0.82 – 1.95)	0.36	1.28 (0.68 – 2.42)	0.14	1.25 (0.62 – 2.53)	0.12

* = Presence and number life event categories (Disease/Injury, Significant loss, Serious Interpersonal, Job Related, Financial Difficulties or other) examined on the hazard of IA overall and first appearing IAA and GADA until 6 years of age adjusting for family history with type 1 diabetes, gender, country of residence, HLA and SNPs as shown in ESM Table 3.

[$] = full models included 6364 total children including 523 of 532 IA positives, 219 of 222 IAA-first and 206 of 209 GADA-first.

+ = life event is associated differently with hazard of IAA-first as compared to GADA-first (p-value<0.05)

ESM Table 5. Multivariate Logistic Regression Model of Prenatal Factors in Relation to Job-related or Serious Interpersonal Life Events.

		Prenatal factors in relation to odds of mother reporting a job related life event during pregnancy [a]		Prenatal factors in relation to odds of mother reporting a serious interpersonal life event during pregnancy [a]	
Maternal prenatal factors	**N or mean (SD)**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Maternal age (/year)[a]					
up to 29 years of age	2591	**0.96 (0.94 – 0.98)**	0.0005	**0.89 (0.87 – 0.91)**	<0.0001
29 years of age and older	4573	**0.97 (0.05 – 0.98)**	0.0002	0.99 (0.97 – 1.01)	0.21
Residence of mother					
US	3011	1.00 (reference)		1.00 (reference)	
EU	4143	**0.82 (0.74 – 0.92)**	0.0007	**0.84 (0.76 – 0.94)**	0.002
Number of maternal illnesses reported during pregnancy					
Illness	2.3 (1.8)	**1.06 (1.03 – 1.09)**	0.0001	**1.06 (1.03 – 1.10)**	<0.0001
Mother's first child					
no	3556	1.00 (reference)		1.00 (reference)	
Yes	2913	**1.22 (1.09 – 1.38)**	0.0009	**1.46 (1.30 – 1.63)**	<0.0001
No information	695	0.99 (0.82 – 1.21)	0.95	**1.32 (1.10 – 1.58)**	0.003
Mother smoked during pregnancy					
No	6205	1.00 (reference)		1.00 (reference)	
yes	959	0.96 (0.82 – 1.13)	0.58	**1.69 (1.46 – 1.96)**	<0.0001
Mother drank alcohol during pregnancy					
No	4716	1.00 (reference)		1.00 (reference)	
Yes	2448	**1.22 (1.08 – 1.36)**	0.001	**1.24 (1.11 – 1.38)**	0.0002

a = the predicted probability from each logistic regression model was used to calculate the propensity of having a job related or serious interpersonal life event during pregnancy. Stabilized weights for Inverse Probability Treatment (exposure) Weighting (IPTW) analysis were calculated from the propensity scores to reduce selection bias (1). The calculation was as followed; when mothers had the life event the stabilized weight was the proportion of mothers with the life event (pLE) divided by the propensity score (PS); when mothers did not have the life event the stabilized weight was (1- pLE)/(1-PS). Maternal age was included in logistic model as a linear spline centered around 29 months of age and for serious interpersonal life even as the outcome, an interaction between maternal age and maternal smoking was included to ensure both variables were correctly controlled for when examining life event on risk of islet autoimmunity. An Inverse Probability of Treatment (Life event) Weighting analysis was performed by weighting children in the Proportional Hazard models by the stabilized weight created from the LE propensity score. Since the propensity score was an estimate and not a known quantity, variances were calculated

from performing analysis on 1000 bootstrap samples. Job related LE remained associated with IAA-first, HR (95%CI) = 0.59 (0.40 – 0.88), and Serious Interpersonal life event with GADA-first HR (95%CI) = 1.55 (1.13 – 2.11).

References

1. Xu S, Ross C, Raebel M, Shetterly S, Blanchette C, Smith D. Use of stabilized inverse propensity scores as weights to directly estimate relative risk and its confidence intervals. Value Health 2010;13:273-7

ESM Table 6. Respiratory Illness and Job-related Life Event During Pregnancy Examined in Univariate and Multivariate Models on Risk of IAA-first Stratified by Genetic Subgroups of Children Showing Where Respiratory Illness Has Strongest Influence on IAA-first.

Genetic subgroup of children	**Gestational Predictor (yes vs. no)**	**Univariate[+] on IAA-first**		**Multivariate[+] On IAA-first**	
		HR(95%CI)	**p-value**	**HR(95%CI)**	**p-value**
All children	Respiratory Illness	0.87 (0.66 – 1.15)	0.34	0.89 (0.67 – 1.18)	0.41
	Job related LE	0.58 (0.40 – 0.84)	0.004	0.54 (0.37 – 0.80)	0.002
CTLA-4-AA genotype	Respiratory Illness	1.51 (0.92 – 2.47)	0.10	1.52 (0.93 – 2.49)	0.10
	Job related LE	0.77 (0.41 – 1.43)	0.41	0.77 (0.41 – 1.43)	0.41
CTLA-4-(AG, GG) genotype	Respiratory Illness	0.64 (0.45 – 0.91)	0.01	0.66 (0.46 – 0.94)	0.02
	Job related LE	0.50 (0.32 – 0.80)	0.004	0.45 (0.27 – 0.74)	0.002
CTLA-4-(AG, GG) & HLA-DR4/8 genotypes	Respiratory Illness	0.28 (0.12 – 0.65)	0.003	0.27 (0.12 – 0.64)	0.003
	Job related LE	0.31 (0.11 – 0.94)	0.04	0.22 (0.06 – 0.79)	0.02

[+] = All models adjusted for factors shown in ESM table 3 including life events other than job-related. Univariate includes respiratory illness and job related LE in separate models. Multivariate includes both predictors in same model.

Note: Job related LE is associated with IAA-first in offspring of mother who have a respiratory illness (HR = 0.51, 95%CI = 0.45 – 0.90, p=0.02) and mothers who did not (HR = 0.58, 95%CI = 0.34 – 1.00, p=0.049)

References

1. Lynch KF, Hye-Seung L, Torn C, et al (2018) Gestational respiratory infections interacting with offspring HLA and *CTLA-4* modifies incident B-cell autoantibodies. J Autoimmun 86:93-103. doi: 10.1016/j.jaut.2017.09.005.

ESM Table 7. Genetic Factors and Job-related Life Event During Pregnancy on the Risk of IAA as the First-appearing Islet Autoantibody Adjusting for Country, Child Sex, Father or Sibling with T1D

Genetic and environmental component			Total	IAA- first	Genetic (G) and Job related life event (Job-LE) on risk of IAA as first appearing Islet autoantibody			
HLA-DR-DQ or SNP (G)		Job related life event (Job-LE)	N	n (%)	(G) and (Job-LE) combination on IAA-first	Job-LE on IAA-first within strata of genetic factor	Additive interaction	Multiplicative interaction
					HR (95%CI)	HR (95%CI)	RERI (95%CI) p-value	HR (95%CI) p-value
HLA–DR3/3	No	No	4315	172 (4.0%)	1.00 (Reference)	0.61 (0.42 – 0.89)	0.20 (-0.16 – 0.56) p-value = 0.27	0.73 (0.16 – 3.32) p-value = 0.67
Genotype	No	Yes	1449	33 (2.3%)	0.61 (0.41 – 0.89)			
	Yes	No	1174	15 (1.3%)	0.33 (0.20 – 0.57)	0.44 (0.10 – 1.94)		
	Yes	Yes	379	2 (0.5%)	0.15 (0.04 – 0.60)			
HLA–DR4/4	No	No	4380	152 (3.5%)	1.00 (Reference)	0.68 (0.47 – 0.99)	-0.44 (-0.97 – 0.09) p-value = 0.05	0.30 (0.07 – 1.30) p-value = 0.11
Genotype	No	Yes	1502	33 (2.2%)	0.69 (0.47 – 1.00)			
	Yes	No	1109	35 (3.2%)	0.95 (0.66 – 1.37)	0.22 (0.05 – 0.90)		
	Yes	Yes	326	2 (0.6%)	0.19 (0.05 – 0.78)			
HLA–DR3/4	No	No	3370	102 (3.0%)	1.00 (Reference)	0.40 (0.22 – 0.74)	0.33 (-0.30 – 0.96) p-value = 0.30	2.03 (0.96 – 4.32) p-value = 0.07
Genotype	No	Yes	1058	12 (1.1%)	0.40 (0.22 – 0.73)			
	Yes	No	2119	85 (4.0%)	1.44 (1.08 – 1.92)	0.80 (0.50 – 1.27)		
	Yes	Yes	770	23 (3.0%)	1.17 (0.74 – 1.84)			
HLA–DR4/8	No	No	4532	143 (3.2%)	1.00 (Reference)	0.65 (0.43 – 0.97)	-0.49 (-1.26 – 0.28) p-value = 0.21	0.70 (0.28 – 1.70) p-value = 0.43
Genotype	No	Yes	1501	28 (1.9%)	0.65 (0.43 – 0.98)			
	Yes	No	957	44 (4.6%)	1.53 (1.08 – 2.17)	0.56 (0.21 – 1.02)		
	Yes	Yes	327	7 (2.1%)	0.69 (0.32 – 1.49)			
Single Nucleotide Polymorphisms (SNPs) associated with IAA* - Minor Allele								
***PTPN22*-A allele**	No	No	3989	128 (3.2%)	1.00 (Reference)	0.67 (0.44 – 1.01)	-0.59 (-1.37 – 0.20) p-value = 0.14	0.68 (0.29 – 1.58) p-value = 0.37
	No	Yes	1335	27 (2.0%)	0.68 (0.45 – 1.02)			
	Yes	No	1027	58 (5.7%)	1.68 (1.23 – 2.29)	0.47 (0.22 – 0.99)		
	Yes	Yes	329	8 (2.4%)	0.76 (0.37 – 1.56)			
***INS*-T allele**	No	No	2629	134 (5.1%)	1.00 (Reference)	0.67 (0.45 – 1.01)	0.10 (-0.26 – 0.45) p-value = 0.59	0.70 (0.29 – 1.71) p-value = 0.43
	No	Yes	880	28 (3.2%)	0.66 (0.44 – 0.99)			
	Yes	No	2210	50 (2.3%)	0.45 (0.33 – 0.63)	0.45 (0.20 – 0.99)		

	Yes	Yes	729	7 (1.0%)	0.21 (0.10 – 0.45)			
***ERBB3*-T allele**	No	No	2292	70 (3.1%)	1.00 (Reference)	0.62 (0.35 – 1.10)	-0.19 (-0.81 – 0.43) p-value = 0.54	0.95 (0.46 – 2.00) p-value = 0.90
	No	Yes	786	14 (1.8%)	0.63 (0.35 – 1.12)			
	Yes	No	2723	116 (4.3%)	1.41 (1.05 – 1.90	0.60 (0.38 – 0.96)		
	Yes	Yes	878	21 (2.4%)	0.85 (0.52 – 1.39)			
***SH2B3*-T allele**	No	No	1519	50 (3.3%)	1.00 (Reference)	0.58 (0.29 – 1.15)	-0.01 (-0.58 – 0.56) p-value = 0.97	1.08 (0.48 – 2.40) p-value = 0.86
	No	Yes	569	10 (1.8%)	0.58 (0.29 – 1.14)			
	Yes	No	3497	136 (3.9%)	1.15 (0.83 – 1.59)	0.62 (0.41 – 0.95)		
	Yes	Yes	1095	25 (2.3%)	0.72 (0.44 – 1.16)			
***BACH2*-T allele**	No	No	1656	70 (4.2%)	1.00 (Reference)	0.69 (0.40 – 1.22)	-0.01 (-0.50 – 0.48) p-value = 0.97	0.88 (0.42 – 1.83) p-value = 0.73
	No	Yes	578	15 (2.6%)	0.66 (0.38 – 1.15)			
	Yes	No	3340	115 (3.4%)	0.83 (0.62 – 1.12)	0.56 (0.35 – 0.91)		
	Yes	Yes	1079	20 (1.9%)	0.48 (0.29 – 0.80)			
***CTLA4*-G allele**	No	No	1607	60 (3.7%)	1.00 (Reference)	0.76 (0.41 – 1.38)	-0.17 (-0.75 – 0.40) p-value = 0.55	0.72 (0.34 – 1.53) p-value = 0.39
	No	Yes	504	13 (2.6%)	0.76 (0.42 – 1.38)			
	Yes	No	3408	126 (3.7%)	0.91 (0.67 – 1.25)	0.55 (0.35 – 0.86)		
	Yes	Yes	1160	22 (1.9%)	0.50 (0.31 – 0.81)			
***BTNL2*-A allele**	No	No	3624	150 (4.1%)	1.00 (Reference)	0.48 (0.31 – 0.76)	**0.56 (0.05 – 1.08) p-value = 0.03 false discovery rate = 0.37**	**2.19 (1.01 – 4.77) p-value = 0.048 false discovery rate = 0.37**
	No	Yes	1171	22 (1.9%)	0.49 (0.31 – 0.76)			
	Yes	No	1392	36 (2.6%)	0.70 (0.49 – 1.01)	1.03 (0.54 – 1.95)		
	Yes	Yes	493	13 (2.6%)	0.75 (0.42 – 1.32)			

RERI = relative excess risk due to interaction (p-value tested against zero for statistically significant additive interaction) RERI >0 = statistical additive interaction

ESM Table 8. Genetic Factors and Serious Interpersonal Life Events on the Risk of GADA as the First-appearing Islet Autoantibody Adjusting for Country, Child Sex, Father or Sibling with T1D

Genetic and environmental component			**Total**	**GADA- first**	**Genetic (G) and serious interpersonal life event (SI-LE) on risk of GADA as first appearing Islet autoantibody**			
***HLA-DR* or SNP (G)**		**Serious interpersonal life event (SI-LE)**	**N**	**n (%)**	**(G) and (SI-LE) combination on GADA-first**	**SI-LE on GADA-first within strata of genetic factor**	**Additive interaction**	**Multiplicative interaction**
					HR (95%CI)	**HR (95%CI)**	**RERI (95%CI) p-value**	**HR (95%CI) p-value**
HLA–DR3/3	No	No	4068	102 (2.5%)	1.00 (Reference)	1.53 (1.11 – 2.12)	-0.13 (-1.09 – 0.84) p-value = 0.79	0.88 (0.45 – 1.72) p-value = 0.70
Genotype	No	Yes	1696	57 (3.4%)	1.53 (1.10 – 2.11)			
	Yes	No	1078	33 (3.1%)	1.17 (0.79 – 1.74)	1.33 (0.74 – 2.40)		
	Yes	Yes	475	17 (3.6%)	1.57 (0.94 – 2.64)			
HLA–DR4/4	No	No	4145	111 (1.5%)	1.00 (Reference)	1.67 (1.24 – 2.26)	**-0.99 (-1.79 – -0.20) p-value = 0.02 false discovery rate = 0.11**	0.38 (0.15 – 0.98) p-value = 0.05
Genotype	No	Yes	1737	68 (3.9%)	1.68 (1.24 – 2.27)			
	Yes	No	1001	24 (2.4%)	0.89 (0.57 – 1.38)	0.65 (0.26 – 1.58)		
	Yes	Yes	434	6 (1.4%)	0.57 (0.25 – 1.30)			
HLA–DR3/4	No	No	3082	73 (2.4%)	1.00 (Reference)	1.13 (0.75 – 1.72)	**1.13 (0.18 – 2.08) p-value = 0.02 false discovery rate = 0.11**	1.71 (0.96 – 3.02) p-value = 0.07
Genotype	No	Yes	1346	32 (2.4%)	1.14 (0.75 – 1.73)			
	Yes	No	2064	62 (3.0%)	1.34 (0.96 – 1.89)	1.95 (1.32 – 2.90)		
	Yes	Yes	825	42 (5.1%)	2.62 (1.79 – 3.84)			
HLA–DR4/8	No	No	4260	121 (2.8%)	1.00 (Reference)	1.47 (1.09 – 1.99)	-0.10 (-0.93 – 0.72) p-value = 0.80	1.09 (0.45 – 2.65) p-value = 0.85
Genotype	No	Yes	1773	65 (3.7%)	1.47 (1.09 – 1.99)			
	Yes	No	886	14 (1.6%)	0.61 (0.35 – 1.07)	1.71 (0.73 – 4.02)		
	Yes	Yes	398	9 (2.3%)	0.98 (0.50 – 1.94)			
Single Nucleotide Polymorphisms (SNPs) associated with GADA-Minor Allele								
***PTPN22*-A allele**	No	No	3784	94 (2.5%)	1.00 (Reference)	1.55 (1.11 – 2.17)	0.08 (-1.17 – 1.33) p-value = 0.90	0.88 (0.47 – 1.66) p-value = 0.69
	No	Yes	1540	54 (3.5%)	1.55 (1.11 – 2.16)			
	Yes	No	968	40 (4.1%)	1.73 (1.20 – 2.51)	1.32 (0.77 – 2.27)		
	Yes	Yes	388	20 (5.2%)	2.36 (1.45 – 3.82)			
***INS*-T allele**	No	No	2529	69 (2.7%)	1.00 (Reference)	1.55 (1.05 – 2.30)	-0.58 (-0.87 – 0.75) p-value = 0.16	0.93 (0.53 – 1.66) p-value =0.82
	No	Yes	980	39 (4.0%)	1.54 (1.04 – 2.28)			
	Yes	No	2074	64 (3.1%)	1.10 (0.78 – 1.54)	1.45 (0.96 – 2.20)		

	Yes	Yes	865	34 (3.9%)	1.58 (1.05 – 2.38)			
***ERBB3*-T allele**	No	No	2184	52 (2.4%)	1.00 (Reference)	1.76 (1.14 – 2.73)	-0.27 (-1.21 – 0.67) p-value = 0.57	0.77 (0.43 – 1.37) p-value = 0.38
	No	Yes	894	33 (3.7%)	1.73 (1.12 – 2.68)			
	Yes	No	2568	82 (3.2%)	1.38 (0.97 – 1.95)	1.33 (0.91 – 1.94)		
	Yes	Yes	1033	41 (4.0%)	1.84 (1.22 – 2.77)			
***SH2B3*-T allele**	No	No	1427	25 (1.8%)	Reference)	1.88 (1.03 – 3.42)	0.01 (-1.19 – 1.21) p-value = 0.86	0.80 (0.40 – 1.57) p-value = 0.51
	No	Yes	661	19 (2.9%)	1.81 (1.00 – 3.29)			
	Yes	No	3325	109 (3.3%)	1.86 (1.21 – 2.89)	1.43 (1.03 – 1.98)		
	Yes	Yes	1267	55 (4.3%)	2.68 (1.67 – 4.31)			
***BACH2*-T allele**	No	No	1547	38 (2.5%)	1.00 (Reference)	0.77 (0.40 – 1.47)	**1.25 (0.50 – 2.00) p-value = 0.001 false discovery rate = 0.02**	**2.40 (1.16 – 4.94) p-value = 0.02 false discovery rate = 0.11**
	No	Yes	687	12 (1.8%)	0.77 (0.40 – 1.47)			
	Yes	No	3188	96 (3.0%)	1.21 (0.83 – 1.77)	1.85 (1.35 – 2.55)		
	Yes	Yes	1231	62 (5.0%)	2.22 (1.48 – 3.33)			
***CTLA-4*-G allele**	No	No	1528	34 (2.2%)	1.00 (Reference)	1.72 (0.99 – 2.99)	-0.15 (-1.19 – 0.88) p-value = 0.77	0.82 (0.43 – 1.56) p-value = 0.54
	No	Yes	583	20 (3.4%)	1.72 (0.99 – 2.99)			
	Yes	No	3223	100 (3.1%)	1.40 (0.95 – 2.07)	1.39 (1.00 – 1.94)		
	Yes	Yes	1245	54 (4.0%)	1.97 (1.28 – 3.03)			
***BTNL2*-A allele**	No	No	3455	100 (2.9%)	1.00 (Reference)	1.61 (1.16 – 2.23)	-0.41 (-1.20 – 0.38) p-value = 0.31	0.76 (0.39 – 1.47) p-value = 0.41
	No	Yes	1340	57 (4.3%)	1.60 (1.16 – 2.22)			
	Yes	No	1297	34 (2.6%)	0.92 (0.62 – 1.36)	1.22 (0.68 – 2.18)		
	Yes	Yes	588	17 (2.9%)	1.12 (0.67 – 1.87)			

RERI = relative excess risk due to interaction (p-value tested against zero for statistically significant additive interaction) RERI >0 = statistical additive interaction

ESM Table 9. *Cis*-expression Quantitative Trait Loci (*cis*-eQTL) Associations of the rs3763305 in *BTNL2* with Different Proximate Genes in the Various Human Immune Cells

Human immune cells	Proximate genes	p-value	Reference
CD4+ memory regulatory T cells (Tregs)	*HLA-DQA2*	1.2×10^{-5}	Schmiedel BJ. *et al*. (1)
CD4+ memory follicular helper T cells (TFH)	*HLA-DQA2*	2.4×10^{-5}	Schmiedel BJ. *et al*. (1)
CD8+ T cells	*HLA-DQA2*	4.6×10^{-5}	Schmiedel BJ. *et al*. (1)
B-cells	*HLA-DQA2*	6.2×10^{-6}	Schmiedel BJ. *et al*. (1)
Monocytes	*HLA-DQA2*	8.1×10^{-6}	Schmiedel BJ. *et al*. (1)
Monocytes	*HLA-DOB*	6.6×10^{-7}	Fairfax BP. *et al*. (2)
Monocytes	*TAP2*	4.3×10^{-4}	Fairfax BP. *et al*. (2)
Monocytes non-classical	*HLA-DQA2*	4.5×10^{-5}	Schmiedel BJ. *et al*. (1)

ImmuneRegulation (https://immuneregulation.mssm.edu/) was used to identified *cis*-eQTL (3)

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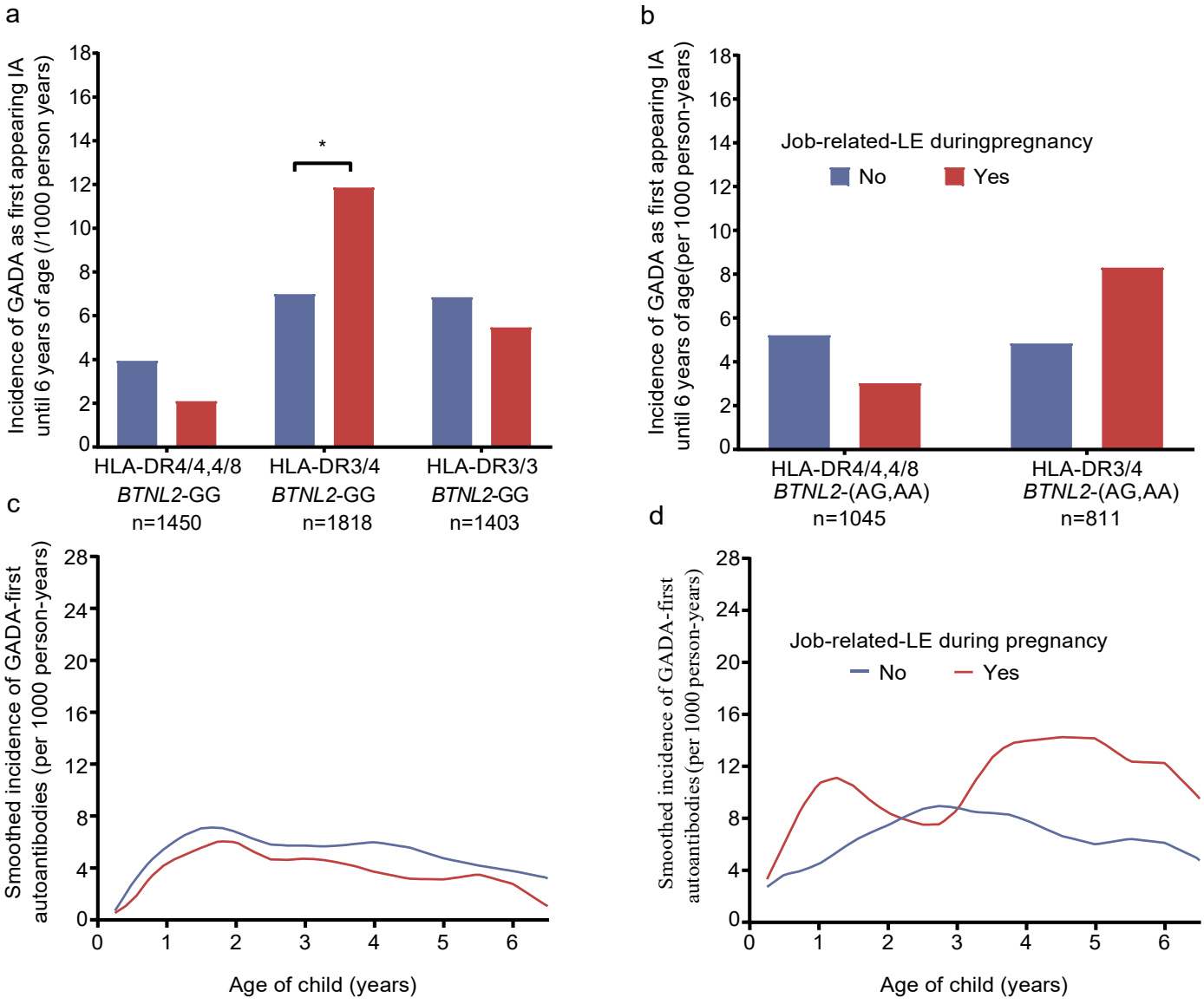
ESM Table 10. Post Hoc Exploratory Analysis of Any IA by Specific Life Event Category and HLA-DR Status

Specific Life Events (LE)	**Reported Maternal Life Events and Risk of Islet Autoimmunity by HLA-DR Genotype of Child**					
	HLA-DR4/4, 4/8, 4/X Children, N= 2509 IA positive, n= 189		**HLA-DR3/4 Children N = 2492, IA positive, n=263**		**HLA-DR3/3, 3/X Children, N = 1363 , IA positive, n= 71**	
	HR*(95%CI)	**p-value**	**HR*(95%CI)**	**p-value**	**HR*(95%CI)**	**p-value**
Disease/Injury(self or others)	1.10 (0.79 – 1.54)	0.57	0.91 (0.68 – 1.21)	0.50	0.73 (0.42 – 1.29)	0.29
Significant loss (death of family member or friend)+	**0.45 (0.24 – 0.86)+**	**0.02**	1.04 (0.70 – 1.55)+	0.86	1.83 (0.95 – 3.53)+	0.07
Serious Interpersonal (marriage, separation, divorce, conflicts with spouse/relative/friend, move or change in family composition)	1.10 (0.79 – 1.53)	0.57	1.27 (0.96 – 1.67)	0.09	1.55 (0.94 – 2.56)	0.08
Job-related (self or spouse quit/lost job, started work/ school**)**	**0.58 (0.58 – 0.86)**	**0.01**	1.18 (0.90 – 1.56)	0.23	0.68 (0.37 – 1.25)	0.21
Financial Difficulties (self or spouse)	1.02 (0.58 – 1.80)	0.95	0.94 (0.59 – 1.50)	0.80	0.75 (0.31 – 1.83)	0.75
Other	1.13 (0.71 – 1.81)	0.60	0.67 (0.42 – 1.08)	0.10	1.41 (0.72 – 2.77)	0.32

* = Specific life event examined together in a multivariate model on the hazard of IA until 6 years of age adjusting for family history with type 1 diabetes, gender, country of residence and SNPs; full models included 6364 total children including 523 IA positives.

+ = Significant loss shows evidence of multiplicative interaction with HLA groups (p-value=0.01)



AG,AA

n

ESM Figure 1. (a) A job-related event in pregnancy was associated with GADA as the first-appearing autoantibody in HLA-DR3/4 children with the *BTNL2*-GG genotype (*p < 0.05) but not for HLA-DR4/4, 4/8 (p = 0.24)) or HLA-DR3/3 (p = 0.52)) with the *BTNL2*-GG genotype. (b)) A job-related event in pregnancy was not associated with GADA as the first-appearing autoantibody in HLA-DR4/4,4/8 children (p = 0.31) or HLA-DR3/4 children (p = 0.17) with the *BTNL2*-AG,AA genotype. (c) Age-specific incidence of GADA as the first-appearing autoantibody by job-related life eve t during pregnancy for HLA-DR4/4,4/8,3/3 children and (D) for HLA DR3/4children.

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