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# Identification of non-HLA genes associated with development of islet autoimmunity and type 1 diabetes in the prospective TEDDY cohort

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ABSTRACT

Traditional linkage analysis and genome-wide association studies have identified HLA and a number of non-HLA genes as genetic factors for islet autoimmunity (IA) and type 1 diabetes (T1D). However, the relative risk associated with previously identified non-HLA genes is usually very small as measured in cases/controls from mixed populations. Genetic associations for IA and T1D may be more accurately assessed in prospective cohorts. In this study, 5806 subjects from the TEDDY (The Environmental Determinants of Diabetes in the Young) study, an international prospective cohort study, were genotyped for 176,586 SNPs on the ImmunoChip. Cox proportional hazards analyses were performed to discover the SNPs associated with the risk for IA, T1D, or both. Three regions were associated with the risk of developing any persistent confirmed islet autoantibody: one known region near *SH2B3* ($HR = 1.35$, $p = 3.58 \times 10^{-7}$) with Bonferroni-corrected significance and another known region near *PTPN22* ($HR = 1.46$, $p = 2.17 \times 10^{-6}$) and one novel region near *PPIL2* ($HR = 2.47$, $p = 9.64 \times 10^{-7}$) with suggestive evidence ($p < 10^{-5}$). Two known regions (*PTPN22*: $p = 2.25 \times 10^{-6}$, *INS*; $p = 1.32 \times 10^{-7}$) and one novel region (*PXK/PDHB:* $p = 8.99 \times 10^{-6}$) were associated with the risk for multiple islet autoantibodies. First appearing islet autoantibodies differ with respect to association. Two regions (*INS*: $p = 5.67 \times 10^{-6}$ and *TTC34/PRDM16*: $6.45 \times 10^{-6}$) were associated if the fist appearing autoantibody was IAA and one region (*RBFOX1*: $p = 8.02 \times 10^{-6}$) was associated if the first appearing autoantibody was GADA. The analysis of T1D identified one region already known to be associated with T1D (*INS*: $p = 3.13 \times 10^{-7}$) and three novel regions (*RNASET2, PLEKHA1,* and *PPIL2*; $5.42 \times 10^{-6} > p > 2.31 \times 10^{-6}$). These results suggest that a

*Abbreviations:* SNP, Single-nucleotide polymorphism; TEDDY, The Environmental Determinants of Diabetes in the Young; IA, Islet autoimmunity; T1D, type 1 diabetes; MAF, minor allele frequency.

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[1] A complete list of the TEDDY Study Group can be found in the Supplementary Appendix online.

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number of low frequency variants influence the risk of developing IA and/or T1D and these variants can be identified by large prospective cohort studies using a survival analysis approach.

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## 1. Introduction

Type 1 Diabetes (T1D) is a multifactorial disease where multiple genes and environmental factors interact in a complex manner to cause the disease. HLA-DR-DQ is known to be the most important genetic factor and a number of non-HLA genes also influence the risk for islet autoimmunity (IA) and clinical disease. Autoantibodies are detectable very early in life and the age and order of occurrence of islet autoantibodies is associated with genetic backgrounds [1]. Most previous genetic studies focused on the clinical disease as the phenotype while few studies focus on IA as an endpoint [2–4]. Previous TEDDY, DAISY and DIPP studies that looked at development of IA and progression from IA to T1D were limited to the known non-HLA genes [4–6]. Interestingly, a previous study has shown associated genes for IA and T1D are not completely overlapping [5]. It is well known that IA is an intermediate phenotype for T1D and not all subjects with autoantibodies will progress to clinical disease within a given time frame. Genetic factors may act at various stages of the disease development, with some genes playing a role in IA development and others playing a critical role in the rate of subsequent disease progression. Therefore, it is important to analyze both stages to fully understand the contribution of genetic factors to the pathogenesis of islet autoimmunity and T1D.

With a few exceptions, all previous genetic studies of T1D used a case-control or family study design. However, cohorts with longitudinal data for survival analysis may be a much more powerful approach to identify genetic factors [7]. The Environmental Determinants of Diabetes in the Young (TEDDY) is a large international prospective cohort study, in which newborns from the US and European general populations and first-degree relatives of T1D patients were screened for specific HLA genotypes. Children with one of nine HLA DR-DQ genotypes associated with increased risk for T1D are followed in a 15 year prospective follow-up study [8]. Furthermore, a major finding in TEDDY is the observation that the first appearing islet autoantibody differ with respect to type and association with HLA. First, IAA as first appearing autoantibody was associated with HLA DR4-DQ8 while GADA first was primarily associated with DR3-DQ2 [1,9]. The incidence rate of IAA first differed from GADA first [1,9]. Also, while IAA first was associated with history of upper respiratory infection, that of GADA first showed a relationship to maternal reported infections dependent of the CTLA-4 genotype of the child [10]. To identify genes associated with T1D, we genotyped 176,586 SNPs in the regions containing autoimmunity and inflammatory response genes using the ImmunoChip.

SNP data were analyzed using Cox proportional hazards analyses to discover the genetic regions influencing the development of IA and/or T1D. Any persistent confirmed islet autoantibody, either IAA first or GADA first, multiple islet autoantibodies (two or more persistent confirmed islet autoantibodies) and T1D were the five outcomes analyzed in this study. Overall, we identified 6 novel regions with suggestive evidence ($9.0 \times 10^{-6} < p < 9.6 \times 10^{-7}$), and confirmed 3 regions with previously known associations. We also found that different genetic regions were associated to IAA or GADA as the first appearing indication of autoimmunity.

## 2. Material and methods

### 2.1. Material

A total of 424,788 newborn children in Finland, Sweden, Germany and the United States (Colorado, Georgia and Washington) were screened for high-risk genotypes for T1D as previously described [11]. Of these, 21,589 had one of nine selected HLA genotypes associated with increased risk for T1D and 8676 eligible children (Supplemental Figure-1) were enrolled to a 15-year prospective follow-up [8]. The TEDDY study was performed according to the principles of the Declaration of Helsinki. Written informed consents were obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and for participation in prospective follow-up. The study was approved by local Institutional Review Boards at 6 clinical research centers (3 in the United States and 3 in Europe): University of Colorado Denver, Augusta University, Pacific Northwest Diabetes Research Institute, Turku University Hospital (Finland), Institute of Diabetes Research (Germany), and Lund University (Sweden). The study is also monitored by an external evaluation committee formed by the National Institutes of Health.

### 2.2. Assessment of study outcomes: islet autoimmunity and type 1 diabetes

The development of islet autoimmunity (presence of GADA, IA-2A and mIAA autoantibodies in serum) was assessed every three months in two reference laboratories using radioimmuno-binding assays [12–15]. In the U.S., all sera were assayed at the Barbara Davis Center for Childhood Diabetes at the University of Colorado Denver; in Europe, all sera were assayed at the University of Bristol, U.K. All samples positive for autoantibodies and 5% of samples negative for islet autoantibodies were re-tested for confirmation in both reference laboratories. Islet autoimmunity (IA) was defined by the presence of persistent confirmed autoantibody for any of three islet autoantibodies (GADA, IA-2A or mIAA) on two or more consecutive visits. The date of any persistent confirmed islet antibody was defined as the draw date of the first of two consecutive samples at which the child was confirmed as “positive” for any autoantibody. The date of multiple islet autoantibodies was defined by the first date when at least two confirmed islet autoantibodies were detected. T1D diagnosis was based on American Diabetes Association criteria [16].

### 2.3. SNP genotyping by ImmunoChip

SNPs were genotyped by the Center for Public Health Genomics at University of Virginia, using the Illumina ImmunoChip Infinium array. Genotype calling and quality control steps were applied to the dataset: (1) individuals with low call rate (<95%), or discordance with reported gender and prior genotyping were not considered in the analysis, (2) SNP markers with low call rates (<95%) were excluded, and (3) markers with allele distributions strongly deviating from Hardy-Weinberg equilibrium in controls

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($p < 10^{-6}$) were discarded (except for chromosome 6 due to HLA eligibility requirements). The numbers of excluded SNPs and subjects for each quality control step are provided in supplementary data (Supplemental File: S2).

### 2.4. Statistical analysis

Cox proportional hazards modeling was used to analyze the effect of each individual SNP (by genotype category 0,1,2) on the risk of developing each of three outcomes (any islet autoantibody, multiple islet autoantibody and T1D), adjusting for country of residence (as strata), sex, family history (first-degree relative) of T1D, HLA-DR-DQ genotype (e.g., DR3/4, DR4/4, DR4/8, DR3/3 and others) [8], HLA-DPB1 genotype, and the top six principal components from ImmunoChip data to account for population stratification (ancestral heterogeneity). The Cox models were fitted using the "survival" package in R 3.2.5 [17]. As the majority of the subjects were Caucasians, and to reduce population stratification further, subjects from other races were excluded and analyses were restricted to the self-reported Caucasians. All subjects from Finland and Sweden were included in the analysis due to the lack of data on ethnicity. However, principal component analysis suggested that Finland and Sweden populations are homogeneous and overlapping with Caucasian cluster. Only one child per family was kept in the analyses. As a result, 5806 subjects were included in the final analysis. Data were analyzed to discover the SNPs associated with five outcomes: (i) time to any persistent confirmed islet autoantibody (time-to-IA), (ii) time to multiple autoantibodies (time-to-multiple IA), (iii) time to IAA as the first appearing antibody, (iv) time to GADA as the first appearing antibody and (v) time to type 1 diabetes (time-to-T1D).

From the 176,586 SNPs that passed quality control filters, the analysis focused on those 131,847 with minor allele frequencies (MAFs) of at least 0.01; thus, chip-wide statistical significance for any single SNP required a Bonferroni-corrected $p < 3.7 \times 10^{-7}$. Significance with suggestive evidence was claimed if $p < 10^{-5}$. A web-based plotting tool *locuszoom* [18] was used to plot HapMap CEU linkage disequilibrium $r^2$ values for proximal SNPs in the discovered genomic regions.

## 3. Results

As of March 31, 2017, the median (interquartile range (IQR)) age at the last follow up of the 5806 children was 8.3 (5.5–10.0) years. A total of 587 children (10.1%) developed any persistent conformed islet autoantibody at the median (IQR) age of 2.8 (1.5–5.5) years. In 224 children, the first appearing confirmed persistent autoantibody was IAA and in 247 children it was GADA. The remaining 116 children had multiple autoantibodies or IA-2A as the first appearing autoantibody. Furthermore, 352 children (6.1%) developed multiple persistent confirmed islet autoantibodies at the median (IQR) age of 2.8 (1.6–5.1) years and 209 children developed T1D at the median (IQR) age of 4.9 (2.7–7.5) years.

### 3.1. SNPs associated with development of any persistent confirmed islet autoantibody

The results from the Cox proportional hazards analyses of the 131,847 SNPs on the risk of developing any persistent confirmed islet autoantibody are presented in Fig. 1. One of these SNPs near *SH2B3* region attained chip-wide association significance, 12 SNPs (Supplemental Table-3) mapped to 3 different regions showed suggestive evidence ($p < 10^{-5}$). Out of these 13 SNPs, 12 SNPs mapped to 2 previously reported T1D regions: rs6679677/*PTPN22* (HR = 1.46; $p = 2.17 \times 10^{-6}$); rs3184504/*SH2B3* (HR = 1.35; $p = 3.58 \times 10^{-7}$) (Table 1) and 1 SNP mapped to a novel region, previously not known to be associated with T1D: rs428595/*PPIL2* (HR = 2.47; $p = 9.64 \times 10^{-7}$). The Kaplan-Meier plots for the lead SNPs with smallest p-values from these 3 regions are shown in Fig. 1B. The novel candidate SNP near *PPIL2* (rs428595) associated with any persistent confirmed islet autoantibody requires further studies to confirm the association. Regional association plots reflecting other nearby genes in these 3 regions are shown in supplementary data (Supplemental File: S4).

### 3.2. SNPs associated with IAA or GADA as the first appearing islet autoantibody

The analysis was divided into either IA-IAA only or IA-GADA only. If the first appearing autoantibody was IAA, it was found that two regions: rs3842727/*INS* (HR = 0.57; $p = 5.67 \times 10^{-6}$) and rs28600853/TTC34/PRDM16 (HR = 3.23; $p = 6.45 \times 10^{-6}$) were associated with suggestive evidence (Table 2). These two regions were not significant in the GADA as first antibody analysis (p = .25 and .53 respectively). In contrast, only one region: rs9934817/*RBFOX1* (HR = 2.66; $p = 8.02 \times 10^{-6}$) was found to be associated if the first appearing autoantibody was GADA (Table 2) and this region was not significant in the IAA as first antibody analysis (p = .61). These results show that there are different genetic factors associated to IAA or GADA as the first appearing islet antibody. Regional association plots reflecting other nearby genes in these 3 regions are shown in supplementary data (Supplemental File: S4).

### 3.3. SNPs associated with development of multiple islet autoantibodies

The analysis results of developing multiple islet autoantibodies are presented in Fig. 2. One of these SNPs near *INS/TH* region attained chip-wide association significance ($p = 1.32 \times 10^{-7}$), 6 SNPs (Supplemental Table-3) mapped to 3 different regions showed suggestive evidence ($P < 10^{-5}$). Two out of these 3 regions are previously reported T1D regions: rs6679677/*PTPN22* (HR = 1.59; $p = 2.25 \times 10^{-6}$); rs3842727/*INS* (HR = 0.50; $p = 3.13 \times 10^{-7}$) and 1 region is a novel region, previously not known to be associated with T1D: rs11705721/*PXK/PDHB* (HR = 1.41; $p = 8.99 \times 10^{-6}$) (Table 1). The novel candidate SNP near *PPIL2* (rs428595) also showed a strong association with the risk of multiple islet autoantibodies (HR = 2.72; $p = 1.06 \times 10^{-5}$). The Kaplan-Meier plots for the lead SNPs with smallest p-values from these 4 regions are shown in Fig. 2B. Regional association plots for these 4 regions are shown in supplementary data (Supplemental File: S4).

### 3.4. SNPs associated with development of T1D

In time-to-T1D analyses, one region previously known to be associated with T1D near *INS* reached the chip-wide significance threshold (HR = 0.504; $p = 3.13 \times 10^{-7}$), and three other novel regions reached the threshold for suggestive evidence ($p < 10^{-5}$): rs73043122/*RNASET2* (HR = 3.35; $p = 4.06 \times 10^{-6}$); rs113306148/*PLEKHA1* (HR = 3.07; $p = 2.54 \times 10^{-6}$), and rs428595/*PPIL2* (HR = 3.42; $p = 2.31 \times 10^{-6}$) (Table 1). The analysis results of T1D are presented in Fig. 3. The Kaplan-Meier plots for the lead SNPs with smallest p-values from these 1 known and 3 novel regions are shown in Fig. 3B. Data for nearby SNPs in each region with the most significant SNPs and genes are shown in the regional association LocusZoom plots (Supplemental File: S4).

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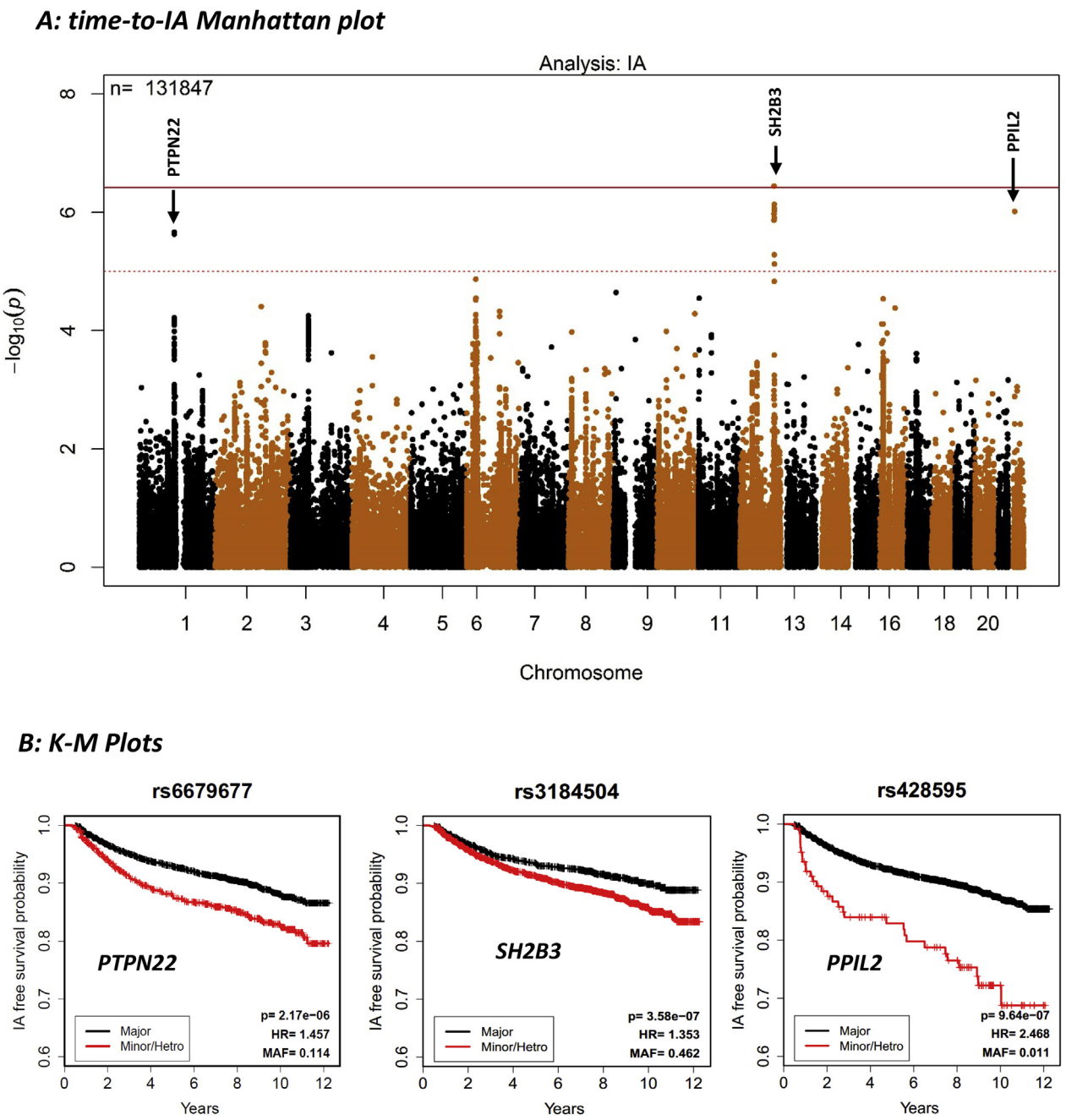


**Fig. 1. Associations with risk of any persistent confirmed islet autoantibody (IA). A:** Manhattan plot of 131,847 SNPs with MAF>0.01, displaying the *p*-values on the $-\log_{10}$ scale for SNP associations with islet autoimmunity. Hazard Ratios and p-values are calculated using the Cox proportional hazards model to analyze each SNP's effect on the risk of IA with adjustment for family history of T1D, HLA-DR-DQ genotype, gender, *HLA-DPB1*, population stratification (ancestral heterogeneity) and country of residence (as strata). The dashed line represents $p = 1 \times 10^{-5}$, the solid line represents Bonferroni correction threshold ($p = 3.7 \times 10^{-7}$). **B:** Kaplan-Meier plot of the most significant SNP associated from 2 known (*PTPN22, SH2B3*) and 1 novel (*PPIL2*) regions are plotted by dividing the subjects in two groups: (i) Major homozygous (black curves) and (ii) Heterozygous combined with minor homozygous (red curves).

### 3.5. Fine mapping of six novel regions

Six novel regions were further examined for nearby SNPs. The first novel region near the PPIL2 gene on chromosome-22 (22q11.21) was found to be associated with development of any islet autoantibody, multiple islet autoantibodies, as well as T1D with suggestive evidence. In addition to the SNP rs428595, five other proximal SNPs showed $p < 10^{-4}$ in time-to-T1D analyses. LocusZoom plots of this region are shown in Fig. 4A (time-to-IA) and 4E (time-to-T1D). Other nearby genes in the region include *UBE2L3, MAPK1, YDJC, YPEL1, CCDC116, SDF2L1* and two microRNAs (*MIR301B* and *MIR130B*). The second novel region is on chromosome-3 (3p14.3) near *PXK/PDHB* genes. LocusZoom plots of this region are shown in Fig. 4B and other nearby genes in the region include *ABHD6*, *RPP14*, *PDHB*, *KCTD6* and *ACOX2*. The third novel region is 6q27 on chromosome-6. As shown in Fig. 4C, genes in this region include *RNASET2, CCR6, RPS6KA2-AS1, GPR31, TCP10L2, FGFR1OP* and microRNA *MIR3939*. We found at least one SNP with suggestive evidence ($p < 10^{-5}$) and several SNPs with $p < 10^{-4}$ mapped to these three regions (*PPIL2, PXK, RNASET2*). The most

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**Table 1**
Lead SNP associations with IA and T1D ($p < 10^{-5}$), mapped to previously known and novel regions.

| Region | Genes | SNP* | Major/Minor allele | CHR | BP | MAF | HR-IA | p-value | HR-Multiple | p-value IA | HR-T1D | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Previously Known Regions** | | | | | | | | | | | | |
| 1 | PTPN22 | rs6679677 | C/A | 1p13.2 | 114105331 | 0.113 | **1.457** | **2.17E-06** | **1.592** | **2.25E-06** | 1.635 | 1.24E-04 |
| 2 | INS/TH | rs3842727 | A/C | 11p15.5 | 2141424 | 0.284 | 0.746 | 2.82E-05 | **0.601** | **1.32E-07** | **0.504** | **3.13E-07** |
| 3 | SH2B3 | rs3184504 | G/A | 12q24.12 | 110368991 | 0.462 | **1.353** | **3.58E-07** | 1.301 | 5.58E-04 | 1.166 | 1.21E-01 |
| **Novel Regions**** | | | | | | | | | | | | |
| 1 | PXK/PDHB | rs11705721 | A/G | 3p14.3 | 58375454 | 0.321 | 1.272 | 8.03E-05 | **1.410** | **8.99E-06** | 1.322 | 5.66E-03 |
| 2 | RNASET2/MIR3939 | rs73043122 | G/C | 6q27 | 167303257 | 0.013 | 1.415 | 1.28E-01 | 1.919 | 1.12E-02 | **3.351** | **4.06E-06** |
| 3 | PLEKHA1/MIR3941 | rs113306148 | G/A | 10q26.13 | 124149828 | 0.013 | 1.965 | 2.59E-04 | 2.463 | 2.00E-05 | **3.067** | **2.54E-06** |
| 4 | PPIL2 | rs428595 | G/A | 22q11.21 | 20346391 | 0.011 | **2.468** | **9.64E-07** | 2.720 | 1.06E-05 | **3.424** | **2.31E-06** |

CHR: Chromosome; BP: Base Pair Position (NCBI 36.3); MAF: Minor Allele Frequency; HR-IA: Hazard Ratio in islet autoimmunity analysis; HR-MIA: Hazard Ratio in multiple islet autoimmunity analysis; HR-T1D: Hazard Ratio in T1D analysis.
*The data for the SNP with smallest p-value is presented from each region. HRs and p-value adjusted for family history of T1D, HLA-DR-DQ genotype, gender, *HLA-DPB1*, population stratification (ancestral heterogeneity) and country of residence (as strata).
** 1. *PXK* was previously associated with susceptibility to systemic lupus erythematosus [19].
2. *RNASET2* gene is known to be associated with Graves disease and vitiligo [34–36].
3. A recent proteome-scale profiling study identified PPIL2 as a novel T1D-associated autoantigens [23].
4. In a recent study, *PLEKHA1* has been associated with T1D with supplementary evidence ($p < 10^{-4}$) [43].

**Table 2**
Lead SNP associations ($p < 10^{-5}$) with either IAA or GADA as first appearing islet autoantibodies.

| Region | Genes | SNP[a] | Major/Minor allele | CHR | BP | MAF | HR IAA- First | p-value | HR GADA- First | p-value | Known/Novel |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *TTC34/PRDM16* | rs28600853 | A/G | 1p36.3 | 2769620 | 0.011 | **3.233** | **6.45E-06** | 0.728 | .529 | Novel |
| 2 | *INS/TH* | rs3842727 | A/C | 11p15.5 | 2141424 | 0.284 | **0.571** | **5.67E-06** | 1.119 | .252 | Known |
| 3 | *RBFOX1* | rs9934817 | A/G | 16p13.3 | 6076220 | 0.019 | 1.181 | .605 | **2.663** | **8.02E-06** | Novel |

CHR: Chromosome; BP: Base Pair Position (NCBI 36.3); MAF: Minor Allele Frequency; HR-IAA-First: Hazard Ratio in IAA as first islet autoimmunity analysis; HR-GAD First: Hazard Ratio in GADA as islet autoimmunity analysis.
[a] The data for the SNP with smallest p-value is presented from each region. HRs and p-value adjusted for family history of T1D, HLA-DR-DQ genotype, gender, *HLA-DPB1*, population stratification (ancestral heterogeneity) and country of residence (as strata).

significant SNPs from these regions are in high linkage disequilibrium ($r^2 > 0.80$) with each other. Another novel region found in time-to-T1D analysis is near *PLEKHA1/MIR3941* on chromosome 10 (10q26.13). Nearby genes in the region are: *BTBD16, ARMS2, HTRA1* and *DMBT1*. However only one SNP (rs113306148) was mapped to this region (Fig. 4D). One novel region near *TTC34* and *PRDM16* genes was associated to IAA as the first appearing islet antibody. Other nearby genes in this region are: *MMEL1, TNFRSF14* and *ACTRT2*. One novel SNP (rs9934817) within *RBFOX1* gene was associated to GADA as the first appearing islet antibody.

## 4. Discussion

Although the genes in the HLA region comprise the most important genetic risk for T1D, other non-HLA genes also contribute to the development of autoantibodies and clinical diabetes. Several non-HLA genes have been associated with T1D with modest effect size [19–21]. In our earlier study, we analyzed 41 non-HLA SNPs that were associated with T1D by the Type 1 Diabetes Genetics Consortium and confirmed four SNPs (rs2476601 in *PTPN22*, rs2292239 in *ERBB3*, rs3184504 in *SH2B3*, and rs1004446 in *INS*) associated with IA and T1D [4]. In the current study, after adjusting for HLA, we used 131,847 SNPs from the TEDDY cohort to identify or confirm non-HLA genes associated with IA and T1D.

We found 3 known T1D regions (*PTPN22, INS, SH2B3*) and 2 novel regions (*PXK/PDHB* and *PPIL2*) associated with the development of any persistent confirmed islet autoantibody and/or multiple islet autoantibodies (Fig. 5). One known (*INS*) and one novel region (*TTC34/PRDM16)* was associated with IAA as first appearing autoantibody and one novel region (*RBFOX1*) was associated if the first appearing autoantibody was GADA. Specific mechanisms linking these loci to islet antibodies development remain to be determined. In analysis of T1D, we confirmed one region (*INS*) previously associated with T1D and discovered three novel regions previously not known to be associated with T1D (*RNASET2, PLEKHA1*, and *PPIL2*) (Fig. 5).

PX domain containing serine/threonine kinase *(PXK)* was previously associated with susceptibility to systemic lupus erythematosus [22]. PXK operates on the B-cell antigen receptor (BCR) and influences the rate of BCR internalization [23]. Individuals carrying the risk haplotype exhibit a decreased rate of BCR internalization, a process known to impact B-cell survival and cell fate. Polymorphisms in the *PXK* gene were associated with autoantibody production, but not disease risk, of systemic lupus erythematosus in a Chinese population [24]. Other nearby genes in the region include *ABHD6*, *RPP14*, *PDHB*, *KCTD6* and *ACOX2*.

One region on chromosome 22 near the Peptidylprolyl Isomerase (Cyclophilin)-Like 2 (*PPIL2*) gene is associated with both IA production and T1D. This gene is a member of the cyclophilin family of peptidylprolyl isomerases. The cyclophilins are a highly conserved ubiquitously expressed family of proteins which play an important role in protein folding, immunosuppression by cyclosporin A, and infection by HIV-1 virions. The PPIL-2 gene product is a protein that interacts with the proteinase inhibitor eglin c and is localized to the nucleus [25]. The highest expression levels of *PPIL2* was observed in thymus, testis, and islet cells in the pancreas. A recent proteome-scale profiling study identified PPIL2 as a novel T1D-associated autoantigens [26]. Other nearby genes in the region are *UBE2L3, MAPK1, YDJC, YPEL1, CCDC116, SDF2L1, MIR301B* and *MIR130B*. A meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis, identified the *CCDC116* locus for both autoimmune disorders, but targeted *UBE2L3* as the most likely candidate gene [27]. The *UBE2L3* gene encodes for ubiquitin-conjugating enzyme E2 L3 and has been functionally linked with celiac disease, rheumatoid arthritis and SLE via the ubiquitination of the NF-κB precursor. This gene plays a key role in

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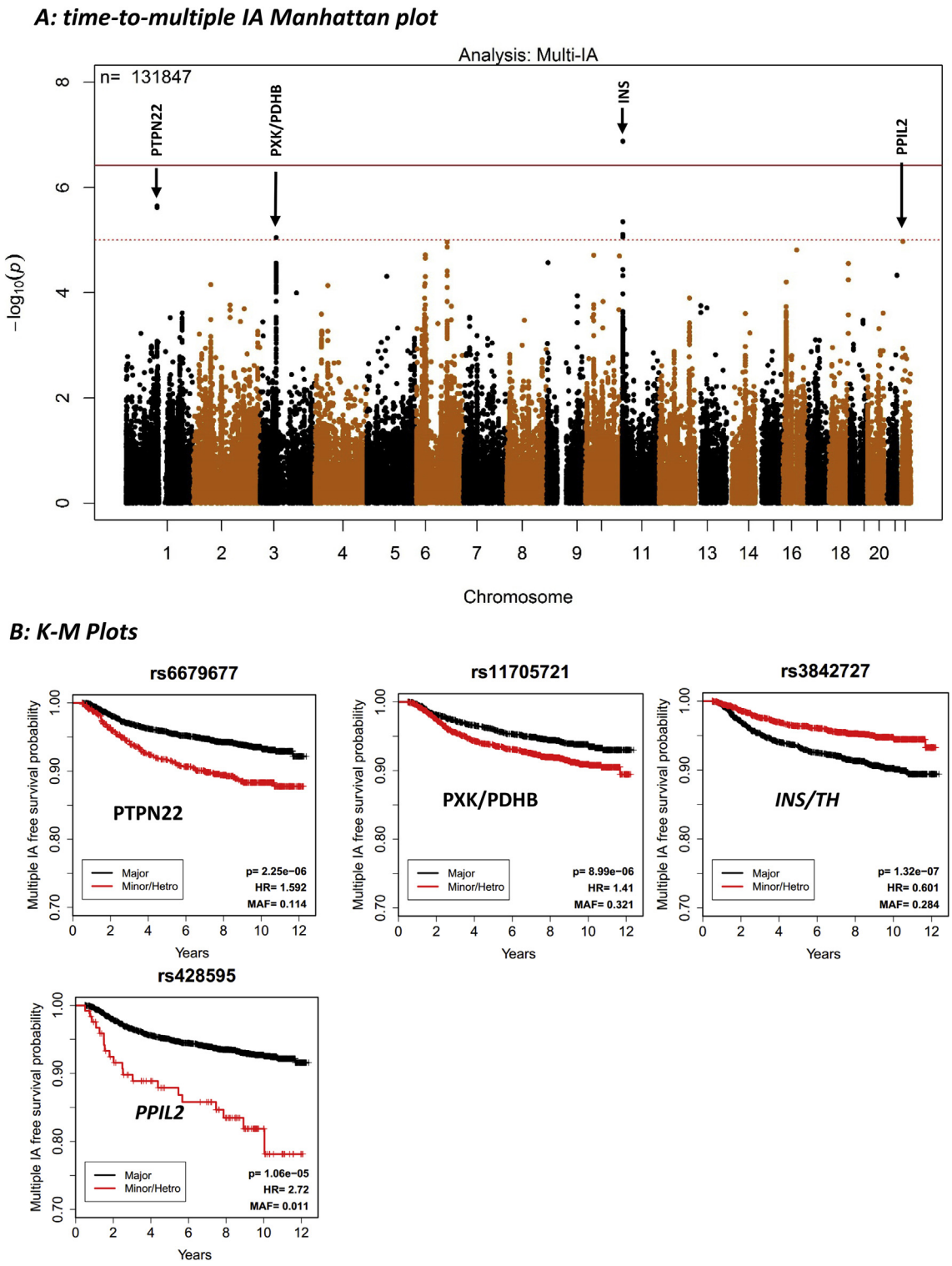


**Fig. 2. Associations with risk of development of multiple islet autoantibodies. A:** Manhattan plot of 131,847 SNPs with MAF>0.01, displaying the *p*-values on the $-\log_{10}$ scale for the SNPs associated with development of multiple islet autoantibodies in the TEDDY study population. Hazard Ratios and p-values are calculated using the Cox proportional hazards model to analyze each SNP's effect on the risk of T1D with adjustment for family history of T1D, HLA-DR-DQ genotype, gender, *HLA-DPB1*, population stratification (ancestral heterogeneity) and country of residence (as strata). The dashed line represents $p = 1 \times 10^{-5}$, the solid line represents Bonferroni correction threshold ($p = 3.7 \times 10^{-7}$). **B:** Kaplan-Meier plot of the most significant SNP from 2 known (*PTPN22, INS*) and 2 novel (*PXK, PPIL2*) regions are plotted by dividing the subjects in two groups: (i) Major homozygous (black curves) and (ii) Heterozygous combined with minor homozygous (red curves).

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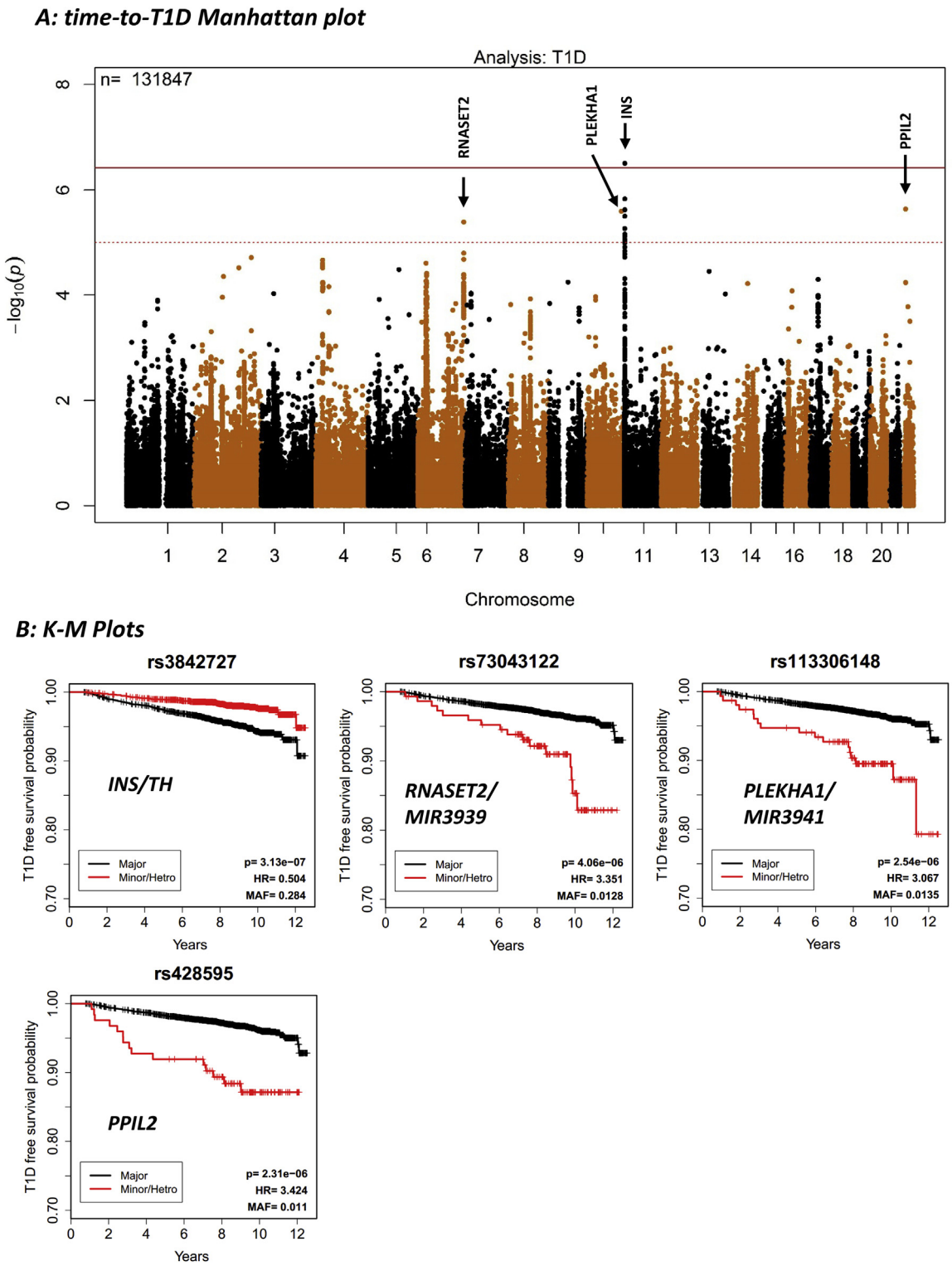


**Fig. 3. Associations with risk of type 1 diabetes. A:** Manhattan plot of 131,847 SNPs with MAF>0.01, displaying the p-values on the $-\log_{10}$ scale for the SNPs associated with **type 1 diabetes** in the TEDDY study population. Hazard Ratios and p-values are calculated using the Cox proportional hazards model to analyze each SNP's effect on the risk of T1D with adjustment for family history of T1D, HLA-DR-DQ genotype, gender, *HLA-DPB1*, population stratification (ancestral heterogeneity) and country of residence (as strata). The dashed line represents $p = 1 \times 10^{-5}$, the solid line represents Bonferroni correction threshold ($p = 3.7 \times 10^{-7}$). **B:** Kaplan-Meier plot of the most significant SNP from 1 known (*INS*) and 3 novel (*RNASET2*, *PLEKHA1* and *PPIL2*) regions are plotted by dividing the subjects in two groups: (i) Major homozygous (black curves) and (ii) Heterozygous combined with minor homozygous (red curves).

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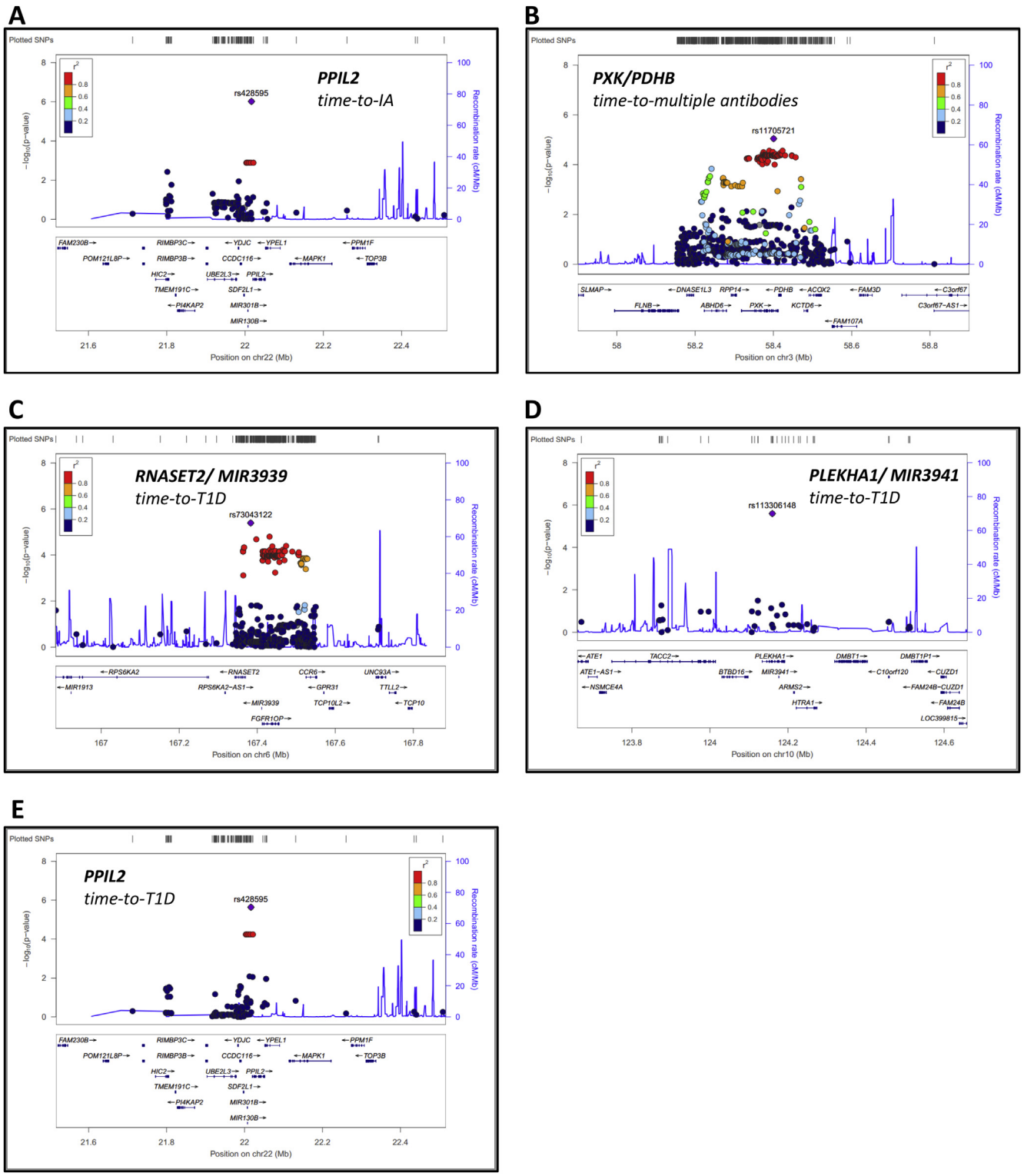


**Fig. 4. Regional plots of four novel regions associated with risk of islet autoimmunity and type 1 diabetes in the TEDDY**. Regional association plots at the four novel regions generated by LocusZoom, showing the significance of association with islet autoimmunity (A) multiple islet autoantibodies (B) type 1 diabetes (C, D, E). Colors represent HapMap CEU linkage disequilibrium $r^2$ values with the most significantly associated SNP (shown in purple).

immune response including antigen processing [28,29]. *MAPK1 (ERK2)* is involved in signaling pathways required for CD8 T-cell proliferation and survival [30] and non-obese diabetic (NOD) mice show defects in signal transduction via these pathways [31]. *SDF2L1* gene encodes endoplasmic reticulum protein involved in miss-folded protein response in beta cells [32]. *MIR301* and *MIR130* are known to play roles in inflammatory response and autoimmunity [33–36].

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| S.No. | Nearby Genes | Lead SNP | IA | MIA | T1D | IAA-First | GADA-First |
|---|---|---|---|---|---|---|---|
| ***Known Regions*** | | | | | | | |
| 1 | PTPN22 | rs6679677 | **2.17E-06** | **2.25E-06** | 1.24E-04 | 1.73E-04 | 0.007 |
| 2 | INS / TH | rs3842727 | 2.82E-05 | **1.32E-07** | **3.13E-07** | **5.67E-06** | 0.252 |
| 3 | SH2B3 | rs3184504 | **3.58E-07** | 5.58E-04 | 0.121 | 0.001 | 2.22E-04 |
| ***Novel Regions*** | | | | | | | |
| 1 | PXK/PDHB | rs11705721 | 8.03E-05 | **8.99E-06** | 0.006 | 0.010 | 0.032 |
| 2 | RNASET2/ MIR3939 | rs73043122 | 0.128 | 0.011 | **4.06E-06** | 0.723 | 0.729 |
| 3 | PLEKHA1/ MIR3941 | rs113306148 | 2.59E-04 | 2.00E-05 | **2.54E-06** | 0.141 | 5.52E-04 |
| 4 | PPIL2 | rs428595 | **9.64E-07** | 1.06E-05 | **2.31E-06** | 0.017 | 0.111 |
| 5 | TTC34 /PRDM16 | rs28600853 | 0.056 | 0.351 | 0.792 | **6.45E-06** | 0.529 |
| 6 | RBFOX1 | rs9934817 | 5.56E-04 | 0.012 | 0.009 | 0.605 | **8.02E-06** |

**Fig. 5. Summary of three known and six novel regions associated with risk of islet autoimmunity and type 1 diabetes in the TEDDY.** Data were analyzed to discover the SNPs associated with five outcomes: (i) time to any persistent confirmed islet autoantibody (IA), (ii) time to multiple autoantibodies (MIA), (iii) time to type 1 diabetes (T1D), (iv) time to IAA as the first appearing antibody (IAA-First), (v) time to GADA as the first appearing antibody (GADA-First). P-values are listed for each SNP and cells with $p < 10^{-5}$ are highlighted.

Another novel region associated with T1D is 6q27 near *RNASET2* (Ribonuclease T2), a member of the Rh/T2/S-glycoprotein class of extracellular ribonucleases. This gene is known to be associated with Graves disease and vitiligo [37–39]. Other genes in this region include *CCR6, RPS6KA2-AS1, GPR31, TCP10L2, FGFR1OP* and microRNA *MIR3939*. There is established evidence on the role of *FGFR1OP* in Crohn's disease, an inflammatory bowel disease of the gastrointestinal tract [40]. *RPS6KA2* is associated with inflammatory bowel disease [41] and epigenetic modification (DNA methylation) in *RPS6KA2* has been associated with proliferative diabetic retinopathy in T1D patients [42]. C-C chemokine receptor type 6 (CCR6) is implicated in a host of immunological responses such as B-cell maturation and differentiation, recruitment of T cells and dendritic cells during an immunological response. In mouse model of T1D, CCR6 expression dramatically decreased upon resveratrol (antioxidant) administration, subsequently inhibiting CCR6 mediated migration and recruitment of inflammatory cells [43]. G-protein coupled receptor 31 (GPR31) is involved in the 12 lipooxygenase (LO) pathway in the β cell. In response to elevated blood glucose levels, free fatty acids and pro-inflammatory cytokines, LO activation engenders pro-inflammatory lipid intermediates such as 12-HPETE and 12-HETE. Activation of GPR31 by 12-HETE ensues signaling pathways that inhibit antioxidant production, increase reactive oxygen species and oxidative stress, and ultimately β cell death [44].

The 10q26.13 region near *PLEKHA1* and microRNA *MIR3941* is also a novel region associated with T1D. Other nearby genes in the region are *BTBD16, ARMS2, HTRA1* and *DMBT1*. We found only one SNP with suggestive evidence in this region. *PLEKHA1* (or TAPP1) encodes a pleckstrin homology domain-containing adapter protein and it is shown that interaction of TAPPs with phosphatidylinositol (3,4)-bisphosphate regulates B-cell activation and autoantibody production [45]. In a recent study, *PLEKHA1* has been associated with T1D with supplementary evidence ($P = 9.92 \times 10^{-05}$) [46]. *DMBT1* is known to mediate interaction between tumor cells and immune response. In addition, DMBT1 is a marker for endocrine (islet cells) differentiation in pancreas [47].

Consistent with findings in other TEDDY publications [1,9,10], this study also supports the observation that the different genomic factors are responsible for first appearing islet autoantibody. IAA as first appearing autoantibody was associated with one known region (*INS*) and one novel region near *MMEL1, TNFRS14, TTC34* and *PRDM16* genes. In a recent study, a non-synonymous SNP within membrane metalloendopeptidase-like 1 (*MMEL1*) was associated with multiple sclerosis [48]. One SNP (rs9934817) within *RBFOX1* found to be associated only with GADA as first islet antibody. RBFOX1 (RNA binding protein fox-1 homolog 1), an mRNA-splicing factor, is well known to play a pivotal role in neuronal development and have been shown to cause neurodevelopmental disorders [49]. Interestingly, a recent study indicate that beta cells share common splicing regulators with neurons and *RBFOX1* regulates the alternative splicing of key genes related to pancreatic beta cell function [50].

There are genetic overlaps across autoimmune disorders and some autoimmunity loci are shared among multiple diseases [51]. Most of the known and novel regions identified in this study are also common with other autoimmune diseases. The *PTPN22* region is known to be associated with T1D, rheumatoid arthritis, SLE, Hashimotos thyroiditis, Graves disease, Addisons disease, Myasthenia Gravis, vitiligo, systemic sclerosis and juvenile idiopathic arthritis [52,53]. The *SH2B3* region is known to be associated with T1D, celiac disease and hepatic autoimmunity [54,55]. The *PXK* region is known to be associated with SLE [22]. The novel region near *RNASET2* and CCR6 on 6q27 is known to be associated with Graves disease, vitiligo and rheumatoid arthritis [37–39,56]. These observations suggest that several common pathways are involved in the loss of tolerance and the development of autoimmunity.

Genetic mapping studies for complex diseases like T1D were traditionally done with affected sibpair analyses and then increasingly by genome wide association studies using case-control study designs. A large number of susceptibility loci for various autoimmune diseases including T1D have been identified using these approaches [2,57–59]. However, a common finding of all these studies is that only a few genes have large effects on the phenotypes of interests. For T1D, over 40 loci have been identified with high statistical significance but none of the non-HLA loci has a large effect size in cross-sectional analyses (usually $RR < 1.5$). In contrast, time-to-event analysis may reveal relatively large effects, which are carried by low frequency (MAF 1–5%) variants [60]. For example, the HR for the *PPIL2* locus is 3.424 and HR for another novel locus, *RNASET2*, is 3.351. The HRs for two previously known regions, *PTPN22* and *INS*, are 1.59 and 0.60, respectively. These results suggest that prospective cohorts and survival analysis are powerful approaches for genetic mapping studies for complex diseases. A time-to-event analysis can have more power than a case-control analysis because time-to-event analysis includes

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information over time for all subjects in the study population, whereas case-control is only a snapshot of time of the selected case and control subjects from the study population. In addition, The Cox proportional hazards model for time-to-event analysis can access instantaneous risk via hazard ratio while the logistic regression model for case-control analysis assesses the likelihood of an event occurring at a particular time via odds ratio. Time-to-event analysis can also estimate the event-free probability over time from the baseline hazard function given observable information.

It is worth noting that the results presented in this study are based on a subset of SNPs included in the Illumina ImmunoChip genotyping platform. Also, due to the limited number of events and short follow-up time, most of the finding have suggestive evidence. However, TEDDY is the largest longitudinal study focusing on the genetic and environmental factors for type 1 diabetes.

Interestingly, our study suggests that there are few genes involved in both IA and T1D. Indeed, in our analysis, only one known region (*INS*) and one novel region (*PPIL2)* were associated with both endpoints. The newly identified region (*PPIL2*) has been identified in association with multiple other autoimmune diseases. Further it contains genes encoding proteins with a plausible pathogenic roles for development of IA and T1D, including signaling pathways involved in inflammation and autoimmunity and the PPIL2 protein, a recently identified T1D autoantigen. Further delineation of the key elements of this region will be an important area for future research.

We could confirm only 3 non-HLA associations and discovered 6 new regions which were previously not found using case/control studies. We hypothesize that as the TEDDY cohort is younger therefore genetic factors found in this study may confer the highest risk for the disease during early childhood. Also, these analyses are based on the high-risk population based HLA susceptibility and the findings may be different from the general population. Time-to-event analysis is more appropriate study design to identify predictors of disease, while case/control studies only identify associations. Replication studies and future analyses of the TEDDY cohort with more events and longer follow-up will likely provide more robust evidence for the newly suggested genetic factors.

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A complete list of the TEDDY Study Group can be found in theSupplementary.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jaut.2017.12.008.

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