The Influence of Type 1 Diabetes Genetic Susceptibility Regions, Age, Sex, and Family History to the Progression from Multiple Autoantibodies to Type 1 Diabetes: A TEDDY Study Report

Running Title: Progressing from Multiple Autoantibodies to T1D

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Abstract

This paper seeks to determine whether factors related to autoimmunity risk remain significant after the initiation of two or more diabetes-related autoantibodies and continue to contribute to T1D risk among autoantibody positive children in The Environmental Determinants of Diabetes in the Young (TEDDY) study. Characteristics included are age at multiple autoantibody positivity, sex, selected high-risk HLA-DR-DQ genotypes, relationship to a family member with T1D, autoantibody at seroconversion, *INS* gene (rs1004446_A), and non-HLA gene polymorphisms identified by the Type 1 Diabetes Genetics Consortium. The risk of progression to T1D was not different among those with or without a family history of T1D (p=0.39) nor HLA-DR-DQ genotypes (p=0.74). Age at developing multiple autoantibodies (HR=0.96 per 1 month increase in age, 95% CI=0.95, 0.97, p<0.001) and the type of first autoantibody (when more than a single autoantibody was the first appearing indication of seroconversion [p=0.006]) were statistically significant. Female sex was also a significant risk factor (p=0.03). Three SNPs were associated with increased diabetes risk (rs10517086_A, [p=0.03], rs1534422_G, [p=0.006], and rs2327832_G in *TNFAIP3* [p=0.03]), and one with decreased risk (rs1004446_A in *INS*, [p=0.006]). The TEDDY data suggest that non-HLA gene polymorphisms may play a different role in the initiation of autoimmunity than they do in progression to T1D once autoimmunity has appeared. The strength of these associations may be related to the age of the population and the high-risk HLA-DR-DQ subtypes studied.

Keywords:

Autoimmunity

Type 1 diabetes

Abbreviations

CI confidence intervals

DNA deoxyribonucleic acid

FDR first degree relative

GADA glutamic acid decarboxylase autoantibodies

GP general population

HLA human leukocyte antigen

HR hazard ratio

IA islet autoimmunity

IAA islet autoantibodies to insulin

IA-2A insulinoma antigen-2

IQR interquartile range

PCR polymerase chain reaction

PH proportional hazard

SNP single nucleotide polymorphism

T1D type 1 diabetes

T1DGC Type 1 Diabetes Genetics Consortium

Introduction

Type 1 diabetes (T1D) is an autoimmune disease preceded by the onset of one of more islet cell autoantibodies. The presence of two or more autoantibodies is generally felt to increase that risk significantly, especially among young children (1,2). Previous studies have shown that the incidence of T1D is increased in individuals with another family member known to have the disease (3,4). The risk of T1D is on the order of 10-fold higher in first degree relatives (FDR) of an individual with T1D as compared to the general population (GP). In addition, it is fairly well established that the incidence of autoimmunity and T1D in individuals with certain human leukocyte antigen (HLA) loci varies considerably with a gradient that spans the range of highly susceptible to protective loci (5,6). This paper examines T1D risk among those individuals who already have developed two or more islet cell autoantibodies (IA) in The Environmental Determinants of Diabetes in the Young (TEDDY) study, a large cohort of genetically at risk individuals followed from birth with uniform sampling from three months of age onwards (7,8). It seeks to determine whether factors significant for autoimmunity risk remain significant after the initiation of autoimmunity and continue to contribute to our understanding of the highly variable rate of progression to T1D among autoantibody positive children.

Research Design and Methods

Participants. TEDDY is a prospective cohort study funded by the National Institutes of Health with the primary goal to identify environmental causes of T1D. It includes six clinical research centers - three in the US: Colorado, Georgia/Florida, Washington and

three in Europe: Finland, Germany, and Sweden. Detailed study design and methods have been previously published (7-9). Written informed consents were obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and participation in the prospective follow-up. The high-risk genotypes for participants screened from the general population were as follows: DRB1*04-DQA1*03-DQB1*03:02/DRB1*03-DQA1*05-DQB1*02:01 (DR3/4), DRB1*04-DQA1*03-DQB1*03:02/DRB1*04-DQA1*03-DQB1*03:02 (DR4/4), DRB1*04-DQA1*03-DQB1*03:02/DRB1*08-DQA1*04-DQB1*04:02 (DR4/8) and DRB1*03-DQA1*05-DQB1*02:01/DRB1*03-DQA1*05-DQB1*02:01 (DR3/3). Additional genotypes were included for first degree relatives (FDRs) of a subject with T1D: DRB1*04-DQA1*03-DQB1*03:02/DRB1*04- DQA1*03-DQB1*02:02 (DR4/4b), DRB1*04-DQA1*03-DQB1*03:02/DRB1*01- DQA1*01-DQB1*05:01 (DR4/1), DRB1*04-DQA1*03-DQB1*03:02/DRB1*13-DQA1*01-DQB1*06:04 (DR4/13), DRB1*04-DQA1*03-DQB1*03:02/DRB1*09- DQA1*03-DQB1*03:03 (DR4/9), and DRB1*03-DQA1*05-DQB1*02:01/DRB1*09- DQA1*03-DQB1*03:03 (DR3/9). The HLA-DR-DQ genotype abbreviations shown in parentheses will be used throughout this paper. Genotyping was confirmed by reverse blot hybridization at the central HLA Reference Laboratory at Roche Molecular Systems, Oakland, CA [9], along with the *INS*-23Hph1 (rs689), *CTLA4* T17A (rs231775) and *PTPN22* R620W (rs2476601) SNP primer pairs. The study was approved by local Institutional Review or Ethics Boards and is monitored by an External Evaluation Committee formed by the National Institutes of Health.

SNP analysis was performed by the Center for Public Health Genomics at University of Virginia, using the Illumina Immunochip which is a custom array for genotyping of SNPs selected from regions of the human genome firmly associated with autoimmune diseases (11). The final selection of SNPs containing ~ 186 000 SNPs in 186 regions, for 12 autoimmune diseases was decided by the Immunochip Consortium. TEDDY previously examined whether any of 41 non-HLA SNPs previously shown to be associated with T1D conferred risk for IA (12). These SNPs were re-examined in relation to the risk of T1D from the time of development of multiple islet autoantibodies.

Islet Autoantibodies. Islet autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA) or insulinoma antigen-2 (IA-2A) were measured in two laboratories by radiobinding assays (7,8). 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. Both laboratories demonstrated high sensitivity and specificity as well as concordance (10). All positive islet autoantibodies and 5% of negative samples were re-tested in the other reference laboratory and deemed confirmed if concordant. Persistent islet autoimmunity was defined as confirmed positive autoantibodies to insulin, GAD65, or IA-2A in at least two consecutive samples.

Statistical Methods. Characteristics of those who progressed to T1D and those who did not are presented for descriptive purposes. Cox proportional hazards (PH) models were applied to examine factors related to the risk of progression from the detection of multiple autoantibodies to T1D. The magnitudes of the associations were described by

hazard ratios (HR) with 95% confidence intervals (CI). Adjustments for population stratification were made by using the top two principal components from the Immunochip SNP data as covariates in the proportional hazards model (15). Data were analyzed using the Statistical Analysis System software (version 9.4; SAS Institute, Cary, NC). Two-tailed p-values less than 0.05 were considered to be statistically significant. No adjustment in type 1 error was made for multiple comparisons except in the context of the multiple Cox regression model.

Results

TEDDY enrolled 8676 children at birth and has followed them quarterly for the appearance of autoantibodies and T1D. Follow up of children with one or more islet autoantibody continued on this schedule, whereas children who were autoantibody negative were followed semiannually after 4 years of age. Excluded from this analysis are 172 children who were either ineligible or whose autoantibody status was indeterminate. The median (interquartile range, IQR) age at last follow up was 8.0 (6.7-9.3) years.

As of June 30, 2016, 412 children (4.8%) have developed multiple persistent confirmed islet autoantibodies and, of these, 190 (46.1%) have progressed to T1D (**Table 1**). The median (IQR) duration of follow up from the appearance of multiple autoantibodies was 3.0 (1.4-5.1) years. The age at which multiple autoantibodies first appeared was associated with increased risk of progression to T1D ($p<0.001$), as was the appearance of multiple autoantibodies at first appearance ($p=0.006$). The risk to progress to T1D was not significantly different when the data were analyzed by country of residence, family

history, sex, and HLA-DR-DQ genotype (p not significant). A multiple Cox regression analysis of these same characteristics confirmed the lack of statistical significance associated with family history (FDR vs. GP) (**Figure 1**) (p=0.39) or HLA-DR-DQ genotype (p=0.74) (**Figure 2**). Relationship of the TEDDY child to the family member with T1D among the FDRs compared to GP was also not significantly different (offspring of father with T1D (p=0.29), mother (p=0.42) or sibling (p=0.96)) (**Table 2**). Age at multiple autoantibodies (HR=0.96 per 1 month increase in age, 95% CI 0.95, 0.97, p<0.001) and when more than a single autoantibody was first appearing indication of seroconversion (HR=1.66 compared to IAA only, p=0.006) were statistically significant (**Figure 3**). In the multiple Cox regression female (as compared to male) sex became a significant risk factor (HR=1.43, 95% CI 1.04, 1.96, p=0.03) (**Figure 4**).

Among those with multiple autoantibodies, SNPs rs10517086_A (p=0.03), rs1534422_G (p=0.006) and rs2327832_G in TNFAIP3 (p=0.03) were significantly associated with increased risk of progression to T1D and SNP rs1004446_A in *INS* (p=0.03) was associated with decreased risk (**Table 2** and **Figure 5**). There was a significant interaction between the SNP rs2327832_G in *TNFAIP3* and the type of first autoantibody (p=0.003), indicating much higher risk of T1D with rs2327832_G polymorphism in the subjects who had the appearance of multiple autoantibodies as the first indication of seroconversion (HR=2.37 95% CI 1.52 3.70, p<0.001) (**Figure 6**). No interaction was found between the other SNPs and first appearing autoantibody. A table of all SNPs included in this analysis appears in the Online Supplemental appendix.

Discussion

While HLA-DR-DQ haplotypes have been shown to be associated with the incidence of autoimmunity, our data does not show that it continues to be related to progression to T1D in the HLA-selected high-risk TEDDY cohort among those who have multiple diabetes related autoantibodies. As well, the risk of progression to T1D was not different among those with or without a family history of T1D, for the high-risk genotypes followed in TEDDY. TEDDY has previously shown (16) that, among those who initially seroconvert to 2 autoantibodies, family history of T1D is a significant risk factor for progression to T1D by 5 years of age, but not among those who initially seroconvert to 3 autoantibodies. The results reported herein, now with additional follow-up to a median of 8 years of age, indicate that family history is no longer significant among those with 2 or more antibodies from the time of becoming multiple autoantibody positive.

Despite the lack of association with HLA-DR-DQ, we did find three SNPs that were associated with increased diabetes risk and one associated with decreased risk. Only SNP rs1004446_A in INS was reported to be significantly protective SNP of T1D from birth in TEDDY overall and in this multiple autoantibody positive subset. The other SNPs tested were not significantly related to T1D in the multiple autoantibody positive population, despite their association with autoimmunity in TEDDY and diabetes in the Type 1 Diabetes Genetics Consortium (T1DGC) (13) suggesting a genetic contribution to progression to diabetes after the appearance of autoantibodies that might be different than in the initiation of autoimmunity.

Of note, the three SNPs associated with an increased diabetes risk in this population of children with multiple diabetes related autoantibodies were not associated with T1D in TEDDY overall despite their significant association reported by others (14). This might be due to the fact that the TEDDY study is limited to certain at risk HLA subgroups or that they play a role in progression of autoimmunity toward T1D, but not in initiation of autoimmunity. SNP rs1534422_G in *TNFAIP3* has also been recently shown to be associated with multiple sclerosis (MS) risk (17) which is also an auto-inflammatory disease with genomic-environmental risk factors involving the HLA locus. SNP rs2327832_G has been reported to be in with a risk factor for rheumatoid arthritis (18) and celiac disease (19,20), whereas SNP rs10517086_A has been shown to have an age-related association with IA with increased risk in children under age 2 (21).

Here we show evidence of increased risk for T1D in multiple autoantibody positive children. These findings are similar to those reported by Lempainen et al (22) in the Finnish Diabetes Prediction and Prevention (DIPP) study which also showed a lack of associated with FDR status or HLA and progression to diabetes, but a positive association with female sex in children positive for two islet autoantibodies. A difference in findings between the two studies is that the DIPP study reports a significant association of the *PTPN22* gene polymorphism with progression from multiple autoantibodies to T1D, whereas the TEDDY study does not. In contrast, the TEDDY study does find an association with the *INS* gene, but the DIPP does not. Similar to TEDDY, the *INS* gene, but not *PTPN22*, was among 5 genes that, together, stratified progression to disease in the German BABYDIAB and BABYDIET studies (23). The reported differences could be

related to the populations, since the Finnish population has a higher prevalence of the *PTPN22* gene polymorphism than elsewhere. DIPP, DAISY and the German BABYDIAB and BABYDIET studies (2,24) report similar findings with regard to appearance of multiple autoantibodies at a young age and the excess risk associated with female sex. Others have speculated a link between the observed protective effect of the *INS* gene and immune tolerance through higher levels of expression in the thymus as a plausible mechanism (25).

The TEDDY data suggest that non-HLA gene polymorphisms may play a different role in the initiation of autoimmunity than they do in progression to T1D once autoimmunity has appeared. The strength of these associations and even their direction (increased vs. decreased risk) may vary by population and the nature of the other characteristics included in multivariate models. While these results extend earlier TEDDY findings by providing additional years of follow-up, it may be that the relationships described are all age related. Cases of T1D diagnosed among older children may share the same mechanisms and strengthen these findings or may be the result of other immunological insults involving other exposures and gene-environmental interactions. Having already published an age effect on the initiation of autoimmunity and differences in the pattern of the types of autoantibodies that arise first (26), it is not inconceivable that there is also an age related association of exposures and both HLA and non-HLA genes. Caution should be exercised in generalizing the results presented here beyond the age range in which they have been discovered and the selected HLA subgroups that constitute the TEDDY population. As well, caution should be exercised in interpreting statistically significant

findings due to the number of comparisons that have been made. Adjusting the significance level for multiple comparisons when conducting epidemiological research, especially in the context of a multivariate analysis has both supporters (27) and detractors (28,29). No matter what side of the argument the reader falls on, the associations reported herein should be viewed in the larger context of the results of other studies and other populations to be properly interpreted.

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Electronic supplementary material: A complete list of the members of the TEDDY Study Group can be found in the online version of this article.

References

1. Orban T, Sosenko JM, Cuthbertson D, et al. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care 2009;32:2269-2274

2. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013;309:2473-2479

3. Cernea S, Dobreanu M, Raz I. Prevention of type 1 diabetes: today and tomorrow. Diabetes-Metab Res 2010;26:602-605

4. Huber A, Menconi F, Corathers S, et al. Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms. Endocr Rev 2008;29:697-725

5. Stankov K, Benc D, Draskovic D. Genetic and epigenetic factors in etiology of diabetes mellitus type 1. Pediatrics 2013;132:1112-1122

6. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet 2016;387:2331-2339

7. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. Pediatr Diabetes 2007;8:286-298

8. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study. Ann N Y Acad Sci 2008;1150:1-13

9. Hagopian WA, Erlich H, Lernmark Å, et al.; TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatr Diabetes 2011;12:733-743

10. Bonifacio E, Yu L, Williams AK, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. J Clin Endocrinol Metab 2010;95:3360-3367

11. Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat Rev Genet 2013;14:661-673

12. Törn C, Hadley D, Lee HS, et al.; TEDDY Study Group. Role of type 1 diabetes associated SNPs on risk of autoantibody positivity in the TEDDY study. Diabetes 2015;64:1818-1829

13. Barrett JC, Clayton DG, Concannon P, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009;41:703-707

14. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38:904-909

15. Concannon P, Chen WM, Julier C, et al. Genome-wide scan for linkage to type 1 diabetes in 2,496 multiplex families from the Type 1 Diabetes Genetics Consortium. Diabetes 2009;58:1018-1022

16. Steck AK, Vehik K, Bonifacio E, et al.; TEDDY Study Group. Predictors of progression from the appearance of islet autoantibodies to early childhood diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). Diabetes Care 2015;38:808-813

17. Lill CM, Luessi F, Alcina A, et al. Genome-wide significant association with seven novel multiple sclerosis risk loci. J Med Genet 2015;52:848-855

18. Elsby LM, Orozco G, Denton J, et al. Functional evaluation of TNFAIP3 (A20) in rheumatoid arthritis. Clin Exp Rheumatol 2010;28:708-714

19. Izzo V, Pinelli M, Tinto N, et al. Improving the estimation of celiac disease sibling risk by non-HLA genes. PLoS One 2011;6:e26920

20. Smyth DJ, Plagnol V, Walker NM, et al. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med 2008;359:2767-2777

21. Frederiksen BN, Steck AK, Kroehl M, et al. Evidence of stage- and age-related heterogeneity of non-HLA SNPs and risk of islet autoimmunity and type 1 diabetes: the diabetes autoimmunity study in the young. Clin Dev Immunol 2013;2013:417657

22. Lempainen J, Hermann R, Veijola R, et al. Effect of the PTPN22 and INS risk genotypes on the progression to clinical type 1 diabetes after the initiation of β-cell autoimmunity. Diabetes 2012;61:963-966

23. Bonifacio E, Krumsiek J, Winkler C, et al. A strategy to find gene combinations that identify children who progress rapidly to type 1 diabetes after islet autoantibody seroconversion. Acta Diabetol 2014;51:403-411

24. Hummel M, Bonifacio E, Schmid S, et al. Brief communication: early appearance of islet autoantibodies predicts childhood type 1 diabetes in offspring of diabetic parents. Ann Intern Med 2004;140:882-886

25. Vafiadis P, Bennett ST, Todd JA, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. Nat Genet 1997;15:289-292

26. Krischer JP, Lynch KF, Schatz DA, et al.; TEDDY Study Group. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015;58:980-987

27. Thompson, J. Invited commentary: Re: "Multiple comparisons and related issues in the interpretation of epidemiologic data." Am J. Epidemiol 1997;147:801-1

28. Savitz DA and Olshan, AF, Multiple Comparisons and Related Issues in the Interpretation of Epidemiologic Data. Am J. Epidemiol 1995; 142:904-8

29. Savitz DA and Olshan, AF, Describing data requires no adjustment for multiple comparisons: a reply from Savitz and Olshan. Am J. Epidemiol 1998; 147:813-14

Table 1

Characteristic	**Did not progress to T1D (%)**	**Progressed to T1D (%)**
Total n	**222 (54)**	**190 (46)**
Country of residence n (%)		
US	82 (59)	57 (41)
Finland	53 (48)	57 (52)
Germany	16 (44)	20 (56)
Sweden	71 (56)	56 (44)
Family History of T1D n (%)		
General Population	171 (55)	140 (45)
FDR: Mother	12 (50)	12(50)
FDR: Father	27 (50)	27 (50)
FDR: Sibling	12 (52)	11 (48)
Gender n (%)		
Female	88 (49)	92 (51)

Male	134 (58)	98 (42)
HLA-DR-DQ genotypes n (%)		
DR3/4	121 (52)	110 (48)
DR4/4	46 (61)	30 (39)
DR4/8	31 (56)	24 (44)
DR3/3	16 (53)	14 (47)
FDR Specific	8 (40)	12 (60)
Median (IQR) Age at multiple persistent confirmed IA (months)	48 (31 -74)	21 (15 -31)
Type of first autoantibody n (%)		
GADA only	85 (66)	43 (34)
IAA only	84 (53)	76 (47)
2+ autoantibodies	49 (42)	68 (58)
IA2A only	4 (57)	3 (43)

Table 2

Cox regression analysis of risk factors for progression from multiple autoantibodies to type 1 diabetes. The top two principal components (PC1 and PC2) from the principal components analysis on Immunochip data were included as covariates to correct for population stratification.

Factor	**HR (95% CI)**	**p**
Age at multiple autoantibodies onset (months)	0.96 (0.95, 0.97)	<0.001
HLA-DR-DQ genotype		0.74
DR3/4	1.24 (0.79, 1.93)	0.35
DR4/4	1 [Reference]	
DR4/8	1.22 (0.68, 2.18)	0.50
DR3/3	1.44 (0.70, 2.96)	0.32
FDR specific	1.58 (0.73, 3.41)	0.25
Family history of T1D		0.69
FDR: Mother	1.34 (0.66, 2.75)	0.42
FDR: Father	1.30 (0.80, 2.09)	0.29
FDR: Sibling	0.98 (0.48, 2.01)	0.96
GP	1 [Reference]	
Type of first autoantibody		0.02
GADA only	1.16 (0.76, 1.78)	0.49
IAA only	1 [Reference]	

2+ autoantibodies	1.66 (1.15, 2.39)	0.006
Sex		
Female	1.43 (1.04, 1.96)	0.03
Male	1 [Reference]	
Country of residence		0.84
US	1 [Reference]	
Finland	1.05 (0.53, 2.10)	0.89
Germany	1.13 (0.59, 2.14)	0.71
Sweden	0.88 (0.58, 1.34)	0.55
SNP rs1004446_A (*INS)*	0.71 (0.55, 0.91)	0.006
SNP rs10517086_A	1.31 (1.03, 1.67)	0.03
SNP rs1534422_G	1.39 (1.10, 1.76)	0.006
SNP rs2327832_G (*TNFAIP3)*	1.34 (1.03, 1.74)	0.03
PC1	1.11 (0.91, 1.35)	0.32
PC2	0.96 (0.72, 1.28)	0.78

Figure legends:

Figure 1. Progression from multiple autoantibodies to type 1 diabetes by FDR status (p=0.39 from Cox regression).

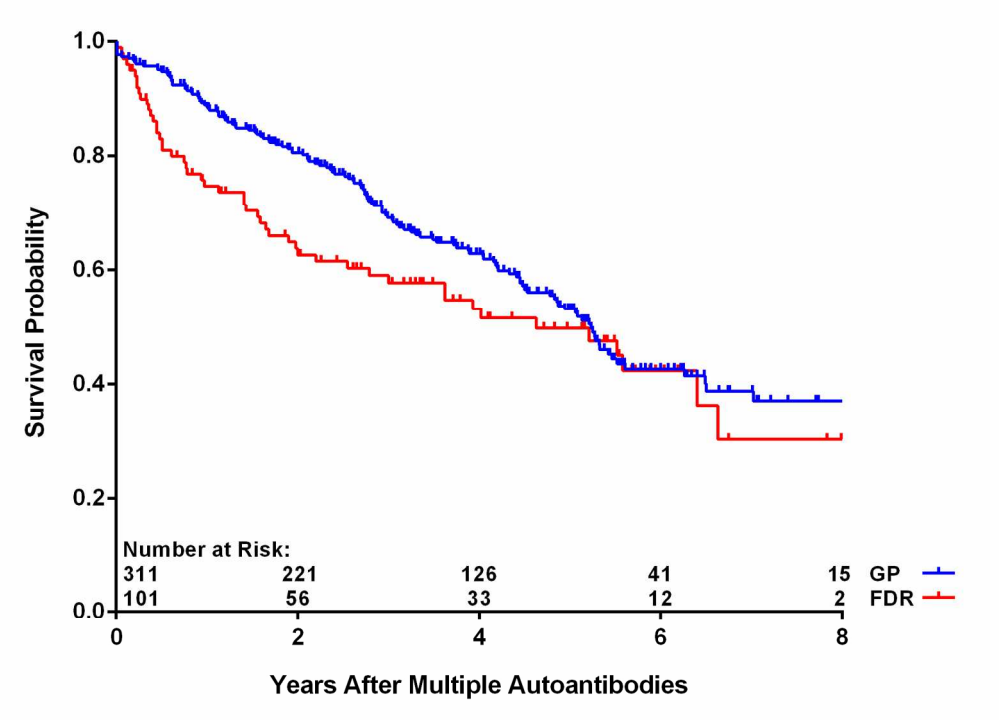
Figure 2. Progression from multiple autoantibodies to type 1 diabetes by HLA-DR-DQ genotypes (p=0.74 from Cox regression). FDR-specific are DR4/4b, DR4/1, DR4/13, DR4/9, and DR3/9.

Figure 3. Progression from multiple autoantibodies to type 1 diabetes by type of first autoantibody (p=0.02 from Cox regression).

Figure 4. Progression from multiple autoantibodies to type 1 diabetes by sex (p=0.03 from Cox regression).

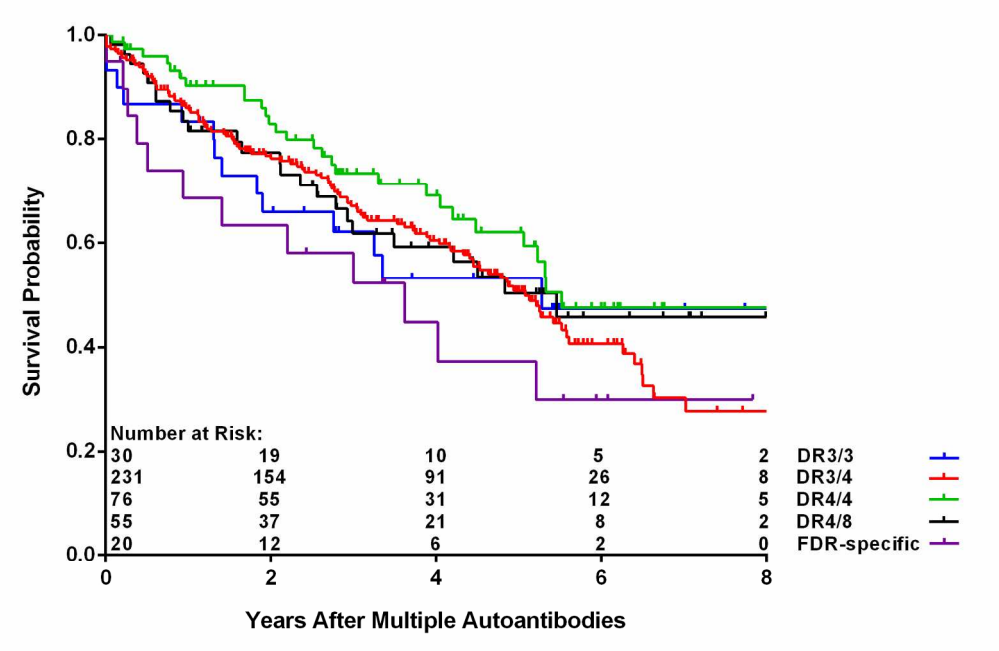
Figure 5. Progression from Multiple Autoantibodies by number of minor alleles of single nucleotide polymorphism within panels (a) rs10517086_A (p=0.03 from Cox regression), (b) rs1004446_A (p=0.006 from Cox regression), (c) rs1534422_G (p=0.006 from Cox regression), and (d) rs2327832_G (p=0.03 from Cox regression).

Figure 6. Progression from multiple autoantibodies to type 1 diabetes by number of minor alleles of SNP rs2327832_G in the subset of more than one autoantibody as first appearing autoantibody (p<0.001 from Cox regression).



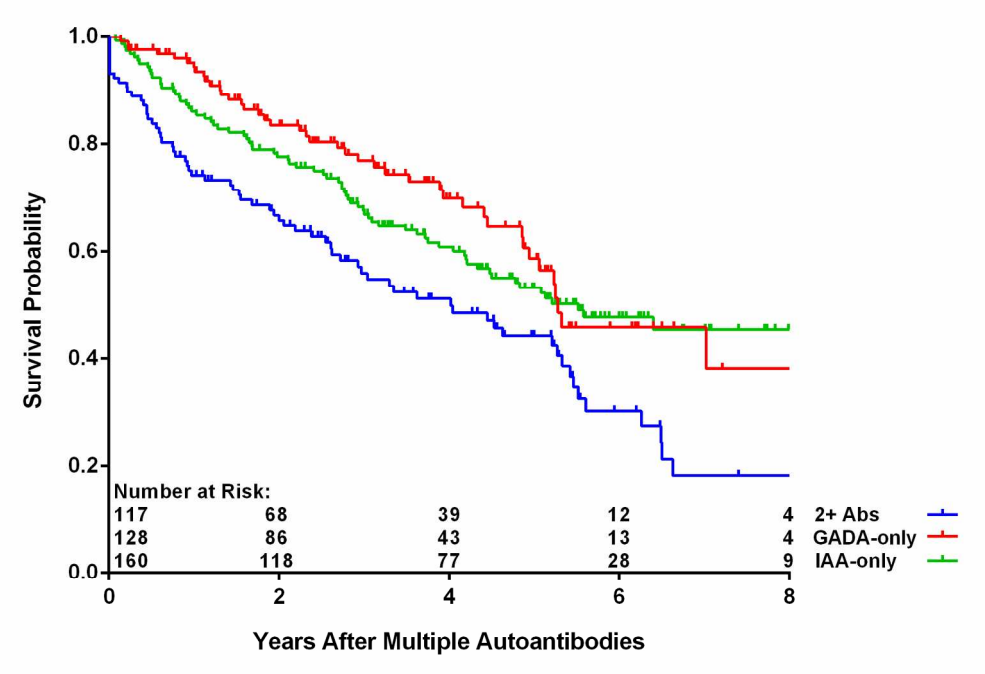
Progression from multiple autoantibodies to type 1 diabetes by FDR status (p=0.39 from Cox regression).

167x120mm (300 x 300 DPI)



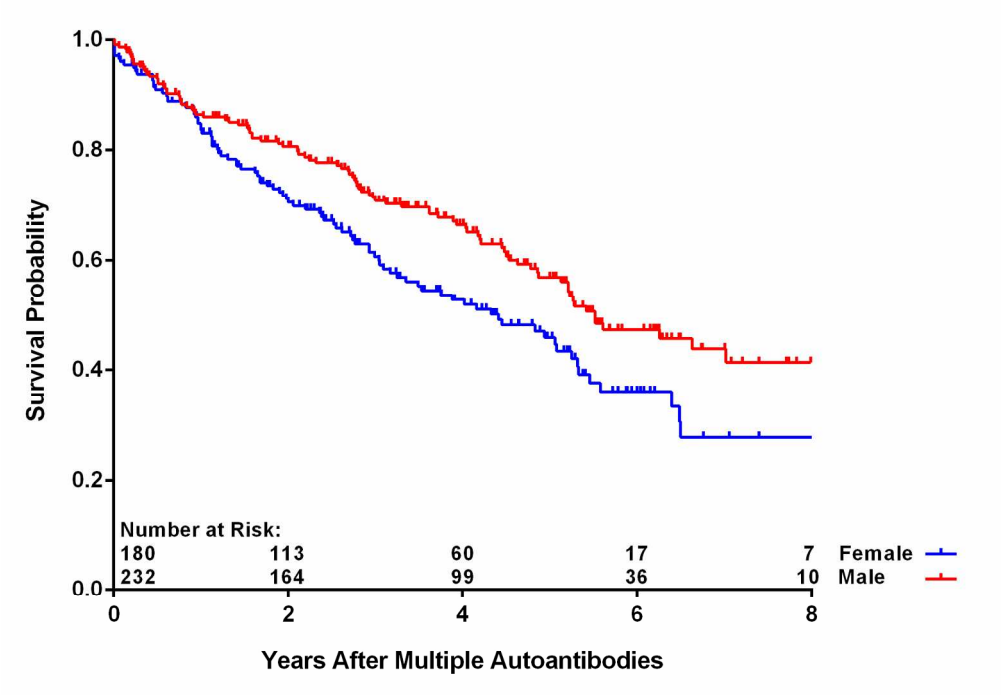
Progression from multiple autoantibodies to type 1 diabetes by HLA-DR-DQ genotypes (p=0.74 from Cox regression). FDR-specific are DR4/4b, DR4/1, DR4/13, DR4/9, and DR3/9.

183x120mm (300 x 300 DPI)



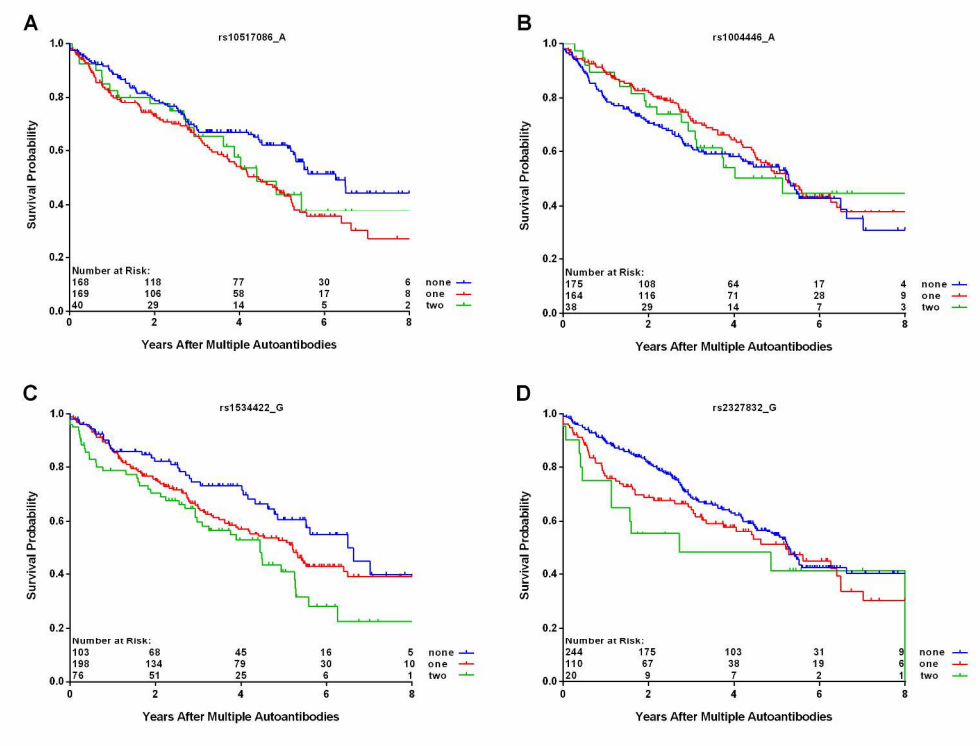
Progression from multiple autoantibodies to type 1 diabetes by type of first autoantibody (p=0.02 from Cox regression).

178x120mm (300 x 300 DPI)



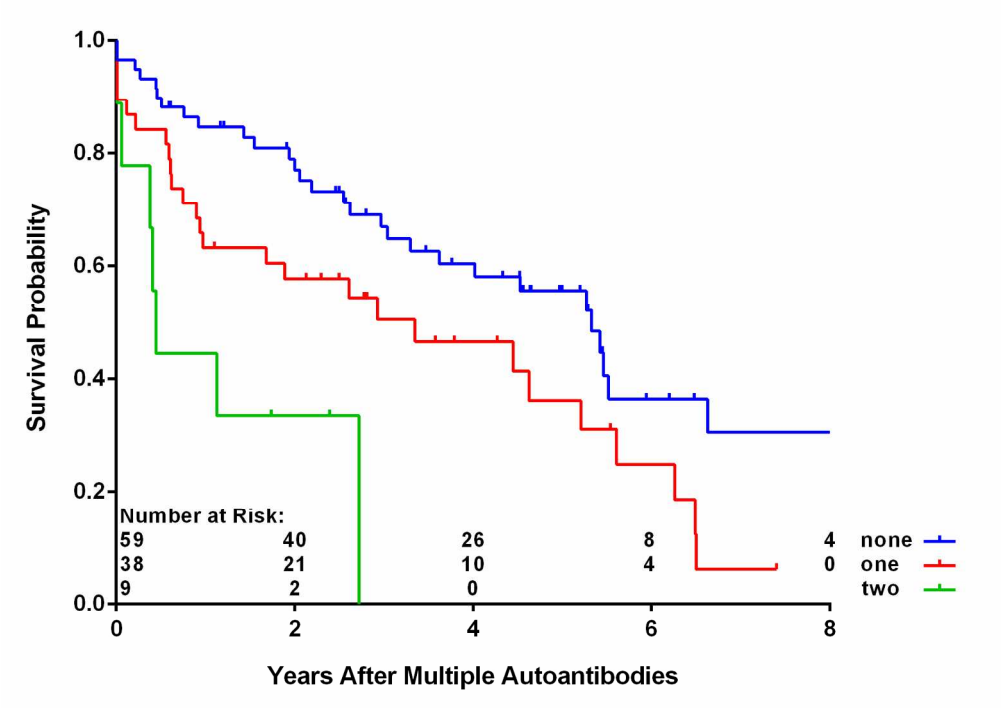
Progression from multiple autoantibodies to type 1 diabetes by sex (p=0.03 from Cox regression).

173x120mm (300 x 300 DPI)



Progression from Multiple Autoantibodies by number of minor alleles of single nucleotide polymorphism within panels (a) rs10517086_A (p=0.03 from Cox regression), (b) rs1004446_A (p=0.006 from Cox regression), (c) rs1534422_G (p=0.006 from Cox regression), and (d) rs2327832_G (p=0.03 from Cox regression).

260x195mm (300 x 300 DPI)



Progression from multiple autoantibodies to type 1 diabetes by number of minor alleles of SNP rs2327832_G in the subset of more than one autoantibody as first appearing autoantibody ($p<0.001$ from Cox regression).

169x120mm (300 x 300 DPI)

Supplemental Table. Cox regression analysis of the 41 type 1 diabetes risk loci on the risk of progression from multiple autoantibodies to type 1 diabetes. Cox model for each SNP was adjusted for age at multiple autoantibodies onset, HLA-DR-DQ genotype, sex, family history of T1D, type of first autoantibody and the top two principal components from the principal components analysis on TEDDY Immunochip data. The minor allele frequency (MAF) for the respective SNP was calculated from the study population.

Chr	SNP	Gene of interest	Minor allele	MAF	HR (95% CI)	p
1p13.2	rs2476601	*PTPN22*	A	0.18	1.21 (0.88, 1.65)	0.241
1p31.3	rs2269241	*PGM1*	C	0.25	0.91 (0.70, 1.18)	0.472
1q31.2	rs2816316	*RGS1*	C	0.18	0.88 (0.67, 1.16)	0.371
1q32.1	rs3024505	*IL10*	A	0.16	1.06 (0.81, 1.41)	0.661
2p25.1	**rs1534422**	**0**	**G**	**0.47**	**1.39 (1.10, 1.76)**	**0.005**
2q24.2	rs1990760	*IF1H1*	C	0.38	1.02 (0.82, 1.28)	0.831
2q33.2	rs3087243	*CTLA4*	A	0.38	1.16 (0.94, 1.44)	0.171
3p21.31	rs11711054	*CCR5*	G	0.31	1.04 (0.82, 1.31)	0.772
4p15.2	**rs10517086**	**0**	**A**	**0.33**	**1.31 (1.03, 1.66)**	**0.026**
4q27	rs4505848	*IL2*	G	0.39	1.19 (0.95, 1.49)	0.122
5p13.2	rs6897932	*IL7R*	T	0.27	1.15 (0.89, 1.49)	0.284
6q22.32	rs9388489	*C6orf173*	G	0.45	0.99 (0.78, 1.25)	0.926
6q23.3	**rs2327832**	***TNFAIP3***	**G**	**0.20**	**1.37 (1.06, 1.78)**	**0.017**
6q25.3	rs1738074	*TAGAP*	T	0.40	1.12 (0.90, 1.41)	0.305
7p12.1	rs4948088	*COBL*	A	0.03	1.11 (0.62, 1.99)	0.718
7p15.2	rs7804356	*SKAP2*	C	0.20	1.21 (0.91, 1.62)	0.194
9p24.2	rs7020673	*GLIS3*	C	0.48	0.95 (0.76, 1.17)	0.613
10p15.1	rs11258747	*PRKCQ*	T	0.26	1.04 (0.82, 1.32)	0.749
10p15.1	rs12251307	*IL2RA*	T	0.11	0.99 (0.71, 1.39)	0.957
10q23.31	rs10509540	*RNLS*	C	0.25	1.04 (0.80, 1.34)	0.780
11p15.5	**rs1004446**	***INS***	**A**	**0.32**	**0.75 (0.59, 0.96)**	**0.021**
11p15.5	rs7111341	*INS*	T	0.23	0.88 (0.67, 1.17)	0.376
12p13.31	rs4763879	*CD69*	A	0.40	1.04 (0.84, 1.29)	0.716
12q13.2	rs2292239	*ERBB3*	T	0.39	1.05 (0.83, 1.33)	0.676

12q24.12	rs3184504	*SH2B3*	T	0.52	0.97 (0.79, 1.20)	0.790
14q24.1	rs1465788	*ZFP36L1,C14orf181*	T	0.29	1.06 (0.84, 1.35)	0.627
14q32.2	rs4900384	C14orf64	G	0.31	0.86 (0.67, 1.10)	0.219
15q25.1	rs3825932	*CTSH*	T	0.32	0.81 (0.63, 1.04)	0.102
16p11.2	rs4788084	*IL27*	T	0.44	1.07 (0.86, 1.34)	0.538
16p13.13	rs12708716	*CLEC16A*	G	0.29	1.13 (0.90, 1.43)	0.287
16q23.1	rs7202877	*CTRB2*	G	0.12	1.04 (0.74, 1.46)	0.832
17p13.1	rs16956936	*2 genes*	T	0.13	1.02 (0.72, 1.44)	0.913
17q12	rs2290400	*ORMDL*	T	0.48	0.97 (0.79, 1.20)	0.771
17q21.2	rs7221109	*CCR7*	T	0.38	1.06 (0.85, 1.32)	0.620
18p11.21	rs1893217	*PTPN2*	G	0.18	1.11 (0.82, 1.49)	0.517
18q22.2	rs763361	*CD226*	T	0.49	1.19 (0.95, 1.50)	0.135
19q13.32	rs425105	*PRKD2*	C	0.15	0.92 (0.67, 1.26)	0.601
20p13	rs2281808	*SIRPG*	T	0.31	1.15 (0.91, 1.46)	0.232
21q22.3	rs11203203	*UBASH3A*	A	0.38	1.04 (0.83, 1.30)	0.732
22q12.2	rs5753037	*HORMAD2*	T	0.37	1.17 (0.92, 1.49)	0.202
22q13.1	rs229541	*C1QTNF6*	A	0.43	1.05 (0.84, 1.31)	0.664

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