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Abbreviations: DIPP, Type 1 Diabetes Prediction and Prevention; GADA, Glutamic acid decarboxylase 65 antibody; HLA, Human leukocyte antigen; HR, Hazard ratio; IAA, Insulin autoantibody; IA-2A, Insulinoma antigen-2 antibody; T1D, Type 1 diabetes; ZnT8A, Zinc transporter 8 antibody

Context: Children with initial autoantibodies to either insulin (IAA) or glutamic acid decarboxylase (GADA) differ in peak age of seroconversion and have different type 1 diabetes (T1D) risk gene associations suggesting heterogeneity in the disease process.

Objective: To compare the associations of age at seroconversion, HLA risk and specificity of secondary autoantibodies with the progression of islet autoimmunity between children with either IAA or GADA as their first autoantibody.

Design and Methods: A cohort of 15,253 children with HLA-associated increased risk of T1D participated in a follow-up programme where islet autoantibodies were regularly measured. The median follow-up time was 6.7 years. Spearman´s correlation, Kaplan-Meier survival plots and Cox proportional-hazard models were used for statistical analyses.

Results: Persistent positivity for at least one of the tested autoantibodies was detected in 998 children and 388 of children progressed to clinical T1D. Young age at initial seroconversion was associated with a high probability of expansion of IAA-initiated autoimmunity and progression to clinical diabetes, whereas expansion of GADA-initiated autoimmunity and progression to diabetes were not dependent on initial seroconversion age. The strength of HLA risk affected the progression of both IAA- and GADA-initiated autoimmunity. The simultaneous appearance of two other autoantibodies increased the rate of progression to diabetes compared to that of a single secondary autoantibody among subjects with GADA-initiated autoimmunity but not among those with IAA as the first autoantibody.

Conclusions: Findings emphasize the differences in the course of islet autoimmunity initiated by either IAA or GADA supporting heterogeneity in the pathogenic process.

Young age at initial islet autoantibody seroconversion in childhood associates with a high risk of further progression to T1D in IAA-initiated autoimmunity but not in GADA-initiated autoimmunity. .

Introduction

The development of type 1 diabetes (T1D) is usually preceded by the appearance of autoantibodies that recognize various autoantigens expressed in the pancreatic islets. Autoantibodies targeting insulin (IAA), glutamic acid decarboxylase (GADA) and the IA-2 antigen (IA-2A) are well characterized and represent the most important predictive markers for T1D (1). A marked difference in the T1D risk has been observed in relation to the presence of only one of these autoantibodies compared to the presence of two or more autoantibodies (2-4). Although substantial variation in the individual progression rate does exist, a joint analysis of three prospective follow-up studies suggests that within 15 years after the appearance of multiple autoantibodies, the proportion of people with clinical diabetes is greater than 80% (5).

 Both genetic susceptibility, mainly defined by the HLA class II gene region, and environmental factors, which remain poorly defined, are important in the development of islet autoimmunity and T1D (6). The heterogeneity of the disease process is supported by the findings of follow-up studies started early in infancy. In most children who develop islet autoimmunity, either IAA or GADA is the first autoantibody to appear alone. IAA as the first single autoantibody peaks before the age of 2 years and rapidly becomes rare thereafter, whereas GADA as the first single autoantibody peaks at the age of 4-5 years and continues to appear at a relatively high level throughout childhood. Genetic markers characterizing IAA-initiated autoimmunity include the HLA DR4-DQ8 haplotype and the T1D risk-associated genotype of the *INS* gene, whereas the GADA-initiated disease process is related to the presence of HLA-DR3-DQ2 and risk markers in the *ERBB3/IKZF4* and *BACH2* gene regions (7-10).

 In the current analysis, we aimed to compare associations of various factors, such as age at seroconversion, HLA risk group and order of appearance of secondary autoantibodies, known to affect the expansion of islet autoimmunity and progression to T1D (3,11-13) between children who had developed IAA as the first single autoantibody and children who had developed GADA as the first single autoantibody. The roles of age at initial seroconversion, genetic susceptibility contributed by the HLA class II genotype and the specificity of the secondary islet autoantibodies in the disease process were separately analysed in children with either IAA or GADA as the initial autoantibody.

Materials and Methods

Participants and serum samples

The DIPP study is an ongoing follow-up study in Finland that has recruited newborn infants with increased HLA-associated genetic risk for T1D since November 1994 (7). The present analysis is based on information collected from 15,253 children followed from a few months of age up to 15 years of age until May 2017 (Table 1). Peripheral blood samples were collected either at 3-month intervals until the age of 24 months and at 6-month intervals thereafter (one participating centre) or at 3, 6, 12, 18 and 24 months and annually thereafter (two participating centres). After initial seroconversion to positivity for islet autoantibodies, the follow-up was continued at 3-month intervals. The diagnosis of T1D was confirmed based on World Health Organization criteria (14).

Islet autoantibodies

In the beginning of the study, classical islet cell antibodies (ICA) defined by immunofluorescence were used for screening, and if the child was found to be positive for ICA, all serum samples collected from this child were also analysed for IAA, GADA and IA-

2A. All sera collected from children enrolled since the beginning of 2003 have been tested systematically for these three biochemical autoantibodies. Additionally, all sera from a cohort of the first 1004 enrolled children who were born between November 1994 and July 1997 were later analyzed for these three biochemical autoantibodies. The methods used in the analyses have been described previously in detail (3). The disease sensitivities and specificities of the assays in our laboratory according to the 2002-2015 DASP/IASP workshops were 44-50% and 96-99% for IAA, 76-92% and 94-99% for GADA, and 64-76% and 97-100% for IA-2A, respectively. Persistent islet autoantibodies were defined by detection in two consecutive samples.

HLA genotyping

HLA genotyping for selection of the follow-up cohort was performed from cord blood and was originally performed using sequence-specific oligonucleotide probes specific for the HLA DQB1*02, DQB1*03:01, DQB1*03:02 and DQB1*06:02/3 alleles. Infants with either DQB1*02/DQB1*03:02 or DQB1*03:02/x (x≠DQB1*02, DQB1*03:01, DQB1*06:02/3) were defined as eligible for the prospective follow-up study. Genotyping was further developed during the study period, and the eligibility criteria were modified to obtain improved specificity as described previously (7).

All study subjects who had developed islet autoantibodies were genotyped using full-house HLA DR/DQ genotyping whenever a DNA sample was available (968/998). This genotyping has been described in detail earlier (15). In short, the common DR-DQ haplotypes were defined based on DQB1 typing expanded by typing of DQA1 and DRB1 alleles needed to deduce the DR-DQ haplotype present and associated risk for T1D. The DR-DQ haplotypes were sorted according to their odds ratio (OR) values based on the comparison of their frequencies in 2991 Finnish children with T1D and affected family based artificial controls formed by the haplotypes, which were not transmitted to the diabetic child in the families. HLA-DRB1*04:01/2/5-DQA1*03-DQB1*03:02 haplotypes were defined to be associated with a strong susceptibility (S), DRB*04:04-DQA1*03-DQB1*03:02 and (DR3)-DQA1*05-DQB1*02 with a weaker susceptibility (s) whereas (DR15)-DQB1*06:01/2, (DR14)-DQB1*05:03 and (DR7)-DQA1*02:01-DQB1*03:03 were defined as strongly protective (P) and (DR11/12/13)-DQA1*05-DQB1*03:01, DRB1*04:03- DQA1*03-DQB1*03:02 and (DR13)-DQB1*06:03 as weakly protective (p) haplotypes. Haplotypes defined as neutral (N) included (DR1/10)-DQB1*05:01, (DR13)-DQB1*06:04/9, (DR16)-DQB1*05:02, (DR4)-DQA1*03-DQB1*03:01, (DR7)-DQA1*02:01-DQB1*02, (DR9)-DQA1*03-DQB1*03:03, (DR8)-DQB1*04. The DR antigens within parentheses were deduced based on fixed combinations within Northern European populations.

Genotypes formed by these haplotypes could be classified into six risk groups with non-overlapping 95% confidence intervals in OR values. Combinations of DR3 and DR4 positive (S/s or s/s) risk haplotypes were associated with the highest risk (group 5), other S/s than DR3/DR4 combinations, S/S, s/s and S/N with a moderately increased risk (group 4). S/N and s/N associated with a slightly increased risk (group 3). N/N, S/P, s/P and s/p combinations were classified as neutral (group 2), p/N as slightly decreased risk (group 1) and further P/N, p/p, P/p, and P/P as strongly decreased risk (group 0).

The more detailed genotyping revealed that a small group of the subjects in the follow-up cohort in fact carried genotypes associated with neutral or even decreased risk of type 1 diabetes. This conclusion is mainly based on the detection of the DRB1*04:03 allele when performing DR4 subtyping of DQB1*03:02-positive subjects and identification of the strongly protective but rare (DR7)-DQA1*02:01-DQB1*03:03 and (DR14)-DQB1*05:03 haplotypes in subjects eligible based on the presence of the DQB1*03:02/x genotype before September 2004.

Statistical analyses

Kaplan-Meier survival plots and Cox proportional hazards models were applied to study and compare clinical T1D and autoantibody development survival functions in different age- and HLA class II genotype groups, as well as IAA- and GADA-initiated models. Participants developing persistent biochemical autoantibodies were divided into four age quartiles based on the age at the first positive sample: Q1 (0-1.48), Q2 (1.48-2.98), Q3 (2.98-5.09) and Q4 (5.09-14.2). For survival analyses of HLA-associated genetic risk, the children were organized into six HLA risk groups from 0 to 5. Because of the small numbers in the neutral or protective genotype groups (0-2) these were combined. The differences between clinical T1D and autoantibody development hazard functions were assessed by the log-rank test. The correlations between age at initial seroconversion and HLA class II genotypes were calculated using the non-parametric Spearman´s test. The statistical significance level was set at 0.05 for all two-sided tests. Kaplan-Meier survival plots and Cox proportional-hazard models were studied using R version 3.4.3 (https://www.R-project.org/) and the "survival" package (https://CRAN.R-project.org/package=survival).

Results

Features of the studied population

The characteristics of the children studied in the follow-up cohort are shown in Table 1. A total of 15,253 children with at least three follow-up samples were included in the study, but the analyses were performed only among children who seroconverted to positivity for islet autoantibodies. Of the total of 998 (402 girls) children who tested positive for at least one persistent biochemical autoantibody, 545 (227 girls) developed at least two autoantibodies, 445 (187 girls) developed three persistent islet autoantibodies and 388 (173 girls) developed T1D. In most cases (754/998, 75.5%), a single autoantibody was detected in the first autoantibody-positive sample. IAA and GADA were the two most common first autoantibodies, 341 (34.2%) children had IAA and 352 (35.3%) GADA as the first autoantibody, whereas IA-2A was observed as the first single autoantibody at seroconversion in only 61 (6.1%) cases. Analyses comparing the groups with different primary autoantibodies were thus performed only for those with either IAA- or GADA-initiated islet autoimmunity.

Effect of age at the initiation of islet autoimmunity on the subsequent appearance of autoantibodies and progression to diabetes

An association between young age at initial seroconversion and rapid expansion of autoimmunity was observed in the group with IAA as the first single autoantibody ($p = 5.1 \times 10^{-11}$, global p-value from the log-rank test). The initial autoantibody seroconversion in the youngest age quartile was associated with a 9.69-fold increased hazard of developing multiple autoantibodies compared to Q4. The hazards of secondary autoantibody development were increased by factors of 6.59 and 6.74 for Q2 and Q3, respectively. Young age at initial seroconversion was also associated with rapid progression to clinical T1D in children with IAA-initiated autoimmunity (*global p* = 5.2×10^{-8}). The initial autoantibody seroconversion in the youngest age quartile was linked to a 7.38-fold higher rate of clinical T1D than that in the oldest age quartile. The hazard ratios of T1D increased by factors of 4.67 and 3.14 for the Q2 and Q3 quartiles, respectively, when compared to the oldest age quartile (Fig. 1).

Only a marginal effect of age at initial seroconversion was observed in children with GADA as the first autoantibody (*global p* = 0.042). The youngest age quartile actually developed fewer multiple autoantibodies than the other groups. No age effect on progression to clinical diabetes was observed (*global p* = 0.410) (Fig. 1).

Effect of HLA class II genotypes on the subsequent appearance of autoantibodies and progression to type 1 diabetes

The expansion of autoimmunity from a single primary autoantibody to the appearance of secondary autoantibodies and progression to T1D was strongly associated with the HLA risk group in both children with IAA-initiated autoimmunity (*global p* = 9.0×10^{-6} for the development of secondary autoantibodies and *global p* = 7.4×10^{-6} for progression to T1D) and children with GADA-initiated autoimmunity (*global p* = 0.0001 for the development of secondary autoantibodies and *global p* = 0.002 for progression to T1D). The hazard ratios for the development of secondary autoantibodies in different HLA genetic risk groups were similar in both IAA- and GADA-initiated models, ranging from the maximum values of 8.65 (IAA-initiated model) to 6.81 (GADA-initiated model) in risk group 5.

Correlation between age at initial seroconversion and HLA class II genotypes

Although both the age at the appearance of the first autoantibody and HLA-associated risk had a strong effect on expansion of autoimmunity and progression to diabetes in children with IAA-initiated autoimmunity, only a weak correlation was detected between these two parameters (Spearman´s ρ = -0.12, *p* = 0.033, Fig. 2).

Progression from secondary autoantibodies to type 1 diabetes

When progression to type 1 diabetes was analysed after the development of secondary islet autoantibodies, progression was slightly faster in children with IAA as the first autoantibody than in those with GADA-initiated autoimmunity (Fig. 3, *global p* = 0.021).

When the effect of the specificities of secondary autoantibodies was tested among those with IAA as the first autoantibody, IA-2A predicted more rapid development of diabetes than GADA (HR= 2.06 with a *p*-value 0.0037), but the further effect of the combination of IA-2A and GADA appearing together was not significant compared to that associated with IA-2A alone as the second autoantibody (HR= 1.56 with a *p*-value 0.0947) (Fig. 4a). The groups with GADA as the first autoantibody and IAA or IA-2A as the second autoantibodies did not differ in their progression rates, but when these antibodies appeared together, progression rate to diabetes was strongly enhanced over that of IAA (HR= 5.37 with a *p*-value 0.0001) alone as the secondary autoantibody (Fig. 4b).

Discussion

Our study revealed important differences in the autoimmune process classified by the specificity of the first single islet autoantibody. The well-established association between young age at the appearance of autoantibodies and rapid progression of the autoimmune process (11,12,16) was observed only in IAA-initiated autoimmunity. The age at initial seroconversion did not influence the subsequent disease course when GADA was the first single autoantibody to appear. This finding may indicate that possible environmental effects that trigger and promote IAA-initiated disease are present mainly during early life, whereas more continuously operative factors are relevant for islet autoimmunity initiated by GADA. This finding is also in accordance with the major early peak in the appearance of IAA as the first autoantibody and the later age peak and continuous appearance of GADA as the first autoantibody through childhood and into adulthood (7,9,10). These differences may also be related to the maturation and development of the immune system in early childhood. Age-associated differences have also been reported in autoantigen-induced cytokine patterns of peripheral blood lymphocytes and lymphocyte populations involved in insulitis (17).

HLA class II region is the major genetic determinant of T1D, and the HLA-DR/DQ genotypes have been used as the basis of genetic screening in multiple prospective follow-up studies, including the DIPP study, where the effect of class II HLA risk grading on the development of islet autoantibodies has been well documented (15,18). The HLA class II

genotype had a very similar effect on the expansion of autoimmunity in children with both IAA- and GADA-initiated autoimmunity. HLA risk group-dependent expansion of islet autoimmunity took place mainly during the first two years after initial seroconversion.

The effect of age on the progression from single to multiple autoantibodies in subjects positive for either IAA or GADA was also recently compared in the TrialNet study, with the conclusion that in relatives with IAA, the spread of autoimmunity was largely limited to early childhood, whereas immune responses initially directed to GADA often also expanded during later age (19). The results are thus congruent, but the studies differ strongly in the age distribution of single autoantibody-positive subjects, with a mean age of 17.6 years in TrialNet and 3.0 years in the DIPP study, and in the relative proportions of subjects with IAA or GADA as the first autoantibody; the studies thus complement each other in this respect. The large differences in the spread of IAA-initiated autoimmunity among the youngest children were well defined in the DIPP study, whereas differences appearing in early adulthood in GADA-initiated autoimmunity were well represented in the TrialNet series.

The original observation of various age peaks in the appearance of IAA and GADA-initiated islet autoimmunity was reported in the DIPP study (7) and has been confirmed in the BABYDIAB and TEDDY studies (9,10). In the current analysis from the DIPP study we also report the different effect of seroconversion age on the progression rate to type 1 diabetes in IAA- and GADA-initiated autoimmunity. This represents novel information and has not been reported earlier in studies, which have followed up children from birth.

The major impact of secondary islet autoantibodies on the probability of T1D progression was evident to a similar extent in children with IAA- and GADA-initiated islet autoimmunity. However, in children with IAA-initiated autoimmunity, IA-2A, as a secondary autoantibody, was associated with slightly faster progression than observed for the GADA-initiated process. The combination of GADA and IA-2A as secondary autoantibodies did not increase the risk of progression compared to that of IA-2A alone in IAA-initiated autoimmunity, but interestingly, the combination of IAA and IA-2A radically increased the progression rate in children with GADA-initiated autoimmunity compared to the appearance of IAA or IA-2A alone as a secondary autoantibody. This observation concerning the importance of additional IA-2A compared to the IAA and GADA combination in children with GADA-initiated autoimmunity is in accordance with the findings from the Belgian Diabetes Registry, in which the majority of the study subjects had GADA as the first autoantibody detected (20).

Our results provide a new method for estimating disease risk in children who have been observed from young age and among whom the initial islet autoantibody has been identified. We now know that if IAA is the first autoantibody to appear, the risk of progression to diabetes is high in the youngest age groups, but we can conclude that the risk decreases rapidly if secondary autoantibodies have not developed within a few years because the probability of their appearance decreases thereafter. However, in children with GADA as the first autoantibody, the age at initial seroconversion does not affect the probability of progression to T1D although also in these subjects, secondary autoantibodies usually appear within a few years after the initial seroconversion. After the development of secondary autoantibodies, the further progression rate can be estimated to a certain extent based on the specificity of these autoantibodies. The level of HLA class II genotype-associated risk affects the disease risk caused by the expansion of autoimmunity in children with both IAA-initiated and GADA-initiated autoimmunity.

This study has some limitations. We had to limit our main comparison to the two main groups, those with either IAA or GADA as the first single autoantibody, since the number of participants with IA-2A as their first autoantibody was too small for reasonable comparisons although the DIPP birth cohort is the largest group of children with increased

genetic risk of type 1 diabetes ever recruited to a follow-up study starting from birth and the number of children with persistent seroconversion for single or multiple islet autoantibodies is also highest reported.

The criteria used in HLA class II typing to determine eligibility for the DIPP study cover only approximately 60% of genotypes found in children diagnosed with T1D in Finland. A considerable proportion of genotypes are thus not included in the DIPP follow-up cohort, and DR3-DQ2-positive subjects with a disease risk are especially underrepresented. In the DIPP study, autoantibody screening was mainly based on ICA until 2003. Therefore, some subjects positive for IAA only were apparently not recognized. Additionally, the visit intervals were longer after 2 years of age, which hampered the identification of the first islet autoantibody in older children, although this parameter could be defined in the majority of the children. Not all of the factors affecting the progression rate to T1D were included in the analyses, e.g. the presence of first-degree relatives with T1D, autoantibodies specific for the zinc transporter 8 molecule, the levels of islet autoantibodies and their affinities, and data from class I HLA alleles and polymorphisms in some non-HLA genes associated with T1D (8,21-24).

These findings should therefore be further expanded by analyzing also children with a lower HLA-inferred risk and subjects developing autoantibodies at an older age. Also, studies on the autoantibody levels and affinities as well as studies on the role of ZnT8 autoantibodies might bring valuable additional information. In the Belgian Diabetes Register study, ZnT8A increased the progression rate over IAA and GADA combination similarly to IA-2A (20) but due to lacking ZnT8A data we were not able to confirm this. Information on the presence of first-degree relatives with T1D (FDR) might also be useful, although their number among newborn children recruited is rather small. In the TEDDY follow-up cohort having a father or sibling with T1D was associated with higher risk of developing islet autoantibodies or T1D, the effect was similar in the groups of either IAA or GADA as the first autoantibody. Having a diabetic sibling was, however, increased only in children with IAA and not in those with GADA as the first autoantibody but the numbers of children with T1D siblings was very small: only 17 among 252 children with IAA and six in 226 children with GADA as the first autoantibody (10). During 2004-2010 when newborn infants were recruited also to the TEDDY study the proportion of FDR positive children was systematically registered in the DIPP centers and was 4.7% in children eligible for either TEDDY or DIPP.

An apparent target of future prevention trials is to stop islet autoimmunity in its early phase. When establishing eligibility criteria and case-control matching for such interventions, it is essential to take into account factors that are known to affect disease progression. Similarly, it is important to include this knowledge in analyses aimed at identifying new environmental or other factors that affect the progression of islet autoimmunity and development of clinical T1D.

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Disclosure summary:
The authors declare that there is no duality of interest associated with this manuscript.

Data Availability
The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Fig. 1 Effect of age at initial seroconversion on spreading of islet autoimmunity from the first single autoantibody to multiple autoantibodies in children with either IAA (a) or GADA (b) as the first autoantibody, and the effect on the progression from initial seroconversion to clinical diabetes in children with either IAA (c) or GADA (d) as the first autoantibody. Number of observations, number of events, the restricted mean survival, hazard ratio and its 95% confidence interval with the log-rank test p-values are presented.

Fig. 2 Correlation between age at seroconversion for IAA as the first autoantibody and HLA class II risk grade. Spearman´s ρ = -0.12, *p* = 0.033.

Fig. 3 Type 1 diabetes-free survival after the appearance of the second autoantibody, in IAA- and GADA-initiated models. Number of observations, number of events, the restricted mean survival, hazard ratio and its 95% confidence interval with the log-rank test p-values are presented.

Fig. 4 Specificity of secondary autoantibodies and progression to clinical diabetes when (a) IAA is the first autoantibody. The second autoantibodies after IAA are either GADA, IA-2A, or IA-2A and GADA (IA-2A:GADA). (b) Specificity of secondary autoantibodies and progression to clinical diabetes when GADA is the first autoantibody. The second autoantibodies after GADA are either IAA, IA-2A, or IAA and IA2A (IAA:IA2A). Number of observations, number of events, the restricted mean survival, hazard ratio and its 95% confidence interval with the log-rank test p-values are presented.
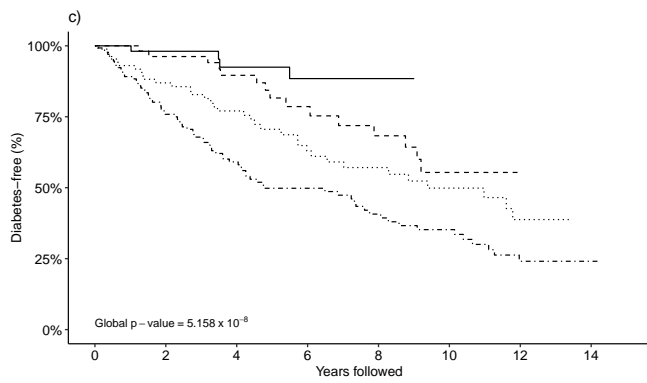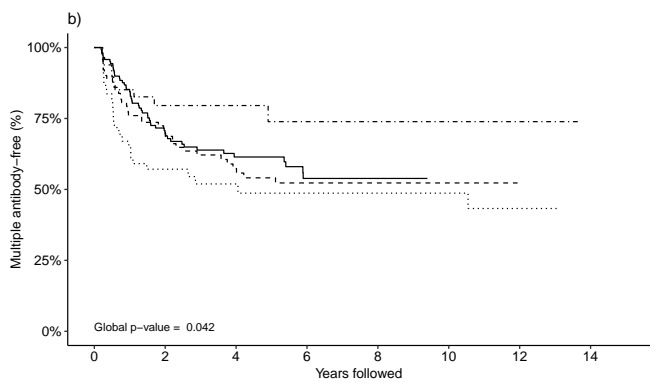
Table 1. Description of the study subjects

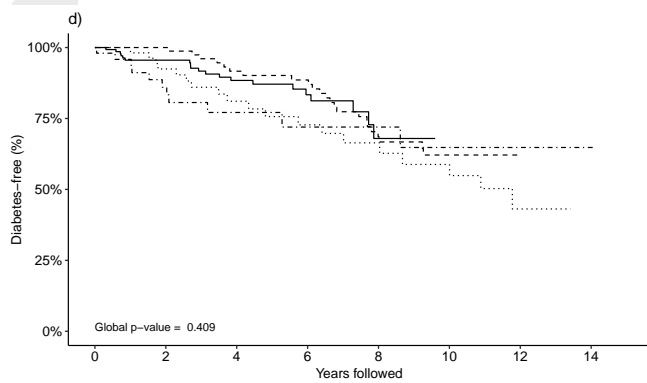| Characteristic	Value
Total number of children in follow-up	15,253
Girls	6,965 (45.7%)
Median follow-up time, years (interquartile range)	6.7 (2.75-12.75)
Developed autoantibodies (aab)	
at least one aab / Girls	998 / 402 (40.3%)
at least two aabs / Girls	545 / 227 (41.7%)
at least three aabs / Girls	445 / 187 (42%)
IAA as the first aab	341 (34.2%)
IA-2A as the first aab	61 (6.1%)
GADA as the first aab	352 (35.3%)
Multiple aabs at initial seroconversion / Girls	244 / 99 (40.6%)
Median age at initial aab seroconversion (interquartile range)	3.0 (1.48-5.1)
Median follow-up time after initial aab seroconversion, years (range)	3.7 (0.0-14.2)
HLA-DR/DQ genotype-associated risk	
High risk (5)	243 (25.1%)
Moderately increased risk (4)	503 (52.0%)
Slightly increased risk (3)	184 (19.0%)
Neutral (2)	27 (2.8%)
Slightly decreased risk (1)	8 (0.8%)
Strongly decreased risk (0)	3 (0.3%)
Incomplete typing	30
Together	998
Developed type 1 diabetes after aab seroconversion	388
Girls	173 (44.6%)
Median age at diagnosis, years (interquartile range)	6.0 (3.47-9.66)

a)

Multiple antibody–free (%)

Global p – value = 5.148×10^{-11}
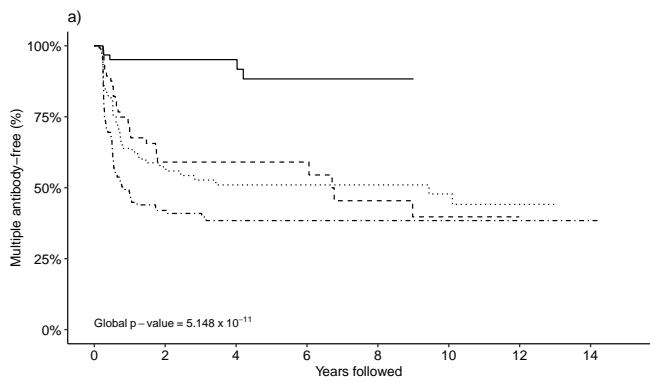
Age group	records	events	*rmean	HR	95% CI	P – value
4th quartile	63	5	11.320	1		
3rd quartile	57	26	6.687	6.741	2.58–17.60	9.77e–05
2nd quartile	87	41	6.602	6.594	2.60–16.73	7.17e–05
1st quartile	134	75	5.181	9.691	3.91–24.00	9.19e–07

b)

Multiple antibody–free (%)

Global p–value = 0.042

Age group	records	events	*rmean	HR	95% CI	P – value
4th quartile	143	50	7.686	1		
3rd quartile	102	40	7.352	1.2	0.79–1.83	0.396
2nd quartile	57	27	6.504	1.742	1.08–2.81	0.023
1st quartile	50	10	9.652	0.699	0.35–1.39	0.305

c)

Diabetes–free (%)

Global p – value = 5.158×10^{-8}

Age group	records	events	*rmean	HR	95% CI	P – value
4th quartile	63	4	11.669	1		
3rd quartile	57	15	9.738	3.137	1.04–9.47	0.043
2nd quartile	87	35	8.348	4.665	1.65–13.18	0.004
1st quartile	134	74	6.573	7.384	2.69–20.26	1.03e–04

d)

Diabetes–free (%)

Global p–value = 0.409

Age group	records	events	*rmean	HR	95% CI	P – value
4th quartile	143	20	10.285	1		
3rd quartile	102	23	10.266	1.04	0.56–1.93	0.901
2nd quartile	57	19	9.136	1.586	0.83–3.02	0.161
1st quartile	50	11	9.476	2.017	0.94–4.31	0.070

Years followed

$R = -0.12$, $p = 0.033$

Global p−value = 0.021
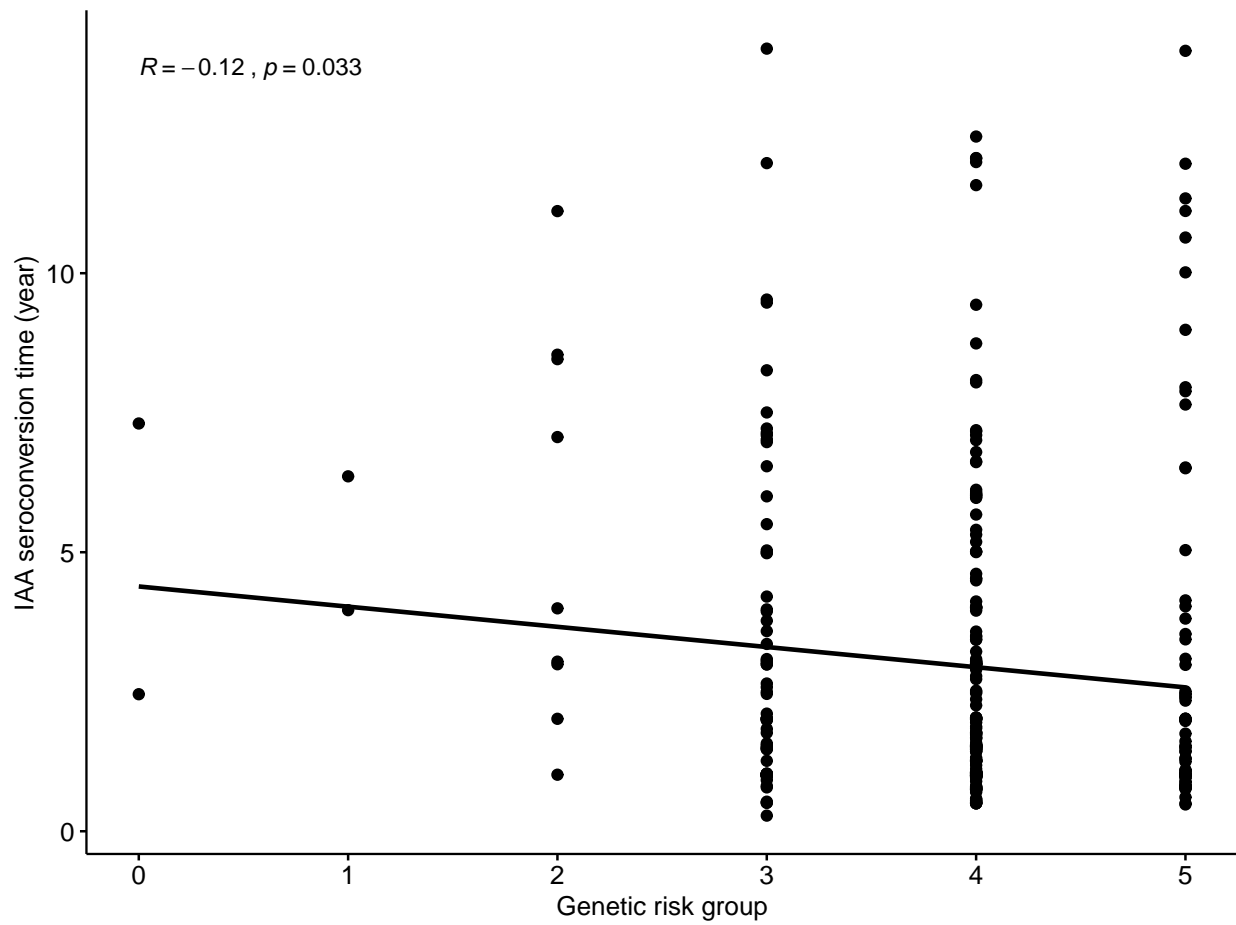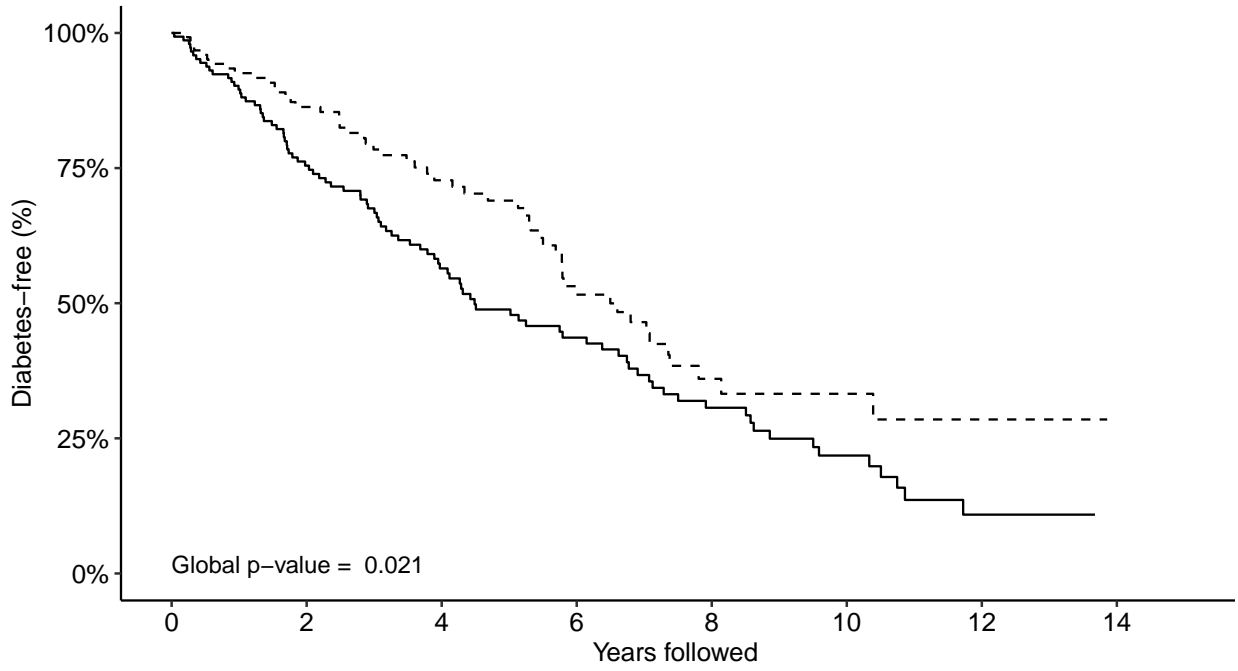
Initial aab.	records	events	*rmean	HR	95% CI	P − value
—— IAA	146	92	5.840	1		
- - GADA	126	54	7.431	0.637	0.45−0.90	0.009

a)
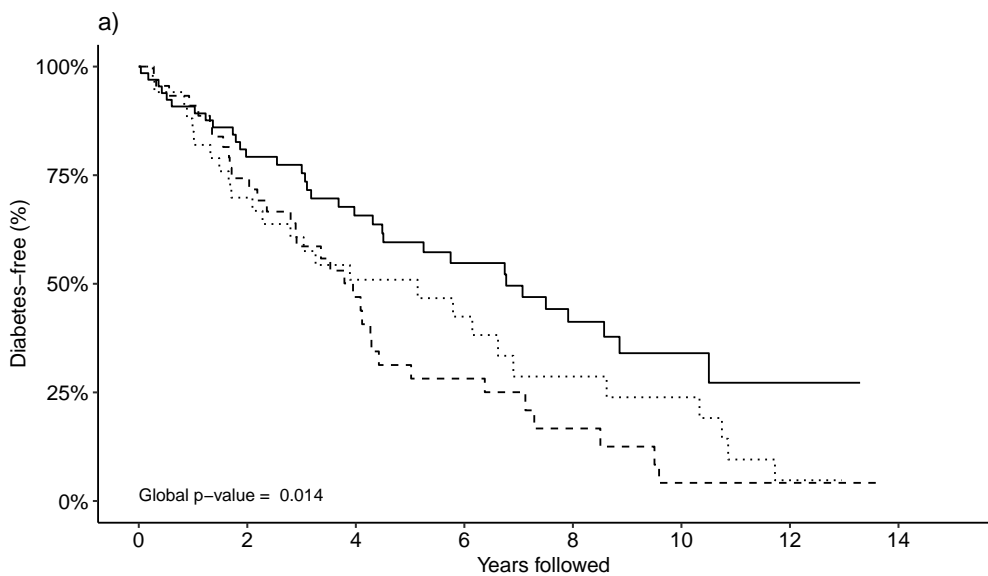
2nd aab.	records	events	*rmean	HR	95% CI	P–value
GADA	66	33	7.128	1		
IA–2A	46	33	4.448	2.062	1.26–3.36	0.004
IA–2A:GADA	34	26	5.369	1.555	0.93–2.61	0.095

b)
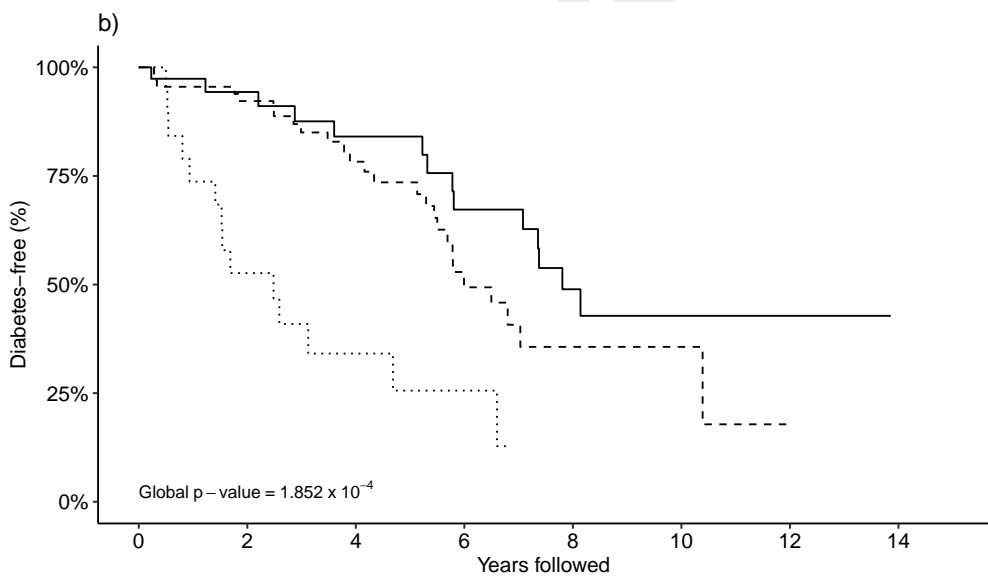
2nd aab.	records	events	*rmean	HR	95% CI	P–value
IAA	38	14	8.238	1		
IA–2A	68	26	6.988	1.506	0.75–3.02	0.249
IAA:IA–2A	20	14	3.779	5.367	2.28–12.61	1.15e–04