Twenty-Year Progression Rate to Clinical Onset According to Autoantibody Profile, Age, and *HLA-DQ* Genotype in a Registry-Based Group of Children and Adults With a First-Degree Relative With Type 1 Diabetes

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OBJECTIVE

We investigated whether islet autoantibody profile, *HLA-DQ* genotype, and age influenced a 20-year progression to diabetes from first autoantibody positivity (autoAb$^+$) in first-degree relatives of patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Persistently islet autoAb$^+$ siblings and offspring (n = 462) under 40 years of age were followed by the Belgian Diabetes Registry. AutoAbs against insulin (IAA), GAD (GADA), IA-2 antigen (IA-2A), and zinc transporter 8 (ZnT8A) were determined by radiobinding assay.

RESULTS

The 20-year progression rate of multiple-autoAb$^+$ relatives (n = 194) was higher than that for single-autoAb$^+$ participants (n = 268) (88% vs. 54%; P < 0.001). Relatives positive for IAA and GADA (n = 54) progressed more slowly than double-autoAb$^+$ individuals carrying IA-2A and/or ZnT8A (n = 38; P = 0.001). In multiple-autoAb$^+$ relatives, Cox regression analysis identified the presence of IA-2A or ZnT8A as the only independent predictors of more rapid progression to diabetes (P < 0.001); in single-autoAb$^+$ relatives, it identified younger age (P < 0.001), *HLA-DQ2/DQ8* genotype (P < 0.001), and IAA (P = 0.028) as independent predictors of seroconversion to multiple positivity for autoAbs. In time-dependent Cox regression, younger age (P = 0.042), *HLA-DQ2/DQ8* genotype (P = 0.009), and the development of additional autoAbs (P = 0.012) were associated with more rapid progression to diabetes.

CONCLUSIONS

In single-autoAb$^+$ relatives, the time to multiple-autoAb positivity increases with age and the absence of IAA and *HLA-DQ2/DQ8* genotype. The majority of multiple-autoAb$^+$ individuals progress to diabetes within 20 years; this occurs more rapidly in the presence of IA-2A or ZnT8A, regardless of age, *HLA-DQ* genotype, and number of autoAbs. These data may help to refine the risk stratification of presymptomatic type 1 diabetes.

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**A complete list of the Members of the Belgian Diabetes Registry can be found in the Supplementary Data online.*

Type 1 diabetes is a chronic autoimmune disease with an incidence peak around puberty (1,2). Observational studies from births in Germany, Finland, and Colorado have followed children at familial or *HLA-DQ/DR* genotype–inferred disease risk for the development of autoantibodies (autoAbs) against insulin (IAA), GAD65 (GADA), and IA-2 antigen (IA-2A) and established that >90% of multiple-autoAb$^+$ individuals progress to clinical onset within 20 years from seroconversion, with the first autoAb appearing most often before 5 years of age (3). Reports that also included adults have, however, indicated that an important fraction of seroconversions to autoAb positivity occurs after 10 years of age (4) and that the disease can become clinically overt at any age, with development of the disease occurring in adulthood in the majority of patients (2,5–7). There is growing consensus that immune interventions in patients with type 1 diabetes should concentrate on the presymptomatic disease phase (8–10). Relatively nonspecific interventions, such as anti-CD3 therapy, may have to include adults first, to evaluate safety and efficacy, before enrolling children (10). Consequently, there is a need to compare criteria for disease staging (11) in adults and children who are at risk.

Since 1989 the Belgian Diabetes Registry (BDR) has recruited first-degree relatives who are <40 years of age and monitored their immunogenetic characteristics (IAA, GADA, IA-2A, zinc transporter 8 autoAbs [ZnT8A], and *HLA-DQ* genotype) in relation to clinical outcome without preselection according to initial autoAb status or genotype (10). We previously reported (12,13) that the presence of IA-2A or ZnT8A plus at least one other diabetes autoAb type conferred a high and age-independent risk of progression to diabetes within 5–10 years in first-degree relatives. Here, we investigated the influence of autoAb profile (number and type) and age at first autoAb positivity on progression to type 1 diabetes in an extended group of siblings and offspring, who were followed over a longer observation period than before (12,13). In addition, we evaluated the impact of changes in autoAb profile during follow-up on long-term diabetes risk and the influence of *HLA-DQ* genotype on the development of multiple autoAbs and diabetes.

RESEARCH DESIGN AND METHODS

Participants

Between March 1989 and December 2015, the BDR consecutively recruited siblings or offspring (under 40 years of age at study entry) of type 1 probands with diabetes according to previously defined criteria (2). The probands are considered representative of the Belgian population of patients with type 1 diabetes (2,6). After obtaining written informed consent from each relative or their parents, a short questionnaire with demographic, familial, and personal information was completed at each visit, and blood samples were taken at study entry and, as a rule, yearly thereafter. Only relatives with two or more contacts during the follow-up period (7,029 individuals), with the last contact being at diagnosis in the case of progression to diabetes, were included in this study. The median intervals between successive visits ranged between 11 and 13 months for the various groups of relatives studied. Diabetes was diagnosed according to the American Diabetes Association criteria (14).

The study was conducted in accordance with the guidelines in the Declaration of Helsinki, as revised in 2013 (accessed on 18 May 2017; https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/), and approved by the ethics committees of the BDR and the participating university hospitals. Random blood samples were collected for sera and buffy coats, and aliquots were stored at −80°C until analyzed for diabetes-associated autoAbs and *HLA-DQ* genotype, respectively, as described previously (12). All relatives were followed regardless of their autoAb status and *HLA-DQ* genotype. Relatives were not prescreened for islet cell cytoplasmic autoAbs (ICA), nor were ICA results analyzed in the current study. AutoAb positivity was defined as being persistent if the next sample was also positive for at least one autoAb regardless of the type. During follow-up, development of diabetes was ascertained through repeated contacts of the BDR with Belgian endocrinologists and pediatricians, self-reporting through yearly questionnaires, and a link with the BDR patient database, where newly diagnosed patients <40 years of age are registered. Follow-up ended at the time of the last blood sampling or, in the case of progression to diabetes, at clinical onset. BMI was expressed as an SD score (BMI-SDS) by comparison with an age- and sex-matched cohort (15).

Analytical Methods

The presence of IAA, GADA, IA-2A, and ZnT8A was determined by liquid-phase radiobinding assay (12), and that of *HLA-DQ* polymorphisms was determined by allele-specific oligonucleotide genotyping (16), as described previously. cDNAs for the preparation of radioligands by in vitro transcription translation were gifts from Å. Lernmark (when at University of Washington, Seattle, WA) for full-length 65-kDa glutamate decarboxylase, M. Christie (King's College School of Medicine and Dentistry, London, UK) for the intracellular portion of IA-2, and J.C. Hutton (Barbara Davis Center for Childhood Diabetes, Aurora, CO) for the dimeric hybrid ZnT8A construct generated by the fusion of CR and CW (zinc transporter-8 carboxy-terminal constructs carrying, respectively, Arg325 and Trp325). ZnT8A were determined in all relatives positive for IAA, GADA, and/or IA-2A but in only a fraction of participants who tested negative for the other three autoAbs (n = 546; 8.7%), in view of the reported low prevalence of solitary ZnT8A positivity (4,12,17).

Statistical Analysis

Statistical differences between groups were assessed by χ^2 test, with Yates correction or Fisher exact test for categorical variables and Mann-Whitney U test for continuous data. Kaplan-Meier analysis was used to estimate diabetes-free survival. The survival curves were compared using the log-rank test. Follow-up for diabetes-free survival started at the time of the first autoAb$^+$ sample and ended at the last contact with the relative or at clinical onset, whichever came first. When assessing multiple-autoAb positivity as the end point, follow-up ended at the time of the first multiple-autoAb$^+$ sample. The persistence of multiple-autoAb positivity was defined as being still positive for at least two autoAbs regardless of the type at the next sampling time. The following age strata were considered: 0–9, 10–19, and 20–39 years of age. The time to diabetes or to seroconversion to multiple-autoAb positivity was assessed by Cox proportional hazards regression analysis, or, in case later development of additional autoAbs was taken into account, by time-dependent Cox regression analysis. The baseline variables listed in

Supplementary Table 1 were first entered into a univariate Cox regression analysis model for predicting the time to diabetes (with age as continuous variable) and those with a univariate P value <0.05 were then entered in a multivariate model. Two-tailed statistical tests were performed using SPSS for Windows version 24.0 (IBM SPSS Statistics, Chicago, IL). GraphPad Prism version 5.00 for Windows (GraphPad, San Diego, CA) was used for the figures.

RESULTS

Overall Progression to Diabetes

Between March 1989 and December 2015 the BDR enrolled 7,029 siblings or offspring (age range 0–39 years; 49% female) of patients with type 1 diabetes (see flowchart in Supplementary Fig. 1). Four hundred sixty-two relatives (45% female) were identified as being persistently positive for IAA, GADA, IA-2A, and/or ZnT8A: 299 were autoAb$^+$ at first sampling, and 163 seroconverted to autoAb positivity during follow-up. In total, diabetes developed in 171 (37%) persistently autoAb$^+$ relatives (40% female) during an overall median follow-up time of 59 months (interquartile range [IQR] 28–102 months) (129 initially autoAb$^+$ relatives and 42 seroconverters after a median follow-up time since first autoAb positivity of 63 months [IQR 29–109 months] and 54 months [IQR 25–88 months], respectively). There were 6,756 persistently autoAb$^-$ or transiently autoAb$^+$ participants, among whom diabetes developed in 10 (median follow-up time 86 months [IQR 73–121 months]; 15-year progression 0.3% [95% CI 0.1–0.5%]).

In the group of persistently autoAb$^+$ relatives, the overall progression rate of individuals who were autoAb$^+$ at first sampling was higher than that for participants who were followed from the time of seroconversion (Supplementary Fig. 2*A*) ($P = 0.025$). However, both groups did not differ in the time to onset of diabetes when compared after stratification according to age (data not shown). Neither did they differ in the time from first multiple-autoAb positivity to onset of diabetes (Supplementary Fig. 2*B*). Therefore, both groups were considered together for further analysis. The overall progression (median) rates of all autoAb$^+$ first-degree relatives were 54% (95% CI 47–60%) after 15 years and 70% (95% CI 55–85%) after 20 years (Supplementary Fig. 2*C*). The characteristics at first autoAb positivity of relatives whose conditions progressed to diabetes and those whose conditions did not (yet) progress during follow-up are shown in Supplementary Table 1.

Progression Rate According to AutoAb Profile at Baseline

Multiple-autoAb$^+$ relatives ($n = 194$) progressed significantly faster to clinical onset than relatives who were positive for only one autoAb at baseline ($n = 268$) (progression after 15 years: 79% [95% CI 72–87%] vs. 30% [95% CI 22–39%]; $P < 0.001$; progression after 20 years: 88% [95% CI 80–95%] vs. 54% [95% CI 26–87%]; $P < 0.001$) (Fig. 1*A*). In multiple-autoAb$^+$ relatives, there was no overall significant difference in progression rate according to increasing numbers of autoAb specificities present ($P = 0.104$) (data not shown). The presence of IA-2A or ZnT8A plus at least one additional autoAb type among the four specificities tested ($n = 139$) was associated with a higher progression rate than in the case of positivity for IAA and GADA alone ($n = 54$, $P = 0.001$) (Fig. 1*B*). In the case of positivity for IA-2A or ZnT8A, the progression rate was not influenced by the total number of other autoAbs present (Fig. 1*B*); neither did it depend on IA-2A or ZnT8A levels (Supplementary Fig. 3*A* and *B*) or on whether only IA-2A, only ZnT8A, or both were present (Supplementary Fig. 3*C*). The progression rate of relatives who were double positive for IAA and GADA was significantly higher than that of single-autoAb$^+$ relatives ($n = 268$, $P < 0.001$) (Fig. 1*B*).

In initially single-autoAb$^+$ relatives, GADA was the most prevalent autoAb (68% vs. 23% for IAA and 9% for IA-2A or ZnT8A), but the progression rate did not vary according to autoAb type (Supplementary Fig. 3*D*). It did, however, increase with higher levels (stratified according to tertiles) for GADA ($P < 0.001$) and IA-2A ($P = 0.017$), but not for IAA ($P = 0.643$) (data not shown). At baseline, IAA levels were inversely correlated and GADA levels were positively correlated with age ($P = 0.001$ and $P = 0.002$, respectively), in line with previous findings at the diagnosis of diabetes (4,6,17); levels of IA-2A and ZnT8A showed no significant correlation with age (data not shown).

Progression Rate According to *HLA-DQ* Genotype

At baseline, the presence of *HLA-DQ2* was not significantly associated with positivity for IAA or ZnT8A, but the susceptibility haplotype was more prevalent in GADA$^+$ than in GADA$^-$ relatives (54% vs. 38%; $P = 0.004$); it tended to be less prevalent in the presence of IA-2A than in its absence (41% vs. 53%; $P = 0.016$) (data not shown). In contrast, *HLA-DQ8* was strongly associated with the development of positivity for IA-2A and ZnT8A (IA-2A$^+$ vs. IA-2A$^-$: 82% vs. 12%; ZnT8A$^+$ vs. ZnT8A$^-$: 74% vs. 26%; $P < 0.001$ for both) but not with the development of positivity for GADA or IAA (data not shown). IA-2A$^+$ or ZnT8A$^+$ relatives presenting with positivity for at least one other autoAb carried *HLA-DQ8* (both *HLA-DQ2/DQ8* and *HLA-DQ8/*non-*DQ2* genotypes) more often than those presenting with positivity only for IAA and GADA (78% vs. 56%; $P = 0.002$) or for a single autoAb (78% vs. 48%; $P = 0.001$); in contrast, the three groups did not differ in frequency of *HLA-DQ2* (50%, 59%, and 45%, respectively; $P = 0.218$) (data not shown). In relatives who were single autoAb$^+$ (any type) or double autoAb$^+$ for IAA and GADA at baseline, the progression rate to diabetes was strongly dependent on the *HLA-DQ* genotype (Fig. 1*C*) ($P = 0.001$), being highest in carriers of the *HLA-DQ2/DQ8* genotype. In contrast, in multiple-autoAb$^+$ relatives in whom IA-2A or ZnT8A had already developed, the progression rate was no longer significantly different according to *HLA-DQ* genotype (Fig. 1*D*) ($P = 0.214$), although there was still a nonsignificant trend to more rapid progression in carriers of *HLA-DQ2/DQ8* genotype ($P = 0.074$ vs. all other genotypes) (data not shown).

Progression Rate According to Changes in AutoAb Profile

Initially, single-autoAb$^+$ relatives in whom positivity for additional autoAb types developed during follow-up ($n = 71$) progressed significantly faster to diabetes than those who remained persistently positive for a single autoAb ($n = 197$) (Fig. 2*A*); likewise, initially autoAb$^+$ individuals who became positive for IA-2A or ZnT8A plus at least one other autoAb during follow-up ($n = 75$) displayed a higher progression rate than relatives who never did acquire this profile ($n = 246$) (Fig. 2*B*). When considering the entire group of

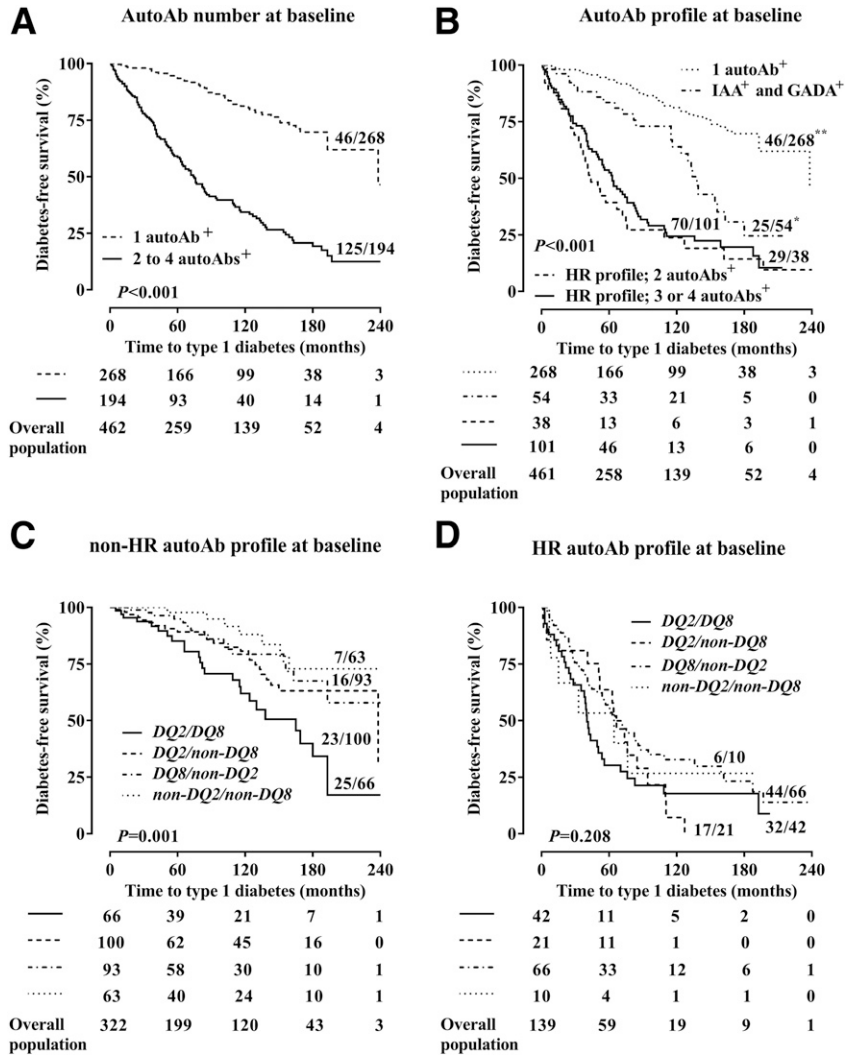

Figure 1—Diabetes-free survival in persistently autoAb$^+$ relatives stratified according to the number of autoAbs at baseline in the whole group (*A*), autoAb type and the number of autoAbs at baseline in the whole group (*B*), *HLA-DQ* genotype in the absence of the high-risk (HR) autoAb profile (HR = IA-2A$^+$ or ZnT8A$^+$ plus at least one other autoAb$^+$) at baseline (*C*), and *HLA-DQ* genotype in the presence of the high-risk autoAb profile at baseline (*D*). Log-rank test in panel *B*: *P = 0.001 vs. both groups with a high-risk autoAb profile (with n = 2 or n = 3 or 4 autoAbs); **$P < 0.001$ vs. IAA$^+$ and GADA$^+$ group. Because of a missing ZnT8A value at baseline, one relative (positive for IAA and GADA but not for IA-2A) was excluded from analysis in panels *B* and *D* because of an uncertain initial risk profile.

relatives (n = 462), the loss of an autoAb marker during follow-up—in most instances IAA (n = 44) or GADA (n = 31)—was associated with an overall decrease in progression rate (P = 0.016) (data not shown); this delay in progression was significant only for the loss of IAA (Fig. 2*C*) (P = 0.014), but not for the loss of any of the other specificities (Fig. 2*D*) (P = 0.307). In particular, diabetes had not developed in any of the 19 single-IAA$^+$ relatives who became IAA$^-$ during follow-up at their last visit (data not shown).

Progression Rate According to Age

The long-term rate of progression to diabetes decreased significantly with age at first autoAb positivity in the group of relatives who were positive for a single autoAb type or were double positive for IAA and GADA (Fig. 3*A*) but was age independent in carriers of IA-2A or ZnT8A presenting with at least one other autoAb (Fig. 3*B*). Likewise, progression to diabetes of single-autoAb$^+$ relatives, but not of multiple-autoAb$^+$ relatives, decreased with age (data not shown).

Multivariate Analysis

In the entire group of autoAb$^+$ relatives (n = 462), the time to diabetes onset was shorter in the case of the presence of IA-2A or ZnT8A plus at least one other autoAb ($P < 0.001$), in the presence of multiple autoAbs (P = 0.001), and in carriers of the *HLA-DQ2/DQ8* genotype (P = 0.014); time to diabetes onset was longer with older age at baseline (P = 0.004) and in offspring of a mother with diabetes (P = 0.007) (Table 1, Model 1). The same exercise was repeated in the group with multiple-autoAb positivity (Table 1, Model 2). The presence of the high-risk autoAb profile was the only significant predictor of more rapid progression to diabetes in this setting ($P < 0.001$).

In initially single-autoAb$^+$ relatives (n = 268), Cox regression confirmed younger age ($P < 0.001$), the presence of *HLA-DQ2/DQ8* genotype ($P < 0.001$), and positivity for IAA (P = 0.028) as independent predictors of seroconversion to multiple-autoAb positivity (Supplementary Table 2, Model 1). A time-dependent Cox regression model for the prediction of diabetes identified seroconversion to multiple autoAbs as an independent risk factor (P = 0.012), in addition to younger age (P = 0.042) and *HLA-DQ2/DQ8* carriership (P = 0.009) (Supplementary Table 2, Model 2). The loss of positivity for IAA during follow-up was not identified as an independent risk factor in single-autoAb$^+$ relatives, but it reached significance when performing time-dependent Cox analysis in the entire group of relatives (P = 0.002) (data not shown).

CONCLUSIONS

By including persistently autoAb$^+$ relatives over a large age range and following them for a longer time than in our previous work (12,13), this study confirms that, in line with earlier findings in children followed from birth (3), the majority of multiple-autoAb$^+$ individuals will progress to the symptomatic disease phase within 20 years and extends this observation to adolescents and young adults. It confirms that multiple-autoAb positivity signals a high risk of progression to clinical onset of diabetes (3,11), which occurs more rapidly in the presence of IA-2A or ZnT8A regardless of age, *HLA-DQ* genotype, the number of additional autoAbs present, and the type of relationship to the proband (12,13). In single-autoAb$^+$ relatives, younger age, the presence of *HLA-DQ2/DQ8* genotype, and IAA positivity are associated with a higher propensity to seroconvert to multiple autoAbs. When they reach that stage, they acquire the risk associated with this new profile. Conversely, the loss of IAA positivity

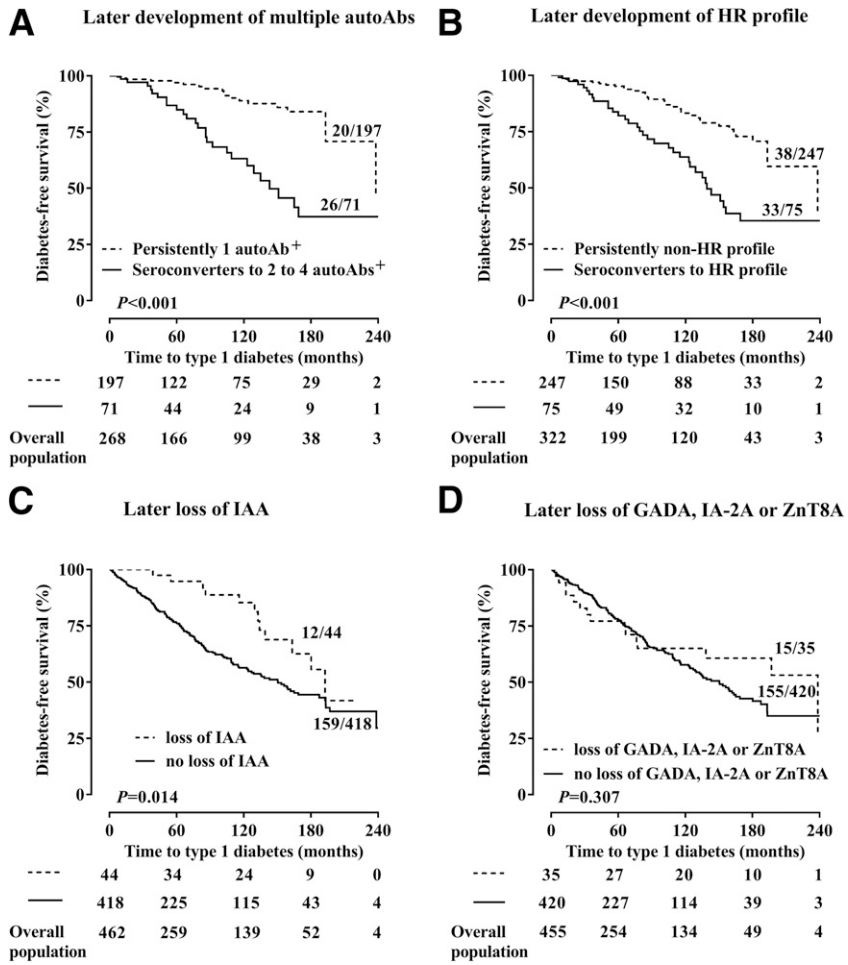

Figure 2—Diabetes-free survival in persistently autoAb$^+$ relatives stratified according to the following changes in autoAb profile during follow-up: later development of at least one additional autoAb in initially single-autoAb$^+$ relatives (*A*); later development of the high-risk (HR) autoAb profile (HR = IA-2A$^+$ or ZnT8A$^+$ plus at least one other autoAb$^+$) in relatives without that profile at baseline (*B*); later loss of initial IAA positivity in the whole group of relatives (*C*); and later loss of initial positivity for GADA, IA-2A, or ZnT8A in the whole group of relatives (*D*). Because of a missing ZnT8A value at baseline, one relative (positive for IAA and GADA but not for IA-2A) was excluded from analysis in panel *B* because of an uncertain initial risk profile. Subjects who lost positivity for IAA together with that for another antibody (n = 7) were not included in panel *D*. Only one of these seven relatives progressed to diabetes.

during follow-up is associated with a lower risk.

The strengths of our study include the recruitment of participants among family members of a representative registry-based group of type 1 diabetes patients (2), the broad age range, and the lack of preselection according to *HLA-DQ* genotype or ICA positivity. The measurement of ZnT8A, which was shown to improve the prediction of the 5-year risk of type 1 diabetes (11–13,18–20), also adds value to long-term risk assessment based on positivity for IAA, GADA, and IA-2A (3). With two exceptions, the ZnT8A assay could be performed in all samples positive for one or more other autoAbs. Our conclusions on the nearly complete 20-year progression to diabetes in multiple-autoAb$^+$ relatives are limited by the low number of individuals followed for 20 years and the fact that not all relatives were followed from the time of seroconversion to autoAb positivity. However, when stratified according to single- or multiple-autoAb positivity, seroconverters to autoAb positivity and initially autoAb$^+$ relatives did not differ in progression rate.

Our data confirm results obtained during a shorter follow-up period of smaller groups that, regardless of the presence of two, three, or four autoAb types, relatives positive for IA-2A and/or ZnT8A at baseline progress more rapidly than those initially positive only for IAA and GADA, which is consistent with observations that autoAb specificities develop sequentially, as a rule, with IA-2A and ZnT8A generally appearing closest to clinical onset (4,21). Relatives who are double positive for IAA and GADA progress more rapidly to disease onset than those who are single positive for an autoAb. In line with recent reports (22,23), the latter were most often GADA$^+$. Their risk of progression to diabetes was independent of the autoAb type present but increased with higher levels (tertiles) of GADA or IA-2A. Using time-dependent Cox regression analysis, we found that the development of positivity for additional autoAbs was an independent determinant of progression to diabetes in single-autoAb$^+$ relatives, which is in agreement with previous reports (22–24) that seroconversion to multiple-autoAb positivity conferred a risk level similar to that for initially multiple-autoAb$^+$ individuals. Such seroconversion occurred more often with younger age; the presence of *HLA-DQ2/DQ8* genotype, similar to a previous study (22); and positivity for IAA, a finding that is at variance with the same study (22). The loss of positivity for IAA—but not for GADA, IA-2A, or ZnT8A—during follow-up tended to delay the progression to diabetes, which is in agreement with a recent report (25) and is consistent with the proposed role of (pro)insulin autoimmunity in directing disease progression in many patients (25). In persistently autoAb$^-$ or transiently autoAb$^+$ relatives, the long-term risk of diabetes was $<$1%, which is in line with earlier studies (4,11).

Our data on the associations among autoAbs and genetic markers are in line with the previously reported (17,26) clustering at disease onset between GADA positivity and *HLA-DQ2/DR3*, on the one hand, and of IA-2A and *HLA-DQ8/DR4*, on the other hand. The association of ZnT8A with *HLA-DQ8*, but not *DQ2*, is somewhat at variance with the association of ZnT8A with both haplotypes in recent-onset diabetes (17). The fact that the prevalence of *HLA-DQ8*, but not of *HLA-DQ2*, was graded according to our autoAb-inferred risk stratification (highest in relatives with positivity for multiple autoAbs carrying IA-2A and/or ZnT8A, intermediate if only positive for IAA and GADA, and lowest if single autoAb$^+$) is a novel finding. Our

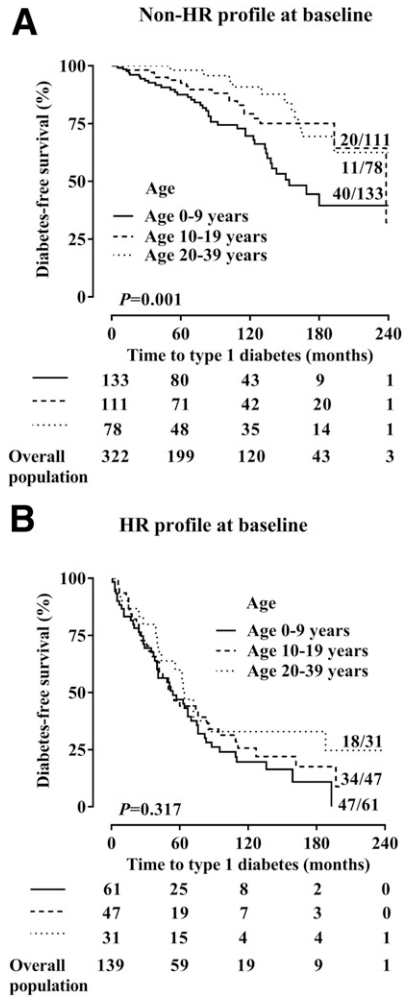

Figure 3—Diabetes-free survival in persistently autoAb$^+$ relatives stratified according to age (0–9, 10–19, and 20–39 years) in the absence (*A*) or presence (*B*) of high-risk (HR) autoAb profile (HR = IA-2A$^+$ or ZnT8A$^+$ plus at least one other autoAb$^+$). Because of a missing ZnT8A value at baseline, one relative (positive for IAA and GADA, but not for IA-2A) was excluded from analysis in panel *B* because of an uncertain initial risk profile.

multivariate analyses showed that *HLA-DQ* susceptibility haplotypes, in particular when combined into the *HLA-DQ2/DQ8* genotype, are important drivers of the development of positivity for multiple autoAbs; however, once that stage is reached, *HLA* class II genotype groups seem to contribute little to the progression from advanced autoimmunity to clinical onset of disease, which is in line with the results of the study by Ilonen et al. (27).

It is now important to carefully investigate further how the development of a higher autoAb-inferred risk profile relates to critical switches in the underlying disease process and to changes in β-cell function, insulin action, and glycemic control (28,29). In addition, the relation to *HLA* class I and non-*HLA* alleles, which have been reported to influence the progression rate (30,31), needs to be explored further. This may allow the identification of episodes during which a large decrease in β-cell function is most likely to occur; such episodes could be investigated further by measurements of markers of β-cell death (32). Of note, careful analysis of pancreas tissue from autoAb$^+$ organ donors revealed that insulitis could be observed only in the presence of multiple-autoAb positivity at an asymptomatic disease stage where β-cell mass appeared still to be unaffected (33). The proposed autoAb-based risk stratification could also be used to investigate episodes of β-cell loss after diagnosis.

Overall, childhood-onset type 1 diabetes is characterized by a more aggressive disease course, more severe clinical presentation, positivity for more autoAbs, and a higher genetic risk load (6,34). However, regardless of age the presence of IA-2A or ZnT8A plus at least one other autoAb enables us to identify individuals with a similar subclinical disease course, allowing first, if needed, the enrollment of adults afflicted by a disease process representative of that in children, in terms of intensity and progression rate, in secondary prevention trials before the actual enrollment of children. Additional hormonal/metabolic parameters should then further secure the selection of participants in the same presymptomatic disease phase (11,28,29). Future work should investigate whether our autoAb-based prediction model may be improved

Table 1—Cox proportional hazards regression analysis for prediction of progression to diabetes based on baseline characteristics

	Univariate analysis		Multivariate analysis*	
Covariates	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Model 1: overall study population				
Age	0.96 (0.94–0.98)	**<0.001**	0.97 (0.95–0.99)	**0.004**
Sex, male vs. female (ref.)	1.26 (0.93–1.72)	0.135		NE
BMI-SDS *z* score	1.06 (0.93–1.21)	0.413		NE
HLA-DQ genotype				
DQ2/DQ8	2.09 (1.52–2.88)	**<0.001**	1.51 (1.09–2.09)	**0.014**
DQ8/non-*DQ2*	1.02 (0.75–1.40)	0.904		NE
DQ2/non-*DQ8*	0.78 (0.55–1.11)	0.165		NE
Non-*DQ8*/non-*DQ2*	0.34 (0.22–0.69)	**0.001**	NS	0.158
Relationship with the proband				
Sibling	0.63 (1.20–2.22)	**0.002**	NS	0.527
Offspring mother	0.33 (0.20–0.53)	**<0.001**	0.51 (0.31–0.83)	**0.007**
Offspring father	1.23 (0.88–1.71)	0.228		NE
Number/profile of autoAbs				
Positivity for 2 to 4 autoAbs	5.22 (3.72–7.34)	**<0.001**	2.27 (1.38–3.74)	**0.001**
HR profile	5.03 (3.70–6.83)	**<0.001**	2.38 (1.53–3.72)	**<0.001**
Model 2: relatives with 2 to 4 autoAbs**				
Age	0.98 (0.96–1.01)	0.136		NE
Sex, male vs. female (ref.)	0.99 (0.83–1.19)	0.980		NE
BMI-SDS *z* score	1.00 (0.86–1.16)	0.995		NE
HLA-DQ genotype				
DQ2/DQ8	1.42 (0.98–2.06)	0.065		NE
DQ8/non-*DQ2*	0.73 (0.51–1.04)	0.084		NE
DQ2/non-*DQ8*	1.14 (0.75–1.75)	0.542		NE
Non-*DQ8*/non-*DQ2*	0.75 (0.35–1.61)	0.462		NE
Relationship with the proband				
Sibling	1.30 (0.90–1.87)	0.158		NE
Offspring mother	0.50 (0.27–0.94)	**0.030**		0.059
Offspring father	1.05 (0.71–1.54)	0.813		NE
AutoAb profile				
HR profile	2.23 (1.44–3.45)	**<0.001**	2.23 (1.44–3.45)	**<0.001**

HR, high autoantibody-inferred risk (IA-2A$^+$ or ZnT8A$^+$ plus positivity for one or more other autoAbs); NE, not selected for entry into the multivariate model. *Forward stepwise multiple regression model. **One relative excluded from the analysis because the presence or absence of a high-risk profile at baseline could not be ascertained (missing ZnT8A value). Significant *P* values appear in boldface type.

by including autoAbs against newly identified autoantigens, such as tetraspanin-7 (35), or by replacing radiobinding assays for established markers, in particular IAA and GADA, by novel sensitive nonisotopic assays claiming to recognize high-affinity autoAbs (36,37) or by assays measuring autoAbs against specific autoantigen fragments such as N-terminally truncated GAD65 (38). Positivity for the latter two types of assays has been reported to be associated with a higher rate of progression to diabetes than that observed for conventional IAA or GADA radiobinding assays (36–38). Further improvements may come from analyzing autoAbs against specific autoantigen (neo)epitopes (39,40).

In conclusion, in single-autoAb$^+$ relatives the development of additional immune markers occurs slower with increasing age and the absence of IAA or *HLA-DQ2*/*DQ8* genotype, but once the stage of multiple-autoAb positivity is reached, most individuals progress to the onset of diabetes within 20 years regardless of age, *HLA-DQ* genotype, or the number of autoAbs. Overall progression is more rapid if IA-2A or ZnT8A are present and tends to be delayed in the case of a later loss of positivity for IAA. This autoAb-based risk stratification offers a platform to further characterize and closely follow subgroups in terms of functional β-cell mass, other biomarkers, and glycemic control, both before and after the clinical onset of diabetes. This is ultimately believed to advance our knowledge of the natural disease history and the underlying pathological process and may contribute to the tailoring of selection criteria for participation in prevention trials to the type of intervention and the targeted disease stage.

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