Accelerated Progression to Type 1 Diabetes in the Presence of *HLA-A*24* and *-B*18* Is Restricted to Multiple Islet Autoantibody–Positive Individuals With Distinct *HLA-DQ* and Autoantibody Risk Profiles

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OBJECTIVE

We investigated the effect of HLA class I risk alleles on disease progression in various phases of subclinical islet autoimmunity in first-degree relatives of patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

A registry-based group of siblings/offspring (aged 0–39 years) was monitored from single- to multiple-autoantibody positivity (n = 267) and from multiple-autoantibody positivity to clinical onset (n = 252) according to *HLA-DQ*, *-A*24*, *-B*18*, and *-B*39* status. Genetic markers were determined by PCR sequence-specific oligotyping.

RESULTS

Unlike *HLA-B*18* or *-B*39*, *HLA-A*24* was associated with delayed progression from single- to multiple-autoantibody positivity (P = 0.009) but not to type 1 diabetes. This occurred independently from older age (P < 0.001) and absence of *HLA-DQ2/DQ8* or *-DQ8* (P < 0.001 and P = 0.003, respectively), and only in the presence of GAD autoantibodies. In contrast, *HLA-A*24* was associated with accelerated progression from multiple-autoantibody positivity to clinical onset (P = 0.006), but its effects were restricted to *HLA-DQ8*$^+$ relatives with IA-2 or zinc transporter 8 autoantibodies (P = 0.002). *HLA-B*18,* but not *-B*39,* was also associated with more rapid progression, but only in *HLA-DQ2* carriers with double positivity for GAD and insulin autoantibodies (P = 0.004).

CONCLUSIONS

***HLA-A*24* predisposes to a delayed antigen spreading of humoral autoimmunity, whereas *HLA-A*24* and *-B*18* are associated with accelerated progression of advanced subclinical autoimmunity in distinct risk groups. The relation of these alleles to the underlying disease process requires further investigation. Their typing should be relevant for the preparation and interpretation of observational and interventional studies in asymptomatic type 1 diabetes.**

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The asymptomatic phase of type 1 diabetes is signaled by circulating islet autoantibodies (autoAbs) (1). Its substaging by biomarkers may shed light on the underlying disease process and its conceivable heterogeneity and thus contribute to risk assessment and selection for prevention trials (2,3). The various autoAb specificities tend to develop sequentially, with those directed against insulin (insulin autoantibody [IAA]) or GAD65 (GAD antibody [GADA]) usually appearing first, and those directed against islet antigen 2 (IA-2A) or zinc transporter 8 (ZnT8A) generally arising closer to clinical onset (3–5). We and others have previously reported that progression from single- to multiple-autoAb positivity occurs more rapidly with younger age, presence of the *HLA-DQ2/DQ8* high-risk genotype, or development of IAA as the first autoAb (6–9). In contrast, from the stage of multiple-autoAb positivity onward, progression to clinical onset appears largely independent of age and HLA class II–inferred risk (6,9,10). Rather, the development of multiple autoAbs appears to signal a state of advanced autoimmunity that almost inevitably progresses to clinical onset within the next 20 years, with development of IA-2A or ZnT8A as indicators of an accelerated disease course (3,6,11–14).

Although genome-wide association studies have identified a large number of non-HLA susceptibility loci for type 1 diabetes, *HLA-DQ/DR* genotypes and, to a lesser degree, HLA class I alleles remain major genetic risk determinants of the disease (15–20). Several reports have associated *HLA-A*24, -B*18*, and/or *-B*39* with increased disease risk, but their effects seem largely restricted to carriers of specific HLA class II haplotypes (18,19,21–24). However, the influence of HLA class I risk alleles on the various asymptomatic stages of type 1 diabetes and their relation to other biomarkers is overall less well documented than for HLA class II. The current study therefore assessed the respective effect of *HLA-A*24, -B*18*, and *-B*39* on subsequent stages of islet autoimmunity (i.e., from single- to multiple-autoAb positivity and from multiple-autoAb positivity to clinical onset of type 1 diabetes), considering differences in *HLA-DQ* risk haplotypes, in autoAb profile, and in age. The study was undertaken in a registry-based cohort of Belgian persistently autoAb$^+$ first-degree relatives of patients with type 1 diabetes, consisting of children and adults younger than age 40 years without preselection for *HLA-DQ*–inferred risk or islet cell cytoplasmic autoAbs (ICAs).

RESEARCH DESIGN AND METHODS

Participants

Between March 1989 and December 2015, the Belgian Diabetes Registry (BDR) consecutively recruited siblings and offspring (<40 years of age at study entry) of patients with type 1 diabetes according to previously defined criteria (25). The probands are considered representative of the Belgian patient population (25,26). 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. 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 (n = 7,029), 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 (6). Diabetes was diagnosed according to the American Diabetes Association criteria (27).

The study was conducted in accordance with the guidelines in the Declaration of Helsinki, as revised in 2013 (accessed on 16 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 and genetic markers, respectively. All relatives were monitored regardless of their autoAb status and *HLA-DQ* genotype. Relatives were not prescreened for ICA nor were ICA results analyzed in the current study. Initial autoAb positivity was defined as being persistent if the next sample was also positive for at least one autoAb, regardless of the type. Development of diabetes during follow-up 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 case of progression to diabetes, at clinical onset. BMI was expressed as an SD score (BMI z score) by comparison with an age- and sex-matched healthy control group (6).

Analytical Methods

IAA, GADA, IA-2A, and ZnT8A were determined by liquid-phase radiobinding assay, using the 99th percentile of healthy control subjects as the cutoff (11). ZnT8A could be measured in all but one relative positive for at least one other autoAb, but only in a fraction (8.7%; n = 546) of participants who tested negative for the other three autoAbs (4,11,28). *HLA-DQ*, *-A*, and *-B* alleles were typed by PCR sequence-specific oligonucleotide dot-blot methods, as described previously (22,23,29).

Statistical Analysis

Statistical differences between groups were analyzed with χ^2 or Fisher exact tests for categorical variables and with the Mann-Whitney U test for continuous data. Kaplan-Meier analysis with the log-rank test was used to assess progression from the first autoAb$^+$ sample to multiple-autoAb positivity and from multiple-autoAb positivity to type 1 diabetes. When multiple-autoAb positivity was assessed as the end point, follow-up ended at the time of the first multiple-autoAb$^+$ sample (before or at diagnosis of diabetes at the latest) or at the last single-autoAb$^+$ sample (before or at diagnosis of diabetes at the latest) (6). Follow-up for diabetes-onset started at the time of the first multiple-autoAb$^+$ sample and ended at the last contact with the relative or at clinical onset, whichever came first. The baseline variables listed in Tables 1 and 2 were first entered in a univariate Cox regression analysis model for predicting time to multiple-autoAb positivity or to diabetes, and those with univariate $P < 0.05$ were entered in a multivariate model with respect of the Vittinghof criterion (at least 5–10 events per variable included) (30). Two-tailed statistical tests were performed by SPSS for Windows version 24.0 software (IBM, Armonk, NY), and $P < 0.05$ was considered significant. GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, CA) was used for the figures.

RESULTS

Overall Characteristics and Disease Progression of Participants

Among the 7,029 relatives recruited during the study period, 462 persistently autoAb$^+$ individuals (6.3%) were identified

Table 1—Cox regression analysis in single-autoAb$^+$ relatives (n = 267) for progression to multiple-autoantibody positivity (71 events)

	Univariate		Multivariate			
			Model 1		Model 2	
Independent variable	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
Age (years) (n = 267)	<0.001	0.92 (0.89–0.95)	<0.001	0.91 (0.88–0.94)	<0.001	0.91 (0.88–0.94)
Male/female (n = 142/125)	0.128					
Offspring of parent with diabetes						
Mother (0/1; n = 196/71)†	0.103					
Father (0/1; n = 196/71)†	0.458					
Sibling (0/1; n = 142/125)†	0.391					
BMI z score (n = 267)	0.220					
IAA$^+$ (0/1; n = 200/67)†	0.013	1.85 (1.14–3.00)	0.061			NM‡
GADA$^+$ (0/1; n = 90/177)†	0.013	0.55 (0.35–0.88)	0.097			NM‡
IA-2A$^+$ or ZnT8A$^+$ (0/1; n = 244/23)†	0.707					
*HLA-A*24*$^+$ (0/1; n = 210/57)†	0.020	0.42 (0.20–0.87)	0.009	0.38 (0.18–0.79)	0.529	
*HLA-B*18*$^+$ (0/1; n = 243/24)†	0.468					
*HLA-B*39*$^+$ (0/1; n = 248/19)†	0.964					
HLA-DQ2$^-$/*DQ8*$^-$ (0/1; n = 210/57)†	0.105					
HLA-DQ2$^+$/*DQ8*$^-$ (0/1; n = 185/82)†	0.161					
HLA-DQ2$^-$/*DQ8*$^+$ (0/1; n = 191/76)†	0.664					
HLA-DQ2$^+$/*DQ8*$^+$ (0/1; n = 215/52)†	0.002	2.25 (1.35–3.76)	<0.001	2.84 (1.67–4.83)	<0.001	2.98 (1.75–5.08)
HLA-DQ2$^+$ (0/1; n = 133/134)†	0.315					
HLA-DQ8$^+$ (0/1; n = 139/128)†	0.007	1.92 (1.19–3.09)	0.138			NM‡
*HLA-A*24*$^+$ × GADA$^+$	0.024	0.31 (0.11–0.86)		NM‡	0.009	0.26 (0.09–0.71)
*HLA-A*24*$^+$ × age	0.010	0.91 (0.84–0.98)		NM‡	0.289	
*HLA-A*24*$^+$ × non(*DQ2*$^+$/*DQ8*$^+$)	0.017	0.16 (0.16–0.83)		NM‡	0.593	

HR, hazard ratio. †0/1: no/yes. ‡NM: not included in the multivariate model; no missing data.

(6), of which 461 were typed for *HLA-DQ*, *-A*24*, *-B*18*, and *-B*39* (Supplementary Fig. 1). The median age (interquartile range [IQR]) at the first autoAb$^+$ sample was 11 (6–19) years. The autoAb-inferred risk status could not exactly be determined in 1 of these 461 relatives because of a missing ZnT8A result (6), and this individual was consequently omitted from analyses taking the high-risk autoAb profile (IA-2A or ZnT8A plus at least one other autoAb present) (6,12) as the end point or stratification criterion. *HLA-A*24*, *-B*18*, and *-B*39* were present in 101 (22%), 56 (12%), and 30 (6%) individuals, respectively. Relatives with or without these HLA class I susceptibility alleles did not differ in age, sex, BMI z score, and relationship to the proband or in the prevalence, number, or levels of the four autoAb types tested, except for a tendency toward a lower frequency of ZnT8A positivity (P = 0.074) and lower GADA levels (P = 0.051) in *HLA-A*24* carriers and slightly more multiple-autoAb positivity in the presence of *HLA-B*18* (P = 0.015) (not shown). *HLA-B*39* was significantly more present in *HLA-A*24*$^+$ individuals (14% vs. 4% in absence of *HLA-A*24*; P = 0.001) (not shown), and *HLA-DQ2* was more prevalent in *HLA-B*18*$^+$ relatives (61% vs. 39% in absence of *HLA-B*18*; P = 0.003) (not shown).

During a median (IQR) follow-up time of 60 (24–132) months, 71 of the 267 initially single-autoAb$^+$ relatives developed one or more additional autoAbs (Supplementary Fig. 1). Most events occurred in relatives aged <15 years at baseline (i.e., first autoAb$^+$ sample; 80% vs. 20% aged ≥15 years; P < 0.001). Nineteen initially single-autoAb$^+$ relatives progressed to type 1 diabetes without first developing additional autoAbs. Progression from multiple-autoAb positivity to diabetes could be monitored in 252 individuals with at least one visit after developing the second autoAb (194 multiple-autoAb$^+$ relatives at first sampling and 58 initially single-autoAb$^+$ relatives who seroconverted to multiple-autoAb positivity during follow-up), of whom 147 progressed to clinical onset during a median (IQR) follow-up time of 56 (28–98) months (Supplementary Fig. 1).

Progression From Single- to Multiple-autoAb Positivity

Kaplan-Meier analysis (Supplementary Fig. 2) and multivariate Cox regression analysis (not shown) indicated that progression from single-autoAb positivity to clinical onset was not influenced by *HLA-A*24*, *-B*18*, or *-B*39* status. However, progression from single- to multiple-autoAb positivity was delayed in the presence of *HLA-A*24* but not in the presence of *HLA-B*18* or *-B*39* (Supplementary Fig. 2). Univariate Cox regression analysis (Table 1) confirmed that positivity for *HLA-A*24* (P = 0.020) was associated with less rapid appearance of additional autoAbs, as was also the case for older age (P < 0.001), presence of GADA (P = 0.014), and absence of IAA (P = 0.012) or *HLA-DQ2/DQ8* (P < 0.001) (Table 1). In multivariate analysis, *HLA-A*24* remained an independent predictor of delayed seroconversion to multiple-autoAb positivity (P = 0.009), together with older age and absence of *HLA-DQ2/DQ8* (P < 0.001 for each) (Table 1, model 1). However, the presence of *HLA-A*24* affected mainly initially GADA$^+$ relatives,

Table 2—Cox regression analysis in multiple-autoAb$^+$ relatives (n = 252) for progression to type 1 diabetes (147 events)

	Univariate		Multivariate			
			Model 1		Model 2	
Independent variable	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
Age (years) (n = 252)	0.108					
Male/female (n = 147/105)	0.917					
Offspring of parent with diabetes						
Mother (0/1; n = 216/36)†	0.004	0.43 (0.24–0.76)	0.011	0.48 (0.27–0.84)	0.015	0.49 (0.27–0.87)
Father (0/1; n = 182/70)†	0.421					
Sibling (0/1; n = 106/146)†	0.116					
BMI z score (n = 252)	0.769					
IA-2A$^+$ and/or ZnT8A$^+$ (0/1; n = 112/139)†‡§	<0.001	2.24 (1.58–3.17)	<0.001	2.20 (1.55–3.13)	0.001	1.90 (1.31–2.75)
*HLA-A*24*$^+$ (0/1; n = 202/50)†	0.034	1.52 (1.03–2.25)	0.006	1.73 (1.17–2.57)	0.949	
*HLA-B*18*$^+$ (0/1; n = 213/39)†	0.057					
*HLA-B*39*$^+$ (0/1; n = 236/16)†	0.374					
HLA-DQ2$^-$*/DQ8*$^-$ (0/1; n = 228/24)†	0.216					
HLA-DQ2$^+$*/DQ8*$^-$ (0/1; n = 199/53)†	0.488					
HLA-DQ2$^-$*/DQ8*$^+$ (0/1; n = 151/101)†	0.233					
HLA-DQ2$^+$*/DQ8*$^+$ (0/1; n = 178/74)†	0.128					
HLA-DQ2$^+$ (0/1; n = 125/127)†	0.055					
HLA-DQ8$^+$ (0/1; n = 77/175)†	0.846					
*HLA-A*24*$^+$ × *HLA-DQ8*$^+$	0.010	1.75 (1.15–2.68)		NM‖	0.194	
*HLA-B*18*$^+$ × *HLA-DQ2*$^+$	0.008	1.84 (1.17–2.88)		NM‖	0.003	1.99 (1.26–3.14)
*HLA-A*24*$^+$ × IA-2A$^+$ and/or ZnT8A$^+$‡§	<0.001	2.90 (1.87–4.51)		NM‖	<0.001	2.32 (1.46–3.70)

HR, hazard ratio. †0/1: no/yes. ‡High-risk autoAb profile. §This analysis excluded one multiple-autoAb$^+$ relative because presence or absence of the high-risk autoAb profile at baseline could not be unambiguously determined due to missing result for ZnT8A. ‖NM: Not included in the multivariate model; no missing data (except for §).

as suggested by the significant interaction between both variables in multivariate analysis (Table 1, model 2) and by Kaplan-Meier analysis (Supplementary Fig. 3). There was no significant interaction between *HLA-A*24* and older age or between *HLA-A*24* and absence of *HLA-DQ2/DQ8* (Table 1, model 2).

Progression From Multiple-autoAb Positivity to Clinical Onset of Type 1 Diabetes

In Kaplan-Meier analysis, time from the first multiple-autoAb positivity to diabetes tended overall to be significantly shorter in the presence of *HLA-A*24* (P = 0.033) or *-B*18* (P = 0.055) but not of *HLA-B*39* (P = 0.372) (not shown). The accelerating effect of *HLA-A*24* was restricted to the presence of *HLA-DQ8* (P = 0.008) (Fig. 1*A–D*) and that of *HLA-B*18* to the absence of *HLA-DQ8* (P = 0.018) or presence of *HLA-DQ2* (P = 0.048) (Fig. 1*E–H*). When multiple-autoAb$^+$ relatives were stratified according to the presence or absence of the high-risk autoAb profile (IA-2A or ZnT8A plus at least one other autoAb), the effect of *HLA-A*24* was only significant in relatives with the high-risk profile, in whom 5-year progression was ~80% (P = 0.001) (Fig. 2*A* and *B*). The progression rate in this group was highest in individuals who were double positive for *HLA-DQ8* and *-A*24* compared with relatives carrying all other possible combinations of *HLA-DQ8* and *-A*24* status (P = 0.002) (Fig. 2*C* and Supplementary Fig. 4). In contrast, the accelerating effect of *HLA-B*18* appeared restricted to multiple-autoAb$^+$ relatives without IA-2A and ZnT8 (only double positive for IAA and GADA; P = 0.016) (Fig. 2*D* and *E*), in particular those who were also *HLA-DQ2*$^+$ (P = 0.004) (Fig. 2*F* and Supplementary Fig. 4).

Multivariate Cox regression analysis identified the presence of *HLA-A*24* (P = 0.006) and of the high-risk autoAb profile ($P < 0.001$) as independent risk factors for accelerated progression from multiple-autoAb positivity to clinical onset, while being the offspring of a mother with diabetes increased time to diagnosis (P = 0.011) (Table 2, model 1). There was a significant interaction between *HLA-A*24* and the high-risk autoAb profile ($P < 0.001$) but not between *HLA-A*24* and *HLA-DQ8* (Table 2, model 2). When considered separately, *HLA-B*18* and *-DQ2* did not affect the progression rate to clinical onset, but in multivariate analysis, their interaction was significantly associated with an acceleration (P = 0.003) (Table 2, model 2).

CONCLUSIONS

In this cohort of 461 Belgian children and adults with persistent autoAb positivity, we found that *HLA-A*24* and *-B*18* predispose to accelerated progression of advanced—but not of early—islet autoimmunity. Their respective effect occurs in different subgroups distinguished by *HLA-DQ* and autoAb risk profiles. At variance with these HLA class I risk alleles, *HLA-DQ2/DQ8* predisposes only to more rapid progression from single- to multiple-autoAb positivity. At this early stage of immune activation, *HLA-A*24* is associated with a tendency toward delayed intermolecular epitope spreading of the humoral autoimmune response in single-GADA$^+$ relatives.

Strengths of our study include the long-term recruitment and follow-up of first-degree relatives of registered patients with type 1 diabetes who are representative of the Belgian patient population, the very low amount of missing data, the lack of preselection based on *HLA-DQ* susceptibility haplotypes or ICA positivity, and the broad age range for eligibility, given

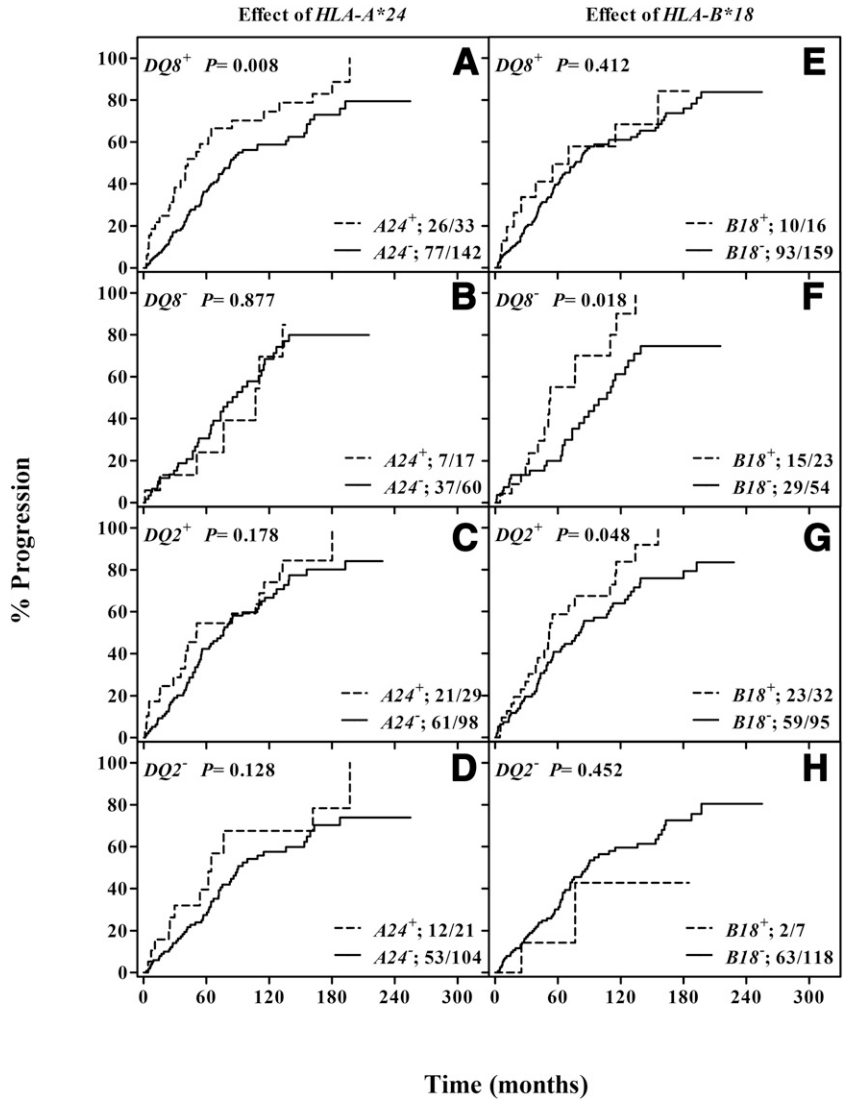

Figure 1—Progression from multiple-autoAb positivity to type 1 diabetes according to the presence (broken line) or absence (full line) of *HLA-A*24* (*A*–*D*) or *HLA-B*18* (*E*–*H*) in first-degree relatives positive for *HLA-DQ8* (*A* and *E*), negative for *HLA-DQ8* (*B* and *F*), positive for *HLA-DQ2* (*C* and *G*), and negative for *HLA-DQ2* (*D* and *H*). In each panel, the number of relatives who are positive or negative for the marker under study is indicated, together with the number of events in each arm.

that most patients develop the disease in adolescence or adulthood (25).

The limited median (IQR) follow-up time of 72 (35–131) months for the whole group constitutes a weakness, indicating that about half of the relatives were monitored for <6 years. However, this did not preclude our ability to unveil strong determinants of accelerated disease progression, particularly when considering appropriate subgroups. Other weaknesses include the absence of extended haplotype analysis and that only a few individuals were monitored from a very young age, at variance with studies such as The Environmental Determinants of Diabetes in the Young (TEDDY) (10).

Most of the relatives in our cohort presented autoAbs at the first sampling and could thus have seroconverted earlier in life. However, after stratification according to initial single- or multiple-autoAb positivity, seroconverters and relatives who were already positive at inclusion did not differ in the progression rate to clinical onset (6). Determining the exact age at seroconversion is useful to further document the natural history of subclinical type 1 diabetes and its mechanistic investigation but is less relevant for the risk assessment of individual relatives and for their possible inclusion in prevention studies (31). Because autoAbs can develop at any age (4) and most patients progress to clinical onset in adolescence or adulthood (25,26), we believe that our results usefully complement those of birth cohort studies (10,13). Indeed, notwithstanding the differences in study protocol between both approaches, our investigations yielded results that were in many aspects similar to findings from birth cohort studies, extending them to a broader age range. For instance, largely in line with the TEDDY study (32), relatives only positive for IAA were younger ($P < 0.001$), leaner ($P = 0.035$), and less often *HLA-DQ2*$^+$ ($P = 0.029$) than relatives presenting with GADA only. Compared with the latter, single-IAA$^+$ positivity tended to be more associated with *HLA-DQ8*/non-*DQ2* ($P = 0.058$) and male sex (58% vs. 52%; not significant) (results not shown). At variance with TEDDY, single-IAA$^+$ and single-GADA$^+$ relatives did not differ in the type of relationship to their proband (32). We and the TEDDY Study Group both found that the *HLA-DQ/DR* genotype did influence progression of early—but not of late (multiple-autoAb positivity)—autoimmune reactivity (6,10,32).

While taking advantage of a large start population, our observations could have been strengthened by larger numbers in particular subgroups, especially the group with *HLA-B*39*, an allele that is less common in the Belgian than in the Finnish population (24). In line with previous work (22,23), the prevalence of *HLA-B*39* was much lower than that of *HLA-A*24* and *-B*18* in the present cohort, which may explain why, at variance with studies in Finland (21), we did not find its association with accelerated disease progression. In addition, *HLA-B*39* subtypes (*B*39:01* and *B*39:06*) are often in linkage disequilibrium with *HLA-A*24:02* (24). This might explain why *HLA-B*39* occurred significantly more often in the presence of *HLA-A*24* in the current study. In *HLA-A*24*$^+$ relatives, however, neither progression to multiple-autoAb positivity nor development of diabetes could be related to the presence or absence of *HLA-B*39* (not shown).

The observation that the presence of *HLA-DQ2/DQ8*, but not of HLA class I susceptibility alleles, is associated with accelerated progression of early-stage islet-specific immune activation is in line with previous reports (6,7,9,10,21,24) and with the decreasing prevalence of *HLA-DQ2/DQ8* with age at diagnosis (26). Our finding of delayed intermolecular epitope spreading,

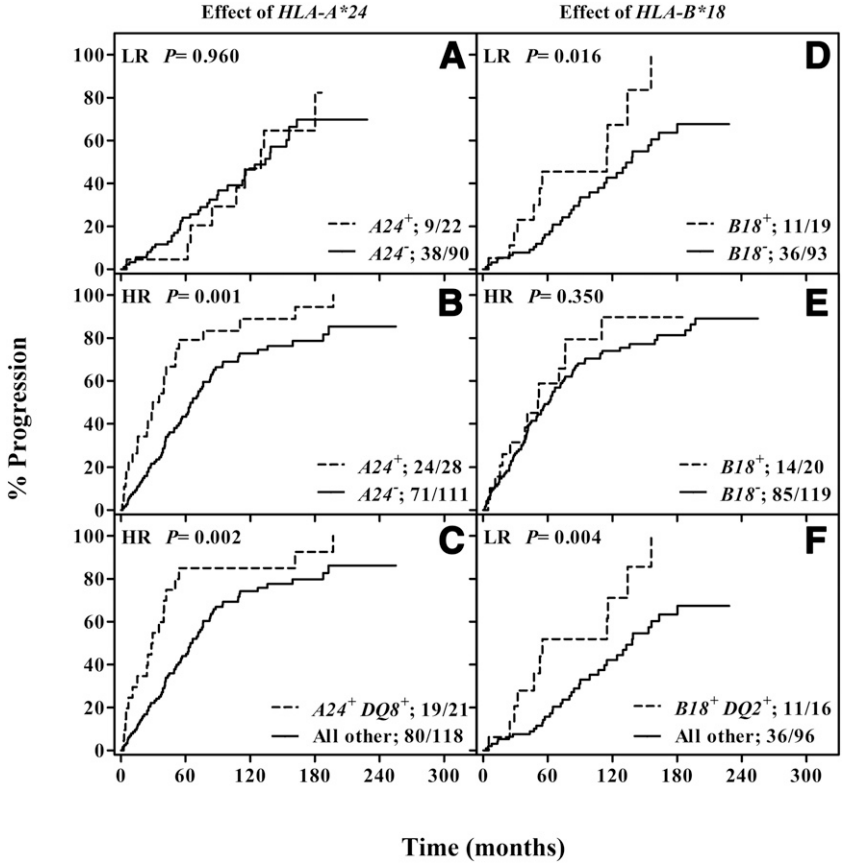

Figure 2—Progression from multiple-autoAb positivity to type 1 diabetes according to the presence (broken line) or absence (full line) of *HLA-A*24* (*A* and *B*) or *HLA-B*18* (*D* and *E*) in first-degree relatives only double positive for IAA and GADA (low-risk [LR]) (*A* and *D*) and those positive for IA-2A and/or ZnT8A (high-risk [HR]) (*B* and *E*). *C*: Progression from positivity for the HR autoAb profile (IA-2A$^+$ and/or ZnT8A$^+$ plus at least one other autoAb) to type 1 diabetes according to the presence (broken line) or absence (full line) of double positivity for *HLA-A*24* and *-DQ8*. *F*: Progression from double positivity for IAA and GADA only (LR profile) to type 1 diabetes according to the presence (broken line) or absence (full line) of double positivity for *HLA-B*18* and *-DQ2*. In each panel, the number of relatives who are positive or negative for the marker under study is indicated, together with the number of events in each arm. This analysis excluded one multiple-autoAb$^+$ relative because autoAb-inferred risk at baseline could not be unambiguously determined due to missing result for ZnT8A.

but not of progression to diabetes, in single-GADA$^+$ relatives carrying *HLA-A*24* is consistent with earlier studies reporting an association between this allele and attenuated humoral responses at the diagnosis of type 1 diabetes or in risk groups (18,33–35). Initial single-GADA positivity has been previously associated with older age at seroconversion and slower spread of islet autoimmunity (7,8,31,35,36). Our results do not allow us to identify a precise mech-anism for the interaction between GADA positivity and *HLA-A*24* in this process. The latter cannot be explained by differences in age because it remained independent of age in multivariate analysis. Moreover, single-GADA$^+$ relatives were overall younger at baseline in the presence of *HLA-A*24* than in its absence (not shown). The reported interaction cannot distinguish whether *HLA-A*24* attenuates spreading of the humoral immune response directly or only indirectly, for example, by causing more rapid β-cell killing leaving less opportunity for developing multiple-autoAb specificities (33,34). Once the stage of multiple-autoAb positivity is reached, however, HLA class I risk alleles are associated with accelerated progression toward clinical onset, which is in line with observations in Finnish risk groups (21,24). At the stage of advanced autoimmunity, the protective effect of being offspring of a mother with type 1 diabetes (37) reached significance in the present analysis.

The fact that the association between HLA class I risk alleles and more rapid progression of advanced islet autoimmunity is restricted to relatives with distinctive autoAb profiles constitutes a new finding. Although our results confirm that the effects of *HLA-A*24* and *-B*18* are largely confined to presence of *HLA-DQ8* and *-DQ2* (or absence of *-DQ8*), respectively (22), these *HLA-DQ* risk haplotypes do not appear to directly influence progression from multiple-autoAb positivity to clinical onset, as indicated by their failure to serve as an independent predictor of symptomatic disease in multivariate Cox regression. Rather, we believe that the *HLA-DQ* haplotypes determine the likelihood of developing distinct autoAb profiles that are indicative of a particular stage of the underlying disease process. In line with this view is our recent observation that the prevalence of *HLA-DQ8* correlated with autoAb-inferred risk, which was higher in multiple-autoAb$^+$ relatives who developed IA-2A or ZnT8A than in those who did not and, even more so, than in single-autoAb$^+$ relatives (6). Depending on the autoAb profile, the underlying disease process may proceed differently in the presence or absence of specific HLA class I risk alleles. It is in this context interesting to note that autoAb$^+$ organ donors without known diabetes exhibited insulitis when at least two autoAb types were present, mostly including IA-2A and/or ZnT8A, while being absent in the others (38–40). Hyperexpression of HLA class I, but not of HLA class II, molecules and predominance of infiltrating CD8$^+$ T cells in insulin-containing islets are distinctive features of type 1 diabetes (40,41).

Whatever the mechanisms of the HLA class I- and II-mediated effect on disease progression, our observations emphasize that the risk factors involved differ according to the stage of asymptomatic type 1 diabetes. They also indicate that the possible effects and interactions of additional genetic and/or environmental/lifestyle determinants should be studied separately in the different stages of subclinical autoimmunity and for each stage after stratification for the autoAb- and HLA-inferred risk profiles described here. In support of this, we (29) and others (42) showed many years ago that insulin region–encoded susceptibility was most pronounced in individuals with lower *HLA-DQ/DR*–inferred risk. More recently, prospective studies such as TEDDY have identified several single nucleotide polymorphisms in non-HLA genes that modulate disease progression, but their influence can vary according to disease stage and primary autoantibody type (32,43,44). The

non-HLA regions of interest comprise diabetes-specific genes and others shared with different autoimmune diseases, but whether and how they interact with HLA class I and II genes needs to be further investigated.

Taken together, our results support the hypothesis that HLA class II molecules on professional antigen-presenting cells drive the initiation of islet autoimmunity, as judged by the development of autoAbs, through facilitating recognition of autoantigens by $CD4^+$ T-helper cells, whereas hyperexpression of HLA class I molecules on islets may promote presentation of relevant peptides to infiltrating $CD8^+$ cytotoxic T cells and subsequent β-cell destruction (9,24,41). In multiple-$autoAb^+$ relatives, accelerated progression was restricted to *HLA-DQ8*$^+$ relatives who had developed IA-2A and/or ZnT8A in the presence of *HLA-A*24* and to relatives carrying *HLA-DQ2* (or lacking *-DQ8*) who were only double positive for IAA and GADA in the presence of *HLA-B*18*. These results warrant further investigation of their relationship to histopathological findings in the pancreas, associated changes in functional β-cell mass, and subsequent development of dysglycemia. They should also be taken into consideration for individual risk assessment of $autoAb^+$ individuals in the perspective of close metabolic follow-up or inclusion in prevention studies as well as for the interpretation of the outcome of such studies.

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