*HLA-A*24* Is an Independent Predictor of 5-Year Progression to Diabetes in Autoantibody-Positive First-Degree Relatives of Type 1 Diabetic Patients

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We investigated whether *HLA-A*24* typing complements screening for *HLA-DQ* and for antibodies (Abs) against insulin, GAD, IA-2 (IA-2A), and zinc transporter-8 (ZnT8A) for prediction of rapid progression to type 1 diabetes (T1D). Persistently Ab^+ siblings/offspring (n = 288; aged 0–39 years) of T1D patients were genotyped for *HLA-DQA1-DQB1* and *HLA-A*24* and monitored for development of diabetes within 5 years of first Ab^+. *HLA-A*24* (P = 0.009), *HLA-DQ2/DQ8* (P = 0.001), and positivity for IA-2A ± ZnT8A ($P < 0.001$) were associated with development of T1D in multivariate analysis. The 5-year risk increased with the number of the above three markers present (n = 0: 6%; n = 1: 18%; n = 2: 46%; n = 3: 100%). Positivity for one or more markers identified a subgroup of 171 (59%) containing 88% of rapid progressors. The combined presence of *HLA-A*24* and IA-$2A^+$ ± $ZnT8A^+$ defined a subgroup of 18 (6%) with an 82% diabetes risk. Among IA-$2A^+$ ± $ZnT8A^+$ relatives, identification of *HLA-A*24* carriers in addition to *HLA-DQ2/DQ8* carriers increased screening sensitivity for relatives at high Ab- and *HLA*-inferred risk (64% progression; P = 0.002). In conclusion, *HLA-A*24* independently predicts rapid progression to T1D in Ab^+ relatives and complements IA-2A, ZnT8A, and *HLA-DQ2/DQ8* for identifying participants in immunointervention trials. ***Diabetes*** **62:1345–1350, 2013**

Autoimmune type 1 diabetes (T1D) results from the T-cell–mediated destruction of pancreatic insulin-producing β-cells, preferentially in individuals carrying HLA class II susceptibility haplotypes (1). Immunointerventions with anti-CD3 antibodies (Abs) (2,3), rituximab (4), and cytotoxic T-lymphocyte antigen-4-immunoglobulin fusion protein (5) transiently preserved β-cell function, preferentially in a patient subgroup with younger age at diagnosis and with relatively preserved residual functional β-cell mass (3), hereby opening perspectives for future trials at the preclinical stage (3,6). To limit numbers needed to treat and the time to reach significant conclusions, such trials require identification of individuals at high risk to develop diabetes short-term (7,8). Abs against IA-2 (IA-2A) and zinc transporter-8 (ZnT8A) generally appear later than Abs against insulin (IAA) or GAD (GADA) in pre-T1D and have been associated with more rapid disease progression in first-degree relatives (FDRs) (9–12).

Genetic factors can further modulate diabetes risk in Ab^+ relatives; for example, the presence of *HLA-DQA1*0501-DQB1*0201(DQ2)/DQA1*0301-DQB1*0302 (DQ8)* identifies a subgroup of IA-$2A^+$ or multiple Ab^+ relatives with impending diabetes (13–15). The HLA class I region has also been shown to contribute to T1D risk (16–18). The presence of *HLA-A*24* has been associated with progression from autoimmunity to T1D (19) and with early and complete β-cell destruction after diagnosis (20). It is, however, still unclear to what extent genotyping for HLA class I can usefully complement screening for Ab- and *HLA-DQ*–inferred risk in identifying relatives with impending diabetes. We investigated in a large representative group of Ab^+ FDRs aged younger than 40 years whether *1*) the presence of *HLA-A*24* affects progression from autoimmunity to T1D independently from other risk markers; *2*) *HLA-A*24* interacts with *HLA-DQ* and/or auto-Ab-inferred risk of diabetes; and *3*) *HLA-A*24* typing can refine the sensitivity or specificity of screening to identify rapid progressors.

RESEARCH DESIGN AND METHODS

Study population. Offspring or siblings of T1D probands were Caucasians (aged 0–39 years) consecutively recruited by the Belgian Diabetes Registry (BDR), as previously described (13). At entry and during yearly follow-up, blood sampling and completion of a short questionnaire were performed after written informed consent. Only relatives with at least two contacts during follow-up, the last being at diagnosis of diabetes in case of progression to clinically overt disease, were included in this study. Blood, serum, and plasma samples were stored as aliquots at −80°C until analyzed. Screening for auto-Abs against insulin (IAA), GAD 65 kDa (GADA), IA-2 (IA-2A), and ZnT8 (ZnT8A) identified 288 persistently positive FDRs (i.e., ≥1 molecular Abs in ≥2 consecutive samples) with available DNA for *HLA-DQ* and *HLA-A*24* typing (flowchart in Supplementary Fig. 1). At baseline (first Ab^+ sample), the median age (interquartile range [IQR]) of FDRs was 12 (6–19) years. Because we focused in the current study on rapid progression to diabetes, follow-up was truncated at 60 months. The median (IQR) follow-up time was 60 (36–60) months (73% of FDRs completed the 5-year follow-up). Diabetes was diagnosed according to the American Diabetes Association criteria (21). Diagnosis was ascertained through repeated contacts with Belgian diabetologists, self-reporting through yearly questionnaires, and a link to the BDR patient database, where newly diagnosed patients are registered.

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

See accompanying commentary, p. 1020.

The study was carried out according to The Helsinki Declaration as revised in 2008 (http://www.wma.net/en/30publications/10policies/b3/, accessed on 18 April 2012), and the protocol was approved by the ethics committees of the BDR and participating university hospitals.

Analytical methods. Diabetes auto-Abs and *HLA-DQ* genotyping were performed as reported previously (9,13,22). *HLA-A*24* typing was performed by the PCR sequence-specific oligonucleotide dot-blot method. Briefly, genomic DNA was amplified by *HLA-A*24* group-specific primers (23), followed by DNA dot-blot hybridization (22) with a panel of three sequence-specific oligonucleotide probes (probe 1: 5′-GAG-GAG-ACA-GGG-AAA-3′; probe 2: 5′-CGC-TCT-TGG-ACC-GCG-3′; and probe 3: 5′-GCG-GCC-CGT-GTG-GCG-3′), and the signals were subsequently detected with a Digoxigenin Luminescent Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany). Our probe set identified all 255 *HLA-A*24* alleles except *HLA-A*240217/0227/0249/08/24/29/31/42/67/77/89/129/145/156191*.

Statistical methods. Statistical differences in proportions between groups were tested by χ^2 with Yates continuity correction for independent samples or by McNemar test for paired samples. For continuous variables, comparisons were performed by Mann-Whitney *U* test. Follow-up of relatives started at the time of the first Ab$^+$ sample and ended at the last contact with the relative or at clinical onset, whichever came first. For the identification of rapid progressors, follow-up was truncated after 5 years. Kaplan-Meier survival analysis and log-rank test were used to compare differences in diabetes-free survival between groups. Cox regression univariate analysis (enter method) was used to select potential predictors ($P < 0.05$) to be tested in a multivariate model (forward stepwise method) for identifying independent predictors of impending diabetes and calculation of their hazard ratio. Statistical analyses were performed two-tailed by EpiInfo 6.0 (USD Inc., Stone Mountain, GA), IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY), or by GraphPad Prism 5 (GraphPad, San Diego, CA) software. Significance was defined as $P < 0.05$ or $P < 0.05/k$ in case of multiple comparisons (Bonferroni adjustment).

RESULTS

***HLA-A*24* as an independent predictor of progression to diabetes.** We identified 288 persistently IAA$^+$, GADA$^+$, IA-2A$^+$ and/or ZnT8A$^+$ relatives at baseline who were tested for the presence or absence of *HLA-DQ2*, *-DQ8* and *-A*24*. Within 5 years after the first Ab$^+$, 50 relatives (17%) developed diabetes after a median (IQR) follow-up time of 29 (12–42) months. Their baseline characteristics are reported in Table 1. Compared with nonprogressors, rapid progressors were younger and more frequently IA-2A$^+$, ZnT8A$^+$, multiple Ab$^+$, and/or *HLA-DQ2/DQ8*$^+$. They tended to be less frequently offspring of a diabetic mother and to carry more often *HLA-DQ2*, *-DQ8*, and *-A*24*.

Cox regression analysis (Table 2) identified *HLA-A*24* as an independent predictor of rapid progression to diabetes in addition to the established high-risk markers IA-2A$^+$, ZnT8A$^+$, and *HLA-DQ2/DQ8*. When positivity for two or more Abs was included in the first model, it also came out as an independent predictor ($P = 0.024$) in addition to *HLA-A*24* ($P = 0.015$), *HLA-DQ2/DQ8* ($P = 0.001$), and IA-2A$^+$ ± ZnT8A$^+$ ($P = 0.006$; data not shown). A second model looking at interactions between independent predictors revealed a significant interaction between the presence of *HLA-A*24* and IA-2A$^+$ ± ZnT8A$^+$ (Table 2). Similar results were obtained when considering 10-year progression to diabetes (Supplementary Table 1). Except for a tendency toward a higher prevalence of GADA$^+$ in carriers of *HLA-DQ2/DQ8* ($P = 0.062$ vs. absence of *HLA-DQ2/DQ8*) or *HLA-A*24* ($P = 0.077$ vs. absence of *HLA-A*24*), Ab frequency did not differ significantly according to *HLA-DQ2/DQ8* and *-A*24* status (data not shown).

Kaplan-Meier survival analysis. Survival analysis confirmed a more rapid progression to diabetes in presence of *HLA-A*24* (Fig. 1*A*), *HLA-DQ2/DQ8* (Fig. 1*B*), and IA-2A ± ZnT8A (Fig. 1*C*), than in their respective absence (Fig. 1*A–C*). Positivity for *HLA-A*24* selectively and significantly increased the 5-year progression rate to diabetes in presence of *HLA-DQ2/DQ8* (Fig. 1*D*) or IA-2A ± ZnT8A (Fig. 1*E*) but not in their absence (Fig. 1*D* and *E*). Similarly, *HLA-DQ2/DQ8* carrier status increased the 5-year diabetes risk more in relatives with IA-2A$^+$ ± ZnT8A$^+$ than in those without (Fig. 1*F*). Table 3 lists the numbers of relatives presenting various combinations of the three markers (IA-2A$^+$ ± ZnT8A$^+$, *HLA-A*24*, *HLA-DQ2/DQ8*) at baseline, their 5-year progression rate, and the fraction of diabetes cases they identify.

The 5-year risk of diabetes and diagnostic sensitivity were higher in IA-2A$^+$ ± ZnT8A$^+$ relatives than when *HLA-DQ2/DQ8* or *HLA-A*24* carrier status alone was examined (Table 3). The 5-year progression rate increased according to the number of independent risk markers (IA-2A$^+$ ± ZnT8A$^+$, *HLA-A*24*, *HLA-DQ2/DQ8*), reaching 100% in presence of all three markers but with 72% of the future patients belonging to the groups with one or two markers (Table 3).

Positivity for at least one of the three high-risk markers identified a subgroup of 171 relatives (57%) harboring 44 of the 50 (88%) rapid progressors (Table 3). Typing for

TABLE 1
Baseline characteristics of Ab$^+$ FDRs** according to 5-year diabetes status

Characteristic	All relatives $n = 288$	Progressors $n = 50$	Nonprogressors $n = 238$	P†
Median (IQR) age at first Ab$^+$ (years)	12 (6–19)	9 (5–13)	12 (7–20)	0.003
Median (IQR) follow-up time (months)	60 (36–60)	29 (12–42)	60 (55–60)	<0.001
Male, *n* (%)	156 (54)	25 (50)	131 (55)	0.621
Offspring, *n* (%)	134 (47)	18 (36)	116 (49)	0.137
Offspring of diabetic mother, *n* (%)	62 (22)	5 (10)	57 (24)	0.046
IAA$^+$, *n* (%)	127 (44)	26 (52)	101 (42)	0.280
GADA$^+$, *n* (%)	224 (78)	41 (82)	183 (77)	0.547
IA-2A$^+$, *n* (%)	79 (27)	30 (60)	49 (21)	<0.001
ZnT8A$^+$, *n* (%)	72 (25)	30 (60)	42 (18)	<0.001
IA-2A$^+$ ± ZnT8A$^+$, *n* (%)	97 (34)	37 (74)	60 (25)	<0.001
≥2 Ab$^+$, *n* (%)	127 (44)	42 (84)	83 (35)	<0.001
HLA-DQ2, *n* (%)	145 (50)	32 (64)	113 (48)	0.049
HLA-DQ8, *n* (%)	174 (60)	39 (78)	135 (57)	0.008
HLA-DQ2/DQ8, *n* (%)	73 (25)	24 (48)	49 (21)	<0.001
*HLA-A*24*, *n* (%)	56 (19)	16 (32)	40 (17)	0.023

**Persistently positive for IAA, GADA, IA-2A, and/or ZnT8A. †Threshold for significance: $P < 0.05/15$ or $P = 0.0033$ (Bonferroni correction).

TABLE 2
Cox regression analysis for 5-year progression to diabetes in 288 Ab^+ FDRs**

Variable	Univariate P†	Multivariate P‡	Hazard ratio (95% CI)
Model 1			
*HLA-A*24*	0.024	0.009	2.2 (1.2–4.1)
HLA-DQ2	0.048	0.524	1.2 (0.5–2.8)
HLA-DQ8	0.007	0.782	1.3 (0.4–4.5)
HLA-DQ2/DQ8	<0.001	0.001	2.6 (1.5–4.6)
Age§	0.026	0.073	0.6 (0.3–1.1)
Offspring of diabetic mother	0.042	0.649	0.8 (0.3–2.3)
IA-$2A^+$ ± $ZnT8A^+$	<0.001	<0.001	6.7 (3.6–12.7)
Model 2			
*HLA-A*24*	0.024	0.647	0.7 (0.2–3.2)
HLA-DQ2/DQ8	<0.001	0.001	2.5 (1.4–4.4)
IA-$2A^+$ ± $ZnT8A^+$	<0.001	<0.001	4.8 (2.4–9.5)
*HLA-A*24* x *HLA-DQ2/DQ8*	<0.001	0.934	1.1 (0.3–3.9)
*HLA-A*24* x (IA-$2A^+$ ± $ZnT8A^+$)	<0.001	0.001	3.1 (1.6–6.1)
HLA-DQ2/DQ8 x (IA-$2A^+$ ± $ZnT8A^+$)	<0.001	0.589	0.7 (0.2–2.5)

**Ab^+: persistently positive for IAA, GADA, IA-2A, and/or ZnT8A. †Enter method. ‡Forward stepwise method for variables with $P <$ 0.05 in univariate analysis. §Categorical variable (median age, 12 years; age ≤12 years = 0; age >12 years = 1).

*HLA-A*24* in addition to *HLA-DQ* and IA-2A ± ZnT8A only marginally increased screening sensitivity in this group. However, the presence of at least two of the three risk markers (IA-$2A^+$ ± $ZnT8A^+$, *HLA-A*24*, *HLA-DQ2/DQ8*) was associated with 59–82% 5-year progression but at the expense of a low sensitivity between 18% and 48%, depending on the marker combinations (Table 3). The combined presence of *HLA-A*24* and IA-$2A^+$ ± $ZnT8A^+$ (regardless of *HLA-DQ2/DQ8* genotype) conferred the highest 5-year risk (82%) of diabetes but identified only 28% of the rapid progressors (Table 3). The combination of IA-$2A^+$ ± $ZnT8A^+$ and *HLA-DQ2/DQ8* was more sensitive (36%), with a high progression rate (64%), and an additional search for *HLA-A*24* carriers among IA-$2A^+$ ± $ZnT8A^+$ relatives further increased sensitivity (48%) without affecting overall 5-year progression rate. When 10-year progression to diabetes was considered (Supplementary Table 2), results were similar to the observations in Table 3 except for a slight decrease in the fraction of diabetic patients identified and an overall increase in progression rate, as expected.

DISCUSSION

The current study confirms the association of *HLA-A*24* with progression from autoimmunity to T1D (16–18). The identification of this allele as an independent predictor of clinical onset within 5 years and interacting with the presence of *HLA-DQ2/DQ8* and IA-$2A^+$ ± $ZnT8A^+$ constitutes the major finding of our study. Our observation has practical implications for screening strategies in preparation of secondary prevention trials with immune intervention. The joint presence of IA-$2A^+$ ± $ZnT8A^+$ and *HLA-A*24* identifies a small subgroup at extremely high diabetes risk (82% within 5 years). In IA-$2A^+$ ± $ZnT8A^+$ FDRs, however, detection of *HLA-A*24* carrier status significantly increases screening sensitivity for individuals at high Ab- and *HLA*-inferred 5-year risk (64% progression to diabetes) as a useful complement to *HLA-DQ* typing. This selected group of relatives qualifies for precise assessment of β-cell function with standardized tests (e.g., hyperglycemic clamp test) to identify individuals with already decreased stimulated hormone release in the perspective of composing homogeneous groups of high-risk participants in immunointervention trials with an acceptable risk-to-benefit ratio in the light of the potentially harmful drugs used (e.g., 80% 3-year diabetes risk) (7,10,13).

Strengths of this study include its registry-based nature, the broad age range of recruited FDRs, its longitudinal nature, with 73% of nonprogressors followed for 5 years, and the completeness of the dataset for all variables. Given the broad age range, most relatives were not monitored from birth, which is the best approach to study the natural history of diabetes. However, Vermeulen et al. (9) showed that relatives who seroconverted to Ab^+ during follow-up did not differ in baseline characteristics and progression rate toward diabetes from relatives who were Ab^+ at the first sampling. Because we focused on rapid progressors, the number of events is relatively limited. However, our cohort constitutes one of the largest and most representative groups of relatives without preselection on the basis of an initial screening.

The mechanisms by which polymorphic HLA class II and I molecules confer T1D risk are incompletely understood but inferred to involve thymic selection of T-cell repertoire and peripheral selection of peptide repertoire (1). Tait et al. (19) suggested that HLA class II and class I, respectively, mediate initiation and progression of β-cell autoimmunity, although observations by us and others also suggest a major role for *DQ2-DR3/DQ8-DR4* in disease progression (13,24). Recent findings by Lipponen et al. (18) showed a strong effect of *HLA-B*39*, but not of *HLA-A*24*, on progression to T1D after seroconversion to persistent Ab^+, but only in individuals carrying *HLA-DR3/DR4*. In contrast, Tait et al. (19) found that disease progression was associated with *HLA-A*24*, *-A*30*, and *-B*18* in Ab^+ relatives of a diabetic proband. Our observation of an accelerating role of *HLA-A*24* on β-cell loss is in accordance with the results of Tait et al. (19) and with previous reports on its association with early-onset T1D and subsequent complete β-cell destruction (17,20) but at variance with Lipponen et al. (18). In line with the latter Finnish study (18), however, the disease-promoting effect of HLA class I alleles was restricted to individuals carrying the *HLA-DQ2/DQ8* high-risk genotype.

Several reasons may explain this discrepancy between Finnish and Belgian studies, including differences in the background population (Finland vs. Belgium), familial history (general population vs. FDRs), age at inclusion (neonates monitored from birth vs. age 0–39 years), and initial Ab screening (ICA vs. four molecular Abs). Our results do not allow us to reach firm conclusions about the exact nature of the mechanisms involved in the disease-promoting effects of *HLA-A*24* and in its interactions with other genetic and immune risk markers. Nevertheless, the accelerating effect of *HLA-A*24* on the underlying process and its interaction with immune markers of impending diabetes are compatible with a role in efficient HLA class I–mediated presentation of antigenic self-epitopes to cytotoxic $CD8^+$ lymphocytes (25). Our data reiterate the need to stratify according to closely linked HLA class II alleles when looking for class I effects (17,18). Finally and more importantly, they demonstrate for the first time how HLA class I typing may contribute in practice to the refinement of screening

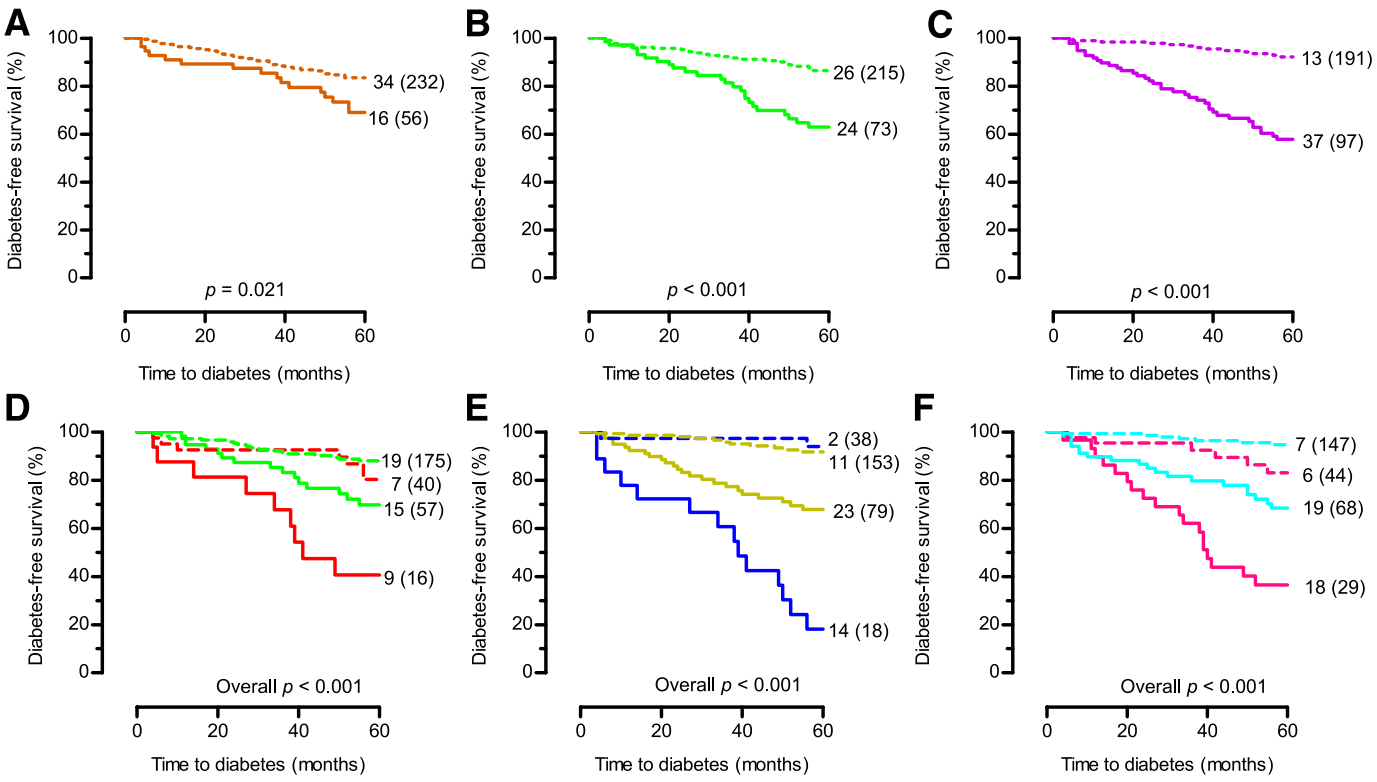

FIG. 1. Diabetes-free survival in persistently Ab$^+$ FDRs at baseline (n = 288) stratified according to *HLA-A*24*, *HLA-DQ2/DQ8*, and IA-2A/ZnT8A status: presence of *HLA-A*24*, solid orange line; absence of *HLA-A*24*, broken orange line (*A*); presence of *HLA-DQ2/DQ8*, solid green line; absence of *HLA-DQ2/DQ8*, broken green line (*B*); presence of IA-2A and/or ZnT8A, solid purple line; absence of IA-2A and ZnT8A, broken purple line (*C*); presence of *HLA-A*24* and *HLA-DQ2/DQ8*, solid red line; presence of *HLA-DQ2/DQ8* and absence of *HLA-A*24*, solid green line; presence of *HLA-A*24* and absence of *HLA-DQ2/DQ8*, broken red line; absence of *HLA-DQ2/DQ8* and of *HLA-A*24*, broken green line (*D*); presence of *HLA-A*24* and (IA-2A and/or ZnT8A), solid blue line; presence of (IA-2A and/or ZnT8A) and absence of *HLA-A*24*, solid yellow line; presence of *HLA-A*24* and absence of (IA-2A and ZnT8A), broken blue line; absence of IA-2A, ZnT8A and of *HLA-A*24*, broken yellow line (*E*); presence of IA-2A and/or ZnT8A and *HLA-DQ2/DQ8*, solid fuchsia line; presence of IA-2A and/or ZnT8A and absence of *HLA-DQ2/DQ8*, solid cyan line; presence of *HLA-DQ2/DQ8* and absence of IA-2A and ZnT8A, broken fuchsia line; absence of IA-2A, ZnT8A, and *HLA-DQ2/DQ8*, broken cyan line (*F*). The numbers in each panel indicate the number of events for each arm (total number at study entry). *P* by log-rank test.

strategies to select suitable candidates for participation in immune interventions in pre-T1D.

In conclusion, we demonstrate that *HLA-A*24* is an independent predictor of 5-year progression to diabetes in Ab$^+$ FDRs of T1D patients. It interacts significantly with the presence of *HLA-DQ2/DQ8* and, in particular, IA-2A$^+$ ± ZnT8A$^+$. Depending on whether the screening goals intend to favor specificity or sensitivity, *HLA-A*24* can help select a subgroup at extremely high risk or increase sensitivity of detecting relatives with high combined Ab- and *HLA*-inferred risk.

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E.M. designed research; acquired data; undertook statistical analysis and interpretation of the results; wrote, reviewed, and edited the manuscript; and approved the final version of the manuscript. B.J.V.d.A. designed research, obtained funding, supervised data collection, interpreted data, reviewed and edited the manuscript, and approved the final version of the manuscript. I.V., S.D., A.V.D., and E.V.B. analyzed and interpreted data, participated in discussions, reviewed and edited the manuscript, and approved the final version of the manuscript. S.V.A., L.D., H.D., and J.D.S. designed the study, recruited FDRs, reviewed and edited the manuscript, and approved the final version of the manuscript. C.v.S. designed the study, participated in discussion, analyzed and interpreted data, reviewed and edited the manuscript, and approved the final version of the manuscript. J.M.W. and J.C.H. contributed new reagents and analytical tools, analyzed and interpreted data, contributed to discussion, reviewed and edited the manuscript, and approved the final version of the manuscript. D.P. designed research, analyzed and interpreted data, contributed to

TABLE 3
Five-year progression to type 1 diabetes according to biomarker profile

Markers present	*n*	Progression** % (95% CI)	Pre-T1D patients with diabetes identified % (*n*)
IAA$^+$, GADA$^+$, IA-2A$^+$, and/or ZnT8A$^+$	288	19 (15–24)	100 (50)
*HLA-A*24* (marker 1)	56	31 (18–44)	32 (16)
HLA-DQ2/DQ8 (marker 2)	73	37 (25–49)	48 (24)
IA-2A$^+$ ± ZnT8A$^+$ (marker 3)	97	42 (32–53)	74 (37)
Number of markers			
0/3	117	6 (1–10)	12 (6)
1/3	124	18 (10–25)	38 (19)
2/3	39	46 (30–62)	34 (17)
3/3	8	100 (100–100)	16 (8)
≥1 type			
Marker 1 and/or marker 2	113	31 (22–40)	62 (31)
Marker 1 and/or marker 3	135	32 (24–40)	78 (39)
Marker 2 and/or marker 3	141	35 (26–43)	86 (43)†
Marker 1, and/or marker 2, and/or marker 3	171	29 (22–36)	88 (44)†
≥2 types			
Marker 1 and marker 2	16	59 (35–84)	18 (9)
Marker 1 and marker 3	18	82 (63–100)	28 (14)‡
Marker 2 and marker 3	29	64 (46–81)	36 (18)‡§
Marker 1, and/or marker 2, and marker 3	39	64 (48–79)	48 (24)§

**Kaplan-Meier survival tables. McNemar test: †$P < 0.001$; ‡$P = 0.072$; §$P = 0.002$.

discussion, reviewed and edited the manuscript, and approved the final version of the manuscript. I.W. designed research, recruited FDRs, supervised statistical analysis, interpreted data, reviewed and edited the manuscript, and approved the final version of the manuscript. F.K.G. designed research; obtained funding; supervised the study; analyzed and interpreted data; drafted, reviewed, and edited the manuscript; and approved the final version of the manuscript. F.K.G. is the guarantor of this work, and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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