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IN ANTIBODY-POSITIVE FIRST-DEGREE RELATIVES OF PATIENTS WITH TYPE 1 DIABETES, *HLA-A*24* AND *HLA-B*18*, BUT NOT *HLA-B*39*, ARE PREDICTORS OF IMPENDING DIABETES WITH DISTINCT *HLA-DQ* INTERACTIONS

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Abstract

Aims/hypothesis—Secondary type 1 diabetes prevention trials require selection of participants with impending diabetes. *HLA-A* and *-B* alleles have been reported to promote disease progression. We investigated whether typing for *HLA-B*18* and *-B*39* may complement screening

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DEDICATION

We dedicate this paper to the memory of J. C. Hutton who passed away on December 18, 2012. He contributed to the design of the study, the discussion of the results and the first version of the manuscript.

DUALITY OF INTEREST

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

CONTRIBUTION STATEMENT

All authors contributed to the design of the study, acquisition of data, statistical analysis, discussion and/or revision of the manuscript. All authors approved the final version. Electronic supplementary material

for *HLA-DQ8*, *-DQ2* and *-A*24* and autoantibodies (Abs) against islet antigen-2 (IA-2) and zinc transporter 8 (ZnT8) for predicting rapid progression to hyperglycaemia.

Methods—A registry-based group of 288 persistently autoantibody-positive (Ab+) offspring/siblings (aged 0–39 years) of known patients (Ab+ against insulin, GAD, IA-2 and/or ZnT8) were typed for *HLA-DQ*, *-A* and *-B* and monitored from the first Ab^+ sample for development of diabetes within 5 years.

Results—Unlike *HLA-B*39*, *HLA-B*18* was associated with accelerated disease progression, but only in *HLA-DQ2* carriers ($p < 0.006$). In contrast, *HLA-A*24* promoted progression preferentially in the presence of *HLA-DQ8* ($p < 0.002$). In *HLA-DQ2*- and/or *HLA-DQ8*-positive relatives (n = 246), *HLA-B*18* predicted impending diabetes ($p = 0.015$) in addition to *HLA-A*24*, *HLA-DQ2/DQ8* and positivity for IA-2A or ZnT8A ($p \leq 0.004$). *HLA-B*18* interacted significantly with *HLA-DQ2/DQ8* and *HLA-A*24* in the presence of IA-2 and/or ZnT8 autoantibodies ($p \leq 0.009$). Additional testing for *HLA-B*18* and *-A*24* significantly improved screening sensitivity for rapid progressors, from 38% to 53%, among relatives at high Ab-inferred risk carrying at least one genetic risk factor. Screening for *HLA-B*18* increased sensitivity for progressors, from 17% to 28%, among individuals carrying ≥3 risk markers conferring >85% 5 year risk.

Conclusions/interpretation—These results reinforce the importance of HLA class I alleles in disease progression and quantify their added value for preparing prevention trials.

Keywords

Autoantibodies; HLA-A; HLA-B; HLA class I; HLA class II; HLA-DQ; Prediction; Prevention; Risk assessment; Type 1 diabetes

INTRODUCTION

*HLA-A*24* was recently shown to complement *HLA-DQ2/DQ8* and islet antigen-2 (IA-2) and zinc transporter 8 (ZnT8) autoantibodies (IA-2A and ZnT8A, respectively) for identifying first-degree relatives (FDRs) with impending diabetes [1] who may qualify for participation in immunointervention trials [2]. *HLA-B* alleles were also reported to promote disease progression in risk groups for type 1 diabetes, some studies identifying *B*18* as an accelerator [3], others implicating *B*39* [4]. There is, however, agreement on the need to adjust for closely linked HLA class II haplotypes when looking for class I effects [4, 5]. *HLA-A*24* was proposed to interact preferentially with *HLA-DQ8* or *DQ8/DQ2* [6], *HLA-B*18* with *HLA-DQ2* [7] and *HLA-B*39* with HLA-DR3/DR4 [4].

While the mechanisms behind the disease-accelerating role of HLA class I alleles remain hypothetical, the added value of typing for *HLA-B*18* and *-B*39* on the selection of participants for immunointervention trials remains largely unknown. Therefore, we investigated, first, whether *HLA-B*18* and *-B*39* are associated with accelerated progression to diabetes in a registry-based group of persistently autoantibody-positive (Ab^+) FDRs younger than 40 years and stratified according to *HLA-DQ* risk haplotype and, second, whether they represent additional predictors of impending diabetes that may usefully assist IA-2A, ZnT8A, *HLA-DQ2/DQ8* and *HLA-A*24* in identifying high risk individuals.

METHODS

Study population

Persistently Ab^+ FDRs (positive for insulin autoantibodies [IAA], GAD autoantibodies [GADA], IA-2A and/or ZnT8A; n = 288 [134 offspring and 154 siblings]) of type 1 diabetes probands were identified and followed by the Belgian Diabetes Registry as reported [1]. At

entry, and during follow-up, blood was sampled and a short questionnaire completed after informed consent. Inclusion criteria are shown in electronic supplementary material (ESM) Fig. 1. At baseline (first Ab^+ sample), the median age (interquartile range [IQR]) of FDRs (156 males and 132 females) was 12 (6–19) years. Of these FDRs, 68% (n = 197) were positive at recruitment and 32% (n = 91) seroconverted to persistent Ab positivity after a median (IQR) follow-up of 36 (24–72) months. The progression rate to diabetes from the first Ab^+ sample was similar in initially Ab^+ relatives and in seroconverters [8]. During a median (IQR) total follow-up time from the first Ab^+ sample of 71 (36–120) months, 33% (n = 96) out of 288 relatives progressed to type 1 diabetes whereas 67% (n = 192) did not. Since we focused on rapid progression to diabetes, the follow-up after the first Ab^+ sample was truncated at 60 months, thereby reducing the proportion of progressors to 17% (n = 50). After truncation, 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 ADA criteria [9] and ascertained as reported [1]. The 288 FDRs were typed for *HLA-B*18* and *HLA-B*39* status and the results were analysed for the entire group with and without stratification for *HLA-DQ2* or *HLA-DQ8*. Further analysis was performed in the subgroup of 246 relatives (85%; ESM Fig. 1) carrying *HLA-DQ2* and/or *-DQ8*. Their baseline characteristics (ESM Table 1) closely resembled those of the entire group [1]. The study protocol was approved by the ethics committees of the Belgian Diabetes Registry and participating university hospitals and was carried out according to The Helsinki Declaration as revised in 2008 (www.wma.net/en/30publications/10policies/b3/, accessed 18 April 2013).

Analytical methods

HLA-DQ and *-A*24* [1] and autoantibodies [8] were previously determined. *HLA-B*39* and *HLA-B*18* were typed by a PCR sequence-specific oligonucleotide dot-blot method as reported for *HLA-A*24* [1, 10], using group-specific primers (ESM Table 2). The probe panels identified all 102 *HLA-B*39* alleles except *HLA-B*39:01:11/30/33/34/36/43* and all *103 HLA-B*18* alleles except *HLA-B*18:29/72* (www.ebi.ac.uk/ipd/imgt/hla/probe.html, release 3.10, accessed 18 April 2013).

Statistical analyses

McNemar and χ^2 tests (Fisher's exact test when appropriate) were used to compare proportions between groups in paired and independent samples, respectively, Mann–Whitney U test for differences between groups for continuous data and Kaplan–Meier survival analysis and logrank test for differences in diabetes-free survival. Multivariate Cox regression analysis was used to assess the independent contribution to 5 year diabetes risk of potential factors identified in univariate analysis ($p < 0.10$). All statistical analyses were performed using IBM SPSS 20.0 (IBM Corporation, Armonk, NY, USA) or GraphPad Prism 5 (GraphPad, San Diego, CA, USA) software. Significance was defined as $p < 0.05$ or $p < 0.05/k$ (Bonferroni correction).

RESULTS

HLA-B*18 and HLA-A*24 as predictors of progression to diabetes with distinct HLA-DQ associations

Within 5 years, 50 relatives (17%; 25 male and 25 female relatives; 18 offspring and 32 siblings) developed diabetes after a median (IQR) follow-up time of 29 (12–42) months. Baseline characteristics (autoantibody, *HLA-DQ2, -DQ8* and *-A*24* status) of progressors and non-progressors were reported previously [1]. Of 288 persistently Ab^+ FDRs, 32 (11%) carried *HLA-B*18* (progressors, 20%; non-progressors, 9%) and 20 (7%) carried *HLA-B*39* (progressors, 4%; non-progressors, 8%). Kaplan–Meier analysis showed slightly more rapid progression to diabetes in relatives with *HLA-B*18* than in those without ($p < 0.021$; Fig. 1a;

ESM Table 3). In contrast, *HLA-B*39* status had no effect on progression rate ($p = 0.377$; Fig. 1b). Cox regression analysis maintained IA-2A and/or ZnT8A, *HLA-DQ2/DQ8* and *HLA-A*24* as predictors of diabetes within 5 years [1] (Table 1). In addition, *HLA-B*18*, but not *B*39*, reached borderline significance ($p = 0.058$; Table 1).

*HLA-A*24* carriers progressed more rapidly to diabetes in the presence of *HLA-DQ8* ($p < 0.003$; Fig. 1c; ESM Table 3) but not in its absence (Fig. 1d). In contrast, positivity for *HLA-B*18* was associated with more rapid progression to diabetes in *HLA-DQ2*-positive relatives ($p < 0.006$; Fig. 1e; ESM Table 3) but not in *HLA-DQ2*-negative ones (Fig. 1f). Multivariate Cox regression analysis confirmed *HLA-A*24* and *HLA-B*18* as predictors of impending diabetes, the effect of *HLA-A*24* being mainly observed in *HLA-DQ8* carriers ($p = 0.002$; ESM Table 4) and that of *HLA-B*18* being limited to carriers of *HLA-DQ2* ($p = 0.004$; ESM Table 4). *HLA-B*39* did not predict diabetes regardless of *HLA-DQ* status.

Added value of HLA-B*18 and -A*24 in predicting 5 year diabetes risk in carriers of HLA-DQ8 and/or HLA-DQ2

Since *HLA-B*18* and *-A*24* were only predictive in the presence of *HLA-DQ2* and/or *HLA-DQ8* we investigated the contribution made by *HLA-B*18* towards the identification of rapid progressors in carriers of ≥1 susceptible *HLA-DQ* haplotype. This group comprised 246 of the 288 (85%) persistently Ab^+ relatives and 47 of the 50 (94%) rapid progressors (ESM Table 1). These 47 progressors (19% of 246) developed diabetes after 33 (17–43) months (median [IQR]). The baseline characteristics (ESM Table 1) of progressors (n = 47) vs non-progressors (n = 199) were similar to those of the entire group (n = 288) [1]. In summary, compared with non-progressors, progressors tended to carry more *HLA-DQ2/DQ8* ($p < 0.001$), *HLA-A*24* ($p < 0.005$) and *HLA-B*18* ($p = 0.024$) but not *HLA-B*39* ($p = 0.355$) (ESM Table 1). In these relatives at *HLA-DQ*-inferred risk, the presence of *HLA-B*18* was associated with a more rapid progression ($p = 0.010$ vs absence; Fig. 2a), as was also the case for the presence of IA-2A and/or ZnT8A, *HLA-DQ2/DQ8* or *HLA-A*24* [1] (not shown). Progression rate gradually increased according to number of these four markers (0 to ≥3) present at baseline (overall $p < 0.001$; Fig. 2b). Multivariate Cox regression analysis confirmed the presence of *HLA-B*18* ($p = 0.015$), *HLA-A*24* ($p = 0.004$) and *HLA-DQ2/DQ8* ($p = 0.001$) and positivity for IA-2A and/or ZnT8A ($p < 0.001$) as predictors of diabetes (Table 1). A significant interaction between *HLA-B*18* and *HLA-DQ2/DQ8* was revealed ($p = 0.009$), while the reported strong interaction of *HLA-A*24* with IA-2A and/or ZnT8A positivity [1] was maintained ($p < 0.001$; ESM Table 5). Borderline significance was reached for younger age at the first Ab^+ sample (Table 1).

In the 246 persistently Ab^+ FDRs the overall 5 year progression increased from 21% to 37–42% in the presence of any of the four predictors (IA-2A and/or ZnT8A, *HLA-DQ2/DQ8*, *-A*24* or *-B*18*) (ESM Table 6) at the expense of a decreased sensitivity (21–72%) for detecting individuals with impending diabetes (ESM Table 6). The presence of *HLA-A*24* and/or *HLA-B*18* in addition to *HLA-DQ2/DQ8*$^+$ and/or IA-2A and/or ZnT8A increased the sensitivity for identifying rapid progressors among individuals with ≥1 risk marker and ≥30% 5 year diabetes risk from 85% to 91% ($p < 0.001$; three additional individuals; ESM Table 6). Moreover, screening for *HLA-B*18*, in addition to IA-2A and/or ZnT8A, *HLA-DQ2/DQ8* and *HLA-A*24* significantly increased the sensitivity of detecting rapid progressors from 38% (18 of 47) to 53% (25 of 47) ($p < 0.001$; ESM Table 6) among relatives at high Ab-inferred risk and carrying at least one genetic susceptibility factor, conferring a ≥60% 5 year risk. The presence of any three markers among IA-2A and/or ZnT8A, *HLA-DQ2/DQ8*, *HLA-A*24* and *HLA-B*18* identified 15 relatives with an 87% 5 year progression rate. Hence additional screening for *HLA-B*18* increased the sensitivity of

detecting relatives carrying three markers and with >85% progression rate from 17% (8 of 47) to 28% (13 of 47) ($p = 0.016$; ESM Table 6).

DISCUSSION

In a representative Belgian group of persistently Ab^+ FDRs, the association of *HLA-B*18*, but not of *HLA-B*39*, with more rapid progression from autoimmunity to type 1 diabetes [3] was confirmed and shown to occur preferentially in the presence of *HLA-DQ2* [7], as opposed to the preferential accelerating effects of *HLA-A*24* in carriers of *HLA-DQ8* [6]. The identification of *HLA-B*18* as a predictor of impending diabetes—complementing the established markers IA-2A and/or ZnT8A, *HLA-DQ2/DQ8* and *HLA-A*24* [1], in Ab^+ FDRs carrying at least one *HLA-DQ* susceptibility haplotype—constitutes the main finding of the present study. Screening for *HLA-B*18* in addition to testing for IA-2A, ZnT8A, *HLA-DQ2/DQ8* and *HLA-A*24* significantly increased the number of rapid progressors identified among individuals positive for IA-2A and/or ZnT8A and carrying at least one genetic risk marker and overall >60% 5 year risk by 39%. Likewise, it expanded by 63% the number of FDRs identified as having impending diabetes among carriers of three risk markers conferring an overall >85% 5 year risk. The complementarity of testing for *HLA-B*18* and *-A*24* is further underscored by their distinct interaction with *HLA-DQ2/DQ8* and IA-2A and/or ZnT8A, respectively. These findings increase the importance of HLA class I alleles in identifying rapid progressors and facilitate the constitution of homogeneous groups of participants in future immunointervention studies. The strengths and weaknesses of our approach have been addressed in a previous paper [1]. Briefly, strengths include the representativeness of the FDRs, their broad age range and completeness of the data set for all variables. Follow-up from birth is lacking but is not relevant in the context of identifying selection criteria for adolescent and adult participants in immunointervention trials. While the distinctive predictor abilities of *HLA-B*18* and *-A*24* are significant, their contribution to the identification of rapid progressors remains modest in absolute numbers. Their inclusion in prediction studies should be weighed against the efficacy of other predictors (e.g. metabolic markers) [11, 12].

The present study does not allow one to decide whether disease acceleration is caused by *HLA-A* and *-B* alleles themselves or whether haplotypic factors are involved. However, the observed preferential effect of *HLA-B*18* in carriers of *HLA-DQ2* is compatible with reports that *HLA-B*18* is often found in a haplotype combined with *DRB1*03-DQB1*02* conferring a higher risk than *B*08-DRB1*03-DQB1*02*, suggesting that specific interactions with *HLA-DR/DQ* might be involved [7]

Our results on the positive association of *HLA-B*18* and *-A*24* with more rapid progression to diabetes are in accordance with the findings of some previous reports [3, 13]. In contrast, Lipponen*et al* documented an *HLA-DR3/DR4*-restricted acceleration of the disease process in the presence of *HLA-B*39* but not of *HLA-B*18* or *-A*24* [4]. These discrepancies might relate to regional differences in genetic background, familial history, gene–environment interactions or age at inclusion of the cohorts followed [1, 3–7, 13]. The lack of a significant accelerating effect of *HLA-B*39* in the present study might relate to the low prevalence of the allele (7%) in our cohort of Ab^+ relatives and in 594 healthy controls (7%; E. Mbunwe, unpublished). The association of *HLA-B*39* with type 1 diabetes (11% in 1,670 patients vs 7% in controls; $p < 0.01$) was also less strong than for *HLA-B*18* (17% in patients vs 10% in controls, $p < 0.001$) (E. Mbunwe, unpublished). Since *HLA-B*39* has been associated with early age at diagnosis [14], the lack of accelerating effect of this allele in our study might in part derive from the overall older age at first Ab^+ sample (median age, 12 years) compared with the individuals studied by Lipponen et al (median age, 1.8 years) [4].

Taken together, results from our group and others [1, 3–7, 13] underscore the importance of HLA class I alleles in disease progression from autoimmunity to overt diabetes, compatible with the role of HLA class I molecules in presentation of antigenic epitopes to cytotoxic $CD8^+$ cells [15–20]. The present study has demonstrated the added value of *HLA-B* and *HLA-A* typing in selection strategies for participants in secondary prevention trials. Further investigations should try to correlate the presence of disease accelerators with changes in functional beta cell mass as assessed by standardised beta cell function tests to better understand the preclinical phase of type 1 diabetes and to further streamline screening strategies [11, 12, 18]. Our results suggest that various pathways may converge to cause beta cell loss and that screening strategies for high-risk individuals need to take regional differences in genetic susceptibility into account. The association of both *HLA-A*24* and IA-2A and/or ZnT8A with rapid progression to diabetes might be reconciled with reports on lower humoral responses to IA-2 or ZnT8 at [19] or after [20] diagnosis of type 1 diabetes in *HLA-A*24* carriers hypothesising more pronounced loss of antigenic stimulus or less opportunity of inter- and intra-molecular spreading of autoimmune response in *HLA-A*24*-positive individuals [19].

In conclusion, *HLA-A*24* and *HLA-B*18*, but not *HLA-B*39*, significantly contributed to prediction of 5 year progression to diabetes in Ab^+ FDRs of patients with type 1 diabetes in Belgium. The effects of *HLA-B*18* are *HLA-DQ2* selective whereas *HLA-A*24* is preferentially effective in the presence of *HLA-DQ8*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

Ab	Autoantibody
Ab+	Autoantibody-positive
BDR	Belgian Diabetes Registry
FDRs	First-degree relatives
GADA	GAD autoantibodies
IAA	Insulin autoantibodies
IA-2	Islet antigen-2
IA-2A	IA-2 autoantibodies
IQR	Interquartile range
ZnT8	Zinc transporter 8
ZnT8A	ZnT8 autoantibodies

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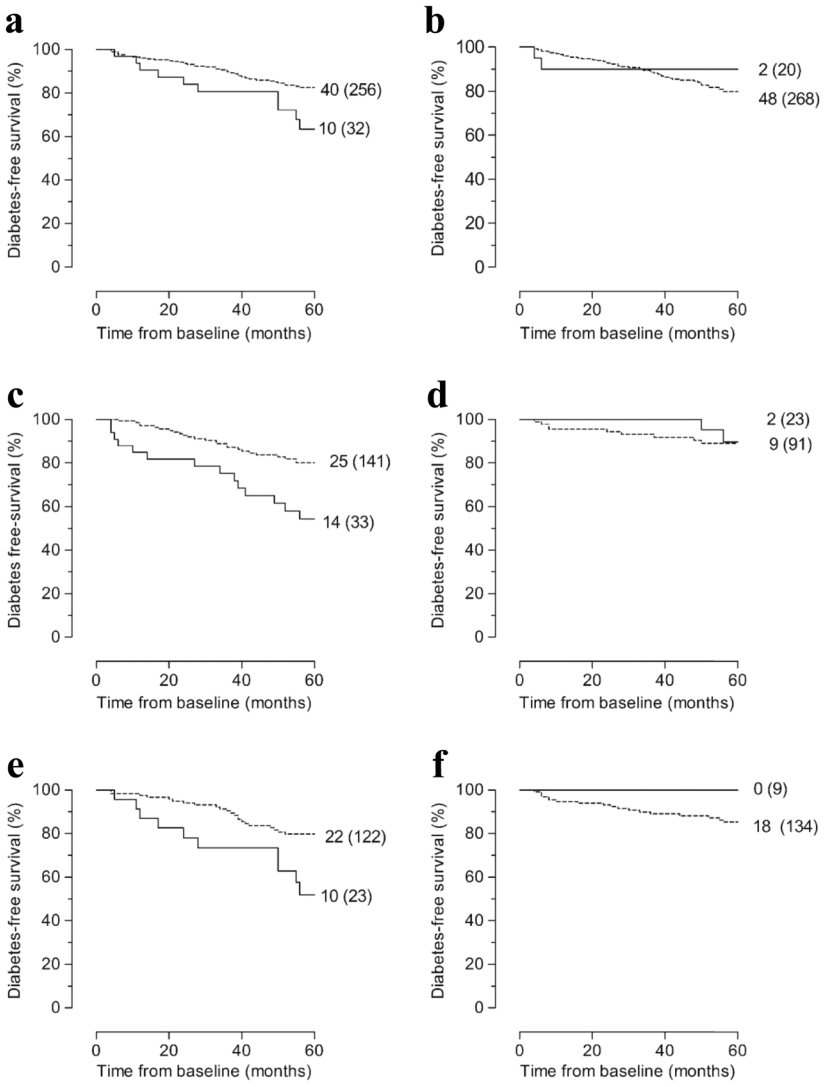

Figure 1. Diabetes-free survival according to *HLA-B*18, HLA-B*39* or *HLA-A*24* status in 288 persistently Ab$^+$ FDRs with or without stratification according to the presence or absence of *HLA-DQ8* or *HLA-DQ2*

(a) Whole group (n = 288) with (solid line) vs without (dashed line) *HLA-B*18* (p = 0.021). (b) Whole group (n = 288) with (solid line) vs without (dashed line) *HLA-B*39* (p = 0.377). (c) *HLA-DQ8*-positive (n = 174) relatives with (solid line) vs without (dashed line) *HLA-A*24* (p = 0.002). (d) *HLA-DQ8*-negative relatives (n = 114) with (solid line) vs without (dashed line) *HLA-A*24* (p = 0.750). (e) *HLA-DQ2*-positive (n = 145) relatives with (solid line) vs without (dashed line) *HLA-B*18* (p = 0.005). (f) *HLA-DQ2*-negative relatives (n = 143) with (solid line) vs without (dashed line) *HLA-B*18* (p = 0.277). The numbers given for each arm are number of events (total number at study entry). For the number of relatives under follow-up at different time points, see ESM Table 3. p by logrank test.

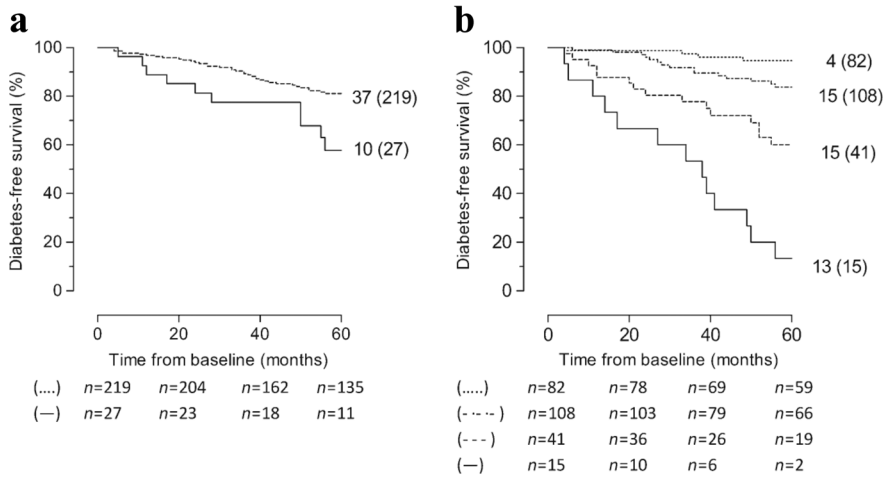

Figure 2.

Diabetes-free survival in 246 persistently Ab^+ FDRs carrying *HLA-DQ2* and/or *HLA-DQ8* according to (a) presence (solid line) vs absence (dashed line) of *HLA-B*18* ($p = 0.010$) or (b) presence of at least three of four markers (solid line) vs two of four markers (dashed line) vs one of four markers (dotted/dashed line) vs absence of all four markers (dotted line) (overall $p < 0.001$). Markers are independent predictors of 5 year diabetes risk in *HLA-DQ2*- and/or *HLA-DQ8*-positive relatives (positive for IA-2A and/or ZnT8A, *HLA-DQ2/DQ8*, *HLA-A*24* and *HLA-B*18*). The numbers given for each arm are number of events (total number at study entry). *p* by logrank test.

Table 1

Cox regression analysis for 5 year progression to diabetes in Ab+ first-degree relatives[a]

Variable	Whole group (n = 288)			HLA-DQ8 and/or HLA-DQ2 carriers (n = 246)		
	p value[b]	HR (95% CI)[b]	HR (95% CI)[c]	*p* value[b]	HR (95% CI)[b]	HR (95% CI)[c]
HLA-A*24	0.024	2.0 (1.1, 3.6)	2.2 (1.2, 4.1)[d]	0.006	2.3 (1.3, 4.3)	2.5 (1.3, 4.5)[d]
HLA-B*18	0.025	2.2 (1.1, 4.4)	2.0 (1.0, 4.0)[e]	0.012	2.4 (1.2, 4.9)	2.4 (1.2, 4.9)[d]
HLA-B*39	0.386	0.5 (0.1, 2.2)	NT	0.257	0.3 (0.0, 2.3)	NT
HLA-DQ2	0.048	1.8 (1.0, 3.2)	1.2 (0.5, 2.8)	0.170	1.5 (0.8, 2.8)	NT
HLA-DQ8	0.007	2.5 (1.3, 4.9)	1.3 (0.4, 4.5)	0.036	2.2 (1.0, 4.8)	1.3 (0.5,3.3)
HLA-DQ2/DQ8	<0.001	3.1 (1.8, 5.4)	2.6 (1.5, 4.6)[f]	<0.001	2.9 (1.6, 5.1)	2.7 (1.5, 4.8)[f]
Age[g] >12 vs≤12 years	0.026	0.5 (0.3, 0.9)	0.6 (0.3, 1.1)	0.010	0.4 (0.2, 0.8)	0.5 (0.3, 1.0)[e]
Offspring of diabetic mother	0.042	0.4 (0.2, 1.0)	0.8 (0.3, 2.3)	0.061	0.4 (0.2, 1.0)	1.0 (0.4, 2.8)
IA-2A and/or ZnT8A positive	<0.001	6.9 (3.7, 13.0)	6.7 (3.6, 12.7)[f]	<0.001	5.6 (3.0, 10.7)	5.8 (3.0, 11.0)[f]

Except indicated as NT (not tested) all variables shown were included in multivariate models

[a] Persistently positive for IAA, GADA, IA-2A and/or ZnT8A;

[b] Univariate analysis (enter method);

[c] Multivariate analysis (forward stepwise method) for variables with $p < 0.10$ in univariate analysis;

[d] $p < 0.02$;

[e] $p < 0.06$;

[f] $p < 0.002$;

[g] Median = 12 years