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Rapid communication

IA-2 autoantibodies predict impending Type I diabetes in siblings of patients

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Abstract

Aims/hypothesis. Multiple islet autoantibody positivity is currently believed to best predict progression to Type I (insulin-dependent) diabetes mellitus. We compared its predictive value with that of positivity for a particular type of islet autoantibody, directed against the IA-2 antigen.
Methods. Autoantibodies against islet cell cytoplasm (ICA), insulin (IAA), GAD (GADA) and IA-2 (IA-2A) were measured at initial sampling in 1724 non-diabetic siblings (median age [range]:16 [0–39] years) of Type I diabetic patients with a median follow-up of 50 months.
Results. On initial sampling 11% of siblings were positive for one antibody type or more and 2.1% for three of more types. During follow-up, 27 antibody-positive siblings developed diabetes. Using survival analysis, the risk for clinical onset within 5 years was 34% in subjects positive for three or more types compared with 13% in those with one type or more. Progression to diabetes amounted to 12% within 5 years among siblings positive for IAA, 20% for ICA, 19% for GADA but 59% for IA-2A ($p<0.001$ vs absence of the respective antibody). IA-2A were detected in 1.7% of all siblings and in 56% of the prediabetic subjects on first sampling. Initial positivity for two or three antibody markers was associated with a higher progression rate in IA-2A positive as compared to IA-2A negative siblings ($p=0.001$). In absence of IA-2A initial positivity for another antibody (IAA, ICA or GADA) conferred a low (<10% within 5 years) risk of diabetes compared to subjects lacking this antibody.
Conclusions/interpretation. In siblings of Type I diabetic patients, IA-2A positivity is a more direct predictor of impending clinical onset than multiple antibody positivity per se. Assessment of IA-2A status allows us to select subjects with homogeneously high risk of diabetes for participation in prevention trials. [Diabetologia (2002) 45:1658–1666]

Keywords Type I diabetes, siblings, prediction, prevention, IA-2 autoantibodies, GAD autoantibodies, insulin autoantibodies, islet cell antibodies, *HLA-DQ*.

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Abbreviations: ICA, Islet cell cytoplasmic antibodies; IAA, insulin autoantibodies; GADA, glutamate decarboxylase antibodies; IA-2A, IA-2 antibodies; BDR, Belgian Diabetes Registry; JDF, Juvenile Diabetes Foundation; IDW, Immunology of Diabetes Workshops; DASP, Diabetes Antibody Standardisation Program.

Clinical onset of Type I (insulin-dependent) diabetes mellitus is often preceded by circulating autoantibodies against islet cell cytoplasm (islet cell antibodies, ICA) or specific islet cell antigens such as insulin (insulin autoantibodies, IAA), the 65,000 M_r isoform of glutamate decarboxylase (GAD antibodies, GADA) or insulinoma-associated protein-2 protein tyrosine phosphatase (IA-2 antibodies, IA-2A) [1, 2, 3, 4, 5]. Although the histopathological correlates of these circulating markers are so far unknown, their detection is widely used to identify subjects at increased risk of developing diabetes, especially in family members of known patients [1, 2, 3, 4, 5]. These subjects could be

Table 1. Demographic and biological findings on first sampling in first degree relatives who developed Type I diabetes during follow-up

Sibling	Age onset (years)	Sex	Time to diagnosis (months)	Antibody activity				*HLA DQA1*-DQB1** genotype	
				ICA (JDFu)	GADA (%)	IA-2A (%)	IAA (%)	Haplotype 1	Haplotype 2
1	4	F	25	0 **(+)**	0.2 **(+)**	0.1	0.3 **(+)**	**0301-0302**	**0501-0201**
2	4	F	9	0	**8.5**	<0.1	**0.7**	*0100-0501/0604*	**0501-0201**
3	6	F	9	**400**	**1303.7**	**282.3**	0.5 **(+)**	*0100-0503/0602/0603*	**0301-0302**
4	7	M	13	0 **(+)**	**8.0**	**<0.1**	**0.9**	*0100-0501/0604*	**0501-0201**
5	8	M	49	**100**	**12.9**	**0.9**	**7.2**	**0301-0302**	**0501-0201**
6	9	M	35	**400**	0.5	<0.1	0.4	**0301-0302**	**0501-0201**
7	9	M	4	0	**25.2**	**0.5**	**0.8**	**0301-0302**	**0501-0201**
8	10	F	12	0	**3.4**	**1.77**	0.4	**0301-0302**	**0501-0201**
9	11	F	4	**12**	**240.4**	<0.1	0.4	**0501-0201**	**0501-0201**
10	12	M	2	**800**	0.4	**78.7**	**0.6**	**0301-0302**	**0501-0201**
11	12	M	82	**200**	**165.4**	**2.9**	**2.4**	**0301-0302**	*0401-0402*
12	13	M	72	0	0.7	<0.1 **(+)**	0.3	**0301-0302**	**0501-0201**
13	13	M	18	**50**	**226.3**	**0.6**	**1.3**	**0301-0302**	**0501-0201**
14	13	F	6	**200**	**508.9**	32.2	0.3	*0100-0501/0604*	**0301-0302**
15	13	F	7	**800**	**7.6**	**508.5**	**1.5**	**0301-0302**	*0301-0303*
16	14	M	13	6	1.6	0.2	**2.0**	**0301-0302**	**0501-0201**
17	15	M	77	0 **(+)**	**4.5**	<0.1 **(+)**	**1.6**	**0301-0302**	**0501-0201**
18	15	F	7	**200**	**46.6**	**445.7**	0.4	*0100-0501/0604*	*0301-0303*
19	16	F	6	**400**	**597.5**	**452.9**	**1.4**	**0301-0302**	**0501-0201**
20	19	M	36	0**(+)**	2.1	**89.6**	0.2	*0201-0201*	**0301-0302**
21	19	M	25	400	1.9	**72.2**	0.5 **(+)**	**0301-0302**	**0301-0302**
22	20	M	32	0 **(+)**	1.0	<0.1 **(+)**	**1.5**	*0201-0201*	*0301-0301*
23	21	F	33	0 **(+)**	**146.6**	<0.1 **(+)**	0.3 **(+)**	**0301-0302**	**0301-0302**
24	24	M	51	0	**11.9**	0.2 **(+)**	0.5	**0301-0302**	**0501-0201**
25	26	M	3	**100**	1.5	**657.7**	**1.0**	**0301-0302**	**0501-0201**
26	27	F	14	**100**	**2181.5**	**9.7**	0.4	**0301-0302**	**0501-0201**
27	29	M	44	**100**	**87.0**	0.11	0.5	**0301-0302**	**0501-0201**

Bold indicates presence of risk haplotypes or antibody activity above cutoff as defined in Methods; (+) indicates seroconversion to positivity before clinical diagnosis of diabetes

offered participation in clinical trials aiming at the suppression of the beta-cell destruction process. Antibody-positive relatives can, however, differ considerably in their individual progression rate towards clinical onset [1, 2, 3, 4, 5]. Siblings of Type I diabetic patients have been reported to carry an overall higher risk of diabetes than their offspring [6]. The risk for developing diabetes is also higher when the individual shows more than one of these antibodies; in fact, multiple antibody positivity is considered as the strongest predictor of impending hyperglycaemia [1, 4, 7, 8].

We noticed that the type of autoantibody could also be important. Positivity for IA-2A seems to have a higher predictive value for impending clinical onset than other diabetes-associated autoantibodies [2]. The interpretation of this finding is, however, complicated by the fact that IA-2A positivity is often noted in patients with multiple autoantibodies [2]. Caution is also needed when examining previous studies as subjects were (partly) selected on the basis of ICA-positivity [1, 7, 9], a state of prediabetes [1, 9], or young age [4]. Most studies did not distinguish subgroups according to the types of first degree relationship [1, 7, 8, 9, 10]. The present study compares the predictive power of diabetes-associated autoantibodies (ICA, IA-2A, GADA, IAA), alone or in combination, in a large group of unselected siblings of Type I diabetic patients, comprised of both children and adults. The purpose is to define more homogeneous groups with different risks for the disease. Such ability should facilitate the design of prevention trials as it is expected to reduce the number of subjects needed to assess intervention efficacy [11].

Subjects and methods

Subjects and data collection. Siblings (n=1724) of patients with Type I (insulin-dependent) diabetes mellitus were consecutively recruited by the Belgian Diabetes Registry (BDR) according to the following criteria: (i) under the age of 40 years at inclusion; (ii) sibling of a Type I diabetic patient diagnosed before the age of 35 years according to the criteria of the National Diabetes Data Group [12] or between the age of 35 and 50 years according to the same criteria with in addition a BMI of less than 28 kg/m^2 and an initial insulin dose of more than 0.25 U kg^{-1} d^{-1}. After informed consent, blood was sampled at inclusion and during follow-up (as a rule yearly) and a short questionnaire with demographic, familial and personal infor-

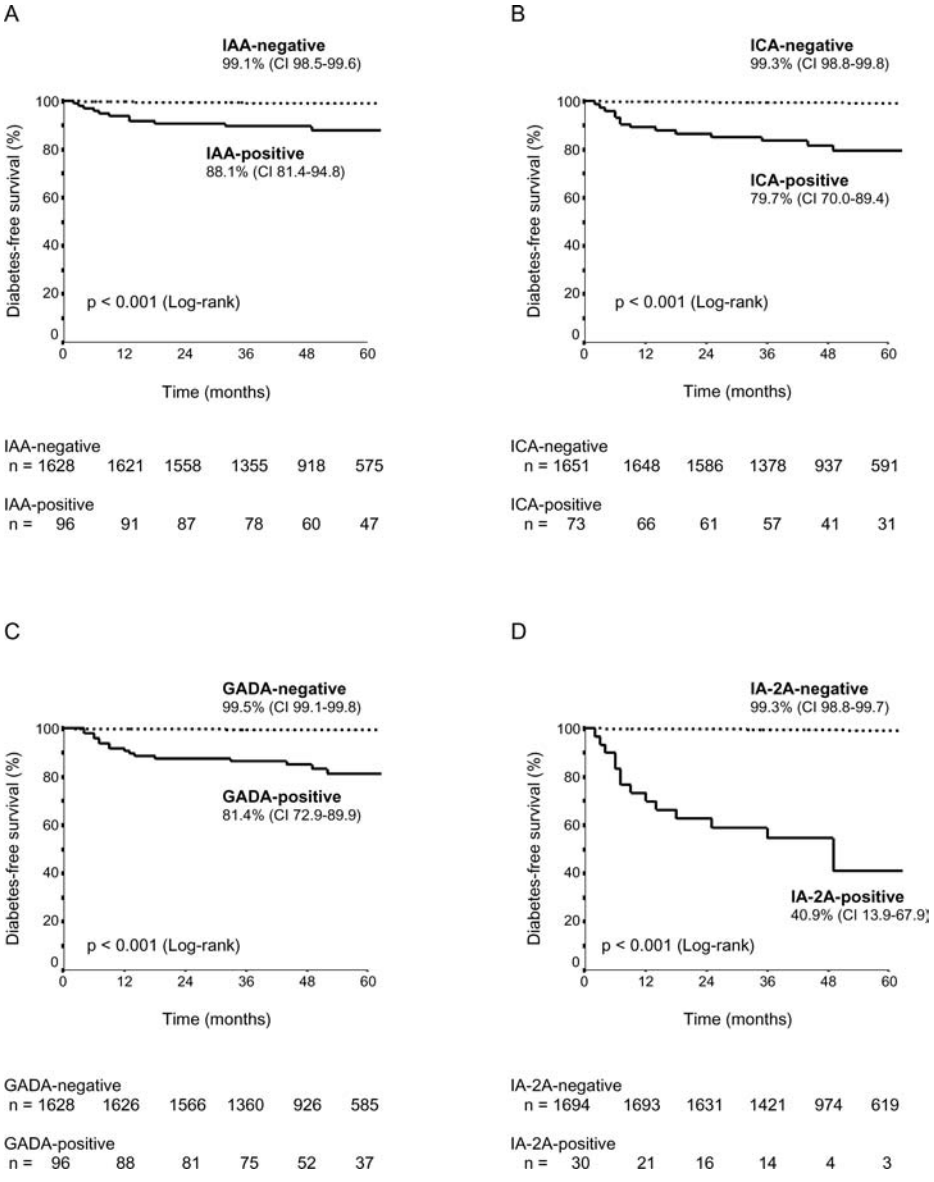

Fig. 1A–D. Diabetes-free survival in siblings (*n*=1724) stratified according to positivity or negativity for IAA (**A**), ICA (**B**), GADA (**C**) and IA-2A (**D**) at first sampling. In each panel the 5-year diabetes-free survival (95% confidence interval) is mentioned for each arm

mation was completed. The study was approved by the ethics committees of the Belgian Diabetes Registry and of participating university hospitals.

Assays. ICA were assessed by indirect immunofluorescence and endpoint titres expressed as Juvenile Diabetes Foundation (JDF) units [13]. IA-2A, GADA and IAA were measured by liquid phase radiobinding assays and expressed as percent tracer bound in haemolysis-free sera [13]. Cut-off values for antibody positivity were calculated as the 99th percentile of antibody activity obtained in 789 nondiabetic control subjects after omission of outlying values, and amounted to more than or equal to 12 JDF units for ICA, 0.6% for IAA, 2.6% for GADA and 0.4% for IA-2A, respectively [13]. The autoantibody assays were tested repeatedly in successive Immunology of Diabetes Workshops (IDW) and proficiency testing of the University of Florida, Gainesville, Florida, USA and the Louisiana State University, New Orleans, Louisiana, USA. In the latter program our four assays achieved 100% diagnostic sensitivity, specificity, validity and consistency. Diagnostic sensitivity adjusted for 99% specificity amounted to 73% for ICA, 85% for GADA and 36% for IAA (1995 IDW program [14]) and to 58% for IA-2A (2001 Diabetes Antibody Standardisation Program [DASP], Centres of Disease Control, Atlanta, Ga, USA). cDNAs for the preparation of radiolabelled GAD and the intracellular domain of IA-2 were kindly donated by Prof. Å. Lernmark (Univ. of Washington, Seattle, Wash., USA) and Dr. M. Christie (King's College School of Medicine and Dentistry, London, UK), respectively. In antibody-positive siblings (*n*=190) DNA-polymorphisms at the *HLA-DQA1* and *DQB1* gene loci were examined as before [15].

Statistical analysis. Diabetes-free survival was investigated by Kaplan-Meier analysis [16] and differences in progression to diabetes between groups, based on the results of testing the initial sample, were assessed by means of the log-rank test [17]. Cox proportional hazards model, done by forward stepwise method, was used to investigate the independent contributions

to diabetes prediction of risk factors identified by univariate analysis [18]. All statistical tests were carried out two-tailed by the "SPSS for Windows 10.0" (SPSS, Chicago, Ill., USA). A p value of less than 0.05 was considered statistically significant.

Results

Characteristics of siblings. We followed 1724 siblings (843 males, 881 females) with a median (range) age of 16 (0–39) years for a median (interquartile range) period of 50 (40–83) months. Antibody prevalence was 4.3% for ICA, 5.6% for GADA, 1.7% for IA-2A and 5.6% for IAA. At first sampling 190 (11%) were positive for at least one type of antibody, 64 (3.7%) for at least two types and 36 (2.1%) for at least three types. After a median (range) follow-up time of 14 (2–82) months, 27 siblings (16 males, 11 females) with a median (range) age of 13 (4–29) years developed diabetes (Table 1). In this group of prediabetic siblings, antibody prevalence on first sampling was 56% for ICA, 67% for GADA, 56% for IA-2A and 48% for IAA; 93% carried at least one antibody type on initial sampling, 70% at least two and 44% at least three types (Table 1). The diagnostic sensitivity of the antibody markers increased during the preclinical phase (Table 1). All but two siblings carried at least one of the *HLA-DQA1-DQB1* risk haplotypes (*0301–0302 or 0501–0201*).

Predictive value of different types of diabetes-autoantibodies. The diabetes-free survival rate was assessed according to the positivity or negativity for a particular antibody at initial sampling (Fig. 1). In the case of ICA, GADA and IAA, less than 25% of the initially antibody-positive siblings (20, 19 and 12% respectively) progressed to diabetes within 5 years (p<0.001 vs siblings lacking the antibody under study). In initially IA-2A positive siblings, however, the progression rate within 5 years was considerably higher and approximated 60% versus less than 1% in case of IA-2A negativity (p<0.001).

Similar to previous reports [1, 4, 7, 8] the progression to clinical onset tended to increase with the number of antibodies detected at first sampling, reaching 13% within 5 years for n greater than or equal to one (not shown), 23% for n=2, 25% for n=3, 60% for n=4 (Fig. 2A) and 34% for n greater than or equal to three (not shown) (in all instances p<0.001 vs no antibodies). However, there was no significant difference in progression rate between subjects positive for two, three or four antibody types (p>0.05). We next pooled the data from subjects carrying two or three antibodies who have similar progression rates (Fig. 2A) and compared survival curves after stratification according to IA-2A status (Fig. 2B). In the absence of IA-2A, initial positivity for two or three other antibody markers did confer less than 15% risk of diabetes within the next 5 years compared to approximately 50% in

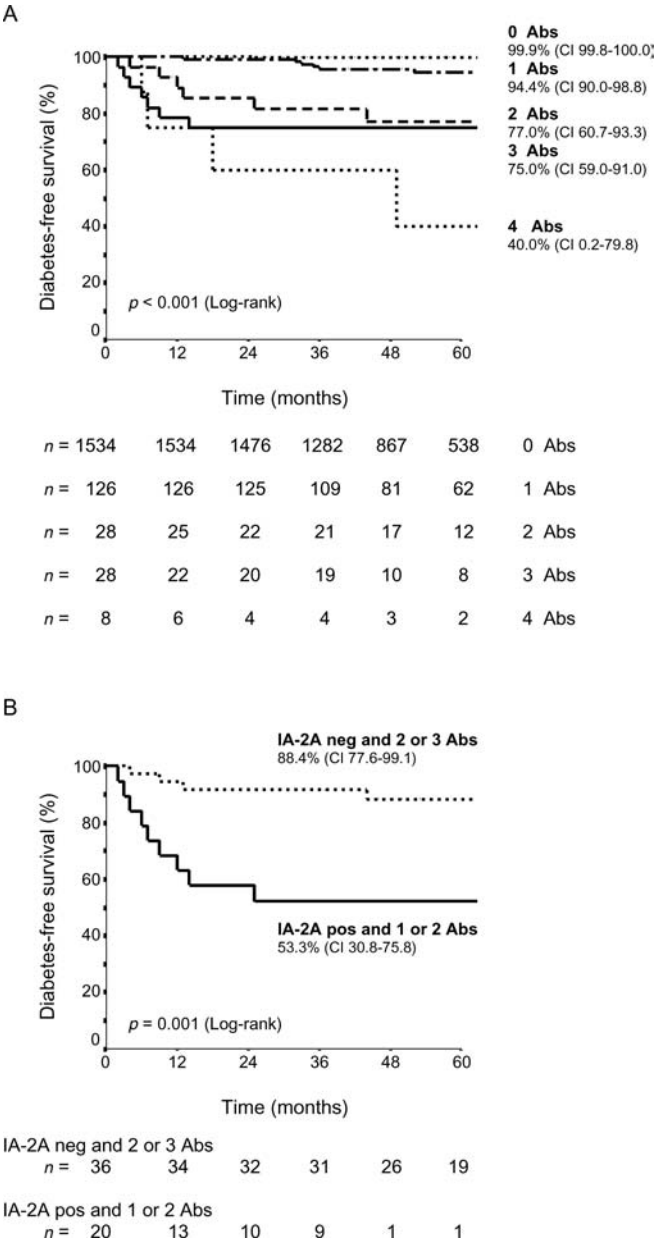

Fig. 2A, B. Diabetes-free survival in siblings (n=1724) stratified according to the number of antibodies (0, 1, 2, 3 or 4) at the first sampling (**A**). Diabetes-free survival after pooling the data from siblings with two or three antibodies (n=56) and stratification according to positivity or negativity for IA-2A (**B**). In each panel the 5-year diabetes-free survival (95% CI) is mentioned for each arm

case of IA-2A positive siblings with no more than two other antibodies detected on first sampling (p=0.001). In case ICA results were omitted from the analysis, progression to diabetes still increased with the number of molecular antibodies (p<0.001), reaching 24% after 5 years for two antibodies and 56% for three antibodies. When siblings with two molecular antibodies were subdivided according to IA-2A status, there was more progression in the presence (58%) than in the absence of IA-2A (10%; p=0.001) (not shown).

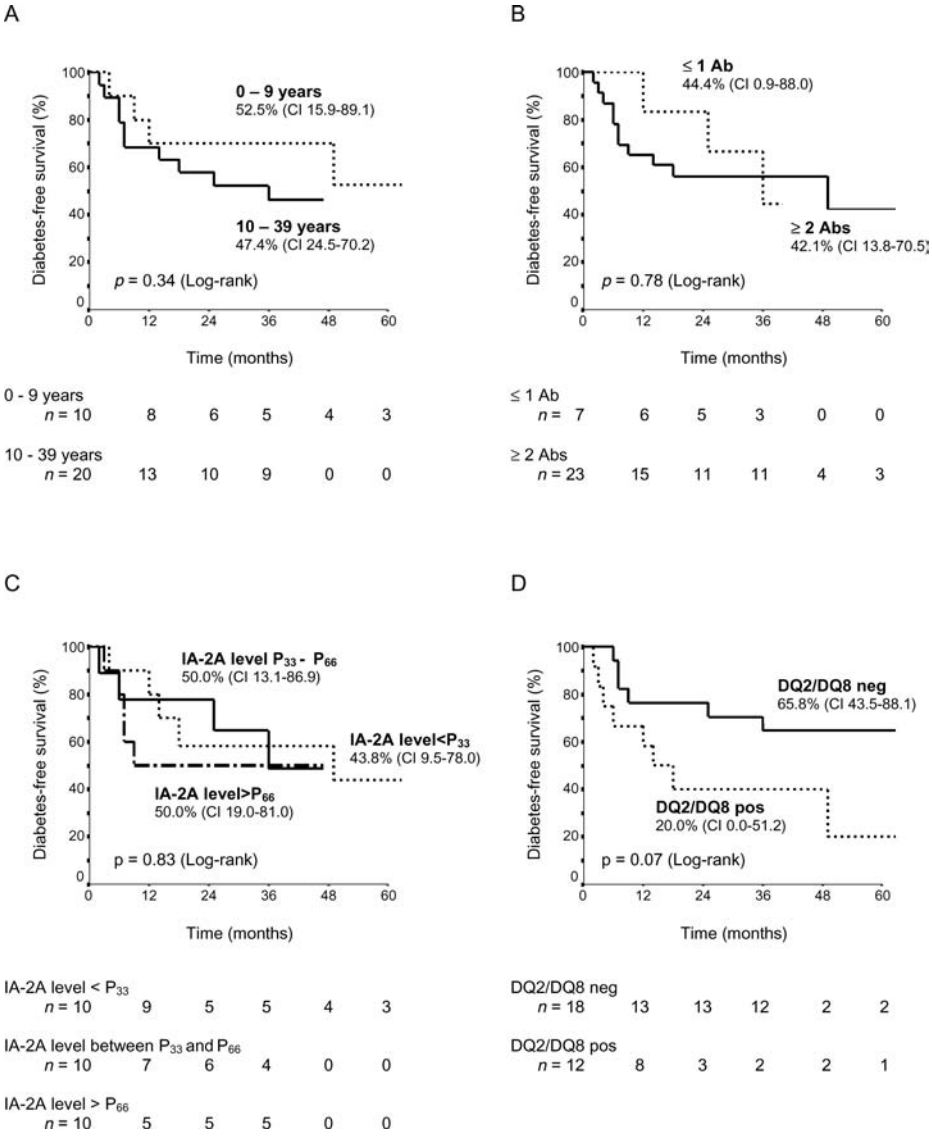

Fig. 3A–D. Diabetes-free survival in IA-2A positive siblings (n=30) after stratification for age at first sampling (0–9 years vs 10–39 years) (**A**), the number of other autoantibodies (≤1 vs ≥2) (**B**), IA-2A activities (tertiles) (**C**) and the positivity or negativity for the *HLA DQ2/DQ8* risk genotype (**D**). In each panel the 5-year diabetes-free survival (95% CI) is mentioned for each arm

Factors affecting progression to diabetes in case of IA-2A positivity. IA-2A positive siblings (n=30) showed no statistical differences in progression rate towards diabetes after stratification for age at first sampling (0–9 years vs 10–39 years, p>0.05), the number of other antibodies detected (n≤1 vs n≥2 antibodies) or for IA-2A activity (subdivided into tertiles, calculated on basis of the levels of the 30 IA-2A positive siblings) (Fig. 3A, B and C) or *HLA DQ* risk status (*DQA1*0501-DQB1*0201* [*DQ2*] positive vs negative; *DQA1*0301-DQB1*0302* [*DQ8*] positive vs negative) (results not shown). A tendency towards more rapid progression was seen in carriers of the heterozygous high risk HLA *DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302* [*DQ2/DQ8*] genotype (Fig. 3D; p=0.07).

Factors affecting progression to diabetes in absence of IA-2A. Since IA-2A was not detected during the preclinical phase in almost 30% of prediabetic siblings, we analysed the predictive value of other antibody markers on first sampling in siblings who remained IA-2A negative throughout follow-up. Progression to diabetes within 5 years occurred in 4% of the siblings with initial IAA positivity, 5% with ICA positivity and 7% with GADA positivity (p<0.001 vs antibody negative siblings for each comparison). In initially GADA positive but IA-2A negative siblings progression to diabetes was not significantly more rapid (p>0.05) according to age at first sampling (0–9 vs 10–39 years), the number of other antibodies detected (0 vs ≥1), GADA activity (tertiles) or *HLA-DQ* risk status.

Table 2. Cox regression analysis

Variable	Univariate analysis (Enter method) *p*	Multivariate analysis (Forward stepwise method)	
		p	Hazard ratio for diabetes (95% CI)[a]
Model 1 (all siblings *n*=1724)			
IA-2A	<0.001	<0.001	199.8 (25.0–1596.7)
IAA	<0.001	–	–
ICA	<0.001	–	–
GADA	<0.001	–	–
Number of antibodies	<0.001	–	–
Number of antibodies without IA-2A	<0.001	<0.001	4.1 (2.6–6.4)
Number of antibodies without IA-2A by IA-2A interaction:	–	<0.001	0.3 (0.1–0.6)
Number of antibodies without IA-2A in IA-2A positive siblings	–	0.840	1.1 (0.6–2.0)
Number of antibodies without IA-2A in IA-2A negative siblings	–	<0.001	4.4 (2.5–7.8)
Model 2 (antibody positive siblings *n*=190)			
IA-2A	<0.001	<0.001	12.5 (5.4–29.0)
HLA DQ2/DQ8 genotype	0.001	0.010	2.8 (1.3–6.3)
Number of antibodies without IA-2A	0.003	–	–
Age	0.163	–	–
Sex	0.439	–	–

[a] Data are hazard ratio (95% CI). Calculations were done with Cox regression models. Within each model, the hazard ratio for each variable is adjusted to the other variables in that model

Cox Regression Analysis. Univariate analyses were first carried out in which each of the potential early antibody markers of diabetes was confirmed to be associated with progression to diabetes (Table 2). To assess the independent contribution of these identified biological predictors (IA-2A, IAA, ICA, GADA and the number of antibodies detected), multivariate forward stepwise Cox regression analysis was carried out.

In a first multivariate model, investigating the relative importance of the number and the type of autoantibodies in the entire group of siblings (*n*=1724) neither IAA, ICA nor GADA were selected (Table 2). IA-2A was selected as the most important predictive factor conferring an almost 200-fold increased hazard ratio of diabetes controlling for the other variables in the model ($p<0.001$). In addition, the number of antibodies (not counting IA-2A to exclude interference with the first selected variable IA-2A) also contributed albeit with a much lower hazard ratio (Table 2; $p<0.001$). The interaction between the number of antibodies (without IA-2A) and positivity for IA-2A was also significant (Table 2; $p<0.001$). Further analysis of this interaction term indicated clearly that the number of antibodies had no additional predictive effect whatsoever in IA-2A positive siblings (Table 2; $p=0.84$) whereas the predictive effect in IA-2A negative siblings was found to be highly significant ($p<0.001$).

In order to confirm the results obtained in univariate analysis in the group of antibody positive siblings (*n*=190), we investigated in model 2 the hazard ratio for diabetes of the following parameters: IA-2A (selected as the most important prognostic factor in model 1), *HLA DQ2/DQ8* genotype, the number of antibodies (without counting IA-2A), age and sex. The latter three factors did not make a contribution in this model (Table 2). However, here again IA-2A were first selected in the model as most predictive factor ($p<0.001$) followed by the presence of the *HLA DQ* high risk genotype ($p=0.01$). Similar results were obtained after omitting ICA results from the Cox regression analysis (both in antibody-positive siblings and in all siblings) and considering only molecular autoantibodies, as well as after using the Williams' microassay for IAA [19] instead of our validated in-house modified Palmer assay [13] (not shown).

Discussion

Our longitudinal study in 1724 siblings of Type I diabetic patients has identified IA-2A positivity as the most potent predictor of impending diabetes. Even if detected only on one occasion, positivity for IA-2A confers a 60% risk of progression to hyperglycaemia within 5 years regardless of the antibody activity. The shape of the survival curves suggests that new diabetes cases could develop beyond this 5-year period. Our study confirms that progression to diabetes increases with the number of autoantibodies detected [1, 4, 7, 8], but in addition objectifies that IA-2A positivity is a better predictor than multiple antibody positivity, at least with respect to rapid progression towards clinical onset. Both by stratifying multiple anti-

body-positive siblings according to the IA-2A status and by Cox regression analysis we could show that it is essentially the appearance of this particular type of antibody – which is often associated with the occurrence of other antibodies – rather than multiple antibody-positivity per se that heralds impending diabetes. The outcome of both Kaplan-Meier and Cox analyses remains unaffected when ICA results are omitted or when IAA results are produced by a microassay [19] instead of by an IAA assay with acid-charcoal extraction [13]. Positivity for other antibodies is also predictive – albeit to a much lower degree and only in the absence of IA-2A.

How the appearance of IA-2A relates to underlying histopathological changes is not known, but our results suggest that IA-2A production coincides with a critical switch in disease progression. Given the fact that the intracellular domain of IA-2 is not expressed on the cell surface unless the cell is damaged it is remarkable that immunoassays using this intracellular domain as radioligand – as is the case in this assay – have shown the best diagnostic sensitivity both in a recent serum exchange programme [20] and in a study comparing various recombinant IA-2 fragments [21]. Furthermore, IA-2 expression is upregulated by glucose and this process was recently suggested to be mediated via paracrine effects of insulin [22]. It is thus conceivable that in a late prediabetic stage increasing blood glucose concentrations and/or beta-cell destruction could lead to higher local insulin discharge and IA-2 expression in residual islet cells activated to meet metabolic demands.

In our group of IA-2A positive siblings the time to clinical onset still ranged between 2 to 82 months. We therefore looked for additional determinants of the progression rate. No influence was seen for the number of antibodies detected in addition to IA-2A nor for the IA-2A activities – the latter finding being at variance with a previous study in children [4]. Positivity for the heterozygous high risk genotype *HLA DQ2/DQ8* tends to be associated with a more rapid progression in IA-2A siblings which is in agreement with observations in multiple antibody-positive Finnish siblings of affected children [23]. When we repeated our analysis after inclusion of 22 IA-2A positive subjects identified among 2378 nonselected offspring of Type I diabetic patients, the difference between *DQ2/DQ8* positive and *DQ2/DQ8* negative relatives became clearly significant (p=0.006) (data not shown). This is also compatible with the finding that in new-onset Type I patients the prevalences of IA-2A and *HLA-DQ2/DQ8* decrease with age at diagnosis [2, 24] and supports the contention that *HLA* class II polymorphisms determine the progression of the subclinical disease process rather than its initiation [25]. After adjustment for IA-2A status, the age of the siblings does not represent an independent determinant of progression to clinical disease phase, which is consistent with observations in multiple antibody-positive relatives [1].

Seroconversion to IA-2A-positivity seems, however, not to be a necessary condition to develop hyperglycaemia since we failed to detect this antibody specificity in almost 30% of prediabetic siblings. The sensitivity of IA-2A-screening for diabetes was 56% at first sampling and needs to be improved by repeated sampling, increasing IA-2A assay performance and/or identifying additional predictors of clinical onset in persistently IA-2A negative subjects. In this subgroup of siblings the initial positivity for IAA, GADA or ICA seems to be associated with a moderate (<10%) but significant progression to diabetes within 5 years (p<0.001). In siblings under age 40, GADA seemed a slightly more specific and sensitive marker of prediabetes in the absence of IA-2A than IAA or ICA. In very young subjects, IAA could be more informative [26, 27, 28]. In GADA positive but IA-2A negative siblings progression to diabetes was not influenced by age at first sampling, the number of other antibodies detected or *HLA-DQ* risk status, but larger numbers might be required to further settle this issue. Using Cox regression analysis the number of (molecular) antibodies was an important – albeit weak – predictor of diabetes but only in the absence of IA-2A.

Our findings challenge the common belief that multiple antibody positivity is the best predictor of Type I diabetes. We believe that the discrepancy with previous reports [1, 4, 7, 8, 9] derives from the unique way we analysed our data. Indeed, the Kaplan-Meier survival curves according to positivity or negativity for one of the four antibodies tested and according to the number of antibodies are very similar to the curves observed by another study [4] in unselected Finnish siblings, but unlike other authors we have stratified subjects with the same number of antibodies according to the type of antibodies detected. Our data are compatible with the observation that IA-2A are often the last to appear before clinical onset in relatives [29]. Moreover, it has been shown earlier that antibodies to 37,000 and 40,000 M_r tryptic fragments of islet antigens, related to IA-2-like proteins [30], are found in up to 80% of recent onset Type I patients and seem closely associated with progression to diabetes in ICA-positive identical twins [31], first degree relatives [32], patients with endocrine autoimmunity [33] or schoolchildren [34]. At variance with previous studies we did not include other types of first or second degree relatives [1, 7, 8, 9, 10] likely to differ in terms of genetic risk for diabetes [6], nor did we preselect subjects on basis of ICA positivity [1, 7, 9] or a state of prediabetes [1, 9]. The use of the intracellular domain of IA-2 as radioligand instead of the sequence derived from the ICA512bdc construct might provide an additional explanation [1, 10].

Taken together our results indicate that IA-2A positive siblings represent a small (<2%) high risk group

(60% risk of diabetes within 5 years); *HLA-DQ* genotyping could further increase the predictive power in these subjects. IA-2A positivity thus identifies relatives with a homogeneously high risk of diabetes and its use as an inclusion criterion in prevention trials is likely to reduce the number of antibody assays and the sample sizes needed. The level of risk conferred by IA-2A positivity is similar to that conferred by the combination of ICA positivity and loss of first phase insulin release during intravenous glucose tolerance testing in the Diabetes Prevention Trial-1 [35]. Preliminary results in IA-2A positive relatives indicate that beta-cell function as measured during a hyperglycaemic clamp followed by a glucagon bolus falls within the range observed for nondiabetic control subjects except for the minority of IA-2A relatives who develop glucose intolerance or diabetes within the next 12 months (E. Vandemeulebroucke et al., unpublished data). Compared to loss of first phase insulin release in ICA positive relatives, IA-2A positivity provides, however, a more reproducible [13, 36] and easily assessed inclusion criterion in prevention trials. Since IA-2A might precede a major loss of beta-cell function in at least part of the relatives its use as inclusion criterion in replacement of ICA positivity plus low first phase insulin release could offer better opportunities for beta-cell preservation in prevention trials. Further studies should also attempt to increase the sensitivity of IA-2A screening or to improve prediction of diabetes in IA-2A negative relatives.

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