An important minority of prediabetic first-degree relatives of type 1 diabetic patients derives from seroconversion to persistent autoantibody positivity after 10 years of age

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Abstract

Aims/hypothesis The appearance of autoantibodies (Abs) before diabetes onset has mainly been studied in young children. However, most patients develop type 1 diabetes after the age of 15 years. In first-degree relatives aged under 40 years, we investigated the frequency of seroconversion to (persistent) Ab positivity, progression to diabetes and baseline characteristics of seroconverters according to age.

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Methods Abs against insulin (IAA), glutamate decarboxylase (GADA), insulinoma-associated protein 2 (IA-2A) and zinc transporter 8 (ZnT8A) were measured during follow-up of 7,170 first-degree relatives.

Results We identified 379 (5.3%) relatives with positivity for IAA, GADA, IA-2A and/or ZnT8A (Ab⁺) at first sampling and 224 (3.1%) at a later time point. Most seroconversions occurred after the age of 10 years (63%). During follow-up, Abs persisted more often in relatives initially Ab⁺ (76%) than in seroconverters (53%; $p < 0.001$). In both groups diabetes developed at a similar pace and almost exclusively with Ab persistence (136 of 139 prediabetic individuals). For both groups, progression was more rapid if Abs appeared before the age of 10 years. Baseline characteristics at seroconversion did not vary significantly according to age.

Conclusions/interpretation Seroconversion to (persistent) Ab⁺ occurs regardless of age. Although the progression rate to diabetes is higher under age 10 years, later seroconverters (up to age 40 years) have similar characteristics when compared with age-matched initially Ab⁺ relatives and generate an important minority of prediabetic relatives, warranting their identification and, eventually, enrolment in prevention trials.

Keywords Autoantibodies · First-degree relatives · GAD · IA-2 · Insulin · Prediabetes · Proinsulin · Seroconversion · Type 1 diabetes · Zinc transporter 8

Abbreviations

Ab	Autoantibody
Ab ⁺	Positivity for IAA, GADA, IA-2A and/or ZnT8A
Ab ⁻	Negativity for IAA, GADA, IA-2A and ZnT8A
BDR	Belgian Diabetes Registry

CRCW	Hybrid ZnT8 construct generated by fusion of CR and CW (zinc transporter-8 carboxy-terminal constructs carrying respectively 325Arg and 325Trp)
GADA	Glutamate decarboxylase autoantibodies
IAA	Insulin autoantibodies
IA-2	Insulinoma-associated protein 2
IA-2A	IA-2 autoantibodies
ICA	Islet cell cytoplasmic antibodies
IQR	Interquartile range
ZnT8	Zinc transporter 8
ZnT8A	ZnT8 autoantibodies

Introduction

Type 1 diabetes is a heterogeneous disease in terms of underlying pathological process, clinical presentation and biological markers [1–3]. Several clinical studies in recent-onset patients have provided proof of principle that selective immuno-interventions may preserve at least temporarily residual beta cell function in subgroups of patients [4, 5]. They have also indicated that future immunomodulation trials should be targeting the preclinical stage, at which beta cell function is better preserved [5, 6]. Such secondary prevention studies are, however, complicated by our incomplete knowledge of the natural history of pre-type 1 diabetes, in particular for the majority of patients who develop the disease after the age of 15 years [7, 8]. Indeed, while several studies have followed newborn infants with or without family history of type 1 diabetes to study the appearance of different autoantibodies (Abs) and their relationship to the development of hyperglycaemia [9–13], data on the frequency of seroconversion to positivity for insulin Abs (IAA), glutamate decarboxylase Abs (GADA), insulinoma-associated protein 2 Abs (IA-2A) and/or zinc transporter 8 Abs (ZnT8A) (Ab^+) and the subsequent risk of diabetes in older children are scarce and data in young adults virtually absent [14, 15]. However, more knowledge on the latter topic is warranted as the launch of immuno-intervention trials in pre-type 1 diabetes will require the screening of large groups of participants in order to enrol sufficient numbers of high-risk individuals. In this context, it is important to know whether it is relevant to continue screening adolescents and young adults who have previously tested negative for Abs.

In the present study, we followed and repeatedly sampled a large representative group of first-degree relatives, initially with negativity for IAA, GADA, IA-2A and ZnT8A (Ab^- ; aged 0–39 years), of patients with type 1 diabetes recruited by the Belgian Diabetes Registry (BDR). The aims were to detect individuals who seroconverted to Ab^+ , to estimate the frequency of this phenomenon over a

wider age range than previously studied and to investigate the baseline characteristics of seroconverters and their progression to diabetes according to age at seroconversion in comparison with initially Ab^+ relatives.

Methods

Participants Between August 1989 and January 2010, the BDR consecutively recruited 9,040 siblings, offspring or parents (under age 40 years at entry) of type 1 diabetic probands according to previously defined criteria. The probands are considered representative of the Belgian population of type 1 diabetic patients [7]. After obtaining written informed consent from each relative or their parents, a short questionnaire with demographic, familial and personal information was completed at each visit and blood samples were taken at entry and, as a rule, yearly thereafter. Relatives who were Ab^- on four consecutive yearly visits were re-invited 4 years later. Only relatives with two or more contacts during follow-up (7,170 of 9,040), the last being at diagnosis in the case of prediabetes, were included in this study. This allowed unambiguous ascertainment of the clinical status of relatives at this last time point.

The study was conducted in accordance with the guidelines in the Declaration of Helsinki as revised in 2008 (www.wma.net/en/30publications/10policies/b3/index.html, accessed 8 September 2011) and approved by the ethics committees of the BDR and the participating university hospitals. Random blood samples were collected for sera, plasma and buffy coats, and aliquots were stored at -80°C until analysed for diabetes-associated autoantibodies, hormonal markers and *HLA-DQ* genotype, respectively, as previously described [16]. Relatives were longitudinally screened for the presence of IAA, GADA and IA-2A. Individuals who had at least one Ab^- sample before the development of IAA, GADA and/or IA-2A—seroconverters—were all sampled at least once after seroconversion to Ab^+ . ZnT8A were also determined in all samples from these relatives. Relatives were not pre-screened for islet cell cytoplasmic antibodies (ICA), nor were ICA results analysed in the present study. During follow-up of initially Ab^- first-degree relatives, the moment of seroconversion was approximated from the sampling time of the serum for which positivity for at least one Ab type was first detected. Both in seroconverters and in initially Ab^+ relatives, Ab^+ was defined as transient if the next sample was negative for all Abs; it was defined as persistent if the next sample was positive for at least one Ab type. The median (interquartile range [IQR]) of the time between the last Ab^- and the first Ab^+ sample was 13 months (12–24 months). During follow-up, development of diabetes was ascertained through repeated contacts with Belgian endocri-

nologists and paediatricians, self-reporting through yearly questionnaires and a link with the BDR patient database, where newly diagnosed patients under 40 years of age are registered. Follow-up ended at the time of the last blood sampling or, in the case of prediabetes, at clinical onset.

Analytical methods IAA, GADA, IA-2A and ZnT8A were determined by liquid-phase radiobinding assays [16], C-peptide by time-resolved fluorescence immunoassay [17], and *HLA-DQ* polymorphisms by allele-specific oligonucleotide genotyping [18], as described previously. The tracers for the Ab assays were purified by ultrafiltration (Amicon Ultra-4 filter units; Millipore, MA, USA) in the case of in vitro transcription/translation or by gel exclusion chromatography for A14-¹²⁵I-labelled insulin. Ab levels were expressed as percentages of bound added tracer (10,000 cpm/tube). cDNAs for the preparation of radioligands by in vitro transcription-translation were kind gifts of Å. Lernmark (when at University of Washington, Seattle, WA, USA) for full-length 65 kDa glutamate decarboxylase, M. Christie (King's College School of Medicine and Dentistry, London, UK) for the intracellular portion of insulinoma-associated protein 2 (IA-2) and J. C. Hutton (Barbara Davis Center for Childhood Diabetes, Aurora, CO, USA) for the dimeric hybrid ZnT8 construct generated by fusion of CR and CW (zinc transporter-8 carboxy-terminal constructs carrying, respectively, Arg325 and Trp325) (CRCW). In the 2009 Diabetes Autoantibody Standardisation Program (DASP) Workshop diagnostic sensitivity and specificity were, respectively, 74% and 97% for GADA, 40% and 98% for IAA, 66% and 99% for IA-2A and 68% and 100% for ZnT8A (CRCW). Cut-off values for Ab⁺ were determined as the 99th percentile of Ab levels in 761 non-diabetic controls, and amounted to $\geq 0.6\%$ tracer binding for IAA, $\geq 2.6\%$ for GADA, $\geq 0.44\%$ for IA-2A and $\geq 1.20\%$ for ZnT8A. Between-day coefficients of variation determined for serum pools within the normal range and within the moderately elevated range were, respectively, 35% (0.3% tracer binding) and 12% (6.9% tracer binding) for IAA, 12% (2.1% tracer binding) and 10% (7.1% tracer binding) for GADA, 18% (0.3% tracer binding) and 9% (2.3% tracer binding) for IA-2A, and 21% (0.7% tracer binding) and 6% (3.9% tracer binding) for ZnT8A.

Statistical analysis The statistical significance of differences between groups was assessed by the χ^2 test, with Yates' correction or Fisher's exact test for categorical variables and by Mann–Whitney *U* test for continuous variables. To estimate diabetes-free survival, Kaplan–Meier analysis was used; the survival curves were compared using the logrank test. In time-to-event analysis, follow-up 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. All statistical tests were performed two-tailed by SPSS for Windows 16.0 (SPSS, Chicago, IL, USA) or by GraphPad Prism version 5.00 for Windows (San Diego, CA, USA) and considered significant at $p < 0.05$ or $p < 0.05/k$ in case of multiple comparisons (Bonferroni correction).

Results

Seroconversion to Ab⁺ and progression to diabetes according to age Among the 7,170 first-degree relatives aged 0–39 years at inclusion and followed for a median (IQR) period of 60 (36–109) months, 603 (8.4%) tested positive at least once for one or more types of Ab (i.e. IAA⁺, GADA⁺, IA-2A⁺ and/or ZnT8A⁺) (Fig. 1). Of these 603 relatives, 379 (5.3% of all relatives) were positive at first sampling. Their median (IQR) age was 13 (7–24) years and the male/female ratio 185/194 (0.95; $p > 0.05$ vs 0.95 in all relatives). The remaining 224 (3.1%) relatives seroconverted at a median (IQR) age of 13 (7–22) years (male/female ratio: 123/101 or 1.22; $p > 0.05$ vs in all relatives) after a median (IQR) follow-up of 27 (15–52) months. The median (IQR) time between their last Ab[−] sample before seroconversion and the first Ab⁺ sample was 13 (12–24) months. Overall, 139/603 (23%) Ab⁺ relatives developed diabetes to date at a median (IQR) age of 14 (10–23) years and after a median (IQR) follow-up of 44 (20–82) months, including 109 (29%) of the 379 relatives who were Ab⁺ at baseline and 30 (13%) of the 224 seroconverters to Ab⁺. Three (0.05%) of the 6,567 persistently Ab[−] relatives also developed diabetes (Fig. 1).

Persistent vs transient Ab⁺ according to age The prevalence of initially Ab⁺ relatives ranged between 4.8% and 5.9% according to age ($p > 0.05$) and most were persistently Ab⁺ (289/379 or 76%), regardless of age (Table 1). In initially Ab[−] relatives most seroconversions (141 of 224 or

First-degree relatives enrolled between 1989 and 2010 with ≥ 2 visits for Ab screening

Ab⁺ in ≥ 1 sample

Ab⁺ in 1st sample

Diabetes

Fig. 1 Disposition diagram showing all participants in the present study, their Ab status and their progression to diabetes

Table 1 Frequency of seroconversion to Ab⁺ and development of diabetes according to age at seroconversion

Relatives	Age group		
	0–9 years	10–19 years	20–39 years
Total number (<i>n</i>)	2,581	2,055	2,534
Initially Ab [−] (<i>n</i>)	2,444	1,934	2,413
Ab ⁺ relatives			
Ab ⁺ at baseline, <i>n</i> (% of total)	137 (5.3)	121 (5.9)	121 (4.8)
Persistently Ab ⁺ , <i>n</i> (% of total)	105 (4.1)	94 (4.6)	90 (3.6)
Transiently Ab ⁺ , <i>n</i> (% of total)	32 (1.2)	27 (1.3)	31 (1.2)
Seroconverters, <i>n</i> (% of initially Ab [−]) ^a	83 (3.4)	79 (4.1)	62 (2.6)
Persistently Ab ⁺ , <i>n</i> (% of initially Ab [−]) ^b	50 (2.0)	39 (2.0)	29 (1.2)
Transiently Ab ⁺ , <i>n</i> (% of initially Ab [−])	33 (1.4)	40 (2.1)	33 (1.4)
Diabetes			
Ab ⁺ at baseline, <i>n</i> (% of group) ^c	56 (41)	32 (26) ^d	21 (17) ^e
Persistently Ab ⁺ , <i>n</i> (% of group) ^c	56 (53)	32 (34) ^f	20 (22) ^d
Transiently Ab ⁺ , <i>n</i> (% of group)	0 (0)	0 (0)	1 (3.2)
Seroconverters, <i>n</i> (% of group) ^g	18 (22)	7 (8.9)	5 (8.1)
Persistently Ab ⁺ , <i>n</i> (% of group) ^g	18 (36)	6 (15)	4 (14)
Transiently Ab ⁺ , <i>n</i> (% of group)	0 (0)	1 (2.5)	1 (3.0)
Median (IQR) ^h follow-up from Ab ⁺			
Ab ⁺ at baseline (months)	74 (43–124)	77 (36–124)	62 (38–112)
Persistently Ab ⁺ (months)	64 (37–110)	62 (36–115)	61 (37–122)
Transiently Ab ⁺ (months)	107 (61–140)	113 (71–139)	64 (48–100) ^h
Seroconverters, <i>n</i> (months) ^b	59 (25–92)	48 (20–96)	59 (24–89)
Persistently Ab ⁺ (months)	53 (21–84)	47 (16–96)	60 (14–115)
Transiently Ab ⁺ (months)	72 (34–100)	49 (23–96)	59 (25–88)

Threshold for significance: overall $p < 0.05/18$ or $p < 0.0028$; for differences among age groups: $p < 0.05/3$ or $p < 0.017$ (Bonferroni correction). ^aOverall $p = 0.020$; ^boverall $p = 0.042$; ^coverall $p < 0.001$; ^d $p = 0.015$; ^e $p < 0.001$; ^f $p < 0.010$ vs age group 0–9 years; ^goverall $p = 0.027$; ^hFollow-up time also limited by age limit

63%) occurred after the age of 10 years. The frequency ranged between 2.6% and 4.1% but did not vary significantly according to age (Table 1); this was also the case when 5-year age groups were considered (Electronic supplementary material [ESM] Fig. 1). Overall, 53% of seroconverters developed persistent Ab⁺ ($p < 0.001$ vs 76% in initially Ab⁺ relatives). This fraction tended to decrease from 60% under age 10 years to 48% after age 20 years, without reaching significance (Table 1). In both initially Ab⁺ relatives (108/109 or 99%) and seroconverters (28/30 or 93%) progression to diabetes occurred almost exclusively in persistently Ab⁺ relatives (Table 1 and ESM Fig. 1). Therefore, follow-up time from Ab⁺ was shorter in this group compared with transiently Ab⁺ relatives (Table 1). Progression to diabetes tended also to be more frequent in those seroconverting before the age of 10 years (Table 1 and ESM Fig. 1). This was not due to differences in follow-up time according to age (Table 1). Among the 109 initially Ab⁺ relatives who progressed to diabetes, at least 56 (51%) had developed Abs before age 10 years compared with 18 of 30 (60%) prediabetic seroconverters (Table 1). Two relatives who developed initially transient Ab⁺ at ages 12 and 24 years, respectively, progressed to diabetes 7 and 8 years later, respectively, but not before having become Ab⁺ again

at an undefined later time point (Table 1). In 21 of the 30 prediabetic seroconverters (70%), diabetes was diagnosed after age 10; 12 of these 21 (57%) had seroconverted after age 10 years and 5 of them (24%) after age 20 years (data not shown).

After seroconversion to persistent Ab⁺, progression to diabetes occurred at a pace that was not significantly different from that in initially Ab⁺ relatives (Fig. 2a). In both groups the progression rate decreased with age at first Ab⁺ (Fig. 2b, c).

Baseline characteristics of seroconverters to persistent Ab⁺ according to age Overall, there was a tendency towards a male excess in the seroconverters (male/female ratio: 69/49 or 1.41; $p = 0.044$ vs 0.95 in all relatives). As shown in Table 2, the male/female ratio tended to decrease with age at seroconversion but significance was lost after correction for multiple comparisons; compared with age-matched Ab[−] relatives the ratio was only higher for seroconversion under age 10 years (2.33 vs 1.14; $p = 0.027$). Young seroconverters more often tended to be IAA⁺, IA-2A⁺ and/or ZnT8A⁺, but in the case of Ab⁺, circulating levels did overall not differ according to age (Table 2). During follow-up of persistently Ab⁺ relatives there was no fixed sequence

Fig. 2 Diabetes-free survival of persistently Ab⁺ relatives as a function of time after first Ab⁺ sample. **a** Ab⁺ relatives at baseline (solid line, $n=289$ at time 0) vs seroconverters to Ab⁺ (dashed line, $n=118$ at time 0) ($p=0.505$ by logrank). **b** Ab⁺ relatives at baseline according to age at inclusion (solid line, 0–9 years; dashed line,

10–19 years; dotted line, 20–39 years; $p=0.003$ by logrank). **c** Seroconverters to Ab⁺ according to age at seroconversion (solid line, 0–9 years; dashed line, 10–19 years; dotted line, 20–39 years; $p=0.042$ by logrank)

for the appearance of the various types of Ab (data not shown), but IA-2A and ZnT8A tended to develop more often after the first biological evidence of the autoimmune process (up to more than 7 years later; ESM Fig. 2) in all age categories (data not shown).

Discussion

During follow-up of over 7,000 first-degree relatives of patients with type 1 diabetes we identified 379 Ab⁺

relatives at first sampling and 224 individuals who seroconverted to islet Ab⁺. Our main finding was that seroconversion can occur at any age between 0 and 40 years at a rate that does not significantly differ according to age, with most events occurring after age 10. During follow-up, Abs persisted more often in initially Ab⁺ relatives than in seroconverters. Both groups developed diabetes at a similar pace and almost exclusively in the presence of Ab persistence. For both groups, progression was more rapid if Abs were first detected under the age of 10 years. The baseline characteristics of relatives at seroconversion did not differ greatly

Table 2 Demographic and biological baseline characteristics of first-degree relatives who seroconverted to persistent Ab⁺ according to age at seroconversion

Characteristic at seroconversion	Persistently Ab ⁺ seroconverters ($n=118$)		
	0–9 years	10–19 years	20–39 years
n (%)	50 (42)	39 (33)	29 (25)
Age (years), P_{50} (IQR)	6 (4–8)	14 (12–16)	29 (22–34)
Sex, n males/ n females (ratio) ^a	35/15 (2.33)	22/17 (1.29)	12/17 (0.70)
Relationship to type 1 diabetes, n (%) ^b			
Parent	0 (0)	0 (0)	19 (31)
Offspring type 1 diabetes mother	12 (24)	12 (31)	6 (21)
Offspring type 1 diabetes father	16 (32)	12 (31)	5 (17)
Sibling	22 (44)	15 (38)	12 (41)
C-peptide (pmol/l), P_{50} (IQR)	645 (374–943)	731 (539–1,155)	867 (583–1,162)
<i>HLA-DQ2/DQ8</i> , n (%)	12 (24)	8 (20)	6 (21)
Ab type, n (%)			
IAA ⁺	26 (52)	15 (38)	10 (34)
GADA ⁺	36 (72)	29 (74)	23 (79)
IA-2A ⁺ ^c	11 (22)	6 (15)	0 (0)
ZnT8A ⁺	7 (14)	6 (15)	0 (0)
Multiple Ab ⁺ , n (%) ^c	21 (42)	10 (26)	4 (14)
Ab levels ^d (% tracer-bound), P_{50} (IQR)			
IAA ⁺	1.3 (0.8–2.3)	1.1 (0.8–2.0)	0.9 (0.8–1.2)
GADA ⁺	9.9 (4.8–36.1)	15.1 (3.6–137)	7.6 (3.6–79.7)
IA-2A ⁺	6.9 (1.7–83.4)	50 (1.1–82.1)	NA
ZnT8A ⁺	5.2 (2.5–14.2)	2.4 (1.7–12.4)	NA

Threshold for significance: overall $p<0.05/14$ or $p<0.0037$, for differences among age groups: $p<0.05/3$ or $p<0.017$ (Bonferroni correction).

^aOverall $p=0.043$; ^boverall $p=0.002$; ^coverall $p<0.030$

^dOnly in relatives positive for that particular Ab

NA, not applicable; P_{50} , median

according to age. In seroconverters, IA-2A and ZnT8A tended to appear later during the subclinical disease process, compatible with their association with—and prediction of—rapid progression to clinical onset [16, 19–21].

The strengths of this study are: (1) its longitudinal nature; (2) the registry-based recruitment of first-degree relatives over a wide age range, when little or no data exist on this topic for adults [14, 15]; (3) the possibility of comparing the clinical outcome of seroconverters with those of Ab⁺ relatives at first sampling; (4) the confirmation of the glycaemic status at the last follow-up point for each relative; (5) the completeness of hormonal, genetic and immunological data for each participant, including results from a sensitive ZnT8A assay, which has so far only rarely been used in longitudinal studies in risk groups; and (6) the lack of selection bias in the absence of pre-screening for ICA.

However, this study also has certain weaknesses. At variance with some other studies in children [8–12] few participants were followed from birth onwards, hence previous transient seroconversions may have been missed. Conceivably, the number of young seroconverters and rapidly progressing prediabetic young children may have been underestimated. However, the fact that seroconversion frequency was largely independent of age and that the rate of progression to diabetes was quite similar to that in age-matched initially Ab⁺ relatives supports the significant contribution of seroconversion after age 10 to incident cases of type 1 diabetes in adolescence and young adulthood. The exact fraction of all 139 Ab⁺ prediabetic relatives who derived from seroconversion after age 10 cannot be precisely determined from the present study: indeed, while we know that all initially Ab⁺ relatives under age 10 must have seroconverted before that age, we do not know how many of the older initially Ab⁺ relatives derived from late seroconversion. Establishing the precise time of seroconversion and the order of appearance of various Ab types was limited by the relatively large intervals between successive blood samples. As a rule these intervals approximated 12 months, but in a minority of cases they spanned several years. Therefore, the age at seroconversion may have been overestimated at times. However, our conclusions remained unchanged if the age at positive seroconversion was approximated from the age at the last Ab[−] sample (not shown).

Our study design may also have been limited by the absolute number of established seroconversions leading to clinical appearance of type 1 diabetes, which may have decreased the power of subgroups analysis. However, because of the size of our registry and the study of first-degree relatives our numbers of seroconverters compare well with those observed in previous studies in young children or in the general population [13, 14, 20]. One may

also criticise the fact that we did not include ICA in our analysis as did some other studies [9, 22]. This was a conscious choice because ICA are not completely independent of GADA, IA-2A and ZnT8A, which are all believed to contribute to ICA reactivity [19, 23, 24]. ZnT8A were only determined in all samples of relatives who tested positive on at least one occasion for other islet Abs, but previous results have shown that their prevalence in relatives lacking the other Abs is virtually zero [16, 25].

To the best of our knowledge, our results are the first to compare seroconversion frequencies for islet Abs in children, adolescents and adults at familial risk of diabetes. The age independency of the seroconversion frequency warrants regular reassessment of Ab-inferred risk of diabetes in first-degree relatives up to 40 years of age in the context of a further in-depth study of the preclinical phase of type 1 diabetes in adults and of identifying additional potential participants in new secondary prevention trials. Our results are also compatible with previous findings indicating that an early appearance of Abs and Ab persistence are preferentially associated with a rapid progression to clinical onset [9, 20, 26–30]. The infrequent progression to diabetes in case of transient Ab⁺ may relate to false-positive results due to the relatively high imprecision of Ab assays in the decision zone, particularly for IAA (see [Methods](#)) or to ‘statistical’ positivity due to the choice of the 99th percentile as cut-off value. However, in some cases Ab levels were clearly transiently elevated and may have reappeared later, suggesting that autoimmunity may at times follow a relapsing/remitting pattern and in other instances a more aggressive and progressive course, similar to that reported for other autoimmune diseases such as multiple sclerosis [31]. IAA and GADA were confirmed as relatively early immune markers in prediabetes, but only GADA or multiple Ab⁺ were predictive of Ab persistence (data not shown). The tendency towards later appearance of IA-2A and ZnT8A is compatible with their reported association with a more rapid progression to diabetes [16, 19, 20, 32–35]. Overall, there was a slight male excess in seroconverters compatible with the male predominance in type 1 diabetes under age 40 [3, 7, 8]; when adjusted for sex ratio in age-matched groups of all included relatives, this male excess was only significant in case of seroconversion under age 10. The trend towards more multiple Ab⁺ in those seroconverting under age 10 years is compatible with the more rapid progression to diabetes in this age group [20, 22]. Finally, the seroconversion rates observed for children and adolescents in the present study are similar to recent data from DPT-1 and TrialNet cohorts [36, 37] but the latter studies were limited to children under age 18, included also second- [36, 37] or third-degree [37] relatives and did not distinguish between seroconversion to transient or persistent Ab⁺. At variance with these reports, the

seroconversion rate in our study only tended to decrease after the age of 20 years.

In conclusion, the frequency of seroconversion approximates to 3% regardless of age in first-degree relatives under age 40 years, with most events occurring after age 10 years. As in initially Ab⁺ relatives, progression rate to diabetes was highest for those seroconverting before age 10 years and occurred almost exclusively in persistently Ab⁺ relatives. However, an important minority of prediabetic relatives derived from seroconversion after age 10 years. As only about 15% of all new patients have a family history of the disease, further studies should investigate whether our conclusions in relatives also hold for the majority of sporadic cases. Reports that patients with or without familial history of type 1 diabetes have quite similar characteristics [38, 39] suggest that this may indeed be the case. Our data on Ab development and persistence should be taken into account when planning further prediction and prevention studies and warrant continued monitoring of Ab status up to at least 40 years in risk groups such as first-degree relatives.

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Contribution statement IV designed research, acquired, analysed and interpreted data, provided statistical analysis and reviewed/edited the manuscript; IW designed research, recruited first-degree relatives, analysed and interpreted data and reviewed/edited the manuscript; OC, MA and KV acquired, analysed and interpreted data and reviewed/edited the manuscript; KD, JR, and KC designed research, recruited

first-degree relatives, contributed clinical data and reviewed/edited the manuscript; JW and JCH contributed new reagents/analytical tools, analysed and interpreted data, contributed to discussion and reviewed/edited the manuscript; DGP designed research, analyzed and interpreted data, contributed to discussion and reviewed/edited the manuscript; FKG designed research, obtained funding, supervised the study, analysed and interpreted data, wrote and reviewed/edited the manuscript. All authors have approved the final version of the manuscript.

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