ARTICLE

Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes

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Abstract

Aims/hypothesis Seroconversion to islet autoantibodies precedes type 1 diabetes. This study aimed to identify periods of high seroconversion incidence, which could be targeted for mechanistic and therapeutic studies.

Methods Incidence of islet autoantibodies was calculated in 1,650 genetically at-risk children followed with measurements of islet autoantibodies and thyroid autoantibodies at age 9 months and 2, 5, 8, 11, 14 and 17 years. Peak incidence periods were confirmed in a second cohort of 150 children followed until age 6 years with three-monthly samples up to age 3 years.

Results Islet autoantibody incidence (per 1,000 person-years) was 18.5 until age 9 months, 21 from 9 months to 2 years and <10 for intervals after age 2 years. The second cohort confirmed peak incidence around age 9 months and demonstrated an absence of seroconversion before this age. Seroconversion to insulin autoantibodies occurred earlier than other autoantibodies ($p<0.01$ against glutamic acid decarboxylase [GAD]-, insulinoma-associated protein 2 [IA-2]- and zinc transporter 8 [ZnT8]-autoantibodies). Early peak seroconversion incidence was most evident in children with high-risk *HLA DR3/4-DQ8* or *DR4/4-DQ8* genotypes.

Conclusion The age period 9 months to 2 years is associated with a high incidence of activation of type 1 diabetes-associated autoimmunity in genetically at-risk children and should be targeted for effective primary prevention strategies.

Abbreviations

GAD	Glutamic acid decarboxylase
GADA	Glutamic acid decarboxylase autoantibodies
IA-2	Insulinoma-associated protein 2
IA-2A	Insulinoma-associated protein 2 autoantibodies
IAA	Insulin autoantibodies
TPO	Thyroid peroxidase
ZnT8	Zinc transporter 8
ZnT8-A	Zinc transporter 8 autoantibodies

A list of the BABYDIAB-BABYDIET Study Group authors is provided in the Appendix.

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Introduction

Type 1 diabetes is an autoimmune disorder in which disease onset is preceded by a pre-clinical period of islet autoimmunity [1]. The initiation of islet autoimmunity is heralded by seroconversion for islet autoantibodies directed against insulin, glutamic acid decarboxylase (GAD), insulinoma-associated protein 2 (IA-2) and zinc transporter 8 (ZnT8) [2–5]. Progression to clinical disease after seroconversion is variable and associated with the diversity and specificity of islet autoantibodies [6, 7] as

well as genetic determinants of type 1 diabetes [8, 9]. While much is known with respect to the incidence of type 1 diabetes with the notable rise over recent decades and a peak age of incidence around puberty [10], little is known about the incidence of islet autoimmunity and its relationship to age. Understanding periods of high and low incidence of seroconversion and their relationships with demographics and genes will provide insights into potential pathogenetic mechanisms and environmental triggers, and identify ages at which prevention strategies are most likely to be needed. We have prospectively followed from birth children who are genetically at risk for type 1 diabetes for more than 20 years, providing the opportunity to examine the incidence of sero-conversion during childhood and adolescence. Here we show that seroconversion is greatest in the age period 9 months to 2 years, is rare at ages 6 months or younger, occurs earlier in boys and occurs earliest for insulin autoantibodies. These data emphasise the importance of developing immune therapies that can be safely used in neonates and infants.

Methods

Study cohort, participants and samples The study was performed in children from the BABYDIAB Study, a longitudinal study examining the natural history of islet autoimmunity and type 1 diabetes in 1,650 children born to a mother or father with type 1 diabetes [11]. Recruitment began in 1989 and ended in 2000. All children (840 boys, 810 girls) were recruited from Germany. The cohort is not population based and 97% of families are German and of European descent. Venous blood samples were obtained from children at study visits scheduled at 9 months and at 2, 5, 8, 11, 14 and 17 years of age. Islet autoantibodies (insulin autoanti-bodies [IAA], GAD autoantibodies [GADA], IA-2 autoanti-bodies [IA-2A] and ZnT8 autoantibodies [ZnT8-A]) were measured in samples taken at all scheduled visits, and every 6 months in children positive for islet autoanti-bodies. The median follow-up time from birth to last sample was 11.1 years (interquartile range, 5.7–14.0 years) and from birth to last contact was 13.9 years (interquartile range, 11.9–15.6 years).

As a confirmatory cohort, islet autoantibody incidence was examined in 150 children who participated in the BABYDIET Study [12]. These children were selected for having both a first-degree relative with type 1 diabetes and type 1 diabetes risk HLA genotypes. Children were recruited between 2000 and 2004 and followed from the age of 3 months with three-monthly blood samples until age 3 years and subsequently with yearly blood samples. The median follow-up time from birth to last sample was 5.2 years (interquartile range, 3.2–6.6 years). The BABYDIET Study was a dietary intervention study in which children were randomised to be first exposed to gluten-containing foods at age 6 or 12 months (ClinicalTrials.gov NCT01115621). No difference in islet autoantibody development was observed with respect to randomisation group [13].

The BABYDIAB and BABYDIET studies were approved by the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer No. 95357 and Ludwig-Maximilians University No. 329/00, respectively). All families gave written informed consent to participate in the studies. Investigations were carried out in accordance with the principles of the Declaration of Helsinki, as revised in 2000.

Autoantibody measurements IAA, GADA, IA-2A and ZnT8-A were determined centrally by the Institute of Diabetes Research Munich using radiobinding assays as previously described [11, 14]. Briefly, IAA were measured by protein A/G radiobinding assays using [^{125}I]-recombinant human insulin labelled at A-14 tyrosine, and GADA, IA-2A and ZnT8-A were measured separately by protein A radiobinding assays using [^{35}S]methionine in vitro transcribed/translated recombinant human GAD65, IA-2ic and the carboxy terminal portion of ZnT8 for the two major variants at amino acid 325 (arginine and tryptophan), respectively. The upper limit of normal for each assay was determined using Q–Q plots and corresponded to the 99th percentile of 836 control children. Islet autoantibody assays were evaluated by the Diabetes Autoantibody Standardization Program (laboratory 121) [15, 16]. Thyroid peroxidase (TPO) autoantibodies were measured using a direct radiobinding assay according to the manufacturer's instructions (CentAK anti-TPO, Medipan, Dahlewitz/Berlin, Germany) as previously described [17].

Genotyping HLA-DRB1, HLA-DQA1 and *HLA-DQB1* alleles were typed using PCR-amplified DNA and non-radioactive sequence-specific oligonucleotide probes as described previously [18]. Children with the high-risk HLA genotypes *DR4-DQA1*030X-DQB1*0302/DR3-DQA1*0501-DQB1*0201* or *DR4-DQA1*030X-DQB1*0302/DR4-DQA1*030X-DQB1*0302* were compared with children with other HLA genotypes.

Statistical analysis Children were classified as islet auto-antibody positive if they were positive for one or more islet autoantibodies in at least two consecutive samples. The age of the first autoantibody-positive sample was considered the seroconversion age in children. If a child was positive in the first sample measured, autoantibodies in the child's mother were compared and, if positive, the child sample was classified as negative on the basis that the positive result was likely to be due to placental transfer of maternal autoantibodies. For some analyses, children were also classi-fied as multiple islet autoantibody positive if they were posi-tive for more than one of the antibodies measured on at least

one occasion, or as single islet autoantibody positive if they were not. For these subanalyses, bias due to different follow-up times in children who seroconverted at different ages was reduced by excluding single islet autoantibody-positive children who had not been followed for at least 3 years after seroconversion (n=19), and children who converted to multiple islet autoantibodies after 3 years of initial seroconversion (n=8).

Islet autoantibody incidence and TPO autoantibody incidence in the BABYDIAB cohort was calculated from the number of children who newly seroconverted to islet autoantibody positivity in samples obtained at 9 months (mean age of sample, 0.85 years), 2 years (mean age of sample, 2.0 years), 5 years (mean age of sample, 5.0 years), 8 years (mean age of sample, 8.0 years), 11 years (mean age of sample, 11.0 years) and 14 years (mean age of sample, 14.0 years) and the number of children followed at each time point, and by subsequently calculating incidence as cases per 1,000 per year (per 1,000 person-years) for each time interval. As an example, if 1,500 children remained negative at their 9 month visit (median age at 9 month visit was 0.85 years) and reached their 2 year follow-up (median age at 2 year visit was 2.0 years), and 25 of these children became positive at age 2 years, the incidence for the period 9 months to 2 years would

Fig. 1 Autoantibody incidence (cases per 1,000 person-years) in offspring of parents with type 1 diabetes (BABYDIAB Study). Incidence is shown at the ages of islet autoantibody testing (9 months and 2, 5, 8, 11 and 14 years) and refers to the age intervals between these time points. a Incidence of islet autoantibodies (AAb; any of IAA, GADA, IA-2A and ZnT8-A). b Incidence of islet autoantibodies in boys (solid line) and girls (dashed line). c Incidence of islet autoantibodies in offspring born between 1989 and 1995 (solid line) and 1996 and 2000 (dashed line). d Incidence of TPO autoantibodies (AAb). e Incidence of TPO autoantibodies in boys (solid line) and girls (dashed line). Numbers under the x-axes are the numbers of children still followed at each time point
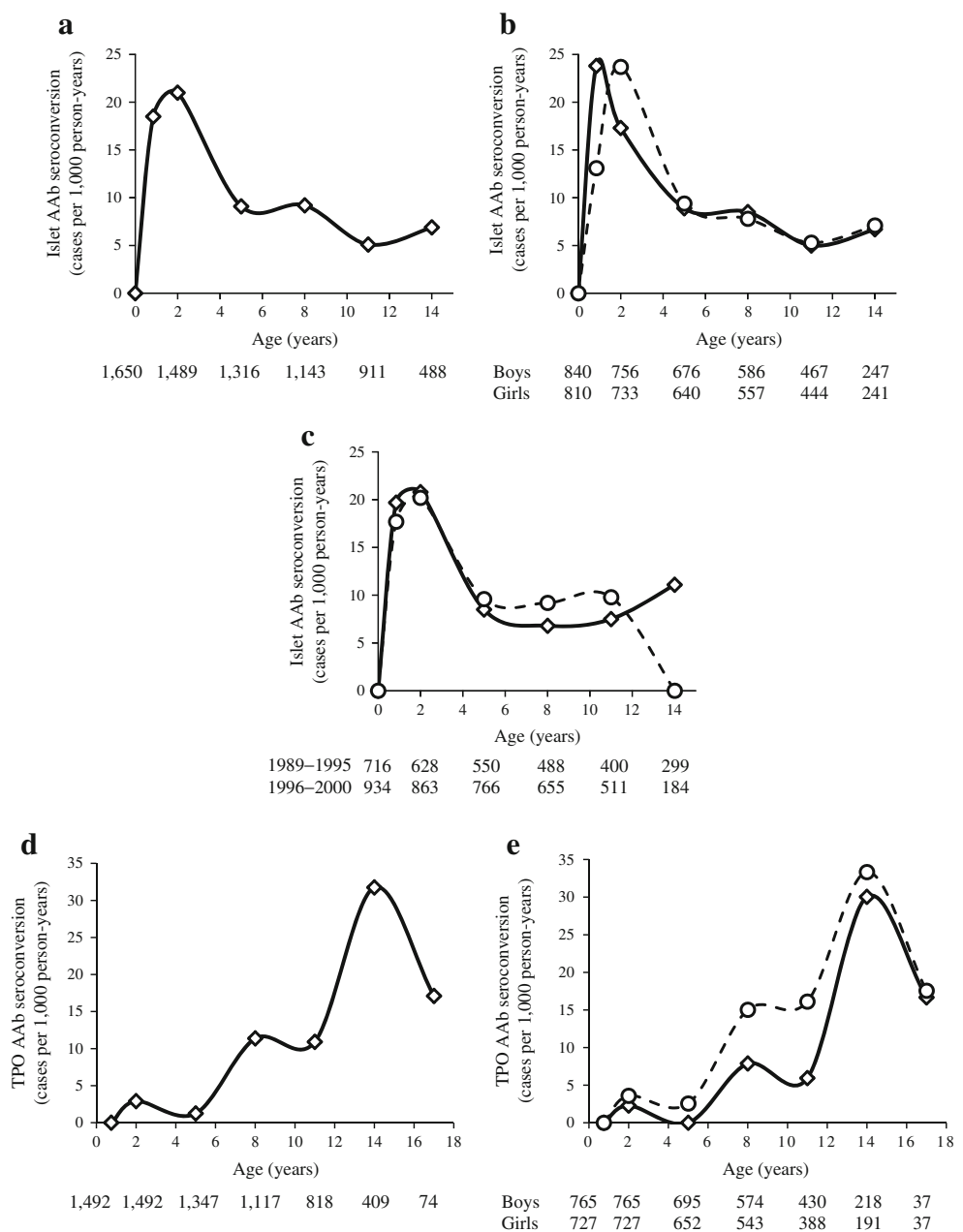
be $25/1{,}500/(2-0.85)\times1{,}000=14.5$ per 1,000 person-years. Intervals used for the BABYDIET cohort were 0 to 6, 6.1 to 12, 12.1 to 18, 18.1 to 24, 24.1 to 36, and 36.1 to 60 months. The 95% CIs are provided around the antibody incidences.

Comparisons of islet autoantibody incidence between groups were performed using Fisher's exact test using the actual number of cases. Time to event analyses (Kaplan–Meier) were used to examine progression from first islet autoantibody to diabetes. Kaplan–Meier analysis was used to calculate risk and to compare probabilities of type 1 diabetes in children stratified for age of seroconversion. The time from the age of seroconversion to the age at diagnosis of diabetes or the age at last follow-up was used as the event time. The logrank test was used to compare categories in the Kaplan–Meier analysis. All statistical analyses were performed using the Statistical Package for Social Science (SPSS 17.0; Chicago, IL, USA).

Results

A total of 152 children in the BABYDIAB cohort developed islet autoantibodies during follow-up; 26 seroconverted at age 9 months, 34 at age 2 years, 38 at 5 years, 28 at 8 years, 14 at 11 years, 10 at 14 years and two after 17 years. The islet autoantibody incidence (95% CI) was high at age 9 months (18.5 [12.7, 27] per 1,000 person-years) and 2 years (21 [15.9, 29] per 1,000 person-years) and decreased thereafter (9.1 [6.3, 12.5] per 1,000 person-years at 5 years; 9.2 [5.7, 11.7] per 1,000 person-years at 8 years; 5.1 [3.1, 8.5] per 1,000 person-years at 11 years; 6.9 [3.8, 12.4] per 1,000 person-years at 14 years; Fig. 1a). Although boys and girls had similar overall risks for islet autoantibodies, the peak

incidence in boys was observed at the 9 month sample and in girls at the 2 year sample (Fig. 1b). The year of birth did not affect islet autoantibody incidence (Fig. 1c). As a comparison, the incidence of seroconversion to thyroid autoimmunity (TPO autoantibodies) did not rise until puberty and reached peak levels at adolescence (Fig. 1d). Unlike islet autoantibodies, the incidence of TPO autoantibodies at early age was higher in girls than in boys ($p=0.002$; Fig. 1e).

Similar changes in incidence by age (95% CI) were seen for all four islet autoantibodies with the highest respective incidence at age 2 years (IAA, 18.7 [13.3, 26.2] per 1,000 person-years; GADA, 18.5 [13.1, 25.9] per 1,000 person-years; IA2A, 13.8 [9.3, 20.4] per 1,000 person-years; ZnT8-A, 13.3 [8.9, 19.8] per 1,000 person-years). However, IAA developed earlier with a higher incidence at age 9 months than other islet autoantibodies (16.4 [10.9, 24.5] per 1,000 person-years vs 5 [2.5, 10.2] for GADA, 2.1 [0.8, 6.2] for IA-2A and 5 [2.5, 10.2] for ZnT8-A; $p=0.005$, $p<0.0001$ and $p=0.005$, respectively; Fig. 2a). The incidence of multiple islet autoantibodies was high and pronounced at 9 months and 2 years compared with age 5, 8, 11 and 14 years (Fig. 2b). In contrast, the incidence of single islet autoantibodies was relatively stable throughout follow-up, and at age 9 months and 2 years was lower that the incidence of multiple islet autoantibodies (Fig. 2b).

Children with the *HLA DR3/4-DQ8* or *DR4/4-DQ8* genotypes had a much higher incidence of islet autoantibodies from age 9 months to 5 years compared with children without these genotypes ($p<0.0001$ for 9 months, $p<0.0001$ for 2 years, $p=0.0006$ for 5 years; Fig. 3a). A clear peak incidence was observed at 9 months to 2 years of age in children with the high-risk genotypes.

IAA	1,650	1,492	1,323	1,163	936	503
GADA	1,650	1,508	1,338	1,171	943	502
IA-2A	1,650	1,512	1,348	1,198	971	523
ZnT8-A	1,650	1,508	1,345	1,186	972	522

| Multiple | 1,650 | 1,489 | 1,316 | 1,143 | 960 | 483 |
| Single | 1,650 | 1,511 | 1,364 | 1,214 | 987 | 500 |
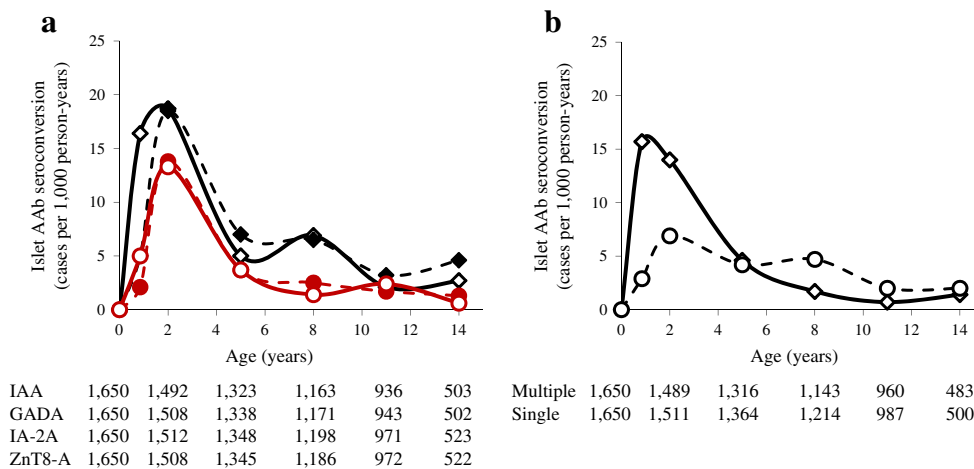
Fig. 2 Incidence (cases per 1,000 person-years) of specific islet autoantibodies (AAb). **a** IAA (solid black line), GADA (dashed black line), IA-2A (solid coloured line) and ZnT8-A (dashed coloured line) in offspring of parents with type 1 diabetes (BABYDIAB Study).

b Incidence of multiple (solid line) and single (dashed line) islet autoantibodies. Incidence is shown at the ages of islet autoantibody testing (9 months and 2, 5, 8, 11 and 14 years). Numbers under the *x*-axes are the numbers of children still followed at each time point
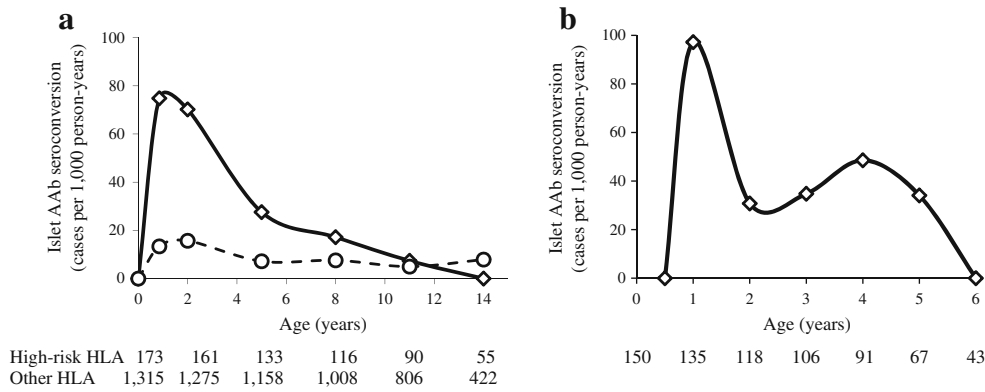
Fig. 3 a Incidence of islet autoantibodies (AAb; cases per 1,000 person-years) in offspring of parents with type 1 diabetes relative to HLA genotype (BABYDIAB Study). Children with the *DR3/4-DQ8* or *DR4/4-DQ8* genotypes (solid line) had higher incidence levels than children with other HLA genotypes (dashed line) during early childhood. Numbers under the *x*-axis are the numbers of children still followed at each time point. **b** Incidence of islet autoantibodies (any of IAA, GADA, IA-2A or ZnT8-A) in 150 children with first-degree relatives with type 1 diabetes and high-risk HLA genotypes participating in the BABYDIET Study. Incidence is shown at ages 6 and 12 months and 2, 3, 4, 5 and 6 years and refer to the age intervals between these points. Numbers under the *x*-axis are the numbers of children still followed at each time point

In order to confirm the findings from the BABYDIAB cohort and to refine incidence from birth to age 3 years, islet autoantibody incidence was determined in the BABYDIET cohort (Fig. 3b). These children had a higher overall increased risk of developing islet autoantibodies than BABYDIAB children and had samples collected at 3 month intervals until age 3 years. No child seroconverted to islet autoantibody positivity at age 3 or 6 months. Peak incidence was observed in the 6.1 to 12 months age period (97 [95% CI 46, 186] per 1,000 person-years; $p=0.006$ vs 0 to 6 months). Incidences for each 12 month period after age 12 months were less than 50 per 100 person-years ($p=0.02$; 6.1 to 12 months vs 12.1 to 72 months). Similarly to observations in the BABYDIAB cohort, the incidence in the 6 to 12 month age period (95% CI) tended to be higher in boys than in girls (159 [66, 326] vs 48 [15, 166] per 1,000 person-years; $p=0.2$).

To determine whether seroconversion during the early peak incidence period was associated with a high risk for type 1 diabetes, we analysed the rate of progression to type 1 diabetes in BABYDIAB children who developed islet autoantibodies at the age of 9 months, at the age of 2 years and after the age of 2 years (Fig. 4). Progression was high and faster in children who seroconverted during the peak incidence period compared with children who developed islet autoantibodies during a low incidence period (progression within 10 years [95% CI]: 46% [27%, 65%] for seroconverters at 9 months; 54% [38%, 70%] for seroconverters at age 2 years; and 31% [16%, 46%] for seroconverters after age 2 years; $p=0.03$ and $p=0.006$ for comparisons against seroconverters after age 2 years, respectively). Moreover, 30 (83%) of 36 children who developed diabetes by age 10 years had seroconverted to islet autoantibody positivity at 9 months or 2 years.

Discussion

The incidence of type 1 diabetes has its peak around puberty. Here, we show that, in genetically at-risk individuals, the incidence of islet autoantibody seroconversion has a peak at 9 months to 2 years of age. This peak incidence period is preceded by a period during which islet autoantibody seroconversion is rare, and is followed by a relatively low incidence period in which seroconversion is often characterised by single islet autoantibodies. The findings imply a heightened propensity to activate type 1 diabetes relevant autoimmunity between the ages of 9 months and 2 years, and suggest an increased susceptibility
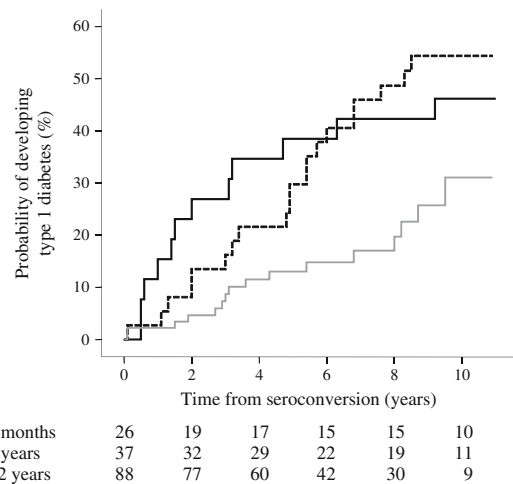
Fig. 4 Risk for type 1 diabetes relative to the age of islet autoantibody seroconversion. Kaplan–Meier analysis for the progression to type 1 diabetes from the age of first appearance of islet antibodies in children who seroconverted at age 9 months (solid line), 2 years (dashed line) and after age 2 years (grey line). Numbers under the *x*-axis are the numbers of children still followed at each time point

Appendix

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