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**The Effect of Age on the Progression and Severity of Type 1 Diabetes:
Potential Effects on Disease Mechanisms**

Pia Leete¹, Roberto Mallone², Sarah J. Richardson³, Jay M. Sosenko⁴, Maria J. Redondo⁵,
and Carmella Evans-Molina⁶

¹Islet Exeter (IBEx), Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, Exeter, UK; P.Leete@exeter.ac.uk

²INSERM U1016, CNRS UMR8104, Cochin Institute, Paris Descartes University, Sorbonne Paris Cité; Assistance Publique Hôpitaux de Paris, Service de Diabétologie, Cochin Hospital, Paris, France; roberto.mallone@inserm.fr

³Islet Exeter (IBEx), Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, Exeter, UK; S.Richardson@exeter.ac.uk

⁴Department of Medicine and the Diabetes Research Institute, Leonard Miller School of Medicine, University of Miami, Miami, FL, USA; jsosenko@med.miami.edu

⁵Department of Pediatrics, Baylor College of Medicine and the Texas Children's Hospital, Houston, TX, USA; redondo@bcm.edu

⁶Departments of Medicine and Pediatrics, and the Herman B Wells Center for Pediatric Research, Indiana University School of Medicine and the Roudebush VA Medical Center, Indianapolis, IN, USA; cevansmo@iu.edu

To Whom Correspondence Should be Addressed: Carmella Evans-Molina, MD, PhD (cevansmo@iu.edu), Indiana University School of Medicine, 635 Barnhill Drive, MS 2031A, Indianapolis, IN 46202; Telephone: (317) 274-4145, Fax (317) 274-4107

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ABSTRACT

Purpose of review: To explore the impact of age on type 1 diabetes (T1D) pathogenesis.

Recent findings: Children progress more rapidly from autoantibody positivity to T1D and have lower C-peptide levels compared to adults. In histological analysis of post-mortem pancreata, younger age of diagnosis is associated with reduced numbers of insulin containing islets and a hyper-immune CD20^{hi} infiltrate. Compared to adults, children exhibit decreased immune regulatory function and increased engagement and trafficking of autoreactive CD8⁺ T-cells, and age-related differences in β -cell vulnerability may also contribute to the more aggressive immune phenotype observed in children. HLA and non-HLA genetic loci that influence multiple disease characteristics, including age of onset, are being increasingly characterized.

Summary: The exception of T1D as an autoimmune disease more prevalent in children than adults results from a combination of immune, metabolic, and genetic factors. Age-related differences in T1D pathology have important implications for better tailoring of immunotherapies.

Introduction

Type 1 diabetes is a disease characterized by autoimmune-mediated destruction of the pancreatic β cell that affects 0.3% of the population (1). The incidence of T1D peaks between 5-9 years of age, with a second peak occurring near puberty (2). Globally, incidence is increasing at rate of approximately 2-3% per year (3, 4), and recent data have identified a disproportionate increase among very young children <5 years of age (2, 5, 6). Thus, T1D stands alone in the pantheon of autoimmune disease for its incidence, which decreases rather than increases with age. In addition to age-related heterogeneity in disease epidemiology, accumulating evidence also suggests a potential contribution of age on T1D pathophysiology and response to disease-modifying therapies. Here, we review the genetic, metabolic, and immunologic underpinnings of the impact of age on T1D risk, progression, and aetiology.

The impact of age on the natural history of T1D

Longitudinally monitoring of birth cohorts of children with high genetic risk based on family history and HLA genotype have offered unique insight into the progression of T1D. Most notably, observations from a variety of these cohorts highlight an important impact of age on the development of islet autoantibodies, which serve as a sentinel of immune activation against the β cell. Analysis of the German BABYDIAB and BABYDIET cohorts identified a peak incidence of autoantibody seroconversion between 9 months and 2 years of age. Children who seroconverted at these younger ages had a higher risk of developing T1D and developed T1D faster than children with a later age of seroconversion (7). Similar findings were seen in the Environmental Determinants of Diabetes in the Young (TEDDY) study, which is a birth cohort study based in the U.S. and Europe. Within the TEDDY cohort, the peak incidence of islet autoantibodies also occurred around 9 months of age. In addition, differences in the timing of autoantibody type were observed in TEDDY participants, with the peak incidence of insulin autoantibodies occurring during the first year of life. In contrast, the peak incidence of GAD autoantibodies occurred later during the second and third years of life (8). Again, age at the time of multiple autoantibody development influenced progression to

T1D, with older children exhibiting a lower hazard ratio for T1D development compared to those developing antibodies at a younger age (9). A similar sequencing of antibody development was observed in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP), where the peak incidence of insulin autoantibodies occurred between 2 and 3 years of age, followed by a peak incidence of GAD autoantibodies between 3 and 5 years (10).

The TrialNet Pathway to Prevention (TNPTP) study and its precursor the Diabetes Prevention Trial-Type 1 (DPT-1) have both undertaken massive screening efforts in relatives of persons with T1D to identify cohorts of autoantibody positive adults and children (11-14). These studies differ from birth cohorts, in that participants are identified in a cross-sectional manner. Therefore, the full duration of autoantibody positivity prior to study enrollment is not known. Nonetheless, data from both cohorts supports the idea that age is inversely predictive of T1D risk once autoantibody positivity is established. Adults are at lower risk of progression compared to children, and even among children, the risk is lower in older children. An inverse association of T1D risk with age is also present among those with abnormal glucose tolerance. In DPT-1, among participants who developed incident dysglycemia, those who were <13 years were at much higher risk than those >13 years to subsequently develop T1D (15). In the TNPTP, the risk according to age among those dysglycemic was not as linear as for the full cohort; the decline in risk for T1D was especially evident among those who were over 20 years (16). Notably, the decreasing risk of T1D with age has had utility as one of the components of a risk score for the prediction of T1D that has been utilized and validated in both studies (17, 18).

Age and metabolic progression in T1D

Age has a clear influence on the rapidity of progression from the time of autoantibody positivity to the development of a clinical diagnosis of T1D. Of additional interest is how patterns of C-peptide decline vary amongst different age groups during disease evolution. Given the inherent challenges in identifying large cohorts of autoantibody-positive individuals around the time of seroconversion, the impact of age on the longitudinal loss of C-peptide in

at-risk individuals during stage 1 (autoantibody-positive, normoglycemic) and 2 (autoantibody-positive, dysglycemic) T1D has been difficult to decipher. Amongst longitudinal studies, differences according to age were either not examined or have been limited by low numbers of subjects for assessing the extent to which progression patterns vary with age (19, 20). In a recent analysis of TNPTP data, patterns of C-peptide loss and glycemia were examined in autoantibody-positive subjects who progressed to T1D after <5 years and ≥5 years of follow-up. Progressors with <5 years of follow-up were younger at study entry had a younger age of diabetes diagnosis compared to progressors with ≥5 years of follow-up (median of 11.6 yrs at T1D onset in progressors<5 vs. a median of 17.0 yrs in progressors≥5). Remarkably, patterns of C-peptide loss and increased glycemia within three years of diagnosis were nearly identical between the two groups, raising the possibility that within this proximity to diagnosis, metabolic progression follows a stereotypical course of decline that is independent of age (21).

At the time of stage 3 T1D onset (i.e. clinical hyperglycemia), absolute values of C-peptide differ between youth and adult populations, with youth exhibiting significantly lower absolute serum and urinary C-peptide levels (22, 23). Similarly, amongst individuals with T1D diagnosed within 10 weeks, higher insulin secretory rates during a 4-hour mixed meal tolerance test correlated with older age at presentation (24). Consistent with this, younger children are more likely to present with ketoacidosis at the time of diagnosis (25, 26). Those with an earlier onset have also been described to have a shorter period of partial clinical remission or a “honeymoon” period (27). Whether these observed differences in C-peptide levels are physiological (i.e. due to differences in adiposity or insulin resistance) or represent differences in disease pathology remain unclear. Interestingly, in an ethnically diverse pediatric cohort, obese and overweight children had higher residual β -cell function compared to lean children at the time of T1D diagnosis (28). Surprisingly, there is very little information regarding C-peptide and age relationships in non-obese, non-diabetic children for comparison.

Longitudinal relationships between age and C-peptide loss after stage 3 disease onset have been examined in several cohorts, and analyses have yielded somewhat differing

results. In subjects who participated in the placebo arms of several TrialNet intervention studies, longitudinal follow-up was initiated within 3 months of diagnosis. Slopes of decline in the C-peptide area under curve values from mixed meal tolerance tests over 2 years of follow-up tended to be parallel for the age groups among children, whereas the slope of decline in adults was flatter (29). In another longitudinal TrialNet study, recently diagnosed participants were followed for up to 4 years. Endpoints included peak stimulated C-peptide levels ≤ 0.2 nmol/L and progression to undetectable C-peptide levels. When groups were separated according to age, the survival curves for those endpoints became less steep with age (30). Thus, both TrialNet post-diagnostic studies showed that C-peptide is better maintained in older individuals, a pattern which appeared to persist for at least 4 years after diagnosis.

A recent analysis of two UK cohorts with diabetes of long duration identified two distinct phases of C-peptide decline. The first was characterized by an initial exponential fall over the first 7 years, followed by a period of relative stability. In contrast to the TrialNet studies, neither the overall pattern or duration of these two phases differed in subjects above and below the median age of diagnosis in the cohort (10.8 years) (31). In a Swedish cohort, Wallensteen et al. examined the slope of C-peptide loss in a cohort of children. Overall, they found the rate of fall of the post-prandial C-peptide was -0.019 nmol/month. Age at onset had no correlation with the observed rate of change. Moreover, the rate of change was nearly parallel in children aged 1-5, 6-11, and 12-17 years of age (32). The reason for these discrepant results amongst different cohorts is unclear, but there are several possibilities to consider. The first is that measures of C-peptide and methods used to model data differ between studies. There are also important differences in the analytic platforms used to measure C-peptide. Finally, differences in the demographic and ethnic characteristics of the cohorts should also be considered, and additional studies are needed.

Analysis of human donor pancreata provides insights into age-related T1D heterogeneity

Notwithstanding these uncertainties, recent studies of human pancreata have provided unique tissue-level insight into the impact of age on T1D pathophysiology. The human pancreas is a challenging organ to study and is only biopsied under exceptional circumstances, for which T1D is not one. The majority of studies of T1D pancreata have therefore been completed on post-mortem (33) or transplant grade organ donor material (34), although few selected donor biopsies have been performed (35). It is these rare samples that have provided insight into striking heterogeneity in the pancreatic pathology and shown these differences are often strongly associated with age. To understand pathology, though, we must first understand the healthy pancreas as this is critical to benchmarking the progression of disease. Whilst research into the healthy human pancreas is not as prolific as it is in disease, we now understand that β -cell mass is relatively stable from 20 years of age; that β -cell replication is low and apoptosis almost undetectable (36-39). Events under the age of 20 years are perhaps less well understood, although it is clear that significant increases in β -cell mass and proliferation are observed in the early years of life (37-39). Finally, the islet size, islet architecture, and connectivity of endocrine cells to one another can change during development (39-42), and the impact of these alterations on the development of disease is still largely unknown.

In T1D, β -cell destruction is linked to a targeted lymphocytic invasion of the islets, termed insulinitis. Insulinitis is more severe at, or close to, the initial diagnosis (33, 43), although prolonged inflammation around islets has been documented (33, 44). Here too, age-related differences in both the proportion of islets affected with insulinitis and the composition of the immune cell infiltrate have been documented (45, 46). For example, people who are older at diagnosis can retain significant and often surprising numbers of insulin containing islets (ICIs) (46-48), despite having an absolute requirement for exogenous insulin. Indeed, isolated islets collected from older onset donor patients partially recovered their insulin secretory profile after

7 days in culture, suggesting that, in a non-diabetogenic environment, these residual islets could be returned to health (49) – a hopeful proposition for the future.

It is widely accepted that, within the insulitic lesion, the CD8⁺ T cells are the main aggressors, whilst CD20⁺ B lymphocytes and CD4⁺ T cells, as well as macrophages, are also present (44-46, 50). However, the number of immune cells present around a given islet, and the proportions of these cells relative to one another, differs markedly between persons with T1D. Intriguingly in those people diagnosed with T1D ≤ 7 y, the majority of the residual ICIs (~75% at diagnosis) have evidence of insulitis (defined as ≥ 15 CD45⁺ cells in or around the islet periphery (51, 52)) that contains high numbers of CD8⁺ T cells and CD20⁺ B cells, but relatively low numbers of CD4⁺ T cells (46). As such the ratio of the average number of CD20⁺ B cells to CD4⁺ T cells is high (>1). These cases have been termed hyper-immune or CD20^{Hi}. In contrast in individuals who are diagnosed beyond their mid-teens, a relatively mild infiltration of only a proportion of residual ICIs (~25% at diagnosis) was found. In these, although the predominant cell type was still CD8⁺ T cells, the CD4⁺ T cells typically outnumbered the CD20⁺ B cells, such that the CD20⁺ B: CD4⁺ T ratio was low (<1) (46). These cases have been termed pauci-immune or CD20^{Lo}. In keeping with these findings, examination of 151 T1D subjects from the EADB cohort and 116 T1D donors from the nPOD cohort, irrespective of disease duration, showed that those diagnosed beyond their mid-teens were far more likely to still contain residual ICIs when compared with those diagnosed ≤ 7 y (46). Interestingly, the histopathological observations appear to mirror those seen when assessing residual β -cell function using C-peptide measurements in the blood or urine, in that C-peptide levels are significantly lower in younger individuals (22-24).

Peripheral immune signatures of T1D vary by age

Peripheral correlates of these histological findings in the pancreas have been tested in a number of studies. Culina *et al.* (53) recently reported that circulating islet-reactive (HLA-A2 multimer-positive) CD8⁺ T cells are strikingly similar between T1D and healthy donors in terms of frequency and history of antigen encounter. Indeed, the other surprising feature of

these islet-reactive CD8⁺ T cells detected in peripheral blood was their predominantly naïve phenotype, implying that this T-cell repertoire is potentially autoreactive, but not actively involved in the autoimmune process. The T-cell fraction participating to disease seemed instead sequestered in the pancreas, where higher densities of islet-reactive CD8⁺ T cells have been found in T1D donors compared to healthy and T2D controls. Importantly, these features applied to islet-reactive CD8⁺ T cells recognizing either known β -cell antigens (53) or novel ones identified as sources of peptides naturally processed and presented by β cells (54). These novel antigens, identified by HLA peptidomics, included urocortin-3, the insulin gene enhancer protein ISL-1, and an islet amyloid polypeptide (IAPP)_{15-17/5-10} epitope generated by the fusion of two non-contiguous amino acid sequences (54). Circulating CD8⁺ T cells recognizing the ZnT8₁₈₆₋₁₉₄ epitope were subsequently compared between new-onset T1D children and adults (53). Their frequency was higher in children versus adults, but again irrespective of T1D status. The same pattern was also observed for CD8⁺ T cells recognizing the extra-pancreatic melanocyte self-epitope MelanA₂₆₋₃₅, but the corresponding ZnT8-reactive populations were more antigen-experienced in T1D children. These results suggest that compared to adults, children harbor a larger autoimmune CD8⁺ T-cell repertoire against different self-antigens, but that the islet-reactive repertoire is preferentially engaged in T1D children.

A similar heterogeneity between T1D children and adults has been described for islet-reactive CD4⁺ T-cell responses detected by ELISpot, which were more polarized toward secretion of the regulatory cytokine interleukin (IL)-10 in patients with older age at T1D diagnosis (55). By studying two distinct groups of children and adolescents with recent-onset T1D, Arif et al. (45) described two clusters of patients characterized by pro-inflammatory (IFN- γ ⁺ CD4⁺ T-cell ELISpot responses, multi-autoantibody⁺) and partially regulated (IL-10⁺, pauci-autoantibody⁺) responses in peripheral blood.

Which mechanisms may underlie the more aggressive islet autoimmunity observed in T1D children? The first possibility is that immune regulation may be more effective in older

patients, resulting in a milder autoimmunity and, possibly, a clinical onset later in life. This hypothesis is supported by the endotypes described in the pancreas (45) and by the observation that the frequency of T regulatory cells increases with age (56). In this respect, the age exception of T1D compared to other autoimmune diseases suggests that tolerance to β cells may critically rely on suppression by T regulatory cells.

The second possibility is that β cells may play a central role of their own. This role may include modulation of the autoimmune aggressiveness, which may be licensed by the vulnerability and 'visibility' that β cells offer to the autoreactive T-cell repertoire. In this scenario, tolerance to β cells may also depend on T-cell ignorance, as suggested in the mouse (57, 58). The loss of such ignorance may be favoured by β -cell stressors, such as islet-tropic enteroviruses and the metabolic demands imposed by growth. These stressors could exert their effects by inducing variable degrees of β -cell death and inflammation, thus making antigens visible to autoreactive T cells under suitable immunogenic conditions. In this respect, the age exception of T1D autoimmunity may be explained by the fact that these β -cell stressors are more frequently encountered during childhood. Consistent with this notion, proinsulin/C-peptide ratios were measured in TrialNet progressors to T1D approximately 12 months prior to the development of Stage 3. Values were highest in the youngest age group, suggesting that levels of β -cell stress may be higher in young children during Stages 1 and 2 disease (59).

Genetic explanations for age-related T1D heterogeneity

Data indicate that the above age-related heterogeneity in T1D may have a genetic basis. Twin studies offered the first evidence of the influence of genetics not only on T1D development but also on the age at diagnosis. Fava et al. studied monozygotic (i.e., identical) and dizygotic (i.e. non-identical) twin pairs and sibling pairs, who were concordant for T1D (i.e., both twins or both siblings had the disease). The correlation in the diagnosis age was stronger in monozygotic than dizygotic twins, but it was not significant between non-twin siblings, (60) underscoring the contribution of genetic factors to age of T1D onset. In a series

of initially non-diabetic monozygotic twins of patients with T1D (n=187) who were followed for a median of 17.7 years (61), younger age at diagnosis in the index twin was a risk factor for T1D in the initially non-diabetic twin; the twins of patients who were diagnosed at age ≥ 25 had approximately half the risk of T1D of twins of patients who were diagnosed at < 14 years.

The effect of specific genetic factors, in particular, the MHC, IL-2 (*IL2*) (4q27, rs2069763) and renalase (*RNLS*) (10q23.31, rs10509540) gene regions, on the age of T1D diagnosis was first reported by Howson et al (62). More recently, using a genome wide approach to address this question, a recent study investigated a large number of SNPs, included in the ImmunoChip, in 16,015 individuals with T1D, 92% diagnosed < 20 years of age, collected through six different international cohorts (63). This study concluded that the HLA complex in the 6p21 region, in particular the rs9273363 SNP that tags the HLA DQB1*03:02 haplotype, and the 6q22.33 region, which contains the genes encoding protein tyrosine phosphatase receptor kappa (*PTPRK*) and thymocyte-expressed molecule involved in selection (*THEMIS*), were associated with younger age of T1D onset. Participants who were homozygous for the allele associated with younger age of onset in both regions were over four years younger at T1D diagnosis than those who were homozygous for the non-risk allele at both loci. Furthermore, while not associated with T1D risk overall in this or prior studies, the SNP most strongly linked with age of diagnosis in the 6q22.33 region, rs72975913, was also associated with T1D risk in children under age 5. This SNP has also been previously reported in association with celiac disease, which might contribute to explain the link between celiac disease and young age at T1D diagnosis (64). Howson et al. also found that DR4 and DR3/DR4, but not DR3, were associated with younger age at onset (65). This is consistent with other reports that individuals who are older at T1D onset are less likely to have T1D-associated HLA alleles (66).

The effect of genetics on the heterogeneity of autoimmune diabetes across ages is also illustrated by latent autoimmune diabetes in adults (LADA). LADA is usually defined as autoimmune diabetes that is diagnosed after age 30 and does not require insulin treatment for

the first 6 months. Mishra et al. studied the associations of T1D and type 2 diabetes (T2D) loci with LADA, compared with childhood onset T1D, adult onset T2D and controls (67). These authors found that although LADA is genetically closer to childhood onset T1D than to T2D, there are differences. For instance, LADA has weaker association with HLA and higher with INS, while PTN22 and SH2B3 were similarly associated with LADA and childhood-onset T1D. Among the 71 T2D-linked loci, only *HNF1A* was associated with LADA in this study, although prior reports showed an association with T2D-linked *TCF7L2* variants as well. T1D and T2D genetic risk scores (GRS) facilitate the comparison of the genetic load by combining either T1D or T2D SNPs, respectively, into a single number (68). The authors found that the T1D GRS was more discriminative of LADA than the T2D GRS, although there was an important T2D genetic load as well.

In a study that evaluated the performance of the T1D GRS to predict progression along pre-clinical T1D stages, the T1D GRS was a significant predictor of progression from single to multiple positive autoantibodies in individuals younger than age 35 but not in older participants who are at a lower risk of T1D overall (69). Thus, T1D loci are stronger predictors in the form of T1D that develops earlier in life, while later onset T1D may have additional genetic determinants. For instance, the association between the T2D-linked transcription factor 7 like-2 (*TCF7L2*) variants and milder autoimmunity, as reflected by expression of a single autoantibody at T1D onset (70, 71) was restricted to participants ≥ 12 years (72). It is possible that in individuals with weaker, slowly progressive islet autoimmunity, this T2D-associated SNP accelerates the progression to diabetes, although still not younger than 12, while the aggressive islet autoimmunity and subsequent profound insulin deficiency often seen at younger ages curtails the potential effect of additional diabetogenic factors such as *TCF7L2*.

Therapeutic implications of age-related heterogeneity in T1D

Over the past 30 years, a number of agents have been tested as disease-modifying therapies for T1D in both secondary and primary prevention strategies. These clinical trial

efforts have focused on induction of tolerance through administration of self-antigen or on the modulation of T-cell, B-cell and cytokine responses. To date, only a handful of interventions have shown efficacy in preserving C-peptide secretion in randomized-placebo controlled trials. Interestingly, in several of these successful trials, important age-related differences in treatment effects have been observed. When administered at T1D onset, anti-CD3 therapy with two different drugs (otelixizumab and tepluzimab) led to greater preservation of C-peptide in younger individuals (73, 74). Younger trial participants were also more likely to exhibit a partial response to alefacept, a fusion protein that binds CD2 and targets CD4+ and CD8+ effector memory T cells (75, 76). Similarly, children exhibited greater responses to rituximab, which targets B cells (77) and the co-stimulatory modulator, abatacept (16, 78).

Conclusions

Age has been shown to have a profound impact on T1D epidemiology, risk, and progression; in all cases, youth are more greatly impacted than adults. These differences likely result from a combination of immunologic, metabolic, and genetic factors. However, at present, we have only a cursory understanding of the underlying mechanisms responsible for these differences. Clinical trial experience suggests that some, but not all, immune interventions exhibit greater effects in children compared to adults. This observation raises several possibilities that must be considered as the field works to identify potential disease-modifying therapies. The first possibility is that pathogenesis is truly different between adults and children and that past interventions have been more effective in addressing pathology present in youth. The second possibility is that the immune system is more pliable in children, therefore accounting for the higher observed efficacy in younger participants. A final possibility is that greater effects are observed in younger subjects, where C-peptide decline after stage 3 diagnosis is more pronounced. Additional mechanistic studies are needed to address these possibilities and to monitor immunologic responses between responders and non-responders in trials where age-related differences in outcome have been observed. In the future, these data should be leveraged to design trials that test distinct interventions between children and

adults and to ensure continued clinical trial efforts in children, where a greater prospect of benefit may exist.

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Compliance with Ethical Standards

Conflict of Interest

Pia Leete, Roberto Mallone, Sarah J. Richardson, Jay M. Sosenko, Maria J. Redondo, and Carmella Evans-Molina declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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Annotated References

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This paper proposes that a universal state of 'benign' autoimmunity is present in all individuals. The key difference between the islet-reactive CD8+ T cells of T1D and healthy donors is not in their circulating frequency or history of antigen encounter, but in their capacity to home to the pancreas.

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**This study demonstrates that age of onset of Type 1 diabetes (in recent-onset cases) is associated with different insulinitic islet immune cell profiles and extent of pancreatic β -cell destruction.

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This study systematically analyzed associations between genetic factors and age of clinical diagnosis of type 1 diabetes.