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Birth and coming of age of islet autoantibodies

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List of abbreviations:

Islet-cell antibodies, ICA; insulin autoantibodies, IAA; insulinoma-associated antigen-2, IA-2; IA-2 autoantibodies, IA-2A; glutamic acid decarboxylase, 65-kDa isoform, GAD65; GAD65 autoantibodies, GADA; zinc transporter 8, ZnT8; ZnT8 autoantibodies, ZnT8A.

Summary

This review takes the reader through 45 years of islet autoantibody research, from the discovery of islet-cell antibodies in 1974 to today's population-based screening for presymptomatic early-stage type 1 diabetes. The review emphasizes the current practical value of, and factors to be considered in, the measurement of islet autoantibodies.

1. Historical perspectives

1.1 Discovery of islet-cell antibodies

The concept of autoimmunity as a pathogenetic mechanism in a subgroup of patients with diabetes was first raised in the 1960s and early 1970s with the observation of insulitis [1], and the association of juvenile-onset diabetes with certain human leukocyte antigen (HLA) alleles and T-cell abnormalities [2-6]. The definitive autoimmune pathogenetic discovery was made in 1974, when two research groups in the UK reported the identification of islet-cell antibodies (ICA) in patients with so-called 'multiple organ-specific autoimmunity' [7, 8].

The first of these publications was by Gian Franco Bottazzo and Deborah Doniach. This research group had discovered thyroid autoimmunity almost 20 years earlier [9] and several other autoantibodies [10-12], and had a treasure chest of samples from patients with various and multiple endocrine autoimmune diseases. Using indirect immunofluorescence, Bottazzo et al. detected ICA in these human pancreas samples (Figure 1a). The manuscript was published in *The Lancet* in November 1974 [7]. The abstract stated: "Antibodies to pancreatic islet cells were found by immunofluorescence in the sera of 13 patients with multiendocrine deficiencies associated with organ-specific autoimmunity. 10 of these patients were diabetic… The presence of organ-specific pancreatic antibodies supports the hypothesis of an autoimmune form of diabetes mellitus put forward to explain the histological 'insulitis' found in selected cases of this disease."

The second publication was by William J. Irvine's research group, which had previously reported T-cell responses against pancreatic antigens in patients with diabetes [6]. His group also examined a collection of samples from polyendocrine patients, and published their work in *The Lancet* one month after the study by Bottazzo et al. [8]. Their abstract stated: "Using an indirect immunofluorescence technique, circulating antibodies to pancreatic islet cells were found in the sera of 5 patients with insulin-dependent diabetes mellitus and coexistent autoimmunity… These findings provide further direct evidence to support the hypothesis of an autoimmune form of diabetes mellitus."

Thus, the discovery and validation of ICA were reported by two independent research groups within the space of a month. These were discovered in polyendocrine patients rather than in typical patients with type 1 diabetes. Moreover, the classification of diabetes into

type 1 and type 2, which had been introduced decades earlier, had not yet taken root, and terms such as 'juvenile', 'adult-onset', 'insulin-dependent', and 'noninsulin-dependent' were used to distinguish age- and therapy-related forms of the disease. In 1975, Lendrum et al. reported ICA in the sera of 51 of 105 children with recent-onset diabetes [13], revealing an autoimmune pathogenetic component in a large proportion of childhood cases of diabetes.

1.2 Prediabetes

Perhaps the most important discoveries were those that led to the notion of a 'prediabetic' stage of the disease. Lendrum et al. examined ICA in the diabetic twin cohort of David Pyke and in 1976, reported that the antibodies could be present years before the onset of diabetes [14]. Also in 1976, Irvine et al. reported that the antibodies could precede diabetes onset by several years [15]. In 1981, Gorsuch et al. measured ICA in the first-degree relatives of patients with insulin-dependent diabetes and discovered that patients who developed insulin-dependent diabetes had ICA up to 30 months before the onset of diabetes [16]. These early findings eventually led to the notion that type 1 diabetes is a chronic autoimmune disease, as described by George Eisenbarth in 1986 [17].

1.3 Standardization

The number of methods for detecting ICA and reports of ICA has increased rapidly since the pivotal studies described above. These reports discussed complement-fixing antibodies [18], bovine-pancreas-positive ICA [19], the two-colour fluorescence detection method [20], the protein-A detection method [21], islet-cell-surface antibodies [22], among many others. What started as a clear concept soon became complex and confused. A workshop to standardize ICA measurements was convened, and after the sobering realization of how

variable these measurements could be [23], an exemplary standardization program that introduced common standards [24] and international units [25, 26] was established and was subsequently used for antigen-specific islet autoantibody measurements [27-29]. Importantly, the program gave credibility to the antibodies as markers of prediabetes [30-32] and many poorly performing detection methods became obsolete.

1.4 Islet-cell antibodies are heterogeneous and target multiple antigens

The ICA immunofluorescence test had become standard, but the identification of their target antigens became an urgent undertaking (Figure 1a). MacCuish et al. had demonstrated T-cell responses to insulin fragments in patients with and without insulin treatment in 1975 [33]. In 1983, Palmer et al. showed that children who developed type 1 diabetes had insulin autoantibodies (IAA) before they were treated with insulin [34]. This was an important breakthrough in the field. It also signalled the presence of multiple autoantibodies because insulin is only expressed in pancreatic islet β cells, whereas all islet cells stained for ICA [7, 8].

In 1982, Baekkeskov et al. reported autoantibodies against a 64-kDa islet protein [35, 36] and in 1990; Christie et al. described autoantibodies against 40-kDa and 37-kDa fragments of islet proteins [37]. The 64-kDa target of autoantibodies was later identified as GAD65 [38], a known antigenic target of autoantibodies in the neurological disorder stiff-person syndrome [39]. The 40-kDa and 37-kDa fragments were identified as ICA512 (now also known as IA-2) [40] and the related protein phogrin (also known as IA-2β) [41], respectively, both of which were identified separately as the targets of autoantibodies in type 1 diabetes [42, 43]. GAD65-directed autoantibodies (GADA) were shown to be part of the ICA reaction,

with a β-cell-specific staining pattern [44]. The IA-2-directed autoantibodies (IA-2A) were shown to be part of the pan-islet-cell staining of ICA [45]. Other proteins, such as ICA69, were claimed to be targets of ICA [46], but were not confirmed by other groups or in standardization workshops [47]. The lipid antigens GM2-1 and sulfatides were also reported to be targeted by ICA [48, 49], but no methods have been developed for robust assessment of their validity. In contrast, the β-cell zinc transporter 8 (ZnT8) protein has been confirmed to be a target of autoantibodies (ZnT8A) in over 50% of patients with type 1 diabetes [50, 51], and tetraspanin 7 was identified as the 38-kDa target of autoantibodies against glima [52, 53]. These autoantibodies were present in over 30% of patients with type 1 diabetes [54].

1.5 Prediction of clinical disease

The notion that ICA and other islet autoantibodies precede the onset of type 1 diabetes allows the prediction of future disease. As early as 1977, Irvine's group showed that the presence of ICA identified adult patients treated with oral hypoglycaemic agents who would later require insulin treatment [55]. Numerous studies, including a prominent study in triplets [56], had identified occasional cases of ICA-positive individuals who later developed diabetes, but it was not until 1988 that an analysis of the Barts-Windsor Family Study showed that it was indeed possible to estimate the risk in ICA-positive relatives of patients with type 1 diabetes [57]. This was followed by the establishment of risk estimates using the standardized international units for ICA [30]. The higher the titre of ICA, the higher the risk that an ICA-positive relative would develop type 1 diabetes. The risk reached 100% within 10 years in relatives who had ICA titres of > 80 JDF units/ml. These studies provided the foundation for later prevention trials in ICA-positive first-degree relatives [58].

The inclusion of IAA, GADA, and IA-2A further improved our ability to stratify the risk of type 1 diabetes. The first reported use of autoantibody combinations to improve diabetes prediction was in twins in 1992, when a combination of ICA, IAA, GADA, and antibodies against the 37 kDa and 40 kDa fragments was used [59]. This was followed in 1994 by a study in relatives of patients with type 1 diabetes, which found that 8% of relatives with ICA only and 88% of those with ICA plus IAA, GADA, or antibodies to the 37 kDa or 40 kDa fragments developed diabetes [60]. It is noteworthy that not all the antibodies are useful in every situation. For example, the prediction of insulin requirement in adult-onset diabetes is made by testing for ICA [61], GADA [62], and IA-2A [63], but IAA are rare in patients in this age group [64].

Antibody combinations were subsequently used to select at-risk relatives for clinical trials [58, 65], and it is now well established that the diabetes risk associated with the presence of multiple islet autoantibodies (two or more of IAA, GADA, IA-2A, and ZnT8A) is markedly greater than the risk in people with a single autoantibody [66-69]. A landmark study that involved combined analysis of over 13,000 individuals from three birth cohorts, demonstrated that almost all children with genetic susceptibility to type 1 diabetes who developed multiple islet autoantibodies progressed to diabetes (Figure 1b) [70]. This has paved the way for population-based screening [71].

1.6 Natural history of islet autoantibodies

Prospective birth-cohort studies have made invaluable contributions to our knowledge of the appearance and progression of islet autoantibodies in childhood, including the German BABYDIAB Study [72], the Finnish DIPP Project [73], the DAISY from Colorado [74], and the

TEDDY Study [75], which have now been running for up to three decades. These studies have shown that in genetically predisposed children, autoantibody seroconversion occurs relatively frequently between the ages of 6 months and 3 years, with the incidence of autoantibodies peaking at an age of 1 year [76-78]. The typical natural history of type 1 diabetes in children is the appearance of the first high-affinity autoantibody [79], which is usually IAA in the youngest children, followed by the appearance of other islet autoantibodies [80], usually within 3 years [81], and eventually the development of diabetes. Two islet-autoimmunity endotypes are distinguished [82]. One is characterized by the first appearance of IAA in children carrying HLA-DR4, and occurs in the first years of life. The second is characterized by the first appearance of GADA in children carrying HLA-DR3, and is the endotype most frequently observed in children who seroconvert after an age of 2 years. Based on the different associations of the two endotypes and environmental factors, it has been suggested that the endotypes have different aetiologies [82]. However, age is an important confounder and it is possible that these differences are merely age related. In contrast to IAA and GADA, IA-2A usually occurs together with autoantibodies against other β-cell antigens and is therefore a very specific and highly predictive immune marker for progression to clinical type 1 diabetes [83, 84], particularly if its reactivity spreads to epitopes on the homologous protein IA-2β [84-86]. ZnT8A also usually appears later in the development of the disease [87].

2. Practical perspectives

2.1 Antibody titre, affinity, and specificity

There are differences in the target autoantigens and epitopes, the titres, affinities, and subclasses of islet autoantibodies. These characteristics are associated with the subject's

age and HLA genotype, and in some cases, can help distinguish diabetes-associated islet autoantibodies from non-disease-associated autoantibody signals [88].

The intensity and maturity of the antibody response are reflected in the antibody titre, affinity, IgG subclass, and target epitopes on single or multiple islet autoantigens. Islet autoantibodies with high titres usually involve multiple IgG subclasses and are directed against multiple epitopes on the target antigen. Similar to ICA [30], high titres of IAA [64, 84] or IA-2A [84] are associated with faster progression to clinical type 1 diabetes. Moreover, IAA or IA-2A responses that include IgG2, IgG3, and/or IgG4 as well as IgG1 are associated with an increased risk, even if the antibody titres are not high. By combining these antibody characteristics, the 5-year diabetes risk in islet-autoantibody-positive relatives can be stratified from less than 10% to over 90% [84].

The affinity (binding strength) of the autoantibody to the target antigen is closely related to the intensity of the antibody response. Accordingly, high-affinity islet autoantibodies are associated with progression to clinical type 1 diabetes, even if the antibody titre is relatively low, whereas low-affinity antibodies are unrelated to the development of diabetes, even if the capacity and titre of the antibody are high [79, 89-92]. Consistent with their high disease specificity, IA-2A are characterized by high affinity [92]. In contrast, both IAA and GADA can range in affinity by more than 1000-fold [79, 89-91]. The highest affinities are > 10^{11} L/mol. For IAA and GADA the low- and high-affinity autoantibodies appear to bind to different epitopes [79, 90, 91]. For example, high-affinity IAA require the preservation of amino acids 8–13 in the insulin A chain to bind to human insulin, and also bind proinsulin. In contrast, the majority of low-affinity IAA are dependent on the COOH-terminal residues of the insulin

B chain and usually do not bind proinsulin [79]. Low-affinity antibodies are seen more frequently in individuals who do not have a strong genetic susceptibility to type 1 diabetes and in children who remain positive for only IAA or GADA [79, 80, 93]. The affinities and epitope specificities of IAA and GADA can be used to stratify the progression to type 1 diabetes [79, 90, 94, 95], and those for GADA can predict insulin therapy in individuals with adult-onset diabetes [96, 97]. The spread of IA-2A reactivity against epitopes on the homologous IA-2β protein is associated with the rapid development of diabetes [84-86].

Therefore, it is useful to identify and/or exclude low-affinity signals in risk screening for clinical trials, particularly in individuals with only IAA or GADA, who may be in an early stage of the disease process and may progress to producing multiple islet autoantibodies [79, 80, 90]. The identification of markers associated with the risk of progression from single to multiple islet autoantibodies has been investigated in studies within the TrialNet Consortium [68, 98-103]. Genetic risk may also be used to select single-islet-autoantibody-positive children who are most likely to progress to producing multiple islet autoantibodies [68, 102, 103]. A low GAD autoantibody titre is associated with a low risk of progression to multiple islet autoantibodies [98].

2.2 Why do multiple antibodies or multiple tests work?

Multiple antibodies and multiple tests have the mathematical advantage of increasing the *a priori* probability of a true result in the samples selected for the second measurement, as may be expected from Bayes' theorem [104]. This can be illustrated theoretically in the example shown in Figure 2. The example assumes that 0.3% of preschool children are true positives and will develop type 1 diabetes. In a population of 100,000, this corresponds to

300 children. A single islet autoantibody measurement (e.g., IAA) with a sensitivity of 70% will identify 1000 children when its threshold is set to the 99th percentile of the population. These children will have a 21% risk (positive predictive value) of developing type 1 diabetes. A second test with similar characteristics will identify a similar number of children with a similar risk. However, there will be a marked enrichment of future cases of type 1 diabetes in the children who have both autoantibodies: there would be 155 children with both IAA and GADA, 147 of whom would develop diabetes (95% risk; 49% sensitivity); and of 1690 with only IAA or GADA, 126 would develop diabetes (7.5% risk; 42% sensitivity). Adding more antibodies, such as IA-2A, would identify multiple islet autoantibodies in another 70% (88) of children with a single IAA or GADA who will develop diabetes, thereby increasing the sensitivity (78%) with only a slight reduction in the risk. Adding another antibody (e.g., ZnT8A) will provide a limited improvement in test performance. In reality, IAA and GADA etc. are not completely independent and there are age relationships, so it is not quite this simple. Nevertheless, for many children who develop the disease, the high risk associated with multiple antibodies has more to do with Bayes' theorem and perhaps less to do with a multiple-hit disease process that progresses from single to multiple antibodies. A similar principle applies when a second test is performed (e.g., an electrochemiluminescence assay [105, 106], luciferase immunoprecipitation system assay [107, 108], or IAA affinity assay [109, 110]) using samples that were previously identified as positive on a radiobinding assay. A very similar outcome would also be expected if the order of the tests were reversed. The thresholds for the different antibodies can also be adjusted to obtain the best possible combination of sensitivity and risk [111].

2.3 Modelling islet autoantibody profiles

Prospective studies have shown that the natural progression to type 1 diabetes is not uniform in children and adolescents. Based on the individuals' different genetic backgrounds and environments, islet autoimmunity may develop at different ages, show different longitudinal autoantibody profiles, and progress to clinical diabetes at various rates. Today, we are unable to predict the individual's progression exactly or to link aetiological factors to the dynamics of islet autoantibody patterns over time. However, recent studies have started to develop mathematical algorithms to model complex longitudinal autoantibody profiles and stratify progression rates [112, 113]. Children who develop multiple islet autoantibodies can be clustered according to their longitudinal profiles, and it has been shown that the likelihood of progressing from seroconversion to clinical diabetes within 5 years ranges in these clusters from below 10% to above 80%. Those children who seroconverted in the first years of life and expressed stable IAA and IA-2A responses had the highest risk of diabetes. Interestingly, this risk was unaffected by the child's GADA status [113]. A cluster analysis also revealed that losing IAA reactivity was associated with delayed progression to type 1 diabetes in children who were positive for multiple islet autoantibodies [112]. Mathematical approaches applied to data from prospective cohorts have strong potential utility as novel tools for the stratification of islet-autoantibody-positive individuals and offer new opportunities to clarify the disease mechanisms.

2.4 Islet autoantibodies used to select for trials and as study outcomes

Clinical trials to investigate the treatment of type 1 diabetes commonly use the participant's autoantibody positivity as an inclusion criterion (Figure 1c) [58, 65, 114]. In trials that recruit individuals with clinical diabetes, islet autoantibodies are used to distinguish type 1 diabetes from other types. In addition to the recruitment of trial participants, islet autoantibodies have been used as outcome markers in several studies (Figure 1c). These include natural history studies such as TEDDY [115] and primary prevention studies such as BABYDIET [116], TRIGR [117] and POInT [118]. The availability of high-quality, high-throughput, and harmonized autoantibody tests [119] mean that stable longitudinal measurements of the outcomes are possible. As discussed in Section 2.2, the definition of outcome can be improved by including confirmation with a second laboratory test or the use of multiple assays.

The age at screening is also important. For first-degree relatives of patients with type 1 diabetes, the risk of developing islet autoantibodies decreases exponentially with age, with a half-life of 3–4 years [120]. This has practical implications. First, if we consider that the peak incidence of islet autoantibody seroconversion occurs in the first 3 years of life, screening is likely to be most effective in preschool years. Second, relatives who remain negative through to their teenage years will have an 8-fold lower risk of developing islet autoantibodies than they had when they were born.

2.5 Extension to the population at large

The development of multiple islet autoantibodies has long been recognized as a critical step in the pathogenesis and diagnosis of type 1 diabetes [59, 60], culminating in the finding that almost all children who develop multiple islet autoantibodies will develop clinical symptomatic diabetes, regardless of whether they have an *a priori* family history of the disease [70]. This has led to a new staging strategy for type 1 diabetes, in which the presence of multiple islet autoantibodies is now used as a criterion for the diagnosis of presymptomatic early-stage type 1 diabetes [121]. An early diagnosis of type 1 diabetes can prevent the severe metabolic decompensation that is frequently observed at the onset of clinical diabetes [122-124]. Screening for islet autoantibodies can be done with capillary blood samples or dried blood spots [125-128]. The Fr1da Study started in 2015 as a model project investigating public-health screening for early-stage type 1 diabetes (confirmed with positivity for multiple islet autoantibodies) in Bavaria, Germany [71]. It assesses: (1) whether the early diagnosis of type 1 diabetes in the context of regular medical check-ups in childhood is feasible and efficient; (2) whether ketoacidosis and the hospitalization of children can be prevented by screening; and (3) whether psychological distress can be reduced with the early diagnosis of diabetes, education, and care. Similar studies have already commenced in Lower Saxony, Germany, with additional screening for low-density lipoprotein–hypercholesterolemia (Fr1dolin Study) [129], and in Colorado, with additional screening for celiac disease (ASK Study) [130].

3. The future

We envisage two areas of activity in the next few years (Figure 1d). From a practical perspective, a technology is required that facilitates the widespread use of islet autoantibody testing for the diagnosis of presymptomatic type 1 diabetes in the public-health context [131]. From the research perspective, activities to identify modified protein targets, both to generate better assays and to identify pathogenetic disease mechanisms, are highly likely.

Technological advances should drive down costs and allow simple high-throughput screening, which will favour its widespread application. Cost is a clear factor because population-based screening requires that tens or hundreds of thousands of children be tested, of whom over 99% will be negative. This will require a sensitive first-line test that covers the majority of the major islet autoantibodies (IAA, GADA, IA-2A, ZnT8A), with follow-up tests for those who are positive to confirm and stratify their risk [71, 127, 132, 133]. Point-of-care testing may be one approach to achieving this. This technology should be coupled to careful application of Bayes' modelling, including additional risk factors such as genetics [134], family history, and age. This sort of modelling will be possible once much larger numbers of children have been tested and followed, emphasizing the need to introduce broad testing programs in many regions and countries.

Modified islet antigens have been reported in the literature [135, 136], but we conclude that a number of these are unlikely to be validated because of weaknesses in the assays. Increased antibody binding to a modified form of the tetraspanin 7 protein was observed in some patients [137], but it is difficult to determine whether this is a favoured *in vivo* target

or an artificial *in vitro* modification. Smart systems that reliably identify antibodies that bind to proteins from unperturbed and perturbed islets should be possible and will probably reveal a range of variations in the autoantibody–autoantigen targets that we know today.

Figure legends

Figure 1. (A) Discovery of ICA and subsequent identification of major β-cell autoantigen targets has provided diagnostic markers with which to distinguish autoimmune type 1 diabetes from other non-autoimmune types. (B) Probability of developing clinical type 1 diabetes increases with increasing numbers of different islet autoantibodies, and children with multiple islet autoantibodies will develop clinical diabetes. (C) Currently, islet autoantibody measurements are used to recruit study participants for natural history studies; as outcome markers in primary prevention trials; and for the recruitment of individuals to prevention trials. (D) Future requirements for islet autoantibody diagnostics are stated.

Figure 2. Illustration of how the application of Bayes' theorem to islet autoantibodies can confer high positive predictive values in the diagnosis of future type 1 diabetes using multiple islet autoantibodies. A population of 100,000 unselected children, including 300 (0.3%) who will develop type 1 diabetes, is tested for IAA with a test that has a threshold selected at the upper 99th percentile (1% positive) and 70% sensitivity. Under these assumptions, 210 of the true future cases of type 1 diabetes will be identified (filled red box) and 790 of the 99,700 who will not develop type 1 diabetes will be positive (filled blue box). This provides a positive predictive value (PPV or risk) of 21%. A second test with a similar threshold and sensitivity (e.g., for GADA), applied to the same 100,000 children, will yield a

similar number of positives and predictive value. However, among the IAA-positive children, there will be a marked enrichment of true positives who are also GADA positive, yielding a PPV of 95%.

References

1. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 1965; **14**:619-33.
2. Cudworth AG, Woodrow JC. Letter: HL-A antigens and diabetes mellitus. Lancet 1974; **2**:1153.
3. Strosberg JM, Harris ED, Jr. Letter: HL-A genotypes and diabetes. Lancet 1974; **2**:1212.
4. Menser MA, Forrest JM, Honeyman MC, Burgess JA. Letter: Diabetes, HL-A antigens, and congenital rubella. Lancet 1974; **2**:1508-9.
5. Cudworth AG, Woodrow JC. Evidence for HL-A-linked genes in "juvenile" diabetes mellitus. British medical journal 1975; **3**:133-5.
6. MacCuish AC, Jordan J, Campbell CJ, Duncan LJ, Irvine WJ. Cell-mediated immunity to human pancreas in diabetes mellitus. Diabetes 1974; **23**:693-7.
7. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; **2**:1279-83.
8. MacCuish AC, Irvine WJ, Barnes EW, Duncan LJ. Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet 1974; **2**:1529-31.
9. Pulvertaft RJ, Doniach D, Roitt IM, Hudson RV. Cytotoxic effects of Hashimoto serum on human thyroid cells in tissue culture. Lancet 1959; **2**:214-6.
10. Forbes IJ, Roitt IM, Doniach D, Solomon IL. The thyroid cytotoxic autoantibody. The Journal of clinical investigation 1962; **41**:996-1006.
11. Taylor KB, Roitt IM, Doniach D, Couchman KG, Shapland C. Autoimmune phenomena in pernicious anaemia: gastric antibodies. British medical journal 1962; **2**:1347-52.
12. Doniach D, Roitt IM, Walker JG, Sherlock S. Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. Clinical and experimental immunology 1966; **1**:237-62.
13. Lendrum R, Walker G, Gamble DR. Islet-cell antibodies in juvenile diabetes mellitus of recent onset. Lancet 1975; **1**:880-2.
14. Lendrum R, Nelson PG, Pyke DA, Walker G, Gamble DR. Islet-cell, thyroid, and gastric autoantibodies in diabetic identical twins. British medical journal 1976; **1**:553-5.
15. Irvine WJ, Gray RS, McCallum CJ. Pancreatic islet-cell antibody as a marker for asymptomatic and latent diabetes and prediabetes. Lancet 1976; **2**:1097-102.
16. Gorsuch AN, Spencer KM, Lister J, McNally JM, Dean BM, Bottazzo GF, Cudworth AG. Evidence for a long prediabetic period in type I (insulin-dependent) diabetes mellitus. Lancet 1981; **2**:1363-5.

17. Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. The New England journal of medicine 1986; **314**:1360-8.
18. Bottazzo GF, Dean BM, Gorsuch AN, Cudworth AG, Doniach D. Complement-fixing islet-cell antibodies in type-I diabetes: possible monitors of active beta-cell damage. Lancet 1980; **1**:668-72.
19. Kawahara DJ, Buckingham B, Kershnar A. Heterogeneity in the specificity of the islet cell cytoplasmic antibody response in insulin-dependent diabetes mellitus. Pancreas 1990; **5**:647-51.
20. Madsen OD, Olsson ML, Bille G, Sundkvist G, Lernmark A, Dahlqvist G, Ludvigsson J. A two-colour immunofluorescence test with a monoclonal human proinsulin antibody improves the assay for islet cell antibodies. Diabetologia 1986; **29**:115-8.
21. Srikanta S, Rabizadeh A, Omar MA, Eisenbarth GS. Assay for islet cell antibodies. Protein A--monoclonal antibody method. Diabetes 1985; **34**:300-5.
22. Lernmark A, Freedman ZR, Hofmann C, Rubenstein AH, Steiner DF, Jackson RL, Winter RJ, Traisman HS. Islet-cell-surface antibodies in juvenile diabetes mellitus. The New England journal of medicine 1978; **299**:375-80.
23. Gleichmann H, Bottazzo GF. Progress toward standardization of cytoplasmic islet cell-antibody assay. Diabetes 1987; **36**:578-84.
24. Bonifacio E, Lernmark A, Dawkins RL. Serum exchange and use of dilutions have improved precision of measurement of islet cell antibodies. Journal of immunological methods 1988; **106**:83-8.
25. Bonifacio E, Boitard C, Gleichmann H, Shattock MA, Molenaar JL, Bottazzo GF. Assessment of precision, concordance, specificity, and sensitivity of islet cell antibody measurement in 41 assays. Diabetologia 1990; **33**:731-6.
26. Greenbaum CJ, Palmer JP, Nagataki S, Yamaguchi Y, Molenaar JL, Van Beers WA, MacLaren NK, Lernmark A. Improved specificity of ICA assays in the Fourth International Immunology of Diabetes Serum Exchange Workshop. Diabetes 1992; **41**:1570-4.
27. Wilkin TJ, Schoenfeld SL, Diaz JL, Kruse V, Bonifacio E, Palmer JP. Systematic variation and differences in insulin-autoantibody measurements. Diabetes 1989; **38**:172-81.
28. Schmidli RS, Colman PG, Bonifacio E, Bottazzo GF, Harrison LC. High level of concordance between assays for glutamic acid decarboxylase antibodies. The First International Glutamic Acid Decarboxylase Antibody Workshop. Diabetes 1994; **43**:1005-9.
29. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS. Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. Diabetes 1998; **47**:1857-66.
30. Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF. Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. Lancet 1990; **335**:147-9.
31. McCulloch DK, Klaff LJ, Kahn SE, Schoenfeld SL, Greenbaum CJ, Mauseth RS, Benson EA, Nepom GT, Shewey L, Palmer JP. Nonprogression of subclinical beta-cell dysfunction among first-degree relatives of IDDM patients. 5-yr follow-up of the Seattle Family Study. Diabetes 1990; **39**:549-56.

32. Bosi E, Becker F, Bonifacio E, Wagner R, Collins P, Gale EA, Bottazzo GF. Progression to type I diabetes in autoimmune endocrine patients with islet cell antibodies. Diabetes 1991; **40**:977-84.
33. MacCuish AC, Jordan J, Campbell CJ, Duncan LJ, Irvine WJ. Cell-mediated immunity in diabetes mellitus; lymphocyte transformation by insulin and insulin fragments in insulin-treated and newly-diagnosed diabetes. Diabetes 1975; **24**:36-43.
34. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983; **222**:1337-9.
35. Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A. Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. Nature 1982; **298**:167-9.
36. Baekkeskov S, Lernmark A. Rodent islet cell antigens recognized by antibodies in sera from diabetic patients. Acta biologica et medica Germanica 1982; **41**:1111-5.
37. Christie MR, Vohra G, Champagne P, Daneman D, Delovitch TL. Distinct antibody specificities to a 64-kD islet cell antigen in type 1 diabetes as revealed by trypsin treatment. The Journal of experimental medicine 1990; **172**:789-94.
38. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990; **347**:151-6.
39. Solimena M, Folli F, Aparisi R, Pozza G, De Camilli P. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. The New England journal of medicine 1990; **322**:1555-60.
40. Payton MA, Hawkes CJ, Christie MR. Relationship of the 37,000- and 40,000-M(r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). The Journal of clinical investigation 1995; **96**:1506-11.
41. Hawkes CJ, Wasmeier C, Christie MR, Hutton JC. Identification of the 37-kDa antigen in IDDM as a tyrosine phosphatase-like protein (phogrin) related to IA-2. Diabetes 1996; **45**:1187-92.
42. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, Goldstein DE, Rae PM. Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. Journal of immunology 1994; **152**:3183-8.
43. Lu J, Li Q, Xie H, Chen ZJ, Borovitskaya AE, Maclaren NK, Notkins AL, Lan MS. Identification of a second transmembrane protein tyrosine phosphatase, IA-2beta, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-kDa tryptic fragment. Proceedings of the National Academy of Sciences of the United States of America 1996; **93**:2307-11.
44. Genovese S, Bonifacio E, McNally JM, Dean BM, Wagner R, Bosi E, Gale EA, Bottazzo GF. Distinct cytoplasmic islet cell antibodies with different risks for type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1992; **35**:385-8.
45. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. Journal of immunology 1995; **155**:5419-26.

46. Pietropaolo M, Castano L, Babu S, Buelow R, Kuo YL, Martin S, Martin A, Powers AC, Prochazka M, Naggert J, et al. Islet cell autoantigen 69 kD (ICA69). Molecular cloning and characterization of a novel diabetes-associated autoantigen. The Journal of clinical investigation 1993; **92**:359-71.
47. Lampasona V, Ferrari M, Bosi E, Pastore MR, Bingley PJ, Bonifacio E. Sera from patients with IDDM and healthy individuals have antibodies to ICA69 on western blots but do not immunoprecipitate liquid phase antigen. Journal of autoimmunity 1994; **7**:665-74.
48. Dotta F, Gianani R, Previti M, Lenti L, Dionisi S, D'Erme M, Eisenbarth GS, Di Mario U. Autoimmunity to the GM2-1 islet ganglioside before and at the onset of type I diabetes. Diabetes 1996; **45**:1193-6.
49. Buschard K, Josefsen K, Horn T, Fredman P. Sulphatide and sulphatide antibodies in insulin-dependent diabetes mellitus. Lancet 1993; **342**:840.
50. Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, Hutton JC. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proceedings of the National Academy of Sciences of the United States of America 2007; **104**:17040-5.
51. Wenzlau JM, Liu Y, Yu L, Moua O, Fowler KT, Rangasamy S, Walters J, Eisenbarth GS, Davidson HW, Hutton JC. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes 2008; **57**:2693-7.
52. McLaughlin KA, Richardson CC, Ravishankar A, Brigatti C, Liberati D, Lampasona V, Piemonti L, Morgan D, Feltbower RG, Christie MR. Identification of Tetraspanin-7 as a Target of Autoantibodies in Type 1 Diabetes. Diabetes 2016; **65**:1690-8.
53. Aanstoot HJ, Kang SM, Kim J, Lindsay LA, Roll U, Knip M, Atkinson M, Mose-Larsen P, Fey S, Ludvigsson J, Landin L, Bruining J, Maclaren N, Akerblom HK, Baekkeskov S. Identification and characterization of glima 38, a glycosylated islet cell membrane antigen, which together with GAD65 and IA2 marks the early phases of autoimmune response in type 1 diabetes. The Journal of clinical investigation 1996; **97**:2772-83.
54. Walther D, Eugster A, Jergens S, Gavrisan A, Weinzierl C, Telieps T, Winkler C, Ziegler AG, Bonifacio E. Tetraspanin 7 autoantibodies in type 1 diabetes. Diabetologia 2016; **59**:1973-6.
55. Irvine WJ, McCallum CJ, Gray RS, Duncan LJ. Clinical and pathogenic significance of pancreatic-islet-cell antibodies in diabetics treated with oral hypoglycaemic agents. Lancet 1977; **1**:1025-7.
56. Srikanta S, Ganda OP, Eisenbarth GS, Soeldner JS. Islet-cell antibodies and beta-cell function in monozygotic triplets and twins initially discordant for Type I diabetes mellitus. The New England journal of medicine 1983; **308**:322-5.
57. Tarn AC, Thomas JM, Dean BM, Ingram D, Schwarz G, Bottazzo GF, Gale EA. Predicting insulin-dependent diabetes. Lancet 1988; **1**:845-50.
58. Gale EA, Bingley PJ, Emmett CL, Collier T, European Nicotinamide Diabetes Intervention Trial G. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet 2004; **363**:925-31.
59. Christie MR, Tun RY, Lo SS, Cassidy D, Brown TJ, Hollands J, Shattock M, Bottazzo GF, Leslie RD. Antibodies to GAD and tryptic fragments of islet 64K antigen as distinct

markers for development of IDDM. Studies with identical twins. Diabetes 1992; **41**:782-7.
60. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, Bottazzo GF, Gale EA. Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. Diabetes 1994; **43**:1304-10.
61. Landin-Olsson M, Nilsson KO, Lernmark A, Sundkvist G. Islet cell antibodies and fasting C-peptide predict insulin requirement at diagnosis of diabetes mellitus. Diabetologia 1990; **33**:561-8.
62. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. Lancet 1997; **350**:1288-93.
63. Bottazzo GF, Bosi E, Cull CA, Bonifacio E, Locatelli M, Zimmet P, Mackay IR, Holman RR. IA-2 antibody prevalence and risk assessment of early insulin requirement in subjects presenting with type 2 diabetes (UKPDS 71). Diabetologia 2005; **48**:703-8.
64. Vardi P, Ziegler AG, Mathews JH, Dib S, Keller RJ, Ricker AT, Wolfsdorf JI, Herskowitz RD, Rabizadeh A, Eisenbarth GS, et al. Concentration of insulin autoantibodies at onset of type I diabetes. Inverse log-linear correlation with age. Diabetes care 1988; **11**:736-9.
65. Diabetes Prevention Trial--Type 1 Diabetes Study G. Effects of insulin in relatives of patients with type 1 diabetes mellitus. The New England journal of medicine 2002; **346**:1685-91.
66. Orban T, Sosenko JM, Cuthbertson D, Krischer JP, Skyler JS, Jackson R, Yu L, Palmer JP, Schatz D, Eisenbarth G, Diabetes Prevention Trial-Type 1 Study G. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes care 2009; **32**:2269-74.
67. Yu L, Boulware DC, Beam CA, Hutton JC, Wenzlau JM, Greenbaum CJ, Bingley PJ, Krischer JP, Sosenko JM, Skyler JS, Eisenbarth GS, Mahon JL, Type 1 Diabetes TrialNet Study G. Zinc transporter-8 autoantibodies improve prediction of type 1 diabetes in relatives positive for the standard biochemical autoantibodies. Diabetes care 2012; **35**:1213-8.
68. Bingley PJ, Boulware DC, Krischer JP, Type 1 Diabetes TrialNet Study G. The implications of autoantibodies to a single islet antigen in relatives with normal glucose tolerance: development of other autoantibodies and progression to type 1 diabetes. Diabetologia 2016; **59**:542-9.
69. Gorus FK, Balti EV, Messaaoui A, Demeester S, Van Dalem A, Costa O, Dorchy H, Mathieu C, Van Gaal L, Keymeulen B, Pipeleers DG, Weets I, Belgian Diabetes R. Twenty-Year Progression Rate to Clinical Onset According to Autoantibody Profile, Age, and HLA-DQ Genotype in a Registry-Based Group of Children and Adults With a First-Degree Relative With Type 1 Diabetes. Diabetes care 2017; **40**:1065-72.
70. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. Jama 2013; **309**:2473-9.
71. Raab J, Haupt F, Scholz M, Matzke C, Warncke K, Lange K, Assfalg R, Weininger K, Wittich S, Lobner S, Beyerlein A, Nennstiel-Ratzel U, Lang M, Laub O, Dunstheimer D, Bonifacio E, Achenbach P, Winkler C, Ziegler AG, Fr1da Study G. Capillary blood islet

autoantibody screening for identifying pre-type 1 diabetes in the general population: design and initial results of the Fr1da study. BMJ open 2016; **6**:e011144.
72. Ziegler AG, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhauer U, Vordemann J, Lenz A, Standl E. On the appearance of islet associated autoimmunity in offspring of diabetic mothers: a prospective study from birth. Diabetologia 1993; **36**:402-8.
73. Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hamalainen AM, Korhonen S, Kimpimaki T, Sjoroos M, Ilonen J, Knip M, Simell O, Juvenile Diabetes Research Foundation Centre for the Prevention of Type IDiF. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. Diabetologia 2001; **44**:290-7.
74. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, McDuffie RS, Jr., Hamman RF, Klingensmith G, Eisenbarth GS, Erlich HA. Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia 1996; **39**:807-12.
75. Hagopian WA, Erlich H, Lernmark A, Rewers M, Ziegler AG, Simell O, Akolkar B, Vogt R, Jr., Blair A, Ilonen J, Krischer J, She J, Group TS. The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatric diabetes 2011; **12**:733-43.
76. Ziegler AG, Bonifacio E, Group B-BS. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. Diabetologia 2012; **55**:1937-43.
77. Parikka V, Nanto-Salonen K, Saarinen M, Simell T, Ilonen J, Hyoty H, Veijola R, Knip M, Simell O. Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. Diabetologia 2012; **55**:1926-36.
78. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark A, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E, Group TS. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015; **58**:980-7.
79. Achenbach P, Koczwara K, Knopff A, Naserke H, Ziegler AG, Bonifacio E. Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. The Journal of clinical investigation 2004; **114**:589-97.
80. Giannopoulou EZ, Winkler C, Chmiel R, Matzke C, Scholz M, Beyerlein A, Achenbach P, Bonifacio E, Ziegler AG. Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. Diabetologia 2015; **58**:2317-23.
81. Chmiel R, Giannopoulou EZ, Winkler C, Achenbach P, Ziegler AG, Bonifacio E. Progression from single to multiple islet autoantibodies often occurs soon after seroconversion: implications for early screening. Diabetologia 2015; **58**:411-3.
82. Krischer JP, Lynch KF, Lernmark A, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B, Group TS. Genetic and Environmental Interactions Modify the Risk of Diabetes-Related Autoimmunity by 6 Years of Age: The TEDDY Study. Diabetes care 2017; **40**:1194-202.
83. Decochez K, De Leeuw IH, Keymeulen B, Mathieu C, Rottiers R, Weets I, Vandemeulebroucke E, Truyen I, Kaufman L, Schuit FC, Pipeleers DG, Gorus FK, Belgian Diabetes R. IA-2 autoantibodies predict impending type I diabetes in siblings of patients. Diabetologia 2002; **45**:1658-66.

84. Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, Bonifacio E, Ziegler AG. Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. Diabetes 2004; **53**:384-92.
85. Achenbach P, Bonifacio E, Williams AJ, Ziegler AG, Gale EA, Bingley PJ, Group E. Autoantibodies to IA-2beta improve diabetes risk assessment in high-risk relatives. Diabetologia 2008; **51**:488-92.
86. De Grijse J, Asanghanwa M, Nouthe B, Albrecher N, Goubert P, Vermeulen I, Van Der Meeren S, Decochez K, Weets I, Keymeulen B, Lampasona V, Wenzlau J, Hutton JC, Pipeleers D, Gorus FK, Belgian Diabetes R. Predictive power of screening for antibodies against insulinoma-associated protein 2 beta (IA-2beta) and zinc transporter-8 to select first-degree relatives of type 1 diabetic patients with risk of rapid progression to clinical onset of the disease: implications for prevention trials. Diabetologia 2010; **53**:517-24.
87. Achenbach P, Lampasona V, Landherr U, Koczwara K, Krause S, Grallert H, Winkler C, Pfluger M, Illig T, Bonifacio E, Ziegler AG. Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. Diabetologia 2009; **52**:1881-8.
88. Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. Immunity 2010; **32**:468-78.
89. Schlosser M, Koczwara K, Kenk H, Strebelow M, Rjasanowski I, Wassmuth R, Achenbach P, Ziegler AG, Bonifacio E. In insulin-autoantibody-positive children from the general population, antibody affinity identifies those at high and low risk. Diabetologia 2005; **48**:1830-2.
90. Mayr A, Schlosser M, Grober N, Kenk H, Ziegler AG, Bonifacio E, Achenbach P. GAD autoantibody affinity and epitope specificity identify distinct immunization profiles in children at risk for type 1 diabetes. Diabetes 2007; **56**:1527-33.
91. Bender C, Schlosser M, Christen U, Ziegler AG, Achenbach P. GAD autoantibody affinity in schoolchildren from the general population. Diabetologia 2014; **57**:1911-8.
92. Krause S, Chmiel R, Bonifacio E, Scholz M, Powell M, Furmaniak J, Rees Smith B, Ziegler AG, Achenbach P. IA-2 autoantibody affinity in children at risk for type 1 diabetes. Clinical immunology 2012; **145**:224-9.
93. Adler K, Mueller DB, Achenbach P, Krause S, Heninger AK, Ziegler AG, Bonifacio E. Insulin autoantibodies with high affinity to the bovine milk protein alpha casein. Clinical and experimental immunology 2011; **164**:42-9.
94. Williams AJ, Lampasona V, Wyatt R, Brigatti C, Gillespie KM, Bingley PJ, Achenbach P. Reactivity to N-Terminally Truncated GAD65(96-585) Identifies GAD Autoantibodies That Are More Closely Associated With Diabetes Progression in Relatives of Patients With Type 1 Diabetes. Diabetes 2015; **64**:3247-52.
95. Wyatt RC, Brigatti C, Liberati D, Grace SL, Gillard BT, Long AE, Marzinotto I, Shoemark DK, Chandler KA, Achenbach P, Gillespie KM, Piemonti L, Lampasona V, Williams AJK. The first 142 amino acids of glutamate decarboxylase do not contribute to epitopes recognized by autoantibodies associated with Type 1 diabetes. Diabetic medicine : a journal of the British Diabetic Association 2018; **35**:954-63.
96. Krause S, Landherr U, Agardh CD, Hausmann S, Link K, Hansen JM, Lynch KF, Powell M, Furmaniak J, Rees-Smith B, Bonifacio E, Ziegler AG, Lernmark A, Achenbach P. GAD autoantibody affinity in adult patients with latent autoimmune diabetes, the study participants of a GAD65 vaccination trial. Diabetes care 2014; **37**:1675-80.

97. Achenbach P, Hawa MI, Krause S, Lampasona V, Jerram ST, Williams AJK, Bonifacio E, Ziegler AG, Leslie RD, Action Lc. Autoantibodies to N-terminally truncated GAD improve clinical phenotyping of individuals with adult-onset diabetes: Action LADA 12. Diabetologia 2018; **61**:1644-9.
98. Xu P, Krischer JP, Type 1 Diabetes TrialNet Study G. Prognostic Classification Factors Associated With Development of Multiple Autoantibodies, Dysglycemia, and Type 1 Diabetes-A Recursive Partitioning Analysis. Diabetes care 2016; **39**:1036-44.
99. Meah FA, DiMeglio LA, Greenbaum CJ, Blum JS, Sosenko JM, Pugliese A, Geyer S, Xu P, Evans-Molina C, Type 1 Diabetes TrialNet Study G. The relationship between BMI and insulin resistance and progression from single to multiple autoantibody positivity and type 1 diabetes among TrialNet Pathway to Prevention participants. Diabetologia 2016; **59**:1186-95.
100. Steck AK, Fouts A, Miao D, Zhao Z, Dong F, Sosenko J, Gottlieb P, Rewers MJ, Yu L, TrialNet Study G. ECL-IAA and ECL-GADA Can Identify High-Risk Single Autoantibody-Positive Relatives in the TrialNet Pathway to Prevention Study. Diabetes technology & therapeutics 2016; **18**:410-4.
101. Bosi E, Boulware DC, Becker DJ, Buckner JH, Geyer S, Gottlieb PA, Henderson C, Kinderman A, Sosenko JM, Steck AK, Bingley PJ, Type 1 Diabetes TrialNet Study G. Impact of Age and Antibody Type on Progression From Single to Multiple Autoantibodies in Type 1 Diabetes Relatives. The Journal of clinical endocrinology and metabolism 2017; **102**:2881-6.
102. Redondo MJ, Geyer S, Steck AK, Sharp S, Wentworth JM, Weedon MN, Antinozzi P, Sosenko J, Atkinson M, Pugliese A, Oram RA, Type 1 Diabetes TrialNet Study G. A Type 1 Diabetes Genetic Risk Score Predicts Progression of Islet Autoimmunity and Development of Type 1 Diabetes in Individuals at Risk. Diabetes care 2018; **41**:1887-94.
103. Redondo MJ, Steck AK, Sosenko J, Anderson M, Antinozzi P, Michels A, Wentworth JM, Atkinson MA, Pugliese A, Geyer S, Type 1 Diabetes TrialNet Study G. Transcription Factor 7-Like 2 (TCF7L2) Gene Polymorphism and Progression From Single to Multiple Autoantibody Positivity in Individuals at Risk for Type 1 Diabetes. Diabetes care 2018; **41**:2480-6.
104. Bayes T. An essay towards solving a problem in the doctrine of chances. 1763. MD computing : computers in medical practice 1991; **8**:157-71.
105. Miao D, Steck AK, Zhang L, Guyer KM, Jiang L, Armstrong T, Muller SM, Krischer J, Rewers M, Yu L, Type 1 Diabetes TrialNet Study G. Electrochemiluminescence assays for insulin and glutamic acid decarboxylase autoantibodies improve prediction of type 1 diabetes risk. Diabetes technology & therapeutics 2015; **17**:119-27.
106. Fouts A, Pyle L, Yu L, Miao D, Michels A, Krischer J, Sosenko J, Gottlieb P, Steck AK, Type 1 Diabetes TrialNet Study G. Do Electrochemiluminescence Assays Improve Prediction of Time to Type 1 Diabetes in Autoantibody-Positive TrialNet Subjects? Diabetes care 2016; **39**:1738-44.
107. Burbelo PD, Hirai H, Issa AT, Kingman A, Lernmark A, Ivarsson SA, Notkins AL, Iadarola MJ. Comparison of radioimmunoprecipitation with luciferase immunoprecipitation for autoantibodies to GAD65 and IA-2beta. Diabetes care 2010; **33**:754-6.
108. Liberati D, Wyatt RC, Brigatti C, Marzinotto I, Ferrari M, Bazzigaluppi E, Bosi E, Gillard BT, Gillespie KM, Gorus F, Weets I, Balti E, Piemonti L, Achenbach P, Williams AJK,

Lampasona V. A novel LIPS assay for insulin autoantibodies. Acta diabetologica 2018; **55**:263-70.
109. Achenbach P, Guo LH, Gick C, Adler K, Krause S, Bonifacio E, Colman PG, Ziegler AG. A simplified method to assess affinity of insulin autoantibodies. Clinical immunology 2010; **137**:415-21.
110. Curnock RM, Reed CR, Rokni S, Broadhurst JW, Bingley PJ, Williams AJ. 'Insulin autoantibody affinity measurement using a single concentration of unlabelled insulin competitor discriminates risk in relatives of patients with type 1 diabetes. Clinical and experimental immunology 2012; **167**:67-72.
111. Bonifacio E. Predicting type 1 diabetes using biomarkers. Diabetes care 2015; **38**:989-96.
112. Endesfelder D, Hagen M, Winkler C, Haupt F, Zillmer S, Knopff A, Bonifacio E, Ziegler AG, Zu Castell W, Achenbach P. A novel approach for the analysis of longitudinal profiles reveals delayed progression to type 1 diabetes in a subgroup of multiple-islet-autoantibody-positive children. Diabetologia 2016; **59**:2172-80.
113. Endesfelder D, Zu Castell W, Bonifacio E, Rewers M, Hagopian WA, She JX, Lernmark A, Toppari J, Vehik K, Williams AJK, Yu L, Akolkar B, Krischer JP, Ziegler AG, Achenbach P, Group TS. Time-Resolved Autoantibody Profiling Facilitates Stratification of Preclinical Type 1 Diabetes in Children. Diabetes 2019; **68**:119-30.
114. Greenbaum C, VanBuecken D, Lord S. Disease-Modifying Therapies in Type 1 Diabetes: A Look into the Future of Diabetes Practice. Drugs 2019; **79**:43-61.
115. Krischer JP, Liu X, Vehik K, Akolkar B, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Lernmark A, Group TS. Predicting Islet Cell Autoimmunity and Type 1 Diabetes: An 8-Year TEDDY Study Progress Report. Diabetes care 2019.
116. Hummel S, Pfluger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes care 2011; **34**:1301-5.
117. Writing Group for the TSG, Knip M, Akerblom HK, Al Taji E, Becker D, Bruining J, Castano L, Danne T, de Beaufort C, Dosch HM, Dupre J, Fraser WD, Howard N, Ilonen J, Konrad D, Kordonouri O, Krischer JP, Lawson ML, Ludvigsson J, Madacsy L, Mahon JL, Ormisson A, Palmer JP, Pozzilli P, Savilahti E, Serrano-Rios M, Songini M, Taback S, Vaarala O, White NH, Virtanen SM, Wasikowa R. Effect of Hydrolyzed Infant Formula vs Conventional Formula on Risk of Type 1 Diabetes: The TRIGR Randomized Clinical Trial. Jama 2018; **319**:38-48.
118. Ziegler AG, Achenbach P, Berner R, Casteels K, Danne T, Gundert M, Hasford J, Hoffmann VS, Kordonouri O, Lange K, Elding Larsson H, Lundgren M, Snape MD, Szypowska A, Todd JA, Bonifacio E, and the GSg. Oral insulin therapy for primary prevention of type 1 diabetes in infants with high genetic risk: the GPPAD-POInT (global platform for the prevention of autoimmune diabetes primary oral insulin trial) study protocol. BMJ open 2019; **9**:e028578.
119. Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, Adler K, Ziegler AG, Mueller PW, Schatz DA, Krischer JP, Steffes MW, Akolkar B. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. The Journal of clinical endocrinology and metabolism 2010; **95**:3360-7.

120. Hoffmann VS, Weiss A, Winkler C, Knopff A, Jolink M, Bonifacio E, Ziegler AG. Landmark models to define the age-adjusted risk of developing stage 1 type 1 diabetes across childhood and adolescence. BMC medicine 2019; **17**:125.
121. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark A, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, Ziegler AG. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes care 2015; **38**:1964-74.
122. Elding Larsson H, Vehik K, Bell R, Dabelea D, Dolan L, Pihoker C, Knip M, Veijola R, Lindblad B, Samuelsson U, Holl R, Haller MJ, Group TS, Group SS, Swediabkids Study G, Group DPVS, Finnish Diabetes Registry Study G. Reduced prevalence of diabetic ketoacidosis at diagnosis of type 1 diabetes in young children participating in longitudinal follow-up. Diabetes care 2011; **34**:2347-52.
123. Winkler C, Schober E, Ziegler AG, Holl RW. Markedly reduced rate of diabetic ketoacidosis at onset of type 1 diabetes in relatives screened for islet autoantibodies. Pediatric diabetes 2012; **13**:308-13.
124. Duca LM, Wang B, Rewers M, Rewers A. Diabetic Ketoacidosis at Diagnosis of Type 1 Diabetes Predicts Poor Long-term Glycemic Control. Diabetes care 2017; **40**:1249-55.
125. Bazzigaluppi E, Bonfanti R, Bingley PJ, Bosi E, Bonifacio E. Capillary whole blood measurement of islet autoantibodies. Diabetes care 1999; **22**:275-9.
126. Bingley PJ, Rafkin LE, Matheson D, Steck AK, Yu L, Henderson C, Beam CA, Boulware DC, TrialNet Study G. Use of Dried Capillary Blood Sampling for Islet Autoantibody Screening in Relatives: A Feasibility Study. Diabetes technology & therapeutics 2015; **17**:867-71.
127. Ziegler AG, Haupt F, Scholz M, Weininger K, Wittich S, Lobner S, Matzke C, Gezginci C, Riethausen S, Beyerlein A, Zillmer S, Amoroso M, Coles R, Powell M, Furmaniak J, Smith BR, Winkler C, Bonifacio E, Achenbach P. 3 Screen ELISA for High-Throughput Detection of Beta Cell Autoantibodies in Capillary Blood. Diabetes technology & therapeutics 2016; **18**:687-93.
128. Liu Y, Rafkin LE, Matheson D, Henderson C, Boulware D, Besser REJ, Ferrara C, Yu L, Steck AK, Bingley PJ, Type 1 Diabetes TrialNet Study G. Use of self-collected capillary blood samples for islet autoantibody screening in relatives: a feasibility and acceptability study. Diabetic medicine : a journal of the British Diabetic Association 2017; **34**:934-7.
129. Kordonouri O, Lange K, Boettcher I, Christoph J, Marquardt E, Tombois C, Galuschka L, Stiller D, Mueller I, Roloff F, Aschemeier B, Danne T. New approach for detection of LDL-hypercholesterolemia in the pediatric population: The Fr1dolin-Trial in Lower Saxony, Germany. Atherosclerosis 2019; **280**:85-91.
130. Rasmussen CRG, Rewers M, Baxter J, Waugh K, Steck A, Frohnert BI, Yu LP, Liu E. Population Screening for T1D and Celiac Disease-Autoimmunity Screening for Kids (ASK). Diabetes 2018; **67**.
131. Ziegler AG, Hoffmann GF, Hasford J, Larsson HE, Danne T, Berner R, Penno M, Koralova A, Dunne J, Bonifacio E. Screening for asymptomatic beta-cell autoimmunity in young children. The Lancet Child & adolescent health 2019; **3**:288-90.
132. Amoroso M, Achenbach P, Powell M, Coles R, Chlebowska M, Carr L, Furmaniak J, Scholz M, Bonifacio E, Ziegler AG, Rees Smith B. 3 Screen islet cell autoantibody

ELISA: A sensitive and specific ELISA for the combined measurement of autoantibodies to GAD65, to IA-2 and to ZnT8. Clinica chimica acta; international journal of clinical chemistry 2016; **462**:60-4.

133. Zhao Z, Miao D, Michels A, Steck A, Dong F, Rewers M, Yu L. A multiplex assay combining insulin, GAD, IA-2 and transglutaminase autoantibodies to facilitate screening for pre-type 1 diabetes and celiac disease. Journal of immunological methods 2016; **430**:28-32.
134. Bonifacio E, Beyerlein A, Hippich M, Winkler C, Vehik K, Weedon MN, Laimighofer M, Hattersley AT, Krumsiek J, Frohnert BI, Steck AK, Hagopian WA, Krischer JP, Lernmark A, Rewers MJ, She JX, Toppari J, Akolkar B, Oram RA, Rich SS, Ziegler AG, Group TS. Genetic scores to stratify risk of developing multiple islet autoantibodies and type 1 diabetes: A prospective study in children. PLoS medicine 2018; **15**:e1002548.
135. Strollo R, Vinci C, Arshad MH, Perrett D, Tiberti C, Chiarelli F, Napoli N, Pozzilli P, Nissim A. Antibodies to post-translationally modified insulin in type 1 diabetes. Diabetologia 2015; **58**:2851-60.
136. Buitinga M, Callebaut A, Marques Camara Sodre F, Crevecoeur I, Blahnik-Fagan G, Yang ML, Bugliani M, Arribas-Layton D, Marre M, Cook DP, Waelkens E, Mallone R, Piganelli JD, Marchetti P, Mamula MJ, Derua R, James EA, Mathieu C, Overbergh L. Inflammation-Induced Citrullinated Glucose-Regulated Protein 78 Elicits Immune Responses in Human Type 1 Diabetes. Diabetes 2018; **67**:2337-48.
137. Eugster A, Kraus G, Lidzba V, Muller D, Jolink M, Ziegler AG, Bonifacio E. Cytoplasmic ends of tetraspanin 7 harbour epitopes recognised by autoantibodies in type 1 diabetes. Diabetologia 2019.