Predicting Type 1 Diabetes Using Biomarkers

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Clinical type 1 diabetes is preceded by an asymptomatic phase that can be identified by serum islet autoantibodies. This perspective proposes that there is now sufficient evidence to allow a broader use of islet autoantibodies as biomarkers to diagnose type 1 diabetes that is already at an asymptomatic stage, so that attempts to prevent clinical hyperglycemia become a feature of disease management. Prediction would first, therefore, shift toward the use of genetic and other biomarkers to determine the likelihood that islet autoimmunity will develop in an infant, and second, toward metabolic assessment to stage and biomarkers to determine the rate of progression to hyperglycemia in children in whom islet autoimmunity is diagnosed. A case is presented for future comprehensive risk assessment that commences at birth and includes attempts to predict, stage, and prevent initiation and progression of the disease process at multiple stages. The biomarkers required achieving this level of sophistication and dissemination are discussed.

The 1995 Immunology of Diabetes Society Congress included a session on the prediction of type 1 diabetes in which the Chair announced that we had the tools to predict this disease, and that there was not much more to do in this field. Twenty years later, that Chair (myself) admits that we have learned much more about predicting type 1 diabetes. Although the available tools have not changed markedly, there has been a substantial increase in our knowledge from applying these tools, and the additional 20 years of follow-up has changed the approach used for the diagnosis and prevention of type 1 diabetes.

We can predict, and indeed do predict, using a combination of islet autoantibodies, genetic markers, and metabolic markers to the point of including children and adults without clinical diabetes in prevention trials conducted through networks such as TrialNet (www.trialnet.org). An important change in the concept is the shift from using biomarkers to predict future clinical type 1 diabetes to using the biomarkers to diagnose an asymptomatic stage of the disease. It is hoped that this shift will lead to wider acceptance and application of the diagnosis and treatment of autoimmunity in order to prevent the onset of hyperglycemia rather than waiting until replacement therapies are required. This article discusses the prediction of type 1 diabetes using biomarkers in order to *1*) identify infants in whom islet autoimmunity is most likely to develop, *2*) diagnose islet autoimmunity, and *3*) determine the rate of progression to overt hyperglycemia or type 1 diabetes.

BIOMARKERS

A biomarker is defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (1). For the purpose of predicting type 1 diabetes, a biomarker should be present in a subset of the population, and this subset should have a bias in the

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proportion of people in whom type 1 diabetes develops. A biomarker should also present a quantifiable risk for the development of type 1 diabetes within a defined period or diagnose a "stage" in the progression to clinical or symptomatic type 1 diabetes. As described in this article, most of the biomarkers used for the prediction of type 1 diabetes are islet specific, supporting the concept that, before overt hyperglycemia, type 1 diabetes is predominantly a targeted disease rather than a systemic disease.

WHY APPLY BIOMARKERS FOR THE PREDICTION OF TYPE 1 DIABETES?

The application of biomarkers should provide benefits greater than its cost. Benefits range from learning about the disease process to preventing complications such as diabetic ketoacidosis at the diagnosis of diabetes, or even the prevention of diabetes entirely. Costs are usually pinned to the anxiety that may be caused in families who have a positive biomarker result. The long-term cost/benefit ratio of estimating the risk of type 1 diabetes risk is still under investigation.

The classic biomarkers that "predict" type 1 diabetes are serum autoantibodies against β-cell antigens, including insulin (2), GAD (3), IA-2 (4), and zinc transporter 8 (5). Autoantibodies to other antigens have been reported, but either occur infrequently or have been inadequately validated and are not used for prediction. Studies in infants followed from birth have found that seroconversion to an islet autoantibody–positive status can be detected as early as 6 months of age (6–8). This event marks a discrete start of the disease process and is associated with a marked increase in the risk of the development of diabetes. The presence of two or more of the four autoantibodies can be considered asymptomatic disease, and usually progresses to hyperglycemia (9). Since broad-scale application of islet autoantibodies for the prediction of type 1 diabetes will be introduced in some regions, there is an obligation that it is done well.

PREDICTING ISLET AUTOIMMUNITY

It is over 30 years since islet autoantibodies were found to precede the onset of type 1 diabetes (10). However, there are still relatively few cases of islet autoimmunity with full genetic typing to allow us to develop comprehensive algorithms for predicting islet autoimmunity, let alone understand or intercalate the role of environmental influences in this disease. To compensate, prediction considers factors that are known to be associated with clinical type 1 diabetes based on the reasonable assumption that they are also associated with islet autoimmunity. While a reasonable assumption, diabetologists need to consider that not all of the genes or environmental factors associated with type 1 diabetes will be useful for predicting islet autoimmunity (11).

HLA and Family History Are Proven Markers

There are several genes and some environmental factors that could be used at or soon after birth to predict the future risk of islet autoimmunity (12). Typing of the HLA DR and DQ loci (13), and knowledge of a family history of type 1 diabetes can stratify the risks of type 1 diabetes and islet autoimmunity from $<$0.01% in infants with no family history of type 1 diabetes and with protective HLA alleles, such as HLA DQB1*0602, to 50% in infants with a multiple first-degree family history of type 1 diabetes and the HLA DRB1*0301/DRB1*04-DQB1*0302 genotype (Fig. 1). Since HLA DR-DQ genotypes largely exert their influence on the risk of islet autoantibodies (11), it is reasonable to assume that they can be used for the selection of newborns and infants into observational and prevention studies.

The largest studies in this setting have been the DIPP (14), TEDDY (8), and TRIGR (15) studies. Each study used HLA genotypes, and the TEDDY and TRIGR studies also included family history of type 1 diabetes. In principle, one selects a risk target and adjusts the HLA and family history eligibility criteria so that the average risk in the selected population reaches the specified target. Because this represents an average risk, some infants show higher and some show lower a priori risk within this group. For example, the TEDDY study included infants without a family history of type 1 diabetes with HLA DR3/4-DQ8, DR4-DQ8/DR4-DQ8, and DR3/DR3 genotypes. The overall risk of type 1 diabetes by age 15 years was estimated to be 3%; but children with HLA DR3/4-DQ8 genotype have a 5% risk of the development of type 1 diabetes by age 15 years, and children with DR3/DR3 genotype have a risk of $<$2% (14). It is also necessary to consider that the estimated risk may vary between geographical regions or ethnic groups, and disease genetics may change over time (16).

Can More Genes Be Incorporated Into Prediction?

Population screening with genetic biomarkers could be improved. There are $>$40 validated markers (single nucleotide polymorphisms [SNPs] that have been confirmed in multiple cohorts [17]). The early interest in finding new genes was most certainly for improving prediction. However, few studies have attempted to combine all of the existing information on genetic risk to the benefit of predicting islet autoimmunity or type 1 diabetes. One reason for this may be the fact that the odds ratios for the non-HLA genes are relatively low, and that any benefit provided by single genes over what is provided by HLA alone is "not much." Indeed, David Clayton (18) was not optimistic in his assessment of the ability to improve prediction by combining genetic markers.

We (19) were more optimistic than Clayton (18) and demonstrated a definite improvement in trial design if additional genetic markers were added to HLA and family history to define eligibility for a primary prevention trial. Admittedly, much of the predictive power comes from HLA DR-DQ genotyping. However, the best estimate provided by HLA typing is the DR3/DR4-DQ8 genotype, which in infants from the general population, confers a 5% risk of the development of islet autoimmunity during childhood. To improve this prediction model, weighted scores for 40 SNPs were included, based on the results of multivariable logistic regression, yielding a mathematical risk score that could select the upper "*n*th" centile as its threshold. This approach should be able to identify infants who are at considerably greater risk than the 5% provided by the HLA DR3/DR4-DQ8 genotype, albeit with a potential cost in the sensitivity of the model. Surprisingly, the main limitation to validating this model was the relatively low number of control subjects who underwent typing of

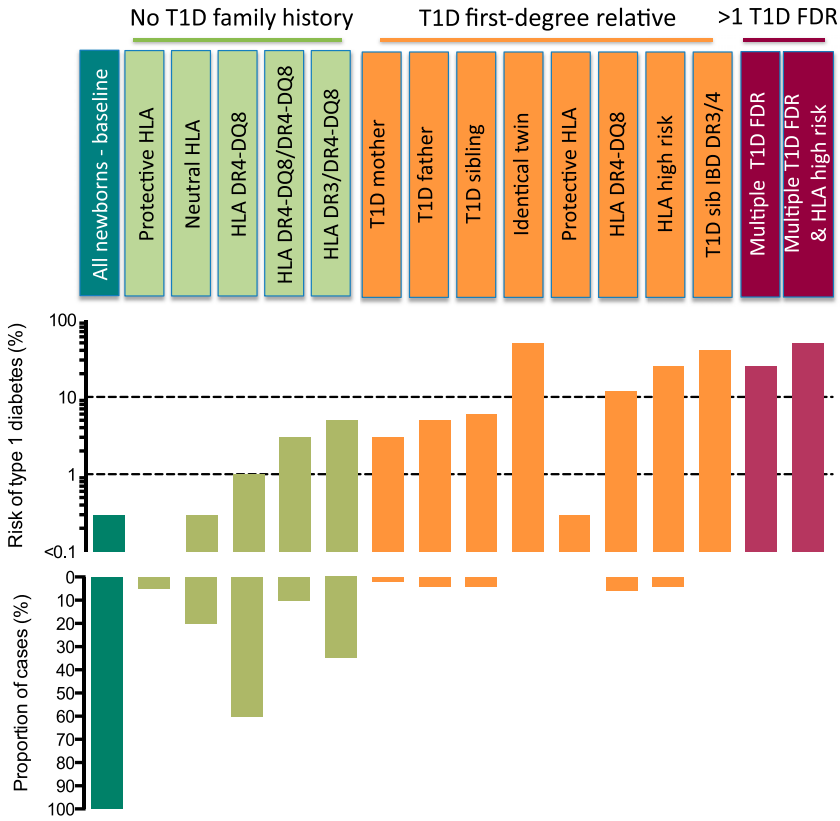

Figure 1—Risk for type 1 diabetes according to HLA and first-degree family history status. The top graph shows the approximate risk for type 1 diabetes by age 20 years for infants of European descent (*y*-axis) in whom the background risk is 0.3%. Risk is stratified by HLA (11) in infants who have no first-degree family history (light green bars), infants who have one first-degree relative with type 1 diabetes (orange bars), and infants who have multiple first-degree relatives with type 1 diabetes (burgundy bars). HLA high risk includes the presence of the HLA DR3/DR4-DQ8 and HLA DR4-DQ8/DR4-DQ8 genotypes. IBD, identical by descent to affected sibling; sib, sibling; T1D, type 1 diabetes. The lower graph shows the corresponding proportion of case patients with type 1 diabetes who are identified by the HLA and/or family history status.

all of the SNPs of interest. In our example, we reasoned that an algorithm that identified 0.5% of the population might be useful. However, with only 2,000 control subjects, the 95% CI for any algorithm that picked up 10 control subjects was 0.24–0.92%, providing wide Bayesian estimates of risk. Thus, population-based prediction with genetic markers still requires additional groundwork, including the following: *1*) efficient methods for genetic typing over all loci; *2*) larger (≥10,000 infants) cohorts of control subjects; *3*) registers of patients with type 1 diabetes containing DNA samples for genetic typing; and *4*) mathematicians who can develop and validate a risk score.

Adding Nongenetic Markers to Predicting Islet Autoimmunity

The biomarkers used to predict islet autoimmunity do not need to be limited to genes. Perinatal factors are also associated with the risk of type 1 diabetes (20). For example, cesarean section is reported to have an odds ratio of 1.3 for type 1 diabetes (21), placing it in the top third of the 40 major genetic risk markers. Such a marker could potentially increase the risk of islet autoimmunity in HLA DR3/4 genotype infants from 5% to 6.5%. Unfortunately, cesarean section does not appear to be associated with islet autoimmunity, but, rather, with a faster rate of progression to hyperglycemia (22). Nevertheless, until we have a better idea of the factors associated with islet autoimmunity, all of the validated factors associated with type 1 diabetes should be combined into a single risk score that can applied soon after birth.

A potentially fertile, but still unexplored, biomarker for predicting islet autoimmunity is experimental exposure to autoantigens. Exposing infant's naive $CD4^+$ T cells to GAD or proinsulin in vitro can lead to specific activation (23). Considering recent developments in T-cell phenotyping methods (24), it is possible that the responsive phenotypes may allow researchers to develop functional assays that stratify the risk of islet autoimmunity in genetically susceptible infants.

DIAGNOSIS OF ISLET AUTOIMMUNITY

Seroconversion to being positive for islet autoantibodies is rare before 6 months of age. Thereafter, seroconversion displays a peak incidence at 1 year of age (6–8), and by 3 years of age the majority of patients in whom clinical type 1 diabetes ultimately will develop during childhood will be islet autoantibody positive. Seroconversion has a major impact on the accuracy of predicting type 1 diabetes. There are currently four islet autoantibodies to consider. Children with seroconversion to any two autoantibodies have a risk of >80% for the development of diabetes during childhood or adolescence (9). Thus, it is important to discuss how islet autoantibodies should be used as a diagnostic tool.

Selecting Thresholds of Positivity for Individual Islet Autoantibodies

The selection of threshold makes a difference, and one can choose a threshold to match one's desired risk. If the biomarker is used to communicate low risk in children with a family history of type 1 diabetes, for example, a low threshold where the large majority of children in whom diabetes develops are negative for the biomarker might be appropriate. However, if the biomarker is used to diagnose a disease state (i.e., islet autoimmunity) or identify children for a clinical trial, it is probably better to use a higher threshold with few false positives.

Researchers usually consider a "yes/no" interpretation rather than thresholds that can be adapted to fulfill the objective of using the biomarker. For islet autoantibodies, the threshold is often set at the 99th centile of control children. However, there are alternative approaches to achieving high specificity and sensitivity. A simple way to improve the measurement of islet autoantibodies is to remeasure positive samples identified in one assay using a confirmation

assay that is sensitive and that uses a slightly different method to measure the antibodies. In this way, a relatively low threshold (e.g., 95th or 90th centile) may be used in the first assay to select samples to be measured in the second assay. The likelihood that both assays will provide results that are above the 95th centile by chance in samples that do not contain the autoantibodies is very low (0.25%). For example, remeasurement of insulin autoantibody (IAA) or GADA radiobinding assay–positive samples by the recently reported enhanced chemiluminescence (ECL) assay increased specificity without a substantial loss in sensitivity (25,26). This should also be true if ECL assays are used as the first-line test and the radiobinding assays are used for confirmation. Improvement will also occur if other assays, such as sensitive commercial ELISAs, are used for confirmation (27).

Selecting Thresholds of Positivity for Multiple Islet Autoantibodies

Using a combination of assays to measure individual islet autoantibodies allows us to compute thresholds to achieve a desired positivity rate in the population tested. The current practice of using a value of >99th centile of control subjects will require a child to be above this threshold for at least two of the four islet autoantibodies in order to meet the diagnosis of multiple islet autoantibodies. By chance alone, this should identify $[(0.01 \times 0.01 \times 3) + (0.01 \times 0.01 \times 2) + (0.01 \times 0.01)] \times 100\%$, or 0.06% (60/100,000), of the population. This is reasonably safe, but may miss some cases, especially if the model is applied in early childhood, when the antibodies may be starting to rise. Using two independent methods to define positivity for each of four antibodies, we will have eight parameters that can be used to define multiple islet autoantibody positivity. When we use a screening test and a confirmatory test using a different assay method for each antibody, the same probability of 0.06% for chance alone would be achieved if the 90th centile of control samples was used as the threshold in each assay. Using the 95th centile, this probability is reduced 16-fold to 0.00375% and will likely yield similar or greater sensitivity. The main point is that the performance of existing biomarkers could and should be improved when they are applied to the population level.

Not All Islet Autoantibodies Are the Same

IAAs are the first to appear, GADAs and IAAs are the most frequent islet autoantibodies in childhood, GADA is the hallmark of adult-onset type 1 diabetes, and IA-2 antigens are very specific for the development of diabetes (12). However, IAAs and GADAs are heterogeneous. They vary in their affinities and epitope specificities, and these variations are associated with different risks for type 1 diabetes. Low-affinity IAAs usually bind to atypical epitopes, do not bind to proinsulin, appear after 2 years of age, and are rarely associated with the progression to diabetes. By contrast, high-affinity IAAs recognize a common epitope that is present on both insulin and proinsulin, usually appear by 2 years of age, and are associated with the progression to multiple islet autoantibodies and diabetes (28). Low-affinity GADAs are not always detected using ELISAs (27) and are rarely found in children in whom diabetes develops. By contrast, high-affinity GADAs are reactive against the middle and C-terminal epitopes, and are associated with the progression to diabetes (29). Thus, it makes sense to include the islet autoantibody phenotype in children with persistent single islet autoantibodies when defining islet autoantibody positive status.

PREDICTING THE PROGRESSION TO HYPERGLYCEMIA OR TYPE 1 DIABETES

A combined analysis of the three longest cohorts of children followed from birth revealed that the risk in children with multiple islet autoantibodies was maintained at about 11% per year over 10 years (Fig. 2*A*). Thus, at any time during the follow-up period, the remaining diabetes-free children have an 11% risk of the development of diabetes within the next 12 months. In other words, the risk over 12 months is the same after 8 years as it was at seroconversion. This also means that, in a cohort of 1,000 3-year-old children with multiple islet autoantibodies, hyperglycemia is expected to develop in 50% of the children within 6 years and in >80% within 12 years and that diabetes would develop in the last individuals in the cohort at 60 years of age (Fig. 2*B*). In terms of diabetes prevention, we must also consider that the risk of the development of diabetes does not appear to change in periods associated with substantial physiological changes, such as puberty.

Titers and Changes in Autoantibodies Over Time

We have long known that the overall islet autoantibody titer is correlated with the risk of progressing to clinical type 1 diabetes (30). This is also true for most individual islet autoantibodies (31,32). However, an increase in the

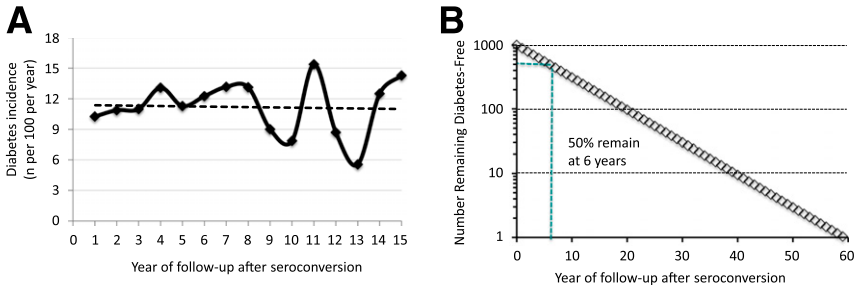

Figure 2—Risk of progression to clinical type 1 diabetes in children with multiple islet autoantibodies. *A*: The incidence of clinical diabetes per 100 children per year (equivalent to 12-month risk) is shown for children with multiple islet autoantibodies at each year after seroconversion to being islet autoantibody positive. The curve is derived from previously published data (9), which were from a combined analysis of the DAISY study from Colorado, the DIPP study from Finland, and the BABYDIAB and BABYDIET studies from Germany. It is presented here with the permission from the lead investigators of those studies. *B*: Based on these data, progression to clinical diabetes remains stable at ~10–12% of the diabetes-free multiple islet autoantibody–positive children per year for at least 10 years after islet autoantibody seroconversion. Using this rate, progression to clinical diabetes is shown for a hypothetical cohort of 1,000 3-year-old children with multiple islet autoantibodies who have a constant risk of 11% for each subsequent 12-month period. Clinical diabetes would develop in half of the cohort within 6 years of follow-up, and clinical diabetes would develop in the last patient at ~60 years of age.

autoantibody titer in an individual is not associated with an increased risk of progressing to clinical diabetes. Instinctively, we tend to interpret a rise in the level of a biomarker as "getting worse," but islet autoantibody titers rise quickly and then decline, and the rise and fall in titers is usually asynchronous for individual autoantibodies in a single child (33). Indeed, some islet autoantibodies "disappear" in some children who eventually progress to clinical diabetes (Fig. 3). The changing titers are also likely to be confounded by the changing profile of multiple islet autoantibodies and epitope specificities (31). Islet autoantibody profiles over time include rapid fulminant autoantibody responses, a waxing and waning response, and a quiescent no-change response (33). However, it is doubtful whether any of these patterns tell us much about the rate of progression to hyperglycemia or clinical type 1 diabetes and, once a child has multiple islet autoantibodies, additional biomarkers are needed to define progression or remission in that child.

Dysglycemia

There is substantial evidence that the loss of glucose tolerance occurs before the diagnosis of type 1 diabetes (34). The most extensive data come from Diabetes Prevention Trial–Type 1 (DPT-1) and the TrialNet Natural History studies (34–36), plus emerging data from prospective studies of children from birth (37). The period of time in which deteriorations in glucose metabolism can be detected varies between islet autoantibody–positive children, but, using current methods, it appears that dysglycemia may occur 2 years before the clinical diagnosis of diabetes. The earliest changes include a delay in the C-peptide response to an oral glucose challenge and elevated blood glucose levels (34). It is also likely that HbA_{1c} levels rise well before the onset of clinical type 1 diabetes, as shown in the DPT-1 study and in the DIPP study (35,37). Using data from the TrialNet Natural History Study, dysglycemia in multiple islet autoantibody–positive children has been defined as an impaired fasting plasma glucose level (>5.6 mmol/L), impaired glucose tolerance in an oral glucose tolerance test (2-h plasma glucose level of ≥7.8 mmol/L, or a value of ≥11.1 mmol/L at 30, 60, or 90 min), and/or an HbA_{1c} level ≥5.7% (36). The rate of progressing to overt clinical type 1 diabetes in children with dysglycemia is 60% within 2 years, which is almost three times greater than the rate in multiple islet autoantibody–positive children. Therefore, a composite metabolic score that includes HbA_{1c}, oral glucose tolerance test results, and possibly C-peptide concentrations may be helpful in predicting the rate of progression to type 1 diabetes in multiple islet autoantibody–positive children. Considering the potential benefits of the early diagnosis of type 1 diabetes (38), it seems reasonable to perform an oral glucose tolerance test every 6–12 months.

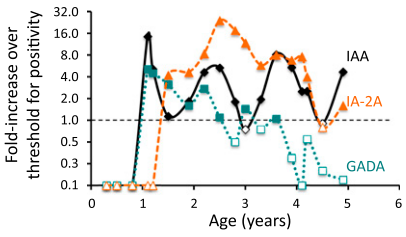

Figure 3—Time course of islet autoantibodies in a child in whom type 1 diabetes develops. Titers of IAA (black solid line), GADA (green dotted line), and IA-2 autoantibodies (IA-2A; orange dashed line) are shown as the fold increase over the threshold for positivity (*y*-axis) against the age of the child (*x*-axis). The dashed black line indicates the threshold for positivity. Open symbols represent negative antibody titers, and filled symbols represent positive antibody titers. This child is an example of autoantibodies decreasing over time to titers that are below the threshold for positivity. The child is a German participant in the TEDDY study (8).

Other Pancreas-Specific Biomarkers—Can Autoreactive T Cells Be "Predictive"?

The biomarkers that have so far been discussed as predictors of type 1 diabetes are directly or indirectly associated with pancreatic islets. The strongest genetic associations are with the *HLA* and *INSULIN* genes that determine islet autoimmunity, the strongest biomarkers are autoantibodies against pancreatic islet antigens, and metabolic biomarkers reflect the production of insulin in response to the metabolic demand. Thus, it makes sense to search for other biomarkers that also reflect pancreas-specific changes. Autoreactive T cells are obvious choices (39). Both autoreactive $CD4^+$ T cells and autoreactive $CD8^+$ T cells can be detected in blood. Moreover, the tools to measure these have improved in recent years (40). However, the practicality and utility of biomarkers that are expected to reside mainly in or around the pancreas, and are only seen in sufficiently high numbers in the circulation during discrete periods of activation and expansion, seem to be limited. Using organ-specific infection as an example (e.g., chronic viral hepatitis), the numbers of circulating $CD4^+$ or $CD8^+$ T cells seem to be too low for their use as biomarkers. In systemic infections (e.g., cytomegalovirus or influenza), the presence of memory virus-specific $CD8^+$ T cells provides evidence of exposure but not of active infection or disease. Active infection is reflected by a marked, but transient expansion of virus-specific $CD8^+$ T cells with an activated phenotype (41–43). Thus, while counting islet-specific T cells in peripheral blood is interesting, it is probably unsuitable as a biomarker for estimating the rate of progression to clinical diabetes. By contrast, the T-cell responses to therapies, such as antigen vaccination, are likely to provide valuable mechanistic biomarkers for therapeutic efficacy.

Biomarkers of β-Cell Death and Stress

Another recently explored field of biomarker discovery is the measurement of pancreatic islet products that are not normally found in the blood. For example, the serum ratio of demethylated insulin DNA, which is thought to be derived from destroyed pancreatic β-cells, to methylated insulin DNA appears promising (44), and may be complemented by other markers of β-cell damage or death (45). Whether these will aid in predicting the rate to hyperglycemia requires evaluation, and, in theory, these markers must eventually be exhausted with the ongoing loss of β-cells, potentially limiting their usefulness once β-cell mass is low.

"OMIC" BIOMARKERS—SHOULD WE REALLY BE LOOKING FOR A SYSTEMIC SIGNATURE?

Omic activities are expected to yield new biomarkers for predicting type 1 diabetes. Apart from genomic studies, omic-like searches have included cell populations in the blood (46), and their gene expression (47,48), blood

metabolomics (49,50), proteomics (51,52), and epigenetics (53). These topics have raised considerable interest, and articles concerning them are often published in highly regarded journals because of their novelty. However, these approaches have yet to identify a validated biomarker suitable for predicting type 1 diabetes. Many of these studies were performed in small, highly selective cross-sectional cohorts and frequently lacked validation cohorts. Moreover, the studies often enrolled patients at the clinical diagnosis of diabetes with severe metabolic instability not seen in the earlier, preclinical stages of type 1 diabetes.

Our own experience in unbiased omic marker identification has been disappointing. Although there have been interesting results using metabolomics and transcriptomics (47,50), none of the findings were strong enough or persisted for a sufficient period of time to be considered disease "signatures." It is also questionable whether the results will be consistently validated in multiple cohorts. We can also provide many examples of unpublished findings regarding general biomarkers that were not validated when applied to a second dataset, and several examples where others have failed to validate their published general omics biomarkers in our samples (again, unfortunately, unpublished). One suspects that, unlike systemic lupus erythematosus, which has a strong systemic type 1 interferon signature, or type 2 diabetes, which is characterized by signatures of insulin resistance and inflammation, type 1 diabetes is ultimately a pancreas-specific immune disease dominated by HLA-associated loss of tolerance to β-cell antigens.

Considering these issues, heading aimlessly into omic biomarkers may not be a wise approach. A targeted approach that considers some of the proven genetic and environmental associations may be more rewarding. In other words, finding biomarkers that are downstream of risk or protective factors may yield stronger biomarkers than the factors themselves, and they may be common to multiple factors.

AGE AS A BIOMARKER

Age is an undervalued biomarker for predicting type 1 diabetes. For example, because there is a significant peak in the incidence of islet autoantibody seroconversion before 3 years of age (6–8), it follows that the risk of the development of islet autoantibodies in a 1-year-old child is substantially greater than that of a 5-year-old child or the same child when he or she reaches 5 years of age without the development of islet autoantibodies. It also makes a difference to the rate of progression to hyperglycemia if a child seroconverts to islet autoantibody positivity at 1 or 5 years of age (9). By contrast, age (or time of follow-up) hardly influences the risk of diabetes in children with multiple islet autoantibodies, with the risk remaining ~11% per year regardless of age (9). This may change in adulthood since it has been reported (54) that the risk is reduced in islet autoantibody–positive adults compared with children.

Another aspect that should be considered is that the biomarkers change with age. Predicting type 1 diabetes in adults is very different to its prediction in children. There are many cases of adult-onset type 1 diabetes, but older patients are less likely to display the biomarkers that are detected in children. First, genes have a smaller impact because patients with disease onset after 20 years of age have lower frequencies of the high-risk HLA DR and DQ haplotypes. A genetic risk score for type 1 diabetes occurring between 20 and 60 years of age will not reach the level of risk seen for a similar score applied to the 0- to 20-year-old age period. Second, the number of islet autoantibodies that are found in patients in whom type 1 diabetes develops in adulthood is less than that in childhood, and many patients with adult-onset type 1 diabetes only present with GADAs (55). This is partly due to the loss of IAAs over time and is possibly related to a milder form of autoimmunity. Regardless of the underlying cause, the smaller number of islet autoantibodies weakens our ability to predict or diagnose adult-onset type 1 diabetes. Multiple islet autoantibodies will confer an important risk, but most patients with adult-onset type 1 diabetes cannot be identified using such stringent criteria (55). Accordingly, it may not be worthwhile to extend testing for islet autoantibodies into adulthood.

POPULATION PREDICTION OF TYPE 1 DIABETES IN THE FUTURE

It is my hope that the prediction of type 1 diabetes will eventually move into a public health setting and become more comprehensive than it is now (Fig. 4). I encourage a combined risk assessment together with an "attempt-to-prevent" approach that starts from birth. Here is a potential outline of population-based "prediction."

First, genetic screening should occur at or soon after birth, and would involve a combined risk score that considers the family history of type 1 diabetes, the HLA DR-DQ genotype, and all validated SNP markers. Infants with a risk score of ≥5%, for example, could be eligible for prevention therapies that are safe but "active," like antigen-specific therapies such as oral insulin. Meanwhile, lower-risk infants might be considered for therapies, such as diet and environmental modifications.

Second, islet autoantibodies should be tested in all children, regardless of their gene score, at 3 years of age, an age when many children in whom multiple islet autoantibodies develop will have seroconverted to being positive for islet autoantibodies. Islet autoantibody tests need to be simple, cheap, and ideally performed using blood spots that can be stored at pediatric ambulatory clinics and shipped to the test center weekly. Unfortunately, such tests are not yet available. Children with multiple islet autoantibodies would be referred for confirmation and metabolic testing to determine whether they have dysglycemia or diabetes. Children with single islet autoantibodies should undergo islet autoantibody phenotyping, and if they are found to be a high-risk phenotype, these children would also undergo metabolic testing. All children with confirmed high-risk islet autoantibodies would be eligible for prevention trials and annual testing to detect changes in their metabolic status. The families of these children should be offered counseling and diabetes education in an attempt to reduce complications associated with diabetes. Throughout the course of testing, a risk score would be calculated that considers all of the applied biomarkers, including age. I believe that such a program will improve the ability to predict the onset of type 1 diabetes and eventually lead to a

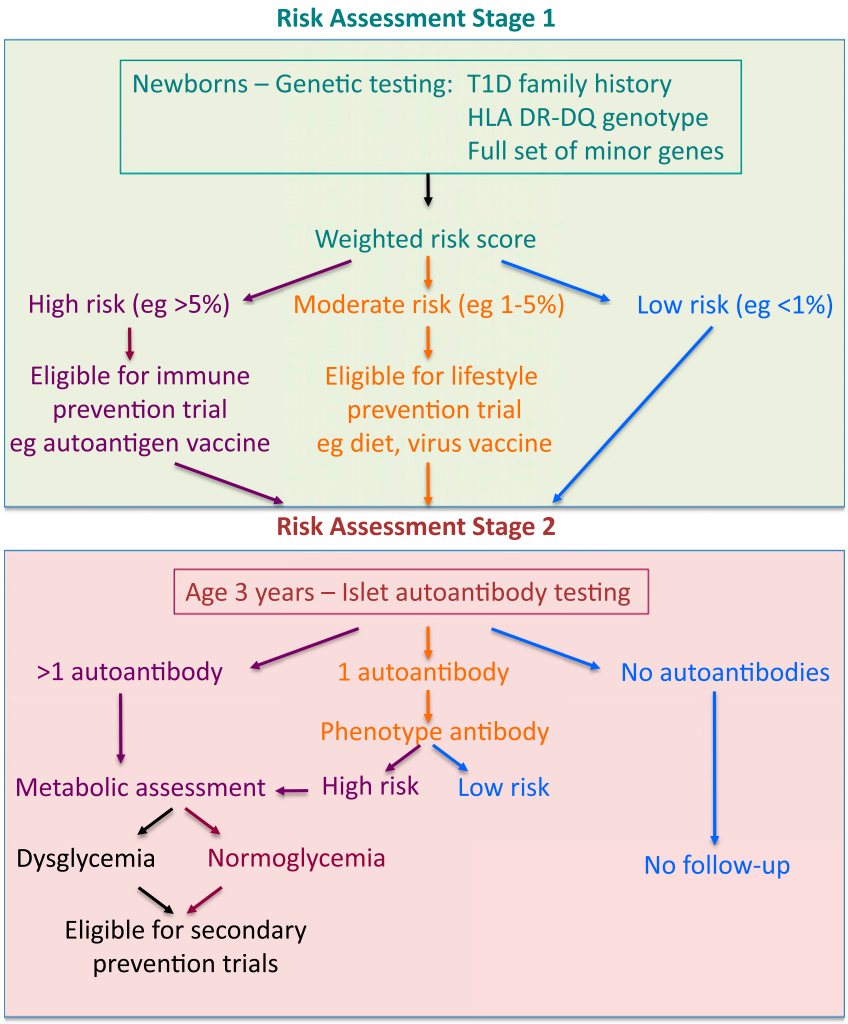

Figure 4—Schematic representation of potential future public health type 1 diabetes risk assessment program.

reduction in diabetes-related complications and the number of patients with type 1 diabetes.

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