

# Immunological Markers in the Diagnosis and Prediction of Autoimmune Type 1a Diabetes

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**D**iabetes mellitus encompasses a family of disorders of carbohydrate metabolism that are characterized by hyperglycemia and the development of long-term macrovascular, microvascular, and neuropathic complications. In 1997, the American Diabetes Association revised the classification of diabetes.<sup>1</sup> Diabetes is now classified as type 1 diabetes, type 2 diabetes, other specific types of diabetes (e.g., secondary diabetes), or gestational diabetes. Type 1 diabetes is primary insulinopenic diabetes that is subclassified as autoimmune type 1 diabetes (type 1a) or idiopathic type 1 diabetes (type 1b).<sup>2</sup> The most important factor differentiating type 1a from type 1b diabetes is the presence of islet autoantibodies.<sup>3</sup>

Our goal is to address two key questions concerning autoimmune type 1 diabetes: 1) Which autoantibodies serve as diagnostic markers of autoimmune type 1 diabetes?, and 2) Can islet autoantibodies that are used to diagnose type 1 diabetes predict the development of type 1 diabetes in nondiabetic individuals?

## ISLET AUTOANTIBODIES AS MARKERS OF AUTOIMMUNE DIABETES

Many autoantibodies are detected at the onset of type 1 diabetes. Islet cell (cytoplasmic) autoantibodies (ICAs) and islet cell surface autoantibodies (ICSAs)<sup>4-9</sup> were initially described in the 1970s. In the 1980s, insulin autoantibodies (IAAs),<sup>10</sup> 64-kDa autoantibodies (64KAs),<sup>11</sup> insulin receptor autoantibodies,<sup>12</sup> carboxypeptidase-H autoantibodies,<sup>13</sup> and heat shock protein

(HSP) autoantibodies<sup>14</sup> were recognized. In the very early 1990s, the nature of the 64KA autoantigen was revealed as glutamic acid decarboxylase (GADs) leading to the recognition of glutamic acid decarboxylase autoantibodies (GADAs).<sup>15</sup> Subsequently, a cascade of various islet autoantibodies were identified including 51-kDa aromatic-L-amino-acid decarboxylase autoantibodies,<sup>16</sup> chymotrypsinogen-related 30-kD pancreatic autoantibodies,<sup>17</sup> DNA topoisomerase II autoantibodies,<sup>18</sup> glima 38 autoantibodies,<sup>19</sup> GLUT2 autoantibodies,<sup>20</sup> glycolipid autoantibodies,<sup>21</sup> GM2-1 islet ganglioside autoantibodies,<sup>22</sup> IA-2 autoantibodies (IA-2As),<sup>23,24</sup> IA-2 $\beta$  autoantibodies (IA-2 $\beta$ As),<sup>25</sup> ICA69 autoantibodies,<sup>26</sup> islet cell-specific 38-kD autoantibodies,<sup>27</sup> proinsulin autoantibodies,<sup>28</sup> and 52-kDa RIN (rat insulinoma) autoantibody (Rubella-related autoantibody).<sup>29</sup> New autoantibodies

associated with type 1 diabetes continue to be discovered.<sup>30,31</sup>

Four autoantibodies have emerged as the most useful autoimmune markers of type 1 diabetes: ICAs, IAAs, GADAs, and IA-2As.<sup>32</sup> IA-2As include ICA512 and IA-2c autoantibodies.

## ICAs

ICAs are detected by indirect immunofluorescence using blood group O human pancreas as substrate. The ICA assay is labor intensive and non-automated and requires a very high level of quality assurance and quality control to produce accurate and precise results.

ICAs are polyclonal autoantibodies that react with all cells of the islet (e.g.,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and PP cells). Lipid and protein autoantigens that are recognized by ICAs include sialoglycoconjugate,<sup>33</sup> GAD,<sup>34</sup> and IA-2.<sup>35</sup>

ICAs, like other islet autoantibodies, do not appear to play an etiological role in  $\beta$ -cell destruction but do serve as important markers of  $\beta$ -cell autoimmunity. At onset of type 1 diabetes, 70% or more of Caucasians are ICA-positive.<sup>36</sup> Only 4 in 10 African Americans with new-onset type 1 diabetes are ICA-positive. This suggests that a considerable proportion of African Americans with new-onset insulin-requiring diabetes do not have autoimmune diabetes.<sup>37</sup> ICA frequency declines following diagnosis, and no more than 5–10% of type 1 diabetic patients remain ICA-positive after 10 years.

The general population frequency of ICAs is low. In the University of Florida Pasco County School study, ~1 in 250 normal schoolchildren were ICA-posi-

## IN BRIEF

Several autoantibodies are associated with autoimmune type 1 diabetes. Measurements of islet autoantibodies can assist in the diagnosis of autoimmune diabetes, and the detection of islet autoantibodies in nondiabetic individuals indicates a significantly increased risk for the subsequent development of type 1 diabetes. When preventive therapies for type 1 diabetes become available, islet autoantibody screening of the general pediatric population should be considered to identify at-risk individuals.

tive.<sup>38</sup> Approximately 2–3% of first-degree relatives of type 1 diabetes patients are ICA-positive. Shortly, we will see that this is a pivotal feature of ICAs that allows the recognition of pre-diabetes.

The detection of ICAs in adults diagnosed clinically with non-insulin-dependent diabetes has revealed the existence of latent autoimmune diabetes of adulthood (LADA), a slowly progressive form of type 1 diabetes.<sup>39–47</sup> After months to years, affected individuals become increasingly insulin-dependent for control of hyperglycemia, which indicates the slow, persistent progress of  $\beta$ -cell damage in LADA.

ICA-positive individuals initially presenting with phenotypic type 2 diabetes display lower C-peptide levels and higher frequencies of the HLA alleles DR3 and DR4, which are associated with type 1 diabetes. Between 4 and 17% of type 2 diabetic patients have LADA based on the presence of islet autoantibodies. The onset of type 2 diabetes in lean individuals also suggests LADA and is an indication for islet autoantibody testing. The pathophysiology of LADA is distinct from that of classic type 2 diabetes.<sup>48,49</sup> ICAs are usually absent in cases of pediatric type 2 diabetes.<sup>50–53</sup>

### Insulin Autoantibodies

The first islet autoantigen and  $\beta$ -cell-specific autoantigen reported was insulin.<sup>10</sup> Autoantibodies to insulin should be sought before the administration of exogenous insulin (either animal or human) because after 5–7 days of exogenous insulin treatment, insulin antibodies will arise. The immunoprecipitation assay for insulin autoantibodies does not distinguish spontaneous autoantibodies from antibodies arising from insulin immunization, which occurs as a consequence of exogenous insulin therapy. IAAs may occur more commonly in individuals who carry HLA-DR4.<sup>54</sup> Techniques that detect insulin autoantibodies by an enzyme-linked immunosorbent assay should not be used because IAAs so detected do

not correlate with autoimmune diabetes.<sup>55,56</sup>

At onset of type 1 diabetes, IAAs occur in 35–60% of children but are decidedly less common in adults.<sup>57</sup> A new IAA microassay has been developed that requires less serum than the traditional IAA assay.<sup>58</sup> At the onset of type 1 diabetes in Australians, Feeney et al.<sup>59</sup> found that IAAs were present in 90% of children <5 years old, 71% of children 5–10 years old, and 50% of children 10–15 years old. Bingley et al.<sup>60</sup> reported IAAs in 83% of children <10 years old and 56% of children 10 years old or older.

IAAs occur in many other autoimmune diseases including autoimmune thyroid disease. IAAs were found in 13–44% of Graves' disease patients and in 16–23% of Hashimoto's thyroiditis patients.<sup>61,62</sup> IAAs have also been detected in Addison's disease (40%), chronic hepatitis (36%), pernicious anemia (40%), systemic lupus erythematosus (29%), and rheumatoid arthritis (25%).<sup>62</sup> Their significance is unknown in the latter conditions.

### GADs

GAD is neither  $\beta$ -cell nor islet specific.<sup>63</sup> GAD is expressed predominantly in the nervous system. Other tissues that express GAD include the testes, ovary, adrenal, pituitary, thyroid, and kidney.

Because GADs are more persistent than ICAs after the diagnosis of type 1 diabetes,<sup>47</sup> GADs may be more often positive than ICAs in LADA. Because the frequency of LADA is ~5–15% based on ICA studies, using GADs as the autoimmune marker, LADA prevalence in phenotypic type 2 diabetes might be even greater. GADs have been a major focus of diabetes research for more than 10 years.<sup>64–70</sup> GADs are detected in 60% or more of new-onset cases of type 1 diabetes and 3–5% of relatives.<sup>71</sup>

### IA-2As

IA-2 is a member of the protein tyrosine phosphatase (PTP) family.<sup>72</sup> Similar to GAD, IA-2 is found in nervous tissue

and other endocrine tissues including the pituitary.<sup>73</sup> Some IA-2 autoantibody assays detect the entire molecule, whereas others detect portions of the molecule (e.g., IA-2c and ICA512 assays).

When testing for autoantibodies against ICA512 or IA-2A in natural history studies of pre-diabetes, some investigators suggest that ICA512 autoantibodies develop later than GADs and thus may serve as markers of higher short-term risk for the clinical onset of type 1 diabetes. Like GADs, IA-2As and related PTP autoantibodies have been a major focus of recent diabetes research.<sup>74–79</sup>

IA-2As and IA-2 $\beta$ As are detected in ~60% or more of cases of new-onset type 1 diabetes.<sup>80</sup> The general population frequencies for these autoantibodies are similar to those of GADs at 2–3%.

A review of 10 representative studies spanning 1995–1998 that include Americans,<sup>81</sup> Australians,<sup>82</sup> Britains,<sup>60</sup> Germans,<sup>83,84</sup> Italians,<sup>71,85</sup> Japanese,<sup>86</sup> and Swedes<sup>87,88</sup> reveals at the onset of type 1 diabetes ICA frequencies of 70–90%, IAA frequencies of 43–69%, GADA frequencies of 52–77%, and IA-2A frequencies of 55–75% (Table 1).

### ISLET AUTOANTIBODIES THAT PREDICT AUTOIMMUNE TYPE 1 DIABETES IN NONDIABETIC INDIVIDUALS

To understand the predictive role of islet autoantibody testing for type 1 diabetes, it is helpful to review the natural history of type 1 diabetes before diagnosis.  $\beta$ -Cell destruction can be viewed as roughly passing through five phases: 1) genetic predisposition, 2) autoantibody positivity, 3) abnormal insulin responses during intravenous glucose tolerance test (IVGTT), 4) glucose intolerance on oral glucose tolerance test (OGTT), and 5) clinical diabetes (Figure 1).<sup>89</sup> The period of time to develop type 1 diabetes may take months (young children) to years (adults).<sup>90,91</sup>

Most cases of type 1 diabetes occur sporadically, e.g., in the absence of a family history of type 1 diabetes in a

**Table 1. Islet Autoantibody Frequency Comparisons in New-Onset Type 1 Diabetes (Single Studies)**

	Swedes <sup>87</sup>	Italians <sup>71</sup>	Germans <sup>83</sup>	Swedes <sup>88</sup>	Australians <sup>82</sup>	Britains <sup>60</sup>	Germans <sup>84</sup>	Americans <sup>81</sup>	Italians <sup>85</sup>	Japanese <sup>86</sup>
ICA	84	88	75	87	70	88	69	90	85	—
IAA	56	—	—	—	65	69	43	—	—	—
GADA	70	70	75	66	69	74	52	72	77	70
IA-2A	—	54	73	68	60	75	55	63	62	58

Data are % positive.

first-degree relative.<sup>92</sup> At most, only 15% of type 1 diabetic patients have an affected first-degree relative. In first-degree relatives of type 1 diabetic patients, there is a risk of ~5% for developing type 1 diabetes. Children born to fathers with type 1 diabetes have been reported to be at higher risk for developing type 1 diabetes than children born to mothers with type 1 diabetes (7 vs. 2%).<sup>93,94</sup>

Two loci have been confirmed as type 1 diabetes susceptibility loci where the specific genes IDDM1 and IDDM2 have been identified.<sup>95-97</sup> IDDM1 includes the HLA-DRB1, HLA-DQB1, and HLA-DQA1 loci within the HLA complex located on the short arm of chromosome 6. HLA alleles associated with proclivity to type 1 diabetes include HLA-DR3, HLA-DR4, HLA-DQB1\*0201, HLA-DQB1\*0302, and HLA-DR1, whereas HLA-DR2 and HLA-DQB1\*0602 are protective of

type 1 diabetes. IDDM2 is the insulin gene located on chromosome 11.<sup>98-100</sup> Insulin gene alleles may affect the degree of insulin expression in the thymus gland affecting immunological tolerance to insulin.<sup>101</sup>

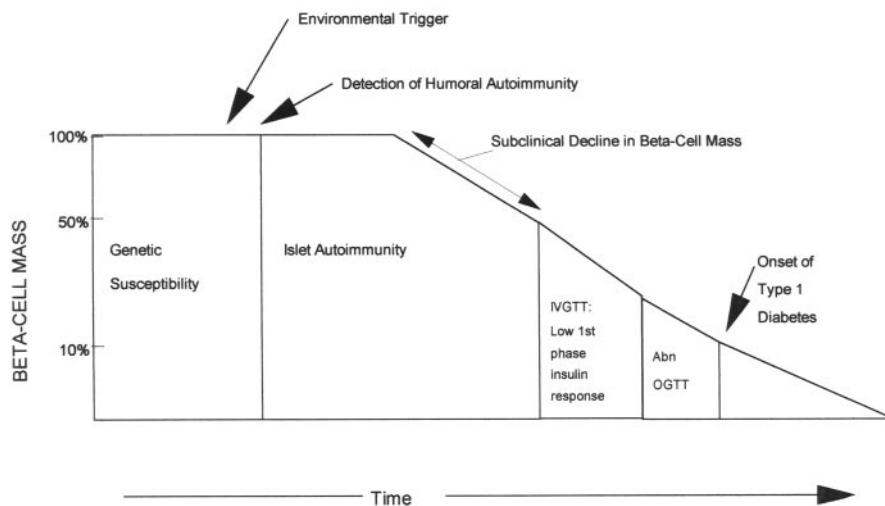
Considering our current understanding of the genetics of type 1 diabetes, most genetically susceptible individuals defined by HLA typing or other genetic typing have a low risk for developing type 1 diabetes. The hope is that by assessing multiple risk loci, patterns of alleles can be identified that substantially increase the predictive value of genetic typing.

Concordance for type 1 diabetes in identical twins ranges between 30 and 50%.<sup>102</sup> About 75% of nondiabetic twins who have a twin with type 1 diabetes are ICA-positive. Thus, up to 75% of the ICA-positive twins go on to develop type 1 diabetes.

Because concordance for type 1 diabetes is 50% at most, environmental factors must play a significant role in the development of type 1 diabetes.<sup>103</sup> Many environmental factors have been explored as possible triggers of  $\beta$ -cell autoimmunity including viral infections,<sup>104</sup> diet (e.g., nitrosamines in smoked meat),<sup>105,106</sup> and breast-feeding and early exposure to cow's milk.<sup>107-112</sup> The specific environmental factor or factors that precipitate  $\beta$ -cell autoimmunity in type 1 diabetes remain elusive.

In genetically at-risk individuals, sometime after exposure to the proposed environmental trigger or triggers,  $\beta$ -cell autoimmunity is first recognized by the detection of islet autoantibodies.<sup>113</sup> Cell-mediated autoimmunity is believed to mediate  $\beta$ -cell destruction. Although it is extremely difficult to reproducibly measure cellular autoimmunity, the assays for humoral autoimmunity are reasonably accurate and precise. Islet autoantibodies can appear throughout childhood in siblings of type 1 diabetic probands.<sup>114</sup>

The cell-mediated immune attack targeted against  $\beta$ -cells is histologically termed "insulinitis."<sup>115-117</sup> Insulinitis has been observed in animal models of autoimmune diabetes including in the BioBreeding rat,<sup>118</sup> in the nonobese diabetic mouse,<sup>119</sup> and as streptozotocin-induced diabetes in mice. It has also been observed in 80% of individuals with clinical type 1 diabetes who come to autopsy within 6 months of diagnosis.<sup>120,121</sup> Using a pancreatic biopsy technique in living human subjects, Japanese investigators have observed insulinitis before the later development of type 1 diabetes.<sup>122</sup> In prospective studies



**Figure 1. Progressive loss of  $\beta$ -cell mass in the natural history of type 1 diabetes.**

of initially nondiabetic individuals, ICAs, IAAs, GADAs, and IA-2As have all been detected before the diagnosis of type 1 diabetes.<sup>123</sup>

The first biochemical evidence of  $\beta$ -cell dysfunction can be detected by a loss of first-phase insulin response to the administration of intravenous glucose.<sup>124–127</sup> IVGTT measures the  $\beta$ -cell's content of preformed insulin and its ability to release that insulin in response to an acute intravenous glucose challenge. Usually, first-phase insulin response is measured as the sum of the plasma insulin concentration at 1 plus 3 minutes after the acute glucose bolus injection.

In an OGTT, there are three forms of  $\beta$ -cell stimulation: autonomic nervous system stimulation of insulin secretion, rising systemic glucose levels, and release of glucagon-like peptide-1 from the intestine. On the other hand, in the IVGTT, there is only one type of insulin stimulus (i.e., rising plasma glucose), and thus with fewer  $\beta$ -cell stimuli, the IVGTT is apparently more sensitive than the OGTT in indicating  $\beta$ -cell dysfunction. In fact,  $\beta$ -cell insulin response to intravenous amino acids (e.g., arginine) is preserved longer than the insulin response to intravenous glucose.<sup>128</sup>

There is a predictable rate of decline in insulin secretion in those people with ICAs that ultimately go on to develop type 1 diabetes.<sup>129</sup> Nevertheless, the course of the development of type 1 diabetes is often most properly described as a waxing-waning pattern of insulin secretion that is typical of many autoimmune diseases (e.g., systemic lupus erythematosus). In this phase of the destructive process, the OGTT is normal and thus there are no detectable abnormalities of glucose tolerance excluding the IVGTT. It is estimated that by the time the first-phase insulin response during IVGTT is low, there has been a 50% decline in  $\beta$ -cell mass.

When more  $\beta$ -cell mass has been lost (substantially <50% but >10% still remaining), the OGTT may display abnormalities at fasting (impaired fasting

glucose, 110–125 mg/dl) or after a glucose challenge (impaired glucose tolerance, 2-hour glucose 140–199 mg/dl), which is more likely in younger children. Such a degree of  $\beta$ -cell impairment is similar to the  $\beta$ -cell dysfunction observed in individuals with type 2 diabetes. Individuals with LADA experience a very gradual decline in  $\beta$ -cell function. However, in individuals who eventually present with typical acute-onset type 1 diabetes, frank clinical diabetes develops within 1–2 years of the onset of oral glucose intolerance. By the time an insulin-dependent state occurs, manifested in acute symptoms of type 1 diabetes,  $\beta$ -cell mass is estimated to have declined by ~90% or more from baseline.

The potential of islet autoantibodies to predict type 1 diabetes is discussed below.<sup>130,131</sup>

#### ICAs: Prediction of Type 1 Diabetes

In a prospective study of first-degree relatives of type 1 diabetic patients from the University of Florida,<sup>132</sup> ICA titers >40 Juvenile Diabetes Foundation (JDF) units predicted a 70% 7-year risk for type 1 diabetes, ICAs of 20–40 JDF units predicted a 20% risk, and ICAs of 10 JDF units predicted a 10% risk. Other studies have also found that a higher ICA titer is associated with a higher risk for type 1 diabetes.<sup>133</sup>

A question of great relevance is whether ICAs predict type 1 diabetes in individuals who do not have a family history of type 1 diabetes. This is an important issue because 85% of people who develop type 1 diabetes are from the general population, i.e., lacking a family history of type 1 diabetes. This question was addressed in the Pasco County, Fla., prospective study of schoolchildren.<sup>38</sup> Approximately 10,000 normal schoolchildren were screened for ICAs. Those with ICAs (0.59% of the all children screened) were entered into a prospective study and followed for the development of type 1 diabetes. The 7-year risk for type 1 diabetes in these otherwise normal ICA-positive

children was 45% compared to 43% in a group of age-matched relatives of type 1 diabetic patients ( $P = 0.3$ ). Thus, ICAs appear to be as valuable for the prediction of type 1 diabetes in the general population as they are in relatives of type 1 diabetic patients.

Age at the time of detection of ICAs influences the risk for type 1 diabetes. In the University of Florida prospective family studies, the 7-year risk for type 1 diabetes in ICA-positive individuals <10 years old was ~80% versus 20% in those ICA-positive individuals >10 years of age.<sup>132,134</sup> The risk in children <2 years old was ~90%. In the Bart's-Windsor, Bart's-Oxford prospective family studies,<sup>133</sup> the risk for type 1 diabetes in the youngest ICA-positive quartile (<13.2 years old) was 62% versus 4% in the oldest quartile (>40.7 years old).

$\beta$ -Cell function assessment in addition to the presence of islet autoantibodies influences the prediction of type 1 diabetes. The presence of decreased first-phase insulin responses during IVGTT in ICA-positive children predicts a 5-year risk for type 1 diabetes of 50–65%. During the Diabetes Prevention Trial–Type 1, these data were borne out in a 60% risk for type 1 diabetes.

#### IAAs, GADAs, and IA-2As: Prediction of Type 1 Diabetes

Most current studies involve investigations of multiple islet autoantibodies. The ability of a single autoantibody by itself to predict type 1 diabetes is limited. In the Florida school study, none of the IAA-positive, ICA-negative children developed type 1 diabetes.<sup>38</sup> However, IAAs in combination with ICAs increased the risk of developing type 1 diabetes. In a study from the Joslin group,<sup>135</sup> the predicted 5-year risk for progression to type 1 diabetes in ICA-negative/IAA-positive relatives was 17 versus 42% for ICA-positive/IAA-negative relatives and 77% for ICA-positive/IAA-positive relatives.

As noted above, some serial autoantibody studies in infants indicate that IAAs may, however, be the first islet

autoantibody to develop during progression to type 1 diabetes.<sup>136-139</sup> The presence of IAAs do influence diabetogenesis.<sup>140</sup> In the Seattle Family Study, which included first-degree relatives of type 1 diabetic probands, GADAs predicted a 50% 5-year risk for type 1 diabetes.<sup>141</sup> In the Munich, Germany, family study,<sup>84</sup> GADA-positive relatives had a 56% 5-year risk for developing type 1 diabetes versus a 24% risk in GADA-negative relatives. Surprisingly, Yu et al.<sup>142</sup> found that very high levels of GADAs are less likely to predict type 1 diabetes. In the Munich, Germany, family study,<sup>84</sup> IA-2A-positive relatives had a 64% 5-year risk for type 1 diabetes versus a 13% risk in IA-2-negative relatives.

As with any assay, the cutpoint chosen to define positivity influences the sensitivity and specificity of the assay. Raising the cutoff increases the predictive power of autoantibodies, although the number of autoantibody-positive people declines as the cutoff increases.

**Multiple Islet Autoantibodies: Prediction of Type 1 Diabetes**

Nondiabetic individuals who express combinations of islet autoantibodies have a much higher risk for type 1 diabetes than individuals who express fewer types of islet autoantibodies.<sup>141-143</sup> Furthermore, the total number of types of islet autoantibodies is usually more important than the specific combination of positive islet autoantibodies. For example, the addition of any positive islet autoantibody to ICA positivity in the Bart's-Windsor, Bart's-Oxford prospective family studies<sup>144</sup> raised the 15-year risk for type 1 diabetes from 47 to 66%. ICAs alone provided a 6% 10-year risk versus a 27% 10-year risk for ICAs plus one other islet autoantibody and an 88% 10-year risk for ICAs plus two other islet autoantibodies. Of the islet autoantibody-positive cohort, 36% were positive for ICAs alone, 38% were positive for ICAs and one other islet autoantibody, and 27% were positive for ICAs and two other islet autoantibodies.

From the Barbara Davis Center in Denver, Colo., first-degree relatives without any islet autoantibodies had a 5-year risk for type 1 diabetes of only 0.2%.<sup>145</sup> When testing for GADAs, ICA512bdc autoantibodies (an IA-2A assay based on immunoprecipitation of the ICA512 autoantigen), and/or IAAs, the risk for type 1 diabetes was 15% with one autoantibody, 44% with two autoantibodies, and 100% with three autoantibodies.

In the Milan, Italy, family studies,<sup>146</sup> the 6-year risk for type 1 diabetes was 26% for ICA positivity, 18.2% for GADA positivity, 17.9% for IA-2A positivity, and only 5.6% for IAA positivity. Whereas the 6-year risk with no islet autoantibodies was 0% and the 6-year risk with any one islet autoantibody was 2.9%, when two or more islet autoantibodies were present, the 6-year risk rose ~10-fold over the risk from any one islet autoantibody to 31.4%.

In the University of Florida study of 15,224 nondiabetic relatives of type 1 diabetic probands,<sup>147</sup> ICAs were the most sensitive marker for the 5-year prediction of type 1 diabetes. ICAs carried a 74% risk for type 1 diabetes, GAD65A (autoantibody to GAD65) carried a 60% risk, IA-2As carried a 54% risk, IAAs carried a 50% risk, and IA-2βs carried a 34% risk.<sup>147</sup> The 5-year risk data demonstrate that as the number of islet autoantibodies increase in any individual, so does the risk for type 1 diabetes (Table 2).

**CONCLUSIONS**

Islet autoantibodies are very common at the time of onset of type 1 diabetes. Islet autoantibody testing can confirm

autoimmunity in cases of new-onset diabetes and can differentiate type 1 from type 2 diabetes: islet autoantibody-positive individuals should be classified as having type 1a diabetes. The absence of islet autoantibodies, however, does not exclude type 1 diabetes.<sup>123</sup> The appearance of islet autoantibodies in pancreas transplant recipients predicts recurrence of type 1 diabetes.<sup>148</sup> Type 1 diabetes can occur after organ donation, and thus living kidney donors from families with histories of type 1 diabetes should be screened for islet autoantibodies.<sup>149</sup> Individuals with phenotypic type 2 diabetes who express islet autoantibodies are affected with LADA. Islet autoantibodies predict type 1 diabetes in relatives of type 1 diabetic probands as well as in the general population.<sup>150</sup> Combinations of autoantibodies enhance predictability.

Once therapies are developed that can prevent type 1 diabetes when applied in the prediabetic phases of β-cell autoimmunity,<sup>151</sup> large screening programs for islet autoantibodies should be undertaken in children. Screening on at least two occasions appears warranted: before age 5 and before puberty. This is because diabetes appears first near age 5 and then peaks with puberty.<sup>152</sup> Combination islet autoantibody assays, e.g., the simultaneous detection of GADAs and IA-2As,<sup>153,154</sup> will likely supplant ICAs testing in future screening programs.

It is potentially valuable to predict type 1 diabetes because 1) early treatment of type 1 diabetes with tight glycemic control preserves β-cell function, prolonging the honeymoon peri-

**Table 2. University of Florida Study of Nondiabetic Relatives of Type 1 Diabetic Probands**

Islet autoantibody status	5-year risk of developing type 1 diabetes (%)
ICA-negative	3.2
ICA alone	5.3
IAA alone	9.1
Any two islet autoantibodies	28.2
ICA-positive + one other islet autoantibody	50.3
IAA-positive + one other islet autoantibody	55.7
Any three or four islet autoantibodies	66.2

od;<sup>155</sup> 2) early diagnosis and treatment of type 1 diabetes should prevent the development of diabetic ketoacidosis; and 3) prediction of type 1 diabetes provides an opportunity for entrance into trials to prevent type 1 diabetes.

REFERENCES

<sup>1</sup>Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 25 (Suppl. 1):S5–S20, 2002

<sup>2</sup>Marker J, Maclaren N: Immunopathology of immune-mediated (type 1) diabetes. *Clin Lab Med* 21:15–30, 2001

<sup>3</sup>Bottazzo GF, Florin-Christensen A, Doniach D: Islet-cell autoantibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2:1279–1283, 1974

<sup>4</sup>Maclaren NK, Huang S-W: Antibody to cultured human insulinoma cells in insulin-dependent diabetes. *Lancet* 1:997–999, 1975

<sup>5</sup>Lernmark A, Freedman ZR, Hofmann C, Rubenstein AH, Steiner DF, Jackson RL, Winter RJ, Traisman HS: Islet-cell-surface antibodies in juvenile diabetes mellitus. *N Engl J Med* 299:375–380, 1978

<sup>6</sup>Eisenbarth GS, Morris MA, Scearce RM: Cytotoxic antibodies to cloned islet cells in serum of patients with diabetes mellitus. *J Clin Invest* 67:403–408, 1981

<sup>7</sup>Sai P, Boitard C, Debray-Sachs M, Pouplard A, Assan R, Hamburger J: Complement-fixing islet cell antibodies from some diabetic patients alter insulin release in vitro. *Diabetes* 30:1051–1057, 1981

<sup>8</sup>Van Der Winkel M, Smets G, Gepts W: Islet cell surface antibodies from insulin-dependent diabetics bind specifically to pancreatic cells. *J Clin Invest* 70:41–49, 1982

<sup>9</sup>Maruyama T, Takei I, Matsuba I, Tsuruoka A, Taniyama M, Ikeda Y, Kataoka K, Abe M, Matsuki S: Cell-mediated cytotoxic islet cell surface antibodies to human pancreatic beta cells. *Diabetologia* 26:30–33, 1984

<sup>10</sup>Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* 222:133–139, 1983

<sup>11</sup>Gerling I, Baekkeskov S, Lernmark A: Islet cell and 64K autoantibodies are associated with plasma IgG in newly diagnosed insulin-dependent diabetic children. *J Immunol* 137:3782–3785, 1986

<sup>12</sup>Maron R, Elias D, DeJongh BM, Bruining GF, VanRood JJ, Shechter Y, Cohen IR: Autoantibodies to the insulin receptor in juvenile onset insulin-dependent diabetes. *Nature* 303:81–88, 1983

<sup>13</sup>Castano L, Russo E, Zhou L, Lipes MA, Eisenbarth GS: Identification and cloning of a granule autoantigen (carboxypeptidase-H) associated with type I diabetes. *J Clin Endocrinol Metab* 73:1197–1201, 1991

<sup>14</sup>Jones DB, Hunter NR, Duff GW: Heat-shock protein 65 as a beta cell antigen of insulin-dependent diabetes. *Lancet* 336:583–585, 1990

<sup>15</sup>Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, DeCamilli P, Camilli PD: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 34:151–156, 1990

<sup>16</sup>Rorsman F, Husebye ES, Winqvist O, Bjork E, Karlsson FA, Kampe O: Aromatic-L-amino-acid decarboxylase, a pyridoxal phosphate-dependent enzyme, is a beta-cell autoantigen. *Proc Natl Acad Sci U S A* 92:8626–8629, 1995

<sup>17</sup>Kim YJ, Zhou Z, Hurtado J, Wood DL, Choi AS, Pescovitz MD, Warfel KA, Vandagriff J, Davis JK, Kwon BS: IDDM patients' sera recognize a novel 30-kD pancreatic autoantigen related to chymotrypsinogen. *Immunol Invest* 22:219–227, 1993

<sup>18</sup>Chang YH, Hwang J, Shang HF, Tsai ST: Characterization of human DNA topoisomerase II as an autoantigen recognized by patients with IDDM. *Diabetes* 45:408–414, 1996

<sup>19</sup>Aanstoot HJ, Kang SM, Kim J, Lindsay LA, Roll U, Knip M, Atkinson M, Mose Larsen P, Fey S, Ludvigsson J, Landin M, 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. *J Clin Invest* 97:2772–2783, 1996

<sup>20</sup>Johnson JH, Crider BP, McCorkle K, Alford M, Unger RH: Inhibition of glucose transport into rat islet cells by immunoglobulins from patients with new-onset insulin-dependent diabetes mellitus. *N Engl J Med* 332:653–659, 1990

<sup>21</sup>Cabrera-Rode E, Diaz-Horta O, Fernandez LE, Carr A, Marquina G, Valiente O, Gonzalez-Suarez RM, Uriarte A: Glycolipids as the major autoantigens of cytoplasmic islet cell antibodies. *Autoimmunity* 20:145–151, 1995

<sup>22</sup>Dotta F, Falorni A, Tiberti C, Dionisi S, Anastasi E, Torresi P, Lernmark A, Di Mario U: Autoantibodies to the GM2-1 islet ganglioside and to GAD-65 at type 1 diabetes onset. *J Autoimmun* 10:585–588, 1997

<sup>23</sup>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. *J Immunol* 152:3183–3188, 1994

<sup>24</sup>Lan MS, Wasserfall C, Maclaren NK, Notkins AL: IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. *Proc Natl Acad Sci U S A* 93:6367–6370, 1996

<sup>25</sup>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* 41:782–787, 1992

<sup>26</sup>Pietro Paolo M, Castaño L, Babu S, Buelow R, Kuo Y-LS, Martin S, Martin A, Powers AC, Prochazka M, Naggert J, Leiter EH, Eisenbarth

GS: Islet cell autoantigen 69 kD (ICA69): molecular cloning and characterization of a novel diabetes-associated autoantigen. *J Clin Invest* 92:359–371, 1993

<sup>27</sup>Pak CY, Cha CY, Rajotte RV, McArthur RG, Yoon JW: Human pancreatic islet cell specific 38 kilodalton autoantigen identified by cytomegalovirus-induced monoclonal islet cell autoantibody. *Diabetologia* 33:569–572, 1990

<sup>28</sup>Bohmer K, Keilacker H, Kuglin B, Hubinger A, Bertrams J, Gries FA, Kolb H: Proinsulin autoantibodies are more closely associated with type 1 (insulin-dependent) diabetes mellitus than insulin autoantibodies. *Diabetologia* 34:830–834, 1991

<sup>29</sup>Karounos DG, Thomas JW: Recognition of common islet antigen by autoantibodies from NOD mice and humans with IDDM. *Diabetes* 39:1085–1090, 1990

<sup>30</sup>Kasimiotis H, Myers MA, Argentaro A, Mertin S, Fida S, Ferraro T, Olsson J, Rowley MJ, Harley VR: Sex-determining region Y-related protein SOX13 is a diabetes autoantigen expressed in pancreatic islets. *Diabetes* 49:555–561, 2000

<sup>31</sup>Taniguchi T, Okazaki K, Okamoto M, Seko S, Uchida K, Seino Y: Presence of autoantibodies to carbonic anhydrase II and lactoferrin in type 1 diabetes: proposal of the concept of autoimmune exocrinopathy and endocrinopathy of the pancreas. *Diabetes Care* 24:1695–1696, 2001

<sup>32</sup>Schatz D, Winter W: Recent advances in the immunopathogenesis of insulin-dependent diabetes mellitus. *Curr Opin Pediatr* 7:459–465, 1995

<sup>33</sup>Nayak RC, Omar MAK, Rabizadeh A, Srikanta S, Eisenbarth GS: "Cytoplasmic" islet cell antibodies: evidence that the target antigen is a sialoglycoconjugate. *Diabetes* 34:617–619, 1985

<sup>34</sup>Atkinson MA, Kaufman DL, Newman D, Tobin AJ, Maclaren NK: Islet cell cytoplasmic autoantibody reactivity to glutamate decarboxylase in insulin-dependent diabetes. *J Clin Invest* 91:350–356, 1993

<sup>35</sup>Myers MA, Rabin DU, Rowley MJ: Pancreatic islet cell cytoplasmic antibody in diabetes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. *Diabetes* 44:1290–1295, 1995

<sup>36</sup>Neufeld M, Maclaren NK, Riley WJ, Lezotte D, McLaughlin JV, Silverstein J, Rosenbloom AL: Islet cell and other organ-specific antibodies in U.S. Caucasians and blacks with insulin-dependent diabetes mellitus. *Diabetes* 29:589–592, 1980

<sup>37</sup>Winter WE, Maclaren NK, Riley WJ, Clarke DW, Kappy MS, Spillar RP: Maturity-onset diabetes of youth in black Americans. *N Engl J Med* 316:285–291, 1987

<sup>38</sup>Schatz D, Krischer J, Horne G, Riley W, Spillar R, Silverstein J, Winter W, Muir A, Derovanessian D, Shah S, Malone J, Maclaren N: Islet cell antibodies predict insulin-dependent diabetes in United States school age children as powerfully as in unaffected relatives. *J Clin Invest* 93:2403–2407, 1994

<sup>39</sup>Scherthaner G, Hink S, Kopp HP, Muzyka B, Streit G, Kroiss A: Progress in the characteri-

- zation of slowly progressive autoimmune diabetes in adult patients (LADA or type 1.5 diabetes). *Exp Clin Endocrinol Diabetes* 109 (Suppl. 2):S94–S108, 2001
- <sup>40</sup>Irvine WJ, McCallum CJ, Gray RS, Duncan LJP: Clinical and pathogenic significance of pancreatic islet-cell antibodies in diabetics treated with oral hypoglycemic agents. *Lancet* 1:1025–1027, 1977
- <sup>41</sup>Di Mario U, Irvine WJ, Borseley DQ, Kyner JL, Weston J, Galfo C: Immune abnormalities in diabetic patients not requiring insulin at diagnosis. *Diabetologia* 25:392–395, 1983
- <sup>42</sup>Niskanen L, Karjalainen J, Sarlund H, Siitonen O, Uusitupa M: Five year follow-up of islet-cell antibodies in (non-insulin dependent) diabetes mellitus. *Diabetologia* 34:402–408, 1991
- <sup>43</sup>Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes* 42:359–362, 1993
- <sup>44</sup>Seissler J, de Sonnaville JJ, Morgenthaler NG, Steinbrenner H, Glawe D, Khoo Morgenthaler UY, Lan MS, Notkins AL, Heine RJ, Scherbaum WA: Immunological heterogeneity in type 1 diabetes: presence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. *Diabetologia* 41:891–897, 1998
- <sup>45</sup>Pietro Paolo M, Barinas-Mitchell E, Pietro Paolo SL, Kuller LH, Trucco M: Evidence of islet cell autoimmunity in elderly patients with type 2 diabetes. *Diabetes* 49:32–38, 2000
- <sup>46</sup>Tan HH, Lim SC: Latent autoimmune diabetes in adults (LADA): a case series. *Singapore Med J* 42:513–516, 2001
- <sup>47</sup>Lohmann T, Kellner K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA, Seissler J: Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). *Diabetologia* 44:1005–1010, 2001
- <sup>48</sup>Unger RH, Grundy S: Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia* 28:119–121, 1985
- <sup>49</sup>DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
- <sup>50</sup>Dean H: Diagnostic criteria for non-insulin dependent diabetes in youth (NIDDM-Y). *Clin Pediatr (Phila)* 37:67–71, 1998
- <sup>51</sup>Ehtisham S, Barrett TG, Shaw NJ: Type 2 diabetes mellitus in UK children: an emerging problem. *Diabet Med* 17:867–871, 2000
- <sup>52</sup>Pinhas-Hamiel O, Dolan LM, Zeitler PS: Diabetic ketoacidosis among obese African-American adolescents with NIDDM. *Diabetes Care* 20:484–486, 1997
- <sup>53</sup>Neufeld ND, Raffel LJ, Landon C, Chen YD, Vadheim CM: Early presentation of type 2 diabetes in Mexican-American youth. *Diabetes Care* 21:80–86, 1998
- <sup>54</sup>Ziegler AG, Standl E, Albert E, Mehnert H: HLA-associated insulin autoantibody formation in newly diagnosed type 1 diabetic patients. *Diabetes* 40:1146–1149, 1991
- <sup>55</sup>Kuglin B, Kolb H, Palmer J: Insulin autoantibodies: which method? *Lancet* 2:622–623, 1989
- <sup>56</sup>Levy-Marchal C, Bridel MP, Sodoyez-Goffaux F, Koch M, Tichet J, Czernichow P, Sodoyez JC: Superiority of radiobinding assay over ELISA for detection of IAAs in newly diagnosed type 1 diabetic children. *Diabetes Care* 14:61–63, 1991
- <sup>57</sup>Atkinson MA, Maclaren NK, Riley WJ, Winter WE, Fisk DD, Spillar RP: Are insulin autoantibodies markers for insulin dependent diabetes? *Diabetes* 35:894–898, 1986
- <sup>58</sup>Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA: A novel micro-assay for insulin autoantibodies. *J Autoimmun* 10:473–478, 1997
- <sup>59</sup>Feeney SJ, Myers MA, Mackay IR, Zimmet PZ, Howard N, Verge CF, Rowley MJ: Evaluation of ICA512As in combination with other islet cell autoantibodies at the onset of IDDM. *Diabetes Care* 20:1403–1407, 1997
- <sup>60</sup>Bingley PJ, Bonifacio E, Williams AJ, Genovese S, Bottazzo GF, Gale EA: Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46:1701–1710, 1997
- <sup>61</sup>Vardi P, Modan-Mozes D, Ish-Shalom S, Soloveitzik L, Barzilai D, Modan M: Low titer, competitive insulin autoantibodies are spontaneously produced in autoimmune diseases of the thyroid. *Diabetes Res Clin Pract* 21:161–166, 1993
- <sup>62</sup>Di Mario U, Perfetti R, Anastasi E, Contreas G, Crisa L, Tiberti C, Amendola MA, Masala C: Autoantibodies to insulin do appear in non-diabetic patients with autoimmune disorders: comparison with anti-immunoglobulin antibodies and other autoimmune phenomena. *Acta Endocrinol (Copenh)* 122:303–308, 1990
- <sup>63</sup>Vives Pi M, Somoza N, Vargas F, Armengol P, Sarri Y, Wu JY, Pujol-Borrell R: Expression of glutamic acid decarboxylase (GAD) in the alpha, beta and delta cells of normal and diabetic pancreas: implications for the pathogenesis of type 1 diabetes. *Clin Exp Immunol* 92:391–396, 1993
- <sup>64</sup>Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 89:283–292, 1992
- <sup>65</sup>Hagopian WA, Michelsen B, Karlens AE, Larsen F, Moody A, Grubin CE, Rowe R, Petersen J, McEvoy R, Lernmark A: Autoantibodies in IDDM primarily recognize the 65,000-M(r) rather than the 67,000-M(r) isoform of glutamic acid decarboxylase. *Diabetes* 42:631–636, 1993
- <sup>66</sup>Luhder F, Schlosser M, Mauch L, Haubruck H, Rjasanowski I, Michaelis D, Kohnert KD, Ziegler M: Autoantibodies against GAD65 rather than GAD67 precede the onset of type 1 diabetes. *Autoimmunity* 19:71–80, 1994
- <sup>67</sup>Mehta HB, Vold BS, Minkin S, Ullman EF: DELISA: sensitive nonisotopic assay for GAD65 autoantibodies, a key risk-assessment marker for insulin-dependent diabetes mellitus. *Clin Chem* 42:263–269, 1996
- <sup>68</sup>Hampe CS, Hammerle LP, Bekris L, Orqvist E, Kockum I, Rolandsson O, Landin-Olsson M, Torn C, Persson B, Lernmark A: Recognition of glutamic acid decarboxylase (GAD) by autoantibodies from different GAD antibody-positive phenotypes. *J Clin Endocrinol Metab* 85:4671–4679, 2000
- <sup>69</sup>Falorni A, Gambelunghe G, Forini F, Kassi G, Cosentino A, Candeloro P, Bolli GB, Brunetti P, Calcinaro F: Autoantibody recognition of COOH-terminal epitopes of GAD65 marks the risk for insulin requirement in adult-onset diabetes mellitus. *J Clin Endocrinol Metab* 85:309–316, 2000
- <sup>70</sup>Ujihara N, Daw K, Gianani R, Boel E, Yu L, Powers AC: Identification of glutamic acid decarboxylase autoantibody heterogeneity and epitope regions in type 1 diabetes. *Diabetes* 43:968–975, 1994
- <sup>71</sup>Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, Gale EAM, Bosi E: Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. *Diabetologia* 38:816–822, 1995
- <sup>72</sup>Lan MS, Lu J, Goto Y, Notkins AL: Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. *DNA Cell Biol* 13:505–514, 1994
- <sup>73</sup>Solimena M, Dirx R Jr, Hermel JM, Pleasic-Williams S, Shapiro JA, Caron L, Rabin DU: ICA 512, an autoantigen of type 1 diabetes, is an intrinsic membrane protein of neurosecretory granules. *EMBO J* 15:2102–2114, 1996
- <sup>74</sup>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. *J Immunol* 152:3183–3188, 1994
- <sup>75</sup>Zhang B, Lan MS, Notkins AL: Autoantibodies to IA-2 in IDDM: location of major antigenic determinants. *Diabetes* 46:40–43, 1997
- <sup>76</sup>Cai T, Xie J, She JX, Notkins AL: Analysis of the coding and promoter regions of the autoantigen IA-2 in subjects with and without autoantibodies to IA-2. *Diabetes* 50:2406–2409, 2001
- <sup>77</sup>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. *Proc Natl Acad Sci U S A* 93:2307–2311, 1996
- <sup>78</sup>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. *J Immunol* 155:5419–5426, 1995
- <sup>79</sup>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). *J Clin Invest* 96:1506–1511, 1995
- <sup>80</sup>Schmidli RS, Colman PG, Cui L, Yu WP,

- Kewming K, Jankulovski C, Harrison LC, Pallen CJ, DeAizpurua HJ: Antibodies to the protein tyrosine phosphatases IAR and IA-2 are associated with progression to insulin-dependent diabetes (IDDM) in first-degree relatives at-risk for IDDM. *Autoimmunity* 28:15-23, 1998
- <sup>81</sup>Libman IM, Pietropaolo M, Trucco M, Dorfman JS, LaPorte RE, Becker D: Islet cell autoimmunity in white and black children and adolescents with IDDM. *Diabetes Care* 21:1824-1827, 1998
- <sup>82</sup>Feeney SJ, Myers MA, Mackay IR, Zimmet PZ, Howard N, Verge CF, Rowley MJ: Evaluation of ICA512As in combination with other islet cell autoantibodies at the onset of IDDM. *Diabetes Care* 20:1403-1407, 1997
- <sup>83</sup>Wiest-Ladenburger U, Hartmann R, Hartmann U, Berling K, Bohm BO, Richter W: Combined analysis and single-step detection of GAD65 and IA2 in IDDM can replace the histochemical islet cell antibody test. *Diabetes* 46:565-571, 1997
- <sup>84</sup>Christie MR, Roll U, Payton MA, Hatfield EC, Ziegler AG: Validity of screening for individuals at risk for type I diabetes by combined analysis of antibodies to recombinant proteins. *Diabetes Care* 20:965-970, 1997
- <sup>85</sup>Hawa M, Rowe R, Lan MS, Notkins AL, Pozzilli P, Christie MR, Leslie RD: Value of antibodies to islet protein tyrosine phosphatase-like molecule in predicting type I diabetes. *Diabetes* 46:1270-1275, 1997
- <sup>86</sup>Yokota I, Matsuda J, Naito E, Ito M, Shima K, Kuroda Y: Comparison of GAD and ICA512/IA-2 antibodies at and after the onset of IDDM. *Diabetes Care* 21:49-52, 1998
- <sup>87</sup>Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlens AE, Sundkvist G, Dahlquist G, Palmer J, Lernmark A: Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest* 95:1505-1511, 1995
- <sup>88</sup>Borg H, Fernlund P, Sundkvist G: Protein tyrosine phosphatase-like protein IA2-antibodies plus glutamic acid decarboxylase 65 antibodies (GADA) indicates autoimmunity as frequently as islet cell antibodies assay in children with recently diagnosed diabetes mellitus. *Clin Chem* 43:2358-2363, 1997
- <sup>89</sup>Schatz D, Winter W: Recent advances in the immunopathogenesis of insulin-dependent diabetes mellitus. *Curr Opin Pediatr* 7:459-465, 1995
- <sup>90</sup>Irvine WJ, Gray RS, McCallum CJ: Pancreatic islet-cell antibody as a marker for asymptomatic and latent diabetes and pre-diabetes. *Lancet* 2:1097-1102, 1976
- <sup>91</sup>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* 2:1363-1365, 1981
- <sup>92</sup>Cudworth AG, Wolf E: The genetic susceptibility to type I (insulin-dependent) diabetes mellitus. *Clin Endocrinol Metab* 11:389-407, 1982
- <sup>93</sup>Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 311:149-152, 1984
- <sup>94</sup>Vadheim CM, Rotter JJ, Maclaren NK, Riley WJ, Anderson CE: Preferential transmission of diabetic alleles within the HLA gene complex. *N Engl J Med* 315:1314-1318, 1986
- <sup>95</sup>Bain SC, Rowe BR, Barnett AH, Todd JA: Parental origin of diabetes-associated HLA types in sibling pairs with type I diabetes. *Diabetes* 43:1462-1468, 1994
- <sup>96</sup>She JX, Marron MP: Genetic susceptibility factors in type I diabetes: linkage, disequilibrium and functional analyses. *Curr Opin Immunol* 10:682-689, 1998
- <sup>97</sup>She JX, Bui MM, Tian XH, Muir A, Wakefield EK, Zorovich B, Zhang LP, Liu MC, Thomson G, Maclaren NK: Additive susceptibility to insulin-dependent diabetes conferred by HLA-DQB1 and insulin genes. *Autoimmunity* 18:195-203, 1994
- <sup>98</sup>Bell GI, Horita, S, Karam JH: A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes. *Diabetes* 33:176-183, 1984
- <sup>99</sup>Julier C, Hyer RN, Davies J, Merlin F, Soularue P, Briant L, Cathelineau G, Deschamps I, Rotter JJ, Froguel P, Boitard C, Bell JI, Lathrop GM: Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. *Nature* 354:155-159, 1991
- <sup>100</sup>Bain SC, Prins JB, Hearne CM, Rodrigues NR, Rowe BR, Pritchard LE, Ritchie RJ, Hall JRS, Unldien DE, Ronningen KS, Dunger DB, Barnett AH, Todd JA: Insulin gene region-encoded susceptibility to type I diabetes is not restricted to HLA-DR4-positive individuals. *Nat Genet* 2:212-215, 1992
- <sup>101</sup>Pugliese A, Zeller M, Fernandez A Jr, Zalberg LJ, Bartlett RJ, Ricordi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD: The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type I diabetes. *Nat Genet* 15:293-297, 1997
- <sup>102</sup>Pyke DA: Diabetes: the genetic connections. *Diabetologia* 17:333-343, 1979
- <sup>103</sup>Maclaren NK, Atkinson MA: Is insulin-dependent diabetes mellitus environmentally induced? *N Engl J Med* 327:347-349, 1992
- <sup>104</sup>Yoon JW, Austin M, Onodera T, Notkins AL: Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. *N Engl J Med* 300:1173-1179, 1979
- <sup>105</sup>Dahlquist G: Non-genetic risk determinants of type I diabetes. *Diabetes Metab* 20:251-257, 1994
- <sup>106</sup>Akerblom HK, Knip M: Putative environmental factors in type I diabetes. *Diabetes/Metab Rev* 14:31-67, 1998
- <sup>107</sup>Fort P, Lanes R, Dahlem S, Recker B, Weyman-Daum M, Pugliese M, Lifshitz F: Breast feeding and insulin-dependent diabetes mellitus in children. *J Am Coll Nutr* 5:439-441, 1986
- <sup>108</sup>Mayer EJ, Hamman RF, Gay EC, Lezotte DC, Savitz DA, Klingensmith GJ: Reduced risk of IDDM among breast-fed children: the Colorado IDDM Registry. *Diabetes* 37:1625-1632, 1988
- <sup>109</sup>Virtanen SM, Rasanen L, Aro A, Lindstrom J, Sippola H, Lounamaa R, Toivanen L, Tuomilehto J, Akerblom HK: Infant feeding in Finnish children less than 7 yr of age with newly diagnosed IDDM. *Diabetes Care* 14:415-417, 1991
- <sup>110</sup>Kyvik KO, Green A, Svendsen A, Mortensen K: Breast feeding and the development of type I diabetes mellitus. *Diabet Med* 9:233-235, 1992
- <sup>111</sup>Bognetti E, Meschi F, Malavasi C, Pastore MR, Sergi A, Illeni MT, Maffei C, Pinelli L, Chiumello G: HLA-antigens in Italian type I diabetic patients: role of DR3/DR4 antigens and breast feeding in the onset of the disease. *Acta Diabetol* 28:229-232, 1992
- <sup>112</sup>Kostraba JN, Cruickshanks KJ, Lawler-Heavner J, Jobim LF, Rewers MJ, Gay EC, Chase HP, Klingensmith G, Hamman RF: Early exposure to cow's milk and solid foods in infancy, genetic predisposition, and risk of IDDM. *Diabetes* 42:288-295, 1993
- <sup>113</sup>Atkinson MA, Maclaren NK: The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 331:1428-1436, 1994
- <sup>114</sup>Savola K, Laara E, Vahasalo P, Kulmala P, Akerblom HK, Knip M: Dynamic pattern of disease-associated autoantibodies in siblings of children with type I diabetes: a population-based study. *Diabetes* 50:2625-2632, 2001
- <sup>115</sup>Von Meyenburg H: Ueber "insulinitis" bei diabetes. *Schweizerische Medizinische Wochenschrift* 24:554-557, 1940
- <sup>116</sup>Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14:619-633, 1965
- <sup>117</sup>Gepts W, Lecompte PM: The pancreatic islets in diabetes. *Am J Med* 70:105-115, 1981
- <sup>118</sup>Logothetopoulos J, Valiquette N, Madura E, Cvet D: The onset and progression of pancreatic insulinitis in the overt, spontaneously diabetic, young adult BB rat studied by pancreatic biopsy. *Diabetes* 33:33-36, 1984
- <sup>119</sup>Tochino Y: The NOD mouse as a model of type I diabetes. *Crit Rev Immunol* 8:49-81, 1987
- <sup>120</sup>Foulis AK, Stewart JA: The pancreas in recent-onset type I (insulin-dependent) diabetes mellitus: insulin content of islets, insulinitis and associated changes in the exocrine acinar tissue. *Diabetologia* 26:456-461, 1984
- <sup>121</sup>Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS: The histopathology of the pancreas in type I (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. *Diabetologia* 29:267-274, 1986
- <sup>122</sup>Imagawa A, Hanafusa T, Tamura S, Moriwaki M, Itoh N, Yamamoto K, Iwahashi H, Yamagata K, Waguri M, Nanmo T, Uno S, Nakajima H, Namba M, Kawata S, Miyagawa JI, Matsuzawa Y: Pancreatic biopsy as a procedure for detecting in situ autoimmune phenomena in type I diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. *Diabetes* 50:1269-1273, 2001

- <sup>123</sup>House DV, Winter WE: Autoimmune diabetes: the role of autoantibody markers in the prediction and prevention of insulin-dependent diabetes mellitus. *Lab Clin North Am* 17:499-545, 1997
- <sup>124</sup>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. *N Engl J Med* 308:322-325, 1983
- <sup>125</sup>Srikanta S, Ganda OP: Chronic progressive beta cell dysfunction in relatives of patients with type I diabetes. *Diabetes* 32:51A, 1983
- <sup>126</sup>Srikanta S, Ganda OP, Jackson RA, Gleason RE, Kaldany A, Garovoy MR, Milford EL, Carpenter CB, Soeldner JS, Eisenbarth GS: Type I diabetes mellitus in monozygotic twins: chronic progressive beta cell dysfunction. *Ann Intern Med* 99:320-326, 1983
- <sup>127</sup>Srikanta S, Ganda OP, Jackson RA, Brink SJ, Fleischnick E, Yunis E, Alpen C, Soeldner JS, Eisenbarth GS: Pre-type 1 (insulin-dependent) diabetes: common endocrinological course despite immunological and immunogenetic heterogeneity. *Diabetologia* 27:146-148, 1984
- <sup>128</sup>Ganda OP, Srikanta S, Brink SJ, Morris MA, Gleason RE, Soeldner JS, Eisenbarth GS: Differential sensitivity to beta-cell secretagogues in early, type 1 diabetes mellitus. *Diabetes* 33:516-521, 1984
- <sup>129</sup>Srikanta S, Ganda OP, Gleason RE, Jackson RA, Soeldner JS, Eisenbarth GS: Pre-type 1 diabetes, linear loss of beta cell response to intravenous glucose. *Diabetes* 33:717-720, 1984
- <sup>130</sup>Tarn AC, Thomas JM, Dean BM, Ingram D, Schwarz G, Bottazzo GF, Gale EA: Predicting insulin-dependent diabetes. *Lancet* 1:845-850, 1988
- <sup>131</sup>Jackson RA, Soeldner JS, Eisenbarth GS: Predicting insulin-dependent diabetes. *Lancet* 2:627-628, 1988
- <sup>132</sup>Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, Schwartz S, Malone J, Shah S, Vadheim C, Rotter JJ: A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *N Engl J Med* 323:1167-1172, 1990
- <sup>133</sup>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* 43:1304-1310, 1994
- <sup>134</sup>Cantor AB, Krischer JP, Cuthbertson DD, Schatz DA, Riley WJ, Malone J, Schwartz S, Quattrin T, Maclaren NK: Age and family relationship accentuate the risk of insulin-dependent diabetes mellitus (IDDM) in relatives of patients with IDDM. *J Clin Endocrinol Metab* 80:3739-3743, 1995
- <sup>135</sup>Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS, Eisenbarth GS: Life-table analysis of progression to diabetes of anti-insulin autoantibody-positive relatives of individuals with type I diabetes. *Diabetes* 38:1320-1325, 1989
- <sup>136</sup>Naserke HE, Bonifacio E, Ziegler AG: Immunoglobulin G insulin autoantibodies in BABYDIAB offspring appear postnatally: sensitive early detection using a protein A/G-based radiobinding assay. *J Clin Endocrinol Metab* 84:1239-1243, 1999
- <sup>137</sup>Ziegler AG, Hummel M, Schenker M, Bonifacio E: Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 48:460-468, 1999
- <sup>138</sup>Yu L, Robles DT, Abiru N, Kaur P, Rewers M, Kelemen K, Eisenbarth GS: Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. *Proc Natl Acad Sci U S A* 97:1701-1706, 2000
- <sup>139</sup>Kimpimaki T, Kupila A, Hamalainen AM, Kukko M, Kulmala P, Savola K, Simell T, Keskinen P, Ilonen J, Simell O, Knip M: The first signs of beta-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish Type 1 Diabetes Prediction and Prevention Study. *J Clin Endocrinol Metab* 86:4782-4788, 2001
- <sup>140</sup>Krischer JP, Schatz D, Riley WJ, Spillar RP, Silverstein JH, Schwartz S, Malone J, Shah S, Vadheim CM, Rotter JJ, Quattrin T, Maclaren NK: Insulin and islet cell autoantibodies as time-dependent covariates in the development of insulin-dependent diabetes: a prospective study in relatives. *J Clin Endocrinol Metab* 77:743-749, 1993
- <sup>141</sup>Greenbaum CJ, Sears KL, Kahn SE, Palmer JP: Relationship of beta-cell function and autoantibodies to progression and nonprogression of subclinical type 1 diabetes: follow-up of the Seattle Family Study. *Diabetes* 48:170-175, 1994
- <sup>142</sup>Yu L, Gianani R, Eisenbarth GS: Quantitation of glutamic acid decarboxylase autoantibody levels in prospectively evaluated relatives of patients with type I diabetes. *Diabetes* 43:1229-1233, 1994
- <sup>143</sup>Knip M, Karjalainen J, Akerblom HK: Islet cell antibodies are less predictive of IDDM among unaffected children in the general population than in sibs of children with diabetes. *Diabetes Care* 21:1670-1673, 1998
- <sup>144</sup>Gardner SG, Gale EA, Williams AJ, Gillespie KM, Lawrence KE, Bottazzo GF, Bingley PJ: Progression to diabetes in relatives with islet autoantibodies: is it inevitable? *Diabetes Care* 22:2049-2054, 1999
- <sup>145</sup>Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Chase HP, Eisenbarth GS: Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than particular autoantibody specificities determines risk of type I diabetes. *J Autoimmun* 9:379-383, 1996
- <sup>146</sup>Pastore MR, Bazzigaluppi E, Bonfanti R, Dozio N, Sergi A, Balini A, Belloni C, Meschi F, Bonifacio E, Bosi E: Two-step islet autoantibody screening for risk assessment of type 1 diabetes in relatives. *Diabetes Care* 21:1445-1450, 1998
- <sup>147</sup>Maclaren N, Lan M, Coutant R, Schatz D, Silverstein J, Muir A, Clare-Salzer M, She JX, Malone J, Crockett S, Schwartz S, Quattrin T, DeSilva M, Vander Vegt P, Notkins A, Krischer J: Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2 and IA-2beta predict immune-mediated (type 1) diabetes in relatives. *J Autoimmun* 12:279-287, 1999
- <sup>148</sup>Bosi E, Braghi S, Maffi P, Scirpoli M, Bertuzzi F, Pozza G, Secchi A, Bonifacio E: Autoantibody response to islet transplantation in type 1 diabetes. *Diabetes* 50:2464-2471, 2001
- <sup>149</sup>Riley WJ, Maclaren NK, Spillar RP: Development of IDDM after donating kidney to diabetic sibling. *Diabetes Care* 13:883-885, 1990
- <sup>150</sup>LaGasse JM, Brantley MS, Leech NJ, Rowe RE, Monks S, Palmer JP, Nepom GT, McCulloch DK, Hagopian WA: Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. *Diabetes Care* 25:505-511, 2002
- <sup>151</sup>Winter WE, House DV, Schatz D: Pharmacologic approaches to the prevention of autoimmune diabetes. *Drugs* 53:943-956, 1997
- <sup>152</sup>Menon RK, Sperling MA: Childhood diabetes. *Med Clin North Am* 72:1565-1576, 1988
- <sup>153</sup>Sacks DB, Lernmark A: Molecular manipulation of autoantibody testing in type 1 diabetes: two for one. *Clin Chem* 47:803-804, 2001
- <sup>154</sup>Kawasaki E, Eisenbarth GS: High-throughput radioassays for autoantibodies to recombinant autoantigens. *Front Biosci* 5:E181-E190, 2000
- <sup>155</sup>Shah SC, Malone JJ, Simpson NE: A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N Engl J Med* 320:550-554, 1989

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