GADA Titer-Related Risk for Organ-Specific Autoimmunity in LADA Subjects Subdivided according to Gender (NIRAD Study 6)

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Context: Latent autoimmune diabetes in adults (LADA) includes a heterogeneous population wherein, based on glutamic acid decarboxylase antibody (GADA) titer, different subgroups of subjects can be identified.

Objective: The aim of the present study was to evaluate GADA titer-related risk for β-cell and other organ-specific autoimmunity in LADA subjects.

Methods: Adult-onset autoimmune diabetes subjects (n = 236) and type 2 diabetes (T2DM) subjects (n = 450) were characterized for protein tyrosine phosphatase (IA-2_{IC} and IA-$2_{256-760}$), zinc transporter 8 (ZnT8), thyroid peroxidase, (TPO), steroid 21-hydroxylase (21-OH), tissue transglutaminase (tTG), and antiparietal cell (APC) antibodies.

Results: High GADA titer compared to low GADA titer showed a significantly higher prevalence of IA-2_{IC}, IA-$2_{256-760}$, ZnT8, TPO, and APC antibodies ($P \leq 0.04$ for all comparison). 21-OH antibodies were detected in 3.4% of high GADA titer. A significant decreasing trend was observed from high GADA to low GADA and to T2DM subjects for IA-$2_{256-760}$, ZnT8, TPO, tTG, and APC antibodies (P for trend ≤ 0.001). TPO was the only antibody showing a different prevalence between gender; low GADA titer and T2DM female patients had a higher frequency of TPO antibody compared to males ($P = 0.0004$ and $P = 0.0006$, respectively), where the presence of high GADA titer conferred an odds ratio of 8.6 for TPO compared to low GADA titer. After subdividing high and low GADA titer subjects according to the number of antibodies, we observed that 73.3% of high GADA titer subjects were positive for at least one or more antibodies, compared to 38.3% of low GADA titer ($P < 0.0001$).

Conclusions: In LADA subjects, high GADA titer was associated with a profile of more severe autoimmunity and, in male gender, specifically predisposed to thyroid autoimmunity. A regular screening for other antibodies is recommended in LADA patients according to GADA titer and gender. **(*J Clin Endocrinol Metab* 97: 3759–3765, 2012)**

Latent autoimmune diabetes in adults (LADA), the slowly progressive form of autoimmune diabetes, is not one clear-cut disease entity but includes a heterogeneous population wherein, based on glutamic acid decarboxylase antibody (GADA) titers, different subgroups of subjects can be identified (1).

In the Non-Insulin Requiring Autoimmune Diabetes (NIRAD) Study 1, we demonstrated the presence of two

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Abbreviations: APC, Antiparietal cell; a.u., arbitrary units; GADA, glutamic acid decarboxylase antibody; LADA, latent autoimmune diabetes in adults; 21-OH, 21-hydroxylase; NIRAD Study, Non-Insulin Requiring Autoimmune Diabetes Study; OR, odds ratio; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TPO, thyroid peroxidase; tTG, tissue transglutaminase; ZnT8, zinc transporter 8.

different populations among individuals with adult-onset autoimmune diabetes (2); analysis of GADA titers showed a bimodal distribution that identified two subgroups of patients with either a low or a high GADA titer.

The potential for several factors to predict progression toward insulin dependence has been previously investigated. However, the only marker that has been widely demonstrated to correlate with a faster decline of islet function in LADA was the presence of multiple β-cell-specific antibodies (3, 4). LADA has also been associated with other autoimmune diseases (5, 6). Gambelunghe *et al.* (7) reported an ongoing thyroid or adrenal autoimmunity in more than one fourth of 67 GAD65 antibody-positive subjects with a prevalence of thyroid peroxidase (TPO) three times higher in female subjects compared with males. The same female/male ratio was reported for thyroid autoimmunity (thyroid microsomal and thyroglobulin antibodies) in Finnish LADA subjects subdivided into tertiles of GADA positivity (8).

More recently, Jin *et al.* (9) reported that a high titer of GADA was a strong predictor for the development of thyroid autoimmunity in Chinese patients with type 1 diabetes mellitus (T1DM) and LADA. It is well known that organ-specific endocrine autoimmunity develops more frequently in women, including T1DM with thyroid autoimmunity (10); the production of TPO antibodies is inheritable in an autosomal fashion in women but not in men (11).

Although an association between LADA and other organ-specific autoimmunity has been previously reported, GADA titer and gender-related risk has not been quantified so far.

In light of these findings, the aim of the present study was to estimate, in LADA subjects, the GADA titer-related risk for organ-specific autoimmunity, diabetes, and non-diabetes-related protein tyrosine phosphatases (IA-2_{IC} and IA-$2_{256-760}$), zinc transporter 8 (ZnT8), TPO, steroid 21-hydroxylases (21-OH), tissue transglutaminase (tTG), and antiparietal cell (APC).

The knowledge of the odds ratio (OR) for organ-specific antibodies in LADA could be useful to identify patients in whom to perform the evaluation of these antibodies, in order to estimate the time to insulin dependence (β-cell-specific antibodies) and to diagnose autoimmune diseases at an early stage (other organ-specific antibodies).

Subjects and Methods

Adult-onset autoimmune diabetic subjects (n = 236; mean age of onset, 50.4 ± 12.9 yr) and age- and sex-matched type 2 diabetes mellitus (T2DM) subjects (n = 450; mean age of onset, 51.6 ± 10.81 yr) were selected from the NIRAD Study cohort of 5330 T2DM subjects (12).

GADA were measured by a radiobinding assay using *in vitro* translated [^{35}S]methionine-labeled GAD65 (13). Results for GADA were converted into arbitrary units (a.u.) by extrapolation from a standard curve with a local standard designated 100 arbitrary units. The thresholds for positivity were determined from the 99th centile of control subjects and corresponded to 3 a.u. for GADA. The distribution of GADA titer in patients with autoimmune diabetes was independent of diabetes duration and showed a bimodal distribution. Consistent with this observation, patients with autoimmune diabetes (GADA titer >3 a.u.) were divided into subgroups representing the two distributions, identified considering as a cutoff the nadir of the distribution and namely low (taken to be ≤32 a.u.) or high (taken to be >32 a.u.) GADA titer as extensively explained in our previous study (2).

Samples with low GADA titer were validated for GAD-specific binding by competition assay with an excess of cold insulin (2). Based on the Diabetes Antibody Standardization Program (14) as a reference, the threshold of 32 a.u. was equivalent to 300 World Health Organization units (2).

IA-2_{IC}, IA-$2_{256-760}$, ZnT8, and tTG antibodies were measured by previously described radioimmunoprecipitation assays (15, 16). TPO and APC antibodies were measured using RIA (Medipan, Berlin, Germany) and ELISA (Axa Diagnostics, Pomezia Italy) commercially available kits, respectively. 21-OH antibodies were analyzed by a radiobinding assay to recombinant human 21-OH radiolabeled with [^{35}S], as previously described (17).

Statistical analyses were performed using SPSS software, version 18 (SPSS Inc., Chicago, IL). Frequency differences were compared using the χ^2 test (with Yates' continuity correction) or Fisher's exact test when appropriate. A P value <0.05 was considered statistically significant.

Results

Table 1 shows the frequency of autoimmune diabetes-specific antibodies in high GADA titer, low GADA titer, and T2DM. Subjects with high GADA titer, compared with low GADA titer, showed a significantly higher prevalence of IA-2_{IC}, IA-$2_{256-760}$, and ZnT8 ($P \leq 0.04$ for all comparisons). Subjects with high GADA titer compared with T2DM showed a significantly higher prevalence of IA-$2_{256-760}$ and ZnT8 ($P \leq 0.0001$ for all comparisons). A significant decreasing trend was also observed from high GADA titer to low GADA titer and to T2DM subjects for IA-$2_{256-760}$ and ZnT8 antibodies ($P < 0.0001$ for all comparisons).

After subdividing LADA patients according to gender, we did not observe any significant difference in the frequency of the three diabetes-specific antibodies between male and female patients. A significantly higher frequency of all antibodies was observed in high GADA titer compared with low GADA titer and T2DM, irrespective of gender—the high GADA titer conferring the highest OR for all the three antibodies compared with T2DM. High

TABLE 1. Prevalence of autoimmune diabetes-specific autoantibodies in high and low GADA titer and in T2DM patients

	High GADA titer	Low GADA titer	T2DM	OR High *vs.* low GADA	OR High *vs.* T2DM	OR Low *vs.* T2DM	*P* for trend
n	116	120	450				
No. of males/females	61/55	62/58	234/216				
IA-2_{IC}	29 (25)	10 (8.3)	0	3.7 (1.7–7.9)[a]	nc	nc	
Male	16 (26.2)	6 (9.7)	0	3.0 (1.1–8.0)	nc	nc	
Female	13 (23.6)	4 (6.9)	0	3.7 (1.1–11.6)	nc	nc	
IA-$2_{256-760}$	41 (35.3)	18 (15)	13 (2.9)	5.8 (3.1–10.8)[a]	18.3 (9.4–35.9)[b]	5.9 (2.8–12.5)[c]	<0.0001
Male	20 (32.8)	8 (12.9)	7 (2.9)	3.2 (1.3–8.2)[a]	15.8 (6.2–39.8)[b]	4.8 (1.6–13.8)[c]	<0.0001
Female	21 (38.2)	10 (17.2)	6 (2.8)	2.9 (1.2–7.0)[a]	21.6 (8.1–57.4)[b]	7.2 (2.5–21)[c]	<0.0001
ZnT8	34 (29.3)	10 (8.3)	7 (1.6)	4.6 (2.1–9.8)[a]	26.2 (11.2–61.2)[b]	5.7 (2.1–15.4)[c]	<0.0001
Male	13 (21.3)	6 (9.7)	1 (0.4)	2.5 (0.9–7.2)	63 (8.1–494.1)[b]	23 (2.8–198.4)[c]	
Female	21 (38.2)	4 (6.9)	6 (2.8)	6.4 (2.1–19.3)[a]	21.6 (8.1–57.4)[b]	2.6 (0.7–9.5)	<0.0001

Data are expressed as number (percentage) or OR (95% confidence interval), unless stated otherwise. IA-2_{IC} + IA-$2_{256-760}$ + ZnT8 in high GADA titer, n = 15 (12.9%); in low GADA titer, n = 7 (5.8%). nc, Not calculable.

[a] For high GADA *vs.* low GADA, $P \leq 0.04$.

[b] For high GADA *vs.* T2DM, $P \leq 0.0001$.

[c] For low GADA *vs.* T2DM, $P \leq 0.004$.

GADA titer males showed an OR of 63 for the presence of ZnT8 antibody.

In Table 2, we reported the prevalence of other organ-specific antibodies in high GADA titer, low GADA titer, and T2DM patients. Subjects with high GADA titer compared with low GADA titer subjects showed a significantly higher prevalence of TPO and APC antibodies ($P \leq 0.004$ for all comparisons). Subjects with high GADA titer, compared with T2DM, showed a significantly higher prevalence of TPO, tTG, and APC antibodies ($P \leq 0.01$ for all comparisons). Antibodies to 21-OH showed a prevalence of 3.4% (4 of 116) in high GADA titer and were not present either in low GADA titer (0 of 120) or in T2DM (0 of 450).

A significant decreasing trend was also observed from high GADA titer to low GADA titer and to T2DM subjects for TPO, tTG, and APC and antibodies ($P \leq 0.001$ for all comparisons). Interestingly, the presence of high GADA titer conferred an OR of 10 for tTG antibody positivity compared with patients with T2DM.

No different gender distribution was observed in LADA subjects, subdivided according to GADA titer, for

TABLE 2. Prevalence of autoantibodies in high and low GADA titer and in T2DM patients

	High GADA titer	Low GADA titer	T2DM	OR High *vs.* low GADA	OR High *vs.* T2DM	OR Low *vs.* T2DM	*P* for trend
n	116	120	450				
No. of males/females	61/55	62/58	234/216				
TPO	43 (37.1)	20 (16.6)	47 (10.4)	2.9 (1.6–5.4)[a]	5.0 (3.1–8.2)[b]	1.7 (1.0–3.0)	<0.0001
Male	21 (34.4)	3 (4.8)	13 (5.5)	8.6 (2.5–29.8)[a]	8.9 (4.1–19.3)[b]	0.9 (0.2–3.1)	<0.0001
Female	22 (40)	17 (29.3)	34 (15.7)	1.6 (0.7–3.5)	3.6 (1.8–6.8)[b]	2.0 (1.1–3.7)[c]	0.0001
21-OH	4 (3.4)	0	0	9.6 (0.5–181.2)	nc	nc	
Male	2 (3.3)	0	0	5.2 (0.2–111.8)	nc	nc	
Female	2 (3.6)	0	0	5.5 (0.2–116.6)	nc	nc	
tTG	5 (4.3)	1 (0.8)	2 (0.4)	5.4 (0.6–46.6)	10 (1.9–52.7)[b]	1.9 (0.17–20.9)	0.001
Male	1 (1.6)	0	0	3.1 (0.1–77.6)	nc	nc	
Female	4 (7.3)	1 (1.7)	2 (0.9)	4.5 (0.5–41.3)	8.4 (1.5–47.1)[b]	2.0 (0.2–22.8)	
APC	29 (25)	11 (9.1)	49 (10.8)	3.0 (1.6–7.0)[a]	2.7 (1.6–5.6)[b]	0.8 (0.4–1.6)	0.0004
Male	13 (21.3)	7 (11.3)	28 (12)	2.0 (0.7–5.3)	2.0 (1.0–4.1)	0.9 (0.9–2.2)	
Female	16 (29.1)	4 (6.9)	21 (9.7)	4.6 (1.5–14.3)[a]	3.3 (1.6–6.5)[b]	0.7 (0.2–2.1)	0.001

Data are expressed as number (percentage) or OR (95% confidence interval), unless stated otherwise. nc, Not calculable.

[a] For high GADA *vs.* low GADA, $P \leq 0.004$.

[b] For high GADA *vs.* T2DM, $P \leq 0.01$.

[c] For low GADA *vs.* T2DM, $P = 0.03$.

TABLE 3. High and low GADA titer patients divided according to the number of autoantibodies

	High GADA titer	Low GADA titer
n	116	120
0 Ab[a]	31 (26.7)	74 (61.7)
1–5 Ab[a]	85 (73.3)	46 (38.3)

Data are expressed as number (percentage).

[a] High GADA titer *vs.* low GADA titer, $P < 0.0001$.

all analyzed antibodies, with the exception of TPO. We found a higher prevalence of TPO antibodies in low GADA titer and T2DM female patients compared with males ($P = 0.0004$ and $P = 0.0006$, respectively; data not shown). In high GADA titer patients, however, we did not observe any difference between female and male subjects for TPO antibody positivity. In females, TPO antibodies were significantly more frequent both in high and low GADA titers compared with T2DM. In males, TPO antibodies were significantly more frequent in patients with high GADA titer compared with patients with low GADA titer and T2DM. Thus, in male patients, the presence of high GADA titer conferred an OR of 8.6 for TPO positivity compared with patients with low GADA titer.

After subdividing high and low GADA titer subjects according to the number of antibodies, we observed that 73.3% of high GADA titer subjects were positive for at least one or more antibodies compared with 38.3% of low GADA titer ($P < 0.0001$) (Table 3).

Discussion

The originality of the present study is due to the characterization of a panel of antibodies directed against different diabetes and non-diabetes-related autoantigens, including IA-2_{IC}, IA-$2_{256–760}$, ZnT8, TPO, 21-OH, tTG, and APC, in LADA patients and in T2DM.

We demonstrated that high GADA titer is associated with a profile of more severe autoimmunity consisting in higher prevalence of organ-specific antibodies compared with low GADA titer and T2DM. This finding not only confirms but also extensively extends our previous observations, showing the presence of a higher frequency of associated thyroid autoimmunity in LADA patients compared with classic T2DM (2, 16).

Although LADA can be considered a major component of the organ-specific autoimmune disease group, few data are available on the risk of these patients for other autoimmune diseases. The presence of ZnT8 antibodies in high GADA titer male patients confers an OR of 63 compared with T2DM patients. Kawasaki *et al.* (18) found that ZnT8, with other diabetes-related antibodies, improves the prediction of a future insulin deficiency in adult-onset autoimmune diabetes. A recent study showed that ZnT8 antibody identified a subgroup of subjects at higher risk of diabetes progression in relatives of patients already positive for one antibody (anti-insulin or GAD65 or IA-2) (19).

ZnT8 is a highly β-cell-specific protein, and its measurement may be useful in monitoring islet destruction after onset and in evaluating therapeutic interventions aimed to limit β-cell-specific autoreactivity or restore β-cell mass (20). This finding supports the importance and utility to evaluate ZnT8 antibodies in high GADA titer male patients because their presence could confer a higher risk for insulin requirement in LADA patients compared with low GADA titer.

In the Botnia Study (5) and in a more recent work (9), LADA patients with high GADA titer showed a higher frequency of TPO antibodies compared with low GADA titer. These findings are in line with our results in which high GADA titer compared with low GADA titer showed a significantly higher prevalence of TPO antibodies (2). Van Deutekom *et al.* (1) reported that the clinical characteristics of LADA patients correlate with the titer and number of diabetes-associated antibodies and that TPO and/or 21-OH antibodies are present in more than one fourth of LADA.

Previous studies performed in T1DM and in LADA (7, 21, 22) showed that the frequency of TPO was higher in females compared with males, suggesting that female gender could be a predisposition factor to the development of organ-specific autoimmunity in T1DM. In view of this consideration, we analyzed whether in LADA patients the TPO distribution, according to GADA titer, was somehow influenced by gender bias.

In agreement with a previous study (7), we found a higher prevalence of TPO antibodies in overall female LADA patients compared with males. The interesting data of the present study is that in patients with high GADA titer, no differences were detected between females and males for TPO antibody positivity. This observation should be taken into account considering the previous evidence of the gender bias for T1DM (higher prevalence in males) (23, 24). In LADA, the gender bias of the autoimmune diabetes could influence the gender bias of another autoantibody according to the level of the immunodominant antibody (GADA titer). This suggests that the autoimmune background of high GADA titer patients in LADA could act as "promoter" for specific thyroid epitope spreading in male patients. The OR of 8.6 for TPO antibodies conferred by high GADA titer in male patients, compared with low GADA titer, indicates the relevance of screening for thyroid autoimmunity in the LADA population to detect asymptomatic thyroid dysfunction.

In a previous Italian study performed on LADA patients, 21-OH antibodies were detected in 5% of cases, compared with 0% in the control group of T2DM patients (7).

Falorni *et al.* (6) found that 21-OH antibodies were detected only in GADA-positive subjects. In agreement with this study, we also observed a quite high frequency of 21-OH antibodies (3.4%) in GADA-positive subjects. To our knowledge, this is the first study to assess the prevalence of 21-OH antibodies in LADA patients subdivided according to GADA titer.

Other reports compared the prevalence of tTG antibodies, a sensitive marker of celiac disease, in LADA and T2DM patients (25–27). Conflicting findings were reported; in one study, LADA patients had a higher prevalence of tTG antibodies compared with T2DM (1), whereas in another study, the frequency of tTGA was similar in LADA and in T2DM patients (28).

Overall, our results provide an additional support to the concept of heterogeneity within autoimmune diabetes in adults, indicating that when GADA titer is high, the disease is characterized by a profile of more severe and extended autoimmunity compared with low GADA titer.

Nonetheless, considering the frequent finding of low GADA titer in nondiabetic individuals (29), the possibility that these antibodies were "assay"-related false-positive was tested by an inhibition assay with an excess of unlabeled antigen in all samples available for retesting. Results showed that in most cases, antibody binding was specific for GAD (2). Furthermore, the coexistence in most of LADA with low GADA titer of mild insulin resistance features with a profile of autoimmunity, although less severe compared with high GADA titer patients (2), tend to confirm that a significant number of low GADA patients can be related to a real anti-islet autoimmunity. These observations, while suggesting that a significant number of low GADA patients should be real LADA, cannot exclude the possibility that some of these patients could be "biological" false positive.

Epitope spreading could be one of the possible mechanisms for the increased frequency of associated antibodies in high GADA titer patients. A self-directed immune response induced by a single epitope could spread to include other epitopes on other self molecules clustered in close vicinity of the target cell (30). Recent evidence from animal models of autoimmune diseases points to epitope spreading as a crucial mechanism in the development of autoimmunity, relapse, and disease progression (31).

We may speculate that in high GADA titer patients a "cascade" of epitopes may contribute to the development of autoimmunity and that the presence of multiple antibodies is a better index of disease progression than the prevalence of antibodies directed against a single antigen.

In conclusion, our findings show a higher frequency of organ-specific antibodies in high GADA titer subjects, confirming the high intensity of the autoimmune process. Furthermore, considering that the risk for other specific antibodies in LADA vary according to GADA titer and gender, knowing the specific OR could help clinicians to perform their screening.

Appendix

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References

1. **van Deutekom AW, Heine RJ, Simsek S** 2008 The islet autoantibody titers: their clinical relevance in latent autoimmune diabetes in adults (LADA) and the classification of diabetes mellitus. Diabet Med 25: 117–125
2. **Buzzetti R, Di Pietro S, Giaccari A, Petrone A, Locatelli M, Suraci C, Capizzi M, Arpi ML, Bazzigaluppi E, Dotta F, Bosi E** 2007 High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care 30:932–938
3. **Desai M, Cull CA, Horton VA, Christie MR, Bonifacio E, Lampasona V, Bingley PJ, Levy JC, Mackay IR, Zimmet P, Holman RR, Clark A** 2007 GAD autoantibodies and epitope reactivities persist after diagnosis in latent autoimmune diabetes in adults but do not predict disease progression: UKPDS 77. Diabetologia 50:2052–2060
4. **Maioli M, Pes GM, Delitala G, Puddu L, Falorni A, Tolu F, Lampis R, Orrù V, Secchi G, Cicalò AM, Floris R, Madau GF, Pilosu RM, Whalen M, Cucca F** 2010 Number of autoantibodies and HLA genotype, more than high titers of glutamic acid decarboxylase autoantibodies, predict insulin dependence in latent autoimmune diabetes of adults. Eur J Endocrinol 163:541–549
5. **Winter WE, Harris N, Schatz D** 2002 Type 1 diabetes islet autoantibody markers. Diabetes Technol Ther 4:817–839
6. **Falorni A, Brozzetti A** 2005 Diabetes-related antibodies in adult diabetic patients. Best Pract Res Clin Endocrinol Metab 19:119–133
7. **Gambelunghe G, Forini F, Laureti S, Murdolo G, Toraldo G, Santeusanio F, Brunetti P, Sanjeevi CB, Falorni A** 2000 Increased risk for endocrine autoimmunity in type 2 diabetic patients with GAD65 autoantibodies. Clin Endocrinol (Oxf) 52:565–573
8. **Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissén M, Ehrnström BO, Forsén B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen MR, Groop LC** 1999 Clinical and genetic characteristics of T2DM with and without GAD antibodies. Diabetes 48:150–157
9. **Jin P, Huang G, Lin J, Yang L, Xiang B, Zhou W, Zhou Z** 2011 High titer of antiglutamic acid decarboxylase autoantibody is a strong predictor of the development of thyroid autoimmunity in patients with type 1 diabetes and latent autoimmune diabetes in adults. Clin Endocrinol (Oxf) 74:587–592
10. **Chuang LM, Wu HP, Chang CC, Tsai WY, Chang HM, Tai TY, Lin BJ** 1996 HLA DRB1/DQA1/DQB1 haplotype determines thyroid autoimmunity in patients with insulin-dependent diabetes mellitus. Clin Endocrinol (Oxf) 45:631–636
11. **Phillips D, Prentice L, Upadhyaya M, Lunt P, Chamberlain S, Roberts DF, McLachlan S, Smith BR** 1991 Autosomal dominant inheritance of autoantibodies to thyroid peroxidase and thyroglobulin studies in families not selected for autoimmune thyroid disease. J Clin Endocrinol Metab 72:973–975
12. **Petrone A, Suraci C, Capizzi M, Giaccari A, Bosi E, Tiberti C, Cossu E, Pozzilli P, Falorni A, Buzzetti R; NIRAD Study Group** 2008 The protein tyrosine phosphatase nonreceptor 22 (PTPN22) is associated with high GAD antibody titer in latent autoimmune diabetes in adults: Non-Insulin Requiring Autoimmune Diabetes (NIRAD) Study 3. Diabetes Care 31:534–538
13. **Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, Gale EAM, Bosi E** 1995 Islet autoantibody markers in insulin dependent diabetes: risk assessment strategies yielding high sensitivity. Diabetologia 38:816–822
14. **Bingley PJ, Bonifacio E, Mueller PW** 2003 Diabetes antibody standardization program: first assay proficiency evaluation. Diabetes 52:1128–1136
15. **Tiberti C, Giordano C, Locatelli M, Bosi E, Bottazzo GF, Buzzetti R, Cucinotta D, Galluzzo A, Falorni A, Dotta F** 2008 Identification of tyrosine phosphatase 2(256–760) construct as a new, sensitive marker for the detection of islet autoimmunity in type 2 diabetic patients: the Non-Insulin Requiring Autoimmune Diabetes (NIRAD) study 2. Diabetes 57:1276–1283
16. **Lampasona V, Petrone A, Tiberti C, Capizzi M, Spoletini M, di Pietro S, Songini M, Bonicchio S, Giorgino F, Bonifacio E, Bosi E, Buzzetti R; Non-Insulin Requiring Autoimmune Diabetes (NIRAD) Study Group** 2010 Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes. Non-Insulin Requiring Autoimmune Diabetes (NIRAD) 4. Diabetes Care 33:104–108
17. **Falorni A, Nikoshkov A, Laureti S, Grenbäck E, Hulting AL, Casucci G, Santeusanio F, Brunetti P, Luthman H, Lernmark A** 1995 High diagnostic accuracy for idiopathic Addison's disease with a sensitive radiobinding assay for autoantibodies against recombinant human 21-hydroxylase. J Clin Endocrinol Metab 80:2752–2755
18. **Kawasaki E, Nakamura K, Kuriya G, Satoh T, Kuwahara H, Kobayashi M, Abiru N, Yamasaki H, Eguchi K** 2010 Autoantibodies to insulin, insulinoma-associated antigen-2, and zinc transporter 8 improve the prediction of early insulin requirement in adult-onset autoimmune diabetes. J Clin Endocrinol Metab 95:707–713
19. **Yu L, Boulware DC, Beam CA, Hutton JC, Wenzlau JM, Greenbaum CJ, Bingley PJ, Krischer JP, Sosenko JM, Skyler JS, Eisenbarth GS, Mahon JL; for the Type 1 Diabetes TrialNet Study Group** 2012 Zinc transporter-8 autoantibodies improve prediction of type 1 diabetes in relatives positive for the standard biochemical autoantibodies. Diabetes Care 35:1213–1218
20. **Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, Hutton JC** 2007 The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA 104:17040–17045
21. **De Block CE, De Leeuw IH, Vertommen JJ, Rooman RP, Du Caju MV, Van Campenhout CM, Weyler JJ, Winnock F, Van Autreve J, Gorus FK; Belgian Diabetes Registry** 2001 β-Cell, thyroid, gastric, adrenal and coeliac autoimmunity and HLA-DQ types in type 1 diabetes. Clin Exp Immunol 126:236–241
22. **Abrams P, De Leeuw I, Vertommen J** 1996 In new-onset insulin-dependent diabetic patients the presence of anti-thyroid peroxidase antibodies is associated with islet cell autoimmunity and the high risk haplotype HLA DQA1*0301-DQB1*0302. Belgian Diabetes Registry. Diabet Med 13:415–419
23. **Skordis N, Efstathiou E, Kyriakides TC, Savvidou A, Savva SC, Phylactou LA, Shammas C, Neocleous V** 2012 Epidemiology of type 1 diabetes mellitus in Cyprus: rising incidence at the dawn of the 21st century. Hormones (Athens) 11:86–93
24. **Blohmé G, Nyström L, Arnqvist HJ, Lithner F, Littorin B, Olsson PO, Scherstén B, Wibell L, Ostman J** 1992 Male predominance of type 1 (insulin-dependent) diabetes mellitus in young adults: results from a 5-year prospective nationwide study of the 15–34-year age group in Sweden. Diabetologia 35:56–62
25. **Kucera P, Nováková D, Behanová M, Novak J, Tlaskalová-Hogenová H, Andel M** 2003 Gliadin, endomysial and thyroid antibodies in patients with latent autoimmune diabetes of adults (LADA). Clin Exp Immunol 133:139–143

26. **Schober E, Granditsch G** 1994 IDDM and celiac disease. Diabetes Care 17:1549–1550
27. **Rewers M, Liu E, Simmons J, Redondo MJ, Hoffenberg EJ** 2004 Celiac disease associated with Type 1 diabetes mellitus. Endocrinol Metab Clin North Am 33:197–214, xi
28. **Sánchez JC, Cabrera-Rode E, Sorell L, Galvan JA, Hernandez A, Molina G, Perich PA, Licea ME, Domínguez E, Díaz-Horta O** 2007 Celiac disease associated antibodies in persons with latent autoimmune diabetes of adult and type 2 diabetes. Autoimmunity 40:103–107
29. **Bonifacio E, Lampasona V, Bernasconi L, Ziegler AG** 2000 Maturation of the humoral autoimmune response to epitopes of GAD in preclinical childhood type 1 diabetes. Diabetes 49: 202–208
30. **You S, Chatenoud L** 2006 Proinsulin: a unique autoantigen triggering autoimmune diabetes. J Clin Invest 116:3108–3110
31. **McRae BL, Vanderlugt CL, Dal Canto MC, Miller SD** 1995 Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis in the Sjl/j mouse. J Exp Med 182:75–85