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Beta-cell, thyroid, gastric, adrenal and coeliac autoimmunity and HLA-DQ types in type 1 diabetes

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SUMMARY

The autoimmune attack in type 1 diabetes is not only targeted to β cells. We assessed the prevalence of thyroid peroxidase (aTPO), parietal cell (PCA), antiadrenal (AAA) and endomysial antibodies (EmA-IgA), and of overt autoimmune disease in type 1 diabetes, in relation to gender, age, duration of disease, age at onset, β-cell antibody status (ICA, GADA, IA2A) and HLA-DQ type. Sera from 399 type 1 diabetic patients (M/F: 188/211; mean age: 26 ± 16 years; duration: 9 ± 8 years) were tested for ICA, PCA, AAA and EmA-IgA by indirect immunofluorescence, and for IA2A (tyrosine phosphatase antibodies), GADA (glutamic acid decarboxylase-65 antibodies) and aTPO by radiobinding assays. The prevalence rates were: GADA 70%; IA2A, 44%; ICA, 39%; aTPO, 22%; PCA, 18%; EmA-IgA, 2%; and AAA, 1%. aTPO status was determined by female gender ($\beta = -1{\cdot}15$, $P = 0{\cdot}002$), age ($\beta = 0{\cdot}02$, $P = 0{\cdot}01$) and GADA + ($\beta = 1{\cdot}06$, $P = 0{\cdot}02$), but not by HLA-DQ type or IA2A status. Dysthyroidism ($P < 0{\cdot}0001$) was more frequent in aTPO + subjects. PCA status was determined by age ($\beta = 0{\cdot}03$, $P = 0{\cdot}002$). We also observed an association between PCA + and GADA + (OR = 1·9, $P = 0{\cdot}049$), aTPO + (OR = 1·9, $P = 0{\cdot}04$) and HLA DQA1*0501-DQB1*0301 status (OR = 2·4, $P = 0{\cdot}045$). Iron deficiency anaemia (OR = 3·0, $P = 0{\cdot}003$) and pernicious anaemia (OR = 40, $P < 0{\cdot}0001$) were more frequent in PCA + subjects. EmA-IgA + was linked to HLA DQA1*0501-DQB1*0201 + (OR = 7·5, $P = 0{\cdot}039$), and coeliac disease was found in three patients. No patient had Addison's disease. In conclusion, GADA but not IA2A indicate the presence of thyrogastric autoimmunity in type 1 diabetes. aTPO have a female preponderance, PCA are weakly associated with HLA DQA1*0501-DQB1*0301 and EmA-IgA + with HLA DQA1*0501-DQB1*0201.

Keywords type 1 diabetes organ-specific antibodies β-cell antibodies HLA-DQ type

INTRODUCTION

Type 1 diabetes is an autoimmune disease arising through a complex interaction of immune, genetic and environmental factors [1]. It is characterized by β-cell destruction and the presence of islet cell antibodies (ICA), antibodies to glutamic acid decarboxylase-65 (GADA) and to tyrosine phosphatase (IA2A) [2,3]. HLA-DQ genes constitute approximately 50% of the genetic risk of type 1 diabetes [4]. Type 1 diabetic patients exhibit an increased risk of other autoimmune disorders, which can severely influence their prognosis [5–11]. Thyroid peroxidase antibodies (aTPO) are associated with Hashimoto's thyroiditis or Graves' disease [12,13], parietal cell antibodies (PCA) with iron deficiency anaemia, pernicious anaemia and autoimmune gastritis [5,14–16], anti-adrenal antibodies (AAA) with Addison's disease [8] and endomysial antibodies (EmA-IgA) with coeliac disease [11]. Determining immune and genetic risk markers may allow a good prediction of associated autoimmune disorders. The relation of β-cell antibodies and HLA-DQ type with aTPO [6,7,17–20] and with EmA-IgA [18,21,22] has been studied before, but we are the first to assess the relation of IA2A, GADA and HLA-DQ type with PCA and with AAA in type 1 diabetes. We investigated the prevalence of thyroid, gastric, adrenal and coeliac autoimmunity in type 1 diabetes in relation to gender, age, duration of diabetes, age at onset, β-cell autoimmunity and HLA-DQ type, to provide an indication of the extent to which these disorders share a common aetiology.

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PATIENTS AND METHODS

Patients

A group of 399 (male/female: 188/211) type 1 diabetic patients was studied, comprising 176 children (≤ 18 years) and 223 adults attending the out-patient Antwerp diabetes clinic and/or listed in the Belgian Diabetes Registry. All patients were of Caucasian ethnicity and, at onset, presented with hyperglycaemia, polydipsia, polyuria and/or ketoacidosis, necessitating insulin treatment from the time of diagnosis. Fasting C-peptide levels were very low at the time of this study (90 ± 80 pmol/l; normal levels: 250–1000 pmol/l). The mean age was 26 ± 16 years and the duration of diabetes averaged 9 ± 8 years (Table 1).

The study was approved by the Ethics Committee of the University Hospital of Antwerp. Informed consent was obtained from each patient and/or parent.

Methods

Islet cell antibodies (ICA) were determined by indirect immunofluorescence on cryosections of human donor pancreas (blood group O) [23]. Antibodies to glutamic acid decarboxylase-65 (GADA) and to tyrosine phosphatase (IA2A) were determined by liquid-phase radiobinding assay using, respectively, Centricon-purified recombinant human ^{35}S-GAD65 and the ^{35}S-labelled intracellular domain of IA-2 as tracer [3]. Cut-off values for antibody positivity were determined as the 99th percentile of antibody levels obtained in 783 non-diabetic control subjects, and amounted to ≥ 12 JDF units for ICA, ≥ 2·6% tracer bound for GADA and ≥ 0·5% tracer bound for IA2A [24]. These assays were validated by repeated participation in Immunology of Diabetes Workshops, and proficiency testing programmes of the University of Florida and the Louisiana State University. In the latter programme, our assays achieved 100% diagnostic sensitivity, specificity, consistency and validity. In the combinational islet autoantibody workshop, assay sensitivity adjusted for 99% specificity amounted to 73% for ICA and 85% for GADA [25]. In the first IA2A proficiency programme, our method achieved a 100% score for laboratory sensitivity, specificity, consistency and validity [3].

Thyroid peroxidase antibodies (aTPO) were measured by radiobinding assay (Henningtest, Brahms, Germany; nl < 100 U/ml). Thyroid function was estimated by assay of TSH (nl: 0·47–4·7 mU/l) and fT4 levels (Vitros, OCD, Amersham, UK; nl: 10·8–21·9 pmol/l). Parietal cell antibodies (PCA) were detected using indirect immunofluorescence on sections of rat gastric mucosa (Medical Diagnostics California, Carlsbad, USA; nl < 1/20 dilution) [5]. This indirect immunofluorescence (IIF) assay correlated well with the enzyme immunoassay for H^+/K^+ATPase antibodies (Varelisa, Pharmacia & Upjohn, GmbH, Germany; nl < 10 U/ml) ($n = 175$; $r = 0{\cdot}85$; $P < 0{\cdot}0001$). Antibodies to intrinsic factor (AIF) were measured by radiobinding assay (Diagnostic Products Corporation, Los Angeles, USA; nl < 1·1). Pernicious anaemia was defined as a megaloblastic anaemia with positive AIF and/or PCA. Iron deficiency anaemia was defined as decreased haemoglobin concentration, microcytic and hypochromic indices, and decreased ferritin levels (M < 20 and F < 12 μg/l). Anti-adrenal antibodies (AAA) and anti-endomysium IgA (EmA-IgA) were determined by indirect immunofluorescence using, respectively, unfixed frozen sections of monkey adrenal tissue and sections of monkey oesophagus as antigen substrate (MeDiCa kit, Cat. no. 6001-AG and 6001-ES, respectively, nl < 1/10 dilution). Quantitative determination of 21-hydroxylase antibodies in 100 patients using ^{125}I-radioassay (DLD, Diagnostika GmbH, Cat. No RA007/50, Hamburg, Germany) gave the same results as those obtained by IIF. HLA-DQ (Human Leucocyte Antigen) typing was performed as described previously [26].

Statistical analysis

Data were analysed using SPSS (SPSS Inc., Chicago, OH, USA).

Table 1 Clinical, genetic and immunological features of 399 type 1 diabetic patients

	Total	Boys/men	Girls/women	*P*-value
n	399	188	211	
Age (years)	26 ± 16	26 ± 13	27 ± 17	n.s.
Age at onset (years)	17 ± 13	17 ± 12	18 ± 13	n.s.
Duration of DM (years)	9 ± 8	9 ± 7	10 ± 8	n.s.
HbA1c (%)	7·9 ± 1·3	7·8 ± 1·2	8·2 ± 1·5	n.s.
C-peptide (pmol/l)	90 ± 80	90 ± 70	100 ± 90	n.s.
ICA + (≥12 JDFU)	157 (39%)	71 (38%)	86 (41%)	n.s.
GADA + (≥ 2·6%)	278 (70%)	121 (64%)	157 (74%)	0·038
IA2A + (≥ 0·5%)	177 (44%)	91 (48%)	86 (41%)	n.s.
aTPO + (> 100 μU/ml)	87 (22%)	27 (14%)	60 (28%)	0·0007
PCA + (≥ 1/20)	73 (18%)	31 (17%)	42 (20%)	n.s.
AAA + (≥ 1/10)	5 (1%)	3 (2%)	2 (1%)	n.s.
EmA-IgA (≥ 1/10)	9 (2%)	3 (2%)	6 (3%)	n.s.
aTPO + PCA +	23 (6%)	10 (5%)	13 (6%)	n.s.
GADA + aTPO + PCA +	19 (5%)	7 (4%)	12 (6%)	n.s.
IA2A + aTPO + PCA +	5 (1%)	2 (1%)	3 (1%)	n.s.
DQA1*0101-DQB1*0501	112 (28%)	54 (29%)	58 (28%)	n.s.
DQA1*0100-DQB1*0600	25 (6%)	7 (4%)	18 (9%)	n.s. ($P = 0{\cdot}06$)
DQA1*0501-DQB1*0201	210 (53%)	103 (55%)	107 (50%)	n.s.
DQA1*0301-DQB1*0302	210 (53%)	94 (50%)	116 (55%)	n.s.
DQA1*0501-DQB1*0301	30 (8%)	16 (9%)	14 (7%)	n.s.

Distributions of continuous data were tested for normality by the Kolmogorov Smirnov test. The unpaired *t*-test, Mann–Whitney *U*-test or ANOVA was used to determine differences between groups. Bonferroni adjustments for multiple comparisons were made. Differences in distributions of categorical data were investigated by χ^2 or Fisher's Exact test. Stepwise forward logistic regression analysis was used to assess the strength and independency of associations. A two-tailed $P < 0{\cdot}05$ was considered significant.

RESULTS

The prevalence of β-cell, thyroid, gastric, adrenal and endomysial autoantibodies is shown in Table 1 and Fig. 1. After a disease duration of 9 ± 8 years, 77% of subjects exhibited β-cell antibodies and 34% had thyrogastric antibodies. Stratification of patients according to disease duration (0–5 years, 6–10 years, > 10 years) showed no significant differences in the prevalence of associated organ-specific autoantibodies. Most patients (83%) were HLA DQA1*0301-DQB1*0302 and/or DQA1*0501-DQB1*0201 positive.

Beta-cell autoimmunity

GADA, but not ICA or IA2A, showed a female preponderance (OR = 1·6 [1·1–2·5], $P = 0{\cdot}038$). ICA ($P < 0{\cdot}0001$) and IA2A ($P < 0{\cdot}0001$), at variance with GADA, were more prevalent in younger subjects. ICA titres ($r = -0{\cdot}43$, $P < 0{\cdot}0001$) and IA2A levels ($r = -0{\cdot}39$, $P < 0{\cdot}0001$) declined with increasing diabetes duration. ICA were less frequent in subjects diagnosed before the age of 10 years, IA2A positivity declined and GADA positivity increased with advancing age at onset (Fig. 1). IA2A status was determined by age ($\beta = -0{\cdot}05$, $P < 0{\cdot}0001$), HLA DQA1*0301-DQB1*0302 + ($\beta = 1{\cdot}3$, $P < 0{\cdot}0001$, OR = 3·4) and DQA1*0501-DQB1*0201– ($\beta = -0{\cdot}6$, $P = 0{\cdot}04$), whereas GADA status was determined by gender ($\beta = -0{\cdot}8$, $P = 0{\cdot}008$) and DQA1*0501-DQB1*0201 ($\beta = 0{\cdot}6$, $P = 0{\cdot}04$).

IA2A (OR = 8·0, $P < 0{\cdot}0001$) and GADA (OR = 5·3, $P < 0{\cdot}0001$) were more prevalent in ICA + than ICA – subjects. GADA +, but not IA2A + subjects, were more prone to exhibit aTPO ($P = 0{\cdot}034$) and PCA ($P = 0{\cdot}049$) than the corresponding antibody-negative patients.

Associated organ-specific autoimmunity

aTPO were found in 22% of subjects, with a female preponderance ($P = 0{\cdot}0007$). There was an association between aTPO and PCA ($P = 0{\cdot}04$) (Table 2a). Logistic regression showed that aTPO status was determined by gender ($\beta = -1{\cdot}2$, $P = 0{\cdot}002$), age ($\beta = 0{\cdot}02$, $P = 0{\cdot}01$) and GADA + ($\beta = 1{\cdot}1$, $P = 0{\cdot}02$), but not by HLA-DQ haplo- or genotype or IA2A status. Four percent of type 1 diabetic patients were treated for hypothyroidism and 3% for hyperthyroidism. aTPO + individuals had a higher frequency of hypothyroidism ($P = 0{\cdot}0004$) and hyperthyroidism ($P < 0{\cdot}0001$) than aTPO –subjects (Table 2a).

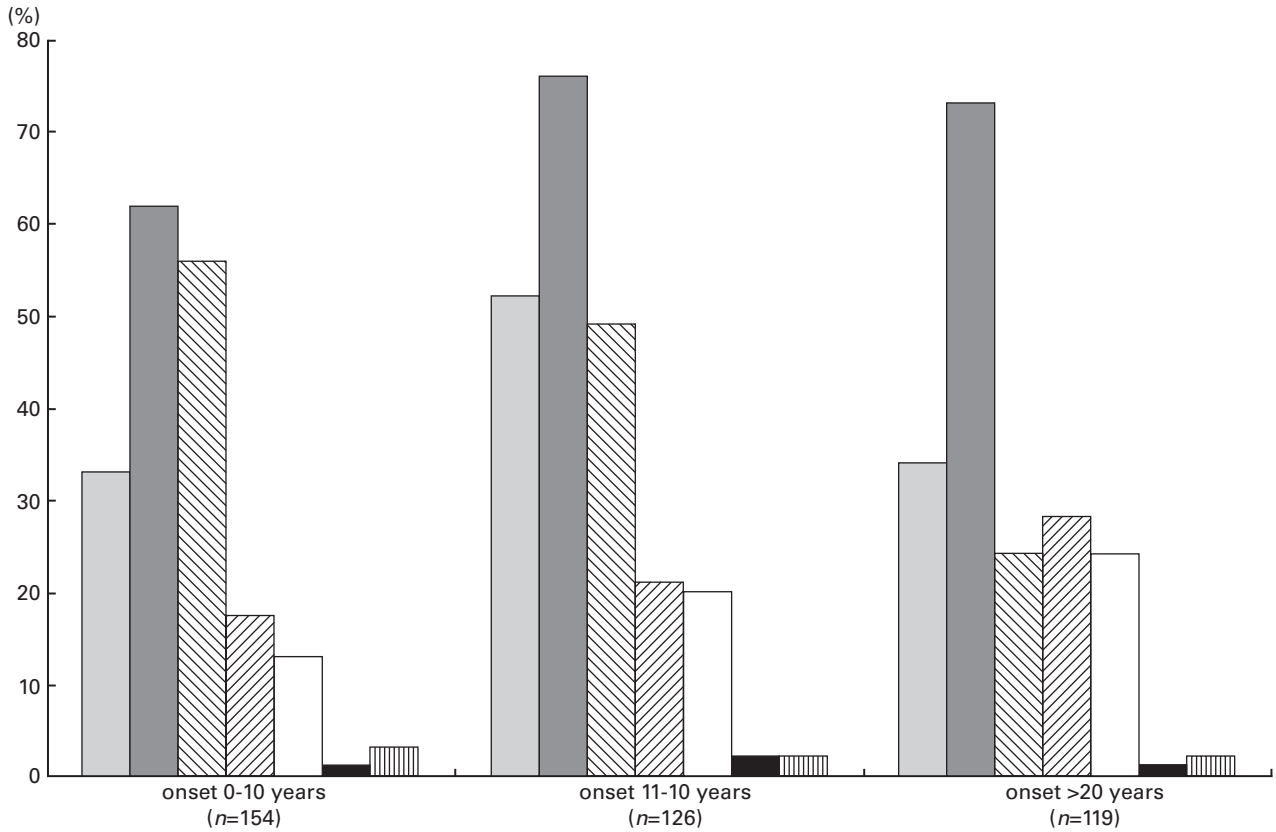

Fig. 1. Frequencies (%) of islet cell antibodies (ICA), antibodies to glutamic acid decarboxylase-65 (GADA), tyrosine phosphatase (IA2A), thyroid peroxidase (aTPO), gastric parietal cells (PCA), anti-adrenal antibodies (AAA) and endomysial antibodies (EmA-IgA) in 399 type 1 diabetic patients stratified per age of onset. χ^2 analysis was performed between the three groups. (▒) ICA+, $P = 0{\cdot}03$; (■) GADA +, $P = 0{\cdot}02$; (▨) IA2A +, $P < 0{\cdot}0001$; (▨) aTPO +, $P = 0{\cdot}09$; (□) PCA +, $P = 0{\cdot}05$; (■) AAA +, (NS); (▥) EmA-IgA +, (NS).

Table 2 Clinical features and prevalence of autoantibodies in thyroid peroxidase (a) and parietal cell antibody (b) positive *versus* negative type 1 diabetic patients

(a)

	aTPO +	aTPO –	*P*-value	OR (95% CI)
n (M/F)	87 (27/60)	312 (161/151)	0·0007	2·4 (1·4–3·9)
Age (years)	30 ± 16	25 ± 16	0·012	
Age at onset (years)	19 ± 13	16 ± 13	n.s. ($P = 0·06$)	
Duration of DM (years)	11 ± 9	9 ± 8	0·048	
ICA + (≥ 12 JDFU)	40 (46%)	117 (38%)	n.s.	
GADA + (≥ 2·6%)	69 (79%)	209 (67%)	0·034	1·9 (1·1–3·3)
IA2A + (≥ 0·5%)	34 (39%)	134 (43%)	n.s.	
PCA + (≥ 1/20)	23 (26%)	50 (16%)	0·04	1·9 (1·1–3·3)
Hypothyroidism (*n*)	10 (11%)	6 (2%)	0·0004	6·6 (2·3–18·7)
Hyperthyroidism (*n*)	9 (10%)	3 (1%)	< 0·0001	11·9 (3·1–44·9)

(b)

	PCA +	PCA –	*P*-value	OR (95% CI)
n (M/F)	73 (31/42)	326 (157/169)	n.s.	
Age (years)	31 ± 17	25 ± 16	0·002	
Age at onset (years)	20 ± 12	16 ± 13	0·023	
Duration of DM (years)	11 ± 10	9 ± 8	0·011	
ICA + (≥ 12 JDFU)	30 (41%)	127 (39%)	n.s.	
GADA + (≥ 2·6%)	58 (80%)	220 (68%)	0·049	1·9 (1·01–3·4)
IA2A + (≥ 0·5%)	26 (36%)	151 (46%)	n.s.	
aTPO + (> 100 μU/ml)	23 (32%)	64 (20%)	0·04	1·9 (1·1–3·3)
DQA1*0501-DQB1*0301	10 (14%)	20 (6%)	0·045	2·4 (1·1–5·4)
Iron deficiency anaemia	16 (22%)	28 (9%)	0·0029	3·0 (1·5–5·9)
Pernicious anaemia (*n*)	8 (11%)	1 (0·3%)	< 0·0001	40·0 (4·9–325·5)

Gastric PCA occurred in 18% of patients, without female preponderance. PCA + increased with advancing age at onset (Fig. 1). PCA positivity was associated with GADA + ($P = 0·049$) and aTPO + ($P = 0·04$), but not with IA2A +. In addition, HLA DQA1*0501-DQB1*0301 was more prevalent in PCA + patients ($P = 0·045$) (Table 2b). Logistic regression analysis, however, showed that PCA status was only determined by age ($\beta = 0·03$, $P = 0·002$). Eleven percent of adults were treated for iron deficiency anaemia and 2·5% for pernicious anaemia. PCA + patients were more prone to have iron deficiency anaemia ($P = 0·003$) and pernicious anaemia ($P < 0·0001$) than PCA – subjects.

AAA were present in 1·3%, but no patients had Addison's disease. EmA-IgA were detected in 2·3%, particularly HLA DQA1*0501-DQB1*0201 + subjects (OR = 7·5 [1·1–60·1], $P = 0·039$), and coeliac disease was diagnosed in three patients.

DISCUSSION

We studied 399 patients after onset of type 1 diabetes and found that 77% exhibited β-cell antibodies; aTPO and PCA were present in ~20%, and adrenal and endomysial antibodies in 1–3%, confirming other data [5,6,8,10,27,28]. Autoimmune thyroid and gastric disease were present in ~10%, and coeliac disease in 1% of patients, mainly antibody-positive subjects. Not all patients develop other autoimmune diseases, and different pathogenic mechanisms may exist between patients with isolated diabetes and those with a more general autoimmune disease. Therefore, we identified risk factors for associated organ-specific autoimmunity and evaluated the extent to which type 1 diabetes and these autoimmune disorders share a common aetiology. We observed that GADA but not IA2A were associated with thyrogastric autoimmunity in type 1 diabetes. PCA were weakly linked to HLA DQA1*0501-DQB1*0301 and EmA-IgA to HLA DQA1*0501-DQB1*0201.

Most autoimmune diseases are more common in females. We confirmed a female preponderance for aTPO [6,8,9,12,13] and GADA, but not for ICA and IA2A [3,17,18], nor for coeliac autoimmunity [29]. No gender associations were found for PCA, confirming most [5,7,9,10] but not all reports [8]. AAA were equally frequent in both sexes, opposing other data [8], but numbers were too low to draw conclusions.

Age at onset, age, and duration of disease may also influence the presence of antibodies. ICA and IA2A levels [9,23,30,31], in contrast to GADA levels [27,32], seem to decline with increasing disease duration, which might reflect a loss of antigenic stimulation due to β-cell depletion. However, the GADA level may be modified by exopancreatic GAD-65 production. We and others found that thyrogastric [5–8,10], but not adrenal [8] or endomysial antibodies [18,29], were more frequent in patients who were older at onset of diabetes, of a higher age and with a longer disease duration. An age-dependent increase in antibody positivity suggests that autoimmune disease is the final phase of a process starting with autorecognition, and passing through immunity with appearance of autoantibodies.

Patients with GADA, in contrast to those with IA2A, were more prone to have aTPO, supporting previous data [19,20,28], and PCA, which we are the first to report. The association of GADA with thyrogastric antibodies might be explained by the fact that GAD-65 is not exclusively present in the brain and pancreas, but can also be found in the thyroid gland and stomach [19]. We and others observed no link between GADA, IA2A and coeliac autoimmunity [22], although the majority of EmA-IgA + patients (7/9) were GADA +. Four out of five AAA + subjects exhibited GADA. The numbers for EmA-IgA and AAA are, however, too low to detect significant associations.

HLA molecules may also influence antibody status and can determine, in part, the tissue to which an autoimmune process develops. Moreover, the position of provisional loci found in type 1 diabetes has been reported to co-localize or overlap with loci found in different autoimmune diseases [33], which might explain the association with organ-specific autoimmunity. We and others found that HLA DQA1*0501-DQB1*0201 (linked to DR3) was associated with GADA +[34] and with EmA-IgA + [20], whereas HLA DQA1*0301-DQB1*0302 (linked to DR4) was associated with IA2A + [3,16,34,35]. At risk haplotypes for Hashimoto's thyroiditis are HLA DQA1*0301, DQB1*0301 and DQB1*0201 [36], and for Graves' disease, DQA1*0501 [37]. Our group previously reported an association between HLA DQA1*0301-DQB1*0302 and aTPO +in new onset type 1 diabetic patients [6]. We are the first to report a link between PCA + and HLA DQA1*0501-DQB1*0301 (linked to DR5), and although the association is weak, this supports our previous data [5].

The association between type 1 diabetes and organ-specific autoimmune diseases can be explained by sharing a common genetic background (HLA antigens), but also by a defective immunoregulation or a poor ability to develop tolerance to autoantigens. We and others suggest that GADA + may represent a propensity for general autoimmunity, while IA2A + may be a more specific marker of β-cell destruction [34].

Combining demographic characteristics (gender, age), immune (GADA) and genetic risk markers (HLA) will improve our capability to predict associated autoimmune diseases. Particularly in GADA + subjects, we suggest screening for aTPO, especially in female patients, and for PCA, repeated yearly because of the possibility of late seroconversion to antibody positivity. Screening for coeliac autoimmunity may be individualized according to HLA DQA1*0501-DQB1*0201 status. In case of positivity, additional work-up, including functional tests or endoscopy, and intervention (T4 or antithyroidea, iron and vitamin B12 supplementation, gluten-free diet) are advocated. These measures can prevent the well known complications and provide a strong rationale for screening strategies.

In conclusion, GADA but not IA2A indicate the presence of thyrogastric autoimmunity in type 1 diabetes. aTPO have a female preponderance, PCA are weakly associated with HLA DQA1*0501-DQB1*0301 and EmA-IgA + with HLA DQA1*0501-DQB1*0201.

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REFERENCES

1 Eisenbarth GS. Type 1 diabetes mellitus – a chronic autoimmune disease. N Engl J Med 1986; **314**:1360–8.
2 Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; **2**:1279–82.
3 Gorus FK, Goubert P, Semakula C *et al.* IA-2 autoantibodies complement GAD-65 autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. Diabetologia 1997; **40**:95–9.
4 Todd JA, Bell JI, McDevitt HO. HLA DQβ-gene contributes to susceptibility and resistance to insulin-dependent diabetic individuals. Nature 1987; **329**:599–604.
5 De Block C, Van Gaal L, Leeuw I, Belgian Diabetes Registry. High prevalence of manifestations of gastric autoimmunity in parietal cell-antibody positive type 1 (insulin-dependent) diabetic patients. J Clin Endocrinol Metab 1999; **84**:4062–7.
6 Abrams P, Leeuw I, Vertommen J, Belgian Diabetes Registry. 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. Diabetic Med 1996; **13**:415–9.
7 Landin-Olsson M, Karlsson FA, Lernmark Ä, Sundkvist G, Diabetes Incidence Study in Sweden Group. Islet cell and thyrogastric antibodies in 633 consecutive 15- to 34-yr-old patients in the Diabetes Incidence Study in Sweden. Diabetes 1992; **41**:1022–7.
8 Maclaren NK, Riley WJ. Thyroid, gastric, and adrenal autoimmunities associated with insulin-dependent diabetes mellitus. Diabetes Care 1985; **8** (Suppl.):34–8.
9 Betterle C, Zanette F, Pedini B *et al.* Clinical and subclinical organ-specific autoimmune manifestations in type 1 (insulin-dependent) diabetic patients and their first-degree relatives. Diabetologia 1984; **26**:431–6.
10 Magzoub MMA, Abdel-Hameed AA, Bottazzo GF. Prevalence of islet cell and thyrogastric autoantibodies in Sudanese patients with type 1 diabetes. Diabetic Med 1994; **11**:188–92.
11 Cronin CC, Shanahan F. Insulin-dependent diabetes mellitus and coeliac disease. Lancet 1997; **349**:1096–7.
12 De Block CEM, Van Campenhout CM, Leeuw IH *et al.* Soluble transferrin receptor level: a new marker of iron deficiency anaemia, a common manifestation of gastric autoimmunity in type 1 diabetes. Diabetes Care 2000; **23**:1384–8.
13 Toh BH, Van Driel IR, Gleeson PA. Mechanisms of disease: Pernicious anemia. N Engl J Med 1997; **337**:1441–8.
14 Ungar B, Stocks AE, Whittingham S, Martin FIR, Mackay IR. Intrinsic-factor antibody, parietal-cell antibody, and latent pernicious anaemia in diabetes mellitus. Lancet 1968; **2**:415–7.
15 Tunbridge WMG, Vanderpump MPJ. Population screening for autoimmune thyroid disease. Endocrinol Metab Clin North Am 2000; **29**:239–53.
16 Mouradian M, Abourizk N. Diabetes mellitus and thyroid disease. Diabetes Care 1983; **6**:512–20.
17 Savola K, Bonifacio E, Sabbah E *et al.* IA-2 antibodies – a sensitive

marker of IDDM with clinical onset in childhood and adolescence. Diabetologia 1998; **41**:424–9.

18 Verge CF, Howard NJ, Rowley MJ *et al.* Anti-glutamate decarboxylase and other antibodies at the onset of childhood IDDM: a population-based study. Diabetologia 1994; **37**:1113–20.

19 Kawasaki E, Takino H, Yano M *et al.* Autoantibodies to glutamic acid decarboxylase in patients with IDDM and autoimmune thyroid disease. Diabetes 1994; **43**:80–6.

20 Martino GV, Tappaz ML, Braghi S *et al.* Autoantibodies to glutamic acid decarboxylase (GAD) detected by an immuno-trapping enzyme activity assay: relation to insulin-dependent diabetes mellitus. J Autoimmunity 1991; **4**:915–23.

21 Sollid LM, Thorsby E. HLA susceptibility genes in coeliac disease: genetic mapping and role in pathogenesis. Gastroenterology 1993; **105**:910–22.

22 Lampasona V, Bonfanti R, Bazzigaluppi E *et al.* Antibodies to tissue transglutaminase C in type I diabetes. Diabetologia 1999; **42**:1195–8.

23 Vandewalle CL, Decraene T, De Schuit FC *et al.* Insulin autoantibodies and high titer islet cell antibodies are preferentially associated with the HLA-DQA1*0301-DQB1*0302 haplotype at clinical onset of type 1 diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years. Diabetologia 1993; **36**:1155–62.

24 Vandewalle CL, Falorni A, Lernmark A *et al.* Associations of GAD65- and IA-2 autoantibodies with genetic risk markers in new-onset IDDM patients and their siblings. Diabetes Care 1997; **20**:1547–52.

25 Verge CF, Stenger D, Bonifacio E *et al.* Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes. Combinatorial Islet Autoantibody Workshop. Diabetes 1998; **47**:1857–66.

26 Heimberg H, Nagy ZP, De Somers G, Leeuw I, Schuit FC. Complementation of HLA DQA and -DQB genes confers susceptibility and protection to insulin-dependent diabetes mellitus. Human Immunol 1992; **33**:10–7.

27 Decochez K, Tits J, Coolens JL *et al.* High frequency of persisting or increasing islet-specific autoantibody levels after diagnosis of type 1 diabetes presenting before 40 years of age. Diabetes Care 2000; **23**:838–44.

28 Yokota I, Matsuda J, Naito E, Ito M, Shima K, Kuroda Y. Comparison of GAD and ICA512/IA-2 antibodies at and after onset of IDDM. Diabetes Care 1998; **21**:49–52.

29 Kordonouri O, Dieterich W, Schuppan D *et al.* Autoantibodies to tissue transglutaminase are sensitive serological parameters for detecting coeliac disease in patients with type 1 diabetes mellitus. Diabet Med 2000; **17**:441–4.

30 Savola K, Sabbah E, Kulmala P, Vähäsalo P, Ilonen J, Knip M. Autoantibodies associated with type I diabetes mellitus persist after diagnosis in children. Diabetologia 1998; **41**:1293–7.

31 Bottazzo GF, Dean BM, Gorsuch AN, Cudworth AG, Doniach D. Complement-fixing islet cell antibodies in type I diabetes: possible monitors of active β-cell damage. Lancet 1980; **i**:668–72.

32 Christie MR, Daneman D, Champagne P, Delovitch TL. Persistence of serum antibodies to 64,000-Mr islet cell protein after onset of type I diabetes. Diabetes 1990; **39**:653–6.

33 Becker KG. Comparative genetics of type 1 diabetes and autoimmune disease. Common loci, common pathways? Diabetes 1999; **48**:1353–8.

34 Sabbah E, Savola K, Kulmala P *et al.* Childhood diabetes in Finland Study Group. Disease-associated autoantibodies and HLA-DQB1 genotypes in children with newly diagnosed insulin-dependent diabetes mellitus (IDDM). Clin Exp Immunol 1999; **116**:78–83.

35 Genovese S, Bonfanti R, Bazzigaluppi E *et al.* Association of IA-2 antibodies with HLA DR4 phenotypes in IDDM. Diabetologia 1996; **39**:1223–6.

36 Barbesino G, Chiovato L. The genetics of Hashimoto's disease. Endocrinol Metab Clin North Am 2000; **29**:357–73.

37 Gough SCL. The genetics of Graves' disease. Endocrinol Metab Clin North Am 2000; **29**:255–66.