Similar Genetic Features and Different Islet Cell Autoantibody Pattern of Latent Autoimmune Diabetes in Adults (LADA) Compared With Adult-Onset Type 1 Diabetes With Rapid Progression

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OBJECTIVE — To compare the clinical parameters, C-peptide levels, pattern of islet cell–specific autoantibodies, and prevalence of predisposing genotypes in subjects with latent autoimmune diabetes in adults (LADA) and those with adult-onset type 1 diabetes with rapid progression.

RESEARCH DESIGN AND METHODS — We evaluated the clinical parameters, C-peptide levels, and islet cell–specific autoantibodies in 54 LADA, 57 adult-onset type 1 diabetic, and 190 type 2 diabetic patients. Islet cell autoantibodies were also compared between subgroups of newly diagnosed patients with LADA and those with newly diagnosed adult-onset and childhood-onset type 1 diabetes. The genetic study was performed in subjects with LADA and those with adult-onset type 1 diabetes in comparison with a control population.

RESULTS — There were no differences in the clinical parameters between LADA and adult-onset type 1 diabetes. Patients with LADA had lower BMI ($P < 0.0001$), waist-to-hip ratio (0.0029), total cholesterol ($P = 0.001$), and triglycerides ($P = 0.001$); higher HDL cholesterol levels ($P < 0.0001$); and lower prevalence of hypertension ($P = 0.0028$) compared with patients with type 2 diabetes. C-peptide levels were similar at onset ($P = 0.403$) but decreased less rapidly in LADA than in adult-onset type 1 diabetes ($P = 0.0253$). Single-autoantibody positivity was more often seen in LADA than in type 1 diabetes ($P = 0.0001$). The prevalence of predisposing HLA-DQB1*0302, -DR4, -DR3, and -DR3/DR4 genotypes and the DR4-DQB1*0302 haplotype were increased in both LADA and adult-onset type 1 diabetic subjects compared with the control population. There were no differences in the frequencies of these risk alleles and haplotypes between the two patient groups.

CONCLUSIONS — Subjects with LADA had clinical characteristics similar to those with adult-onset type 1 diabetes with rapid progression. C-peptide levels did not differ at onset but decreased less rapidly in LADA. Patients with LADA rather had single islet cell–specific autoantibody positivity. The prevalence of HLA-DQB1*0302, -DR4, -DR3, and -DR3/DR4 risk alleles and the DR4-DQB1*0302 high-risk haplotype did not differ in the two forms of autoimmune diabetes.

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Abbreviations: GADA, GAD 65 autoantibody; ICA, islet-cell cytoplasma autoantibody; IA-2A, tyrosine phosphatase-like protein IA-2 autoantibody; JDF, Juvenile Diabetes Foundation; LADA, latent autoimmune diabetes in adults; SSP, sequence-specific polymorphism; UKPDS, U.K. Prospective Diabetes Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

See accompanying editorial, p. 536.

According to the recent diabetes classification, type 1 diabetes has two forms: the immune-mediated and the idiopathic one (1). Immune-mediated type 1 diabetes is a complex autoimmune disease caused by the interaction of environmental factors with an inherited predisposition. Autoimmune β-cell destruction leads to insulin deficiency. Circulating autoantibodies, such as autoantibodies to islet-cell cytoplasma (ICAs) and/or to GAD 65 (GADAs) and/or to the intracytoplasmatic domain of the tyrosine phosphatase-like protein IA-2 (IA-2As), are markers of this process. The disease has strong class II HLA association, with linkage to the DQA and B genes, and it is influenced by the DRB genes (2–4). The encoded HLA-DQ/DR alleles can be either predisposing or protective. HLA-DR3 and -DR4 alleles occur in >95% of Caucasian childhood-onset type 1 diabetic patients, with the heterozygote DR3/DR4 conferring the highest risk (5). The risk presented by the DR4 allele is primarily attributable to an association in a haplotype with HLA-DQB1*0302 (6). Susceptibility associated with HLA-DR3 may be determined directly by HLA-DQB1*0201 (7).

The autoimmune type 1 diabetes is subdivided into a rapidly and a slowly progressive form (1). The latter subgroup is primarily manifested in adulthood and is referred to as latent autoimmune diabetes in adults (LADA) (8,9). The major characteristics of LADA are islet cell–specific autoantibody positivity, age at onset >35 years, and demand for insulin treatment in the early phase of the disease course (9,10). The presence of ICAs and/or GADAs is the best predictor of the early insulin requirement (11). Epidemiological data demonstrate that LADA accounts for 2–12% of all cases of diabetes (11–13). It is not clear whether LADA represents a late manifestation of type 1

diabetes or is a distinct disease entity (10). Furthermore, other authors reported on two clinically different types of LADA: LADA-type 1, with the clinical features of type 1 diabetes, and LADA-type 2, with the phenotype of type 2 diabetes (14). There is little information about the clinical characteristics of LADA compared with the other forms of diabetes (10,15). We have even less data about its genetic background. In the U.K. Prospective Diabetes Study (UKPDS), high-risk alleles for type 1 diabetes were found in the young LADA patients, although the prevalence of these genotypes decreased with age (16). Tuomi et al. (10) published a distinct genetic background of LADA patients in comparison with type 1 and type 2 diabetes.

Therefore, the aims of our study were to investigate *1*) the clinical parameters, including C-peptide; *2*) the pattern of islet cell–specific autoantibodies in patients with LADA compared with patients with adult-onset type 1 and type 2 diabetes; and *3*) the prevalence of predisposing genotypes in patients with LADA and adult-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS

— We studied 54 patients with LADA, 57 patients with type 1 diabetes whose disease onset was >25 years of age (adult onset), and 190 subjects with diabetes considered to be type 2 recruited from our outpatient clinic. The diagnosis of LADA was established if the onset was >35 years of age, any circulating islet cell–specific autoantibody (ICA, GADA, or IA-2A) was detected, and insulin therapy was not indicated in the first 6 months after the diagnosis. To identify patients with LADA, we screened those cases with type 2 diabetes where LADA was clinically suspected, e.g., patients with lower body weight, the presence of other autoimmune disease, and a lack of positive family history of diabetes. We tested 311 patients to find the 54 patients with LADA. The median age at the onset of diabetes was 51.9 years (39.0–61.8) in the LADA group.

Type 1 diabetes was considered to be the diagnosis if the patient had typical diabetic symptoms (polyuria, polydipsia, or weight loss), ketonuria or ketoacidosis was present at the time of the diagnosis, and a prompt insulin treatment was required. The median age at the onset was 39.3 years (30.5–45.5) in the type 1 diabetic group, all the patients had ketonuria ($n = 57$), and 21 patients had ketoacidosis.

Clinical parameters, fasting serum C-peptide levels, and the presence of islet cell–specific autoantibodies were compared in the three patient groups. We also analyzed the islet cell autoantibody pattern in subgroups of patients with newly diagnosed LADA ($n = 21$), new adult-onset type 1 diabetes ($n = 37$), and new childhood-onset type 1 diabetes ($n = 48$, age at onset <16 years). The genetic study was performed in patients with LADA ($n = 50$) and adult-onset type 1 diabetes ($n = 50$). As a control group, we used the registered data of 336 Hungarian kidney donor cadavers.

Measurements and assays

BMI and the waist-to-hip ratio were measured in all subjects. If a patient used an antihypertensive drug, that subject was considered to have hypertension. HbA_{1c} concentrations were tested by immunoturbidimetry (HbA_{1c} Unimates 3, Boehringer Mannheim, Mannheim, Germany; Cobas Mira Plus, F. Hoffmann-La Roche, Basel, Switzerland). The reference values for this assay were 3–6%. Serum total cholesterol, total triglyceride, and HDL cholesterol levels were determined by enzymatic assay methods (Cholesterol PAP Unimate 5, Triglycerides PAP Unimate 5, Roche Diagnosticum, F. Hoffman-La Roche; HDL-koleszterin direct, Diagnosticum Hungary, Budapest, Hungary; Beckman CX7, Beckman Instruments, Fullerton, CA) Fasting serum C-peptide levels were measured by radioimmunoassay (BRAHMS Diagnostica, Berlin, Germany). Ketonuria was tested by a semiquantitative method using sodium nitroprusside (Multistix 10 SG; Bayer Diagnostics, München, Germany) and controlled by the modified Rothera method. Ketoacidosis was established if, besides ketonuria, pH was <7.3 and calculated bicarbonate was <18.0 mmol/l, as revealed by arterial gas analysis (ABL 620/625; Radiometer Medical, Copenhagen, Denmark).

Antibody measurements

ICA was determined by an indirect immunofluorescence test on unfixed cryostat sections of human pancreas (17). The cutoff for our assay was 10 Juvenile Diabetes Foundation (JDF) units. In the 12th Immunology of Diabetes Workshop (IDW) ICA Proficiency Program, our laboratory achieved values of 100% for specificity and consistency, 58% for sensitivity, and 73% for validity. GADAs and IA-2As were detected by radioligand assays (Medipan Diagnostica, Berlin, Germany) (17). The amounts of antibodies were expressed in arbitrary units, with a cutoff of 1.2 GAD units/ml and 1.3 IA-2 units/ml. On the 4th GADA Proficiency Test, our laboratory had values of 100% for sensitivity, 83% for specificity, and 94% for consistency and validity. On the 3rd Anti-IA2 Proficiency Test, we achieved values of 100% for sensitivity, specificity, consistency, and validity. ICA levels of ≥20 JDF units and GADA levels >20 units/ml were considered as high titer levels.

Genotyping

The HLA-DR and -DQ alleles were detected by sequence-specific polymorphism (SSP) PCR method (Olerup SSP DR and DQ kits; Olerup SSP, Saltsjöbaden, Sweden). The following predisposing genotypes were tested: DQB1*0201, DQB1*0302, DR3, and DR4. Total genomic DNA was extracted using the method of Miller et al. (18). Venous blood samples were collected in tubes containing EDTA. The erythrocytes were lysed by ammonium chloride, and the leukocytes were washed with PBS. DNA was isolated from the leukocytes by incubation with proteinase K and 20% SDS. DNA was precipitated with a saturated sodium chloride solution, and the final DNA concentration was 30 ng/μl. HLA-DR and -DQ alleles were determined according to the instructions of the manufacturer. The PCR products were run by agarose (2%) gel electrophoresis and stained with ethidium bromide. The results were detected by UV transilluminator and documented by photography.

Statistical analysis

Statistical analyses were performed using SPSS for Windows program version 9.0 or GraphPad Prism 3.0. Differences between groups in continuous variables were estimated using a nonparametric Mann-Whitney *U* test. For dichotomous variables, a χ^2 test or Fisher's exact test was used. All tests were two-tailed. Logistic regression was used to evaluate potential confounding by covariables. Changes in the C-peptide levels in different groups were compared by two-way ANOVA

Table 1—*Clinical parameters, including fasting C-peptide levels of patients with LADA and those with adult-onset type 1 and type 2 diabetes*

Parameter	LADA (n = 54)	Adult-onset type 1 diabetes (n = 57)	Type 2 diabetes (n = 190, for C-peptide n = 90)	Comparison of groups*		
				LADA-type 1	LADA-type 2	Type 1–type 2
Age (years)	59.0 (47.5–67.0)	44.5 (34.0–53.0)	63.0 (53.0–72.0)	<0.0001†	0.089†	<0.0001†
Sex (M/F)	25/29	30/27	103/87	NS‡	NS‡	NS‡
Duration of diabetes (years)	4.00 (1.0–9.5)	0.10 (0.1–4.5)	8.00 (3.0–15.5)	0.002†	0.0005†	<0.0001†
Treatment with insulin	43 (79.6)	57 (100)	84 (44.2)	—	—	—
HbA_{1c} (%)	8.9 (7.8–11.2)	9.7 (7.3–12.8)	8.80 (7.6–10.6)	NS	NS	0.01
Fasting C-peptide at onset (nmol/l)	0.53 (0.24–1.40)	0.46 (0.24–1.05)	1.23 (0.70–2.55)	NS† (0.403)	0.0226†	0.0009†
Fasting C-peptide at onset (nmol/l)	0.53 (0.24–1.40)	0.46 (0.24–1.05)	1.23 (0.70–2.55)			
Fasting C-peptide after onset, 1–10 years (nmol/l)	0.34 (0.21–1.87)	0.40 (0.24–1.05)	0.68 (0.41–1.02)	0.0253§	NS§ (0.113)	0.0007§
Fasting C-peptide after onset, >10 years (nmol/l)	0.40 (0.16–1.17)	0.03 (0.01–0.56)	0.54 (0.40–0.82)			
BMI (kg/m^2)	23.5 (22.6–27.1)	23.1 (21.2–26.6)	29.1 (26.0–32.0)	NS	<0.0001	<0.0001
Waist-to-hip ratio	0.87 (0.83–0.91)	0.84 (0.77–0.94)	0.95 (0.88–1.01)	NS	0.0029	0.08
Hypertension	17	10	101	NS	0.0028	0.027
Total cholesterol (mmol/l)	5.3 (4.5–6.1)	5.4 (4.6–6.2)	6.0 (5.0–6.9)	NS	0.001	0.02
Triglycerides (mmol/l)	1.2 (0.9–1.8)	1.4 (0.8–2.7)	2.2 (1.5–3.5)	NS	0.001	0.0079
HDL cholesterol (mmol/l)	1.4 (1.1–1.7)	1.2 (1.0–1.5)	1.1 (0.9–1.3)	NS	<0.0001	0.001

Data are median (interquartile range), n, or n (%). *P value by logistic regression analyses, adjusted for age and duration of diabetes. †Mann-Whitney test; ‡Fisher's exact test; §two-way-ANOVA test, for duration of diabetes. NS, not significant.

test. A P value <0.05 was considered as significant.

RESULTS — Table 1 shows the clinical parameters of patients with LADA, adult-onset type 1, and type 2 diabetes. Patients with LADA and type 1 diabetes had lower BMI, total cholesterol, and triglyceride levels and lower prevalence of hypertension compared with patients with type 2 diabetes. There was a lower waist-to-hip ratio in LADA compared with patients with type 2 diabetes. HDL cholesterol levels were higher in the LADA and type 1 diabetic subjects compared with those with type 2 diabetes. There were no differences between the LADA and the adult-onset type 1 diabetes groups in the parameters of the metabolic syndrome mentioned above.

Fasting C-peptide levels did not differ between LADA and adult-onset type 1 diabetes at the onset of diabetes. However, a lower level of C-peptide was observed in type 1 diabetes compared with LADA with longer disease duration (P = 0.0253). We observed the opposite phenomenon between LADA and type 2 diabetes. The patients with LADA had lower fasting C-peptide levels at the time of the diagnosis in comparison with type 2 diabetes (P = 0.0226), but this difference disappeared with prolongation of diabetes (Table 1). HbA_{1c} did not show a difference between LADA compared with either adult-onset type 1 or type 2 diabetes. The high number of newly diagnosed cases in the LADA and the type 1 diabetes groups could explain the relatively elevated HbA_{1c} concentrations.

The prevalence of any circulating autoantibody to islet-cell antigens was higher in patients with LADA and adult-onset type 1 diabetes than in those with type 2 diabetes (Table 2). Single-autoantibody positivity was more often seen in LADA (59%) than in adult-onset type 1 diabetes (23%) (P = 0.0001). Single-autoantibody positivity with low titer was seen in 10 patients in LADA. The presence of ICA detected at onset disappeared in six patients in the LADA group with longer duration of diabetes. IA-2A was only observed in combination with the other autoantibodies in LADA and adult-onset type 1 diabetes. In type 2 diabetic patients, there was only single-antibody positivity: either ICA (2.6%) or GADA (2.1%) with low titer (the highest ICA level was 10 JDF units, and GADA was 2.2 units/ml).

We compared the prevalence of circulating islet cell–specific autoantibodies in a subgroup of patients with newly diagnosed LADA with that in subgroups of new adult-onset and new childhood-onset type 1 diabetes (Table 2). Similar to the whole patient group, the prevalence of single-autoantibody positivity (ICA or GADA) was higher in the subjects with newly diagnosed LADA compared with those with new adult-onset (P = 0.0042) or new childhood-onset type 1 diabetes (P = 0.0008). The titers of ICA and GADA did not differ between LADA and adult-onset (P = 0.224 and P = 0.231, respectively) or childhood-onset type 1 diabetes (P = 0.06 and P = 0.90, respectively) in the newly diagnosed cases (Table 2). Titers of IA-2A were lower in subjects with LADA (P = 0.0028) and in those with adult-onset type 1 diabetes (P = 0.0175) compared with those with childhood-onset type 1 diabetes (Table 2). No difference was found between the titer of IA-2A in subjects with LADA and those with adult-onset type 1 diabetes (P = 0.134) at onset.

Table 3 shows the prevalence of genotypes and haplotypes associated with increased risk for type 1 diabetes in patients with LADA and those with adult-onset type 1 diabetes. The presence of the

Table 2—*Prevalence and titer of circulating islet cell–specific autoantibodies in patients with LADA, adult-onset type 1 diabetes, and type 2 diabetes and in patients with newly diagnosed LADA and those with adult-onset and childhood-onset type 1 diabetes*

Autoantibody	LADA	Adult-onset type 1 diabetes	Type 2 diabetes	Newly diagnosed LADA	Newly diagnosed adult-onset type 1 diabetes	Newly diagnosed childhood-onset type 1 diabetes
n	54	57	190	21	37	48
ICA only	18 (33)	8 (14)	5 (3)	6 (29)	6 (16)	2 (4)
GADA only	14 (26)	5 (9)	4 (2)	6 (29)	1 (3)	2 (4)
IA-2A only	0	0	0	0	0	3 (6)
ICA + GADA	12 (22)	11 (19)	0	4 (19)	8 (22)	10 (21)
ICA + IA-2A	0	1 (2)	0	0	1 (3)	6 (13)
GADA + IA-2A	1 (2)	2 (3)	0	1 (5)	0	1 (2)
ICA + GADA + IA-2A	9 (17)	18 (32)	0	4 (19)	17 (46)	23 (48)
Antibody negative	0	12 (21)	181 (95)	0	4 (11)	1 (2)
ICA and/or GADA and/or IA-2A	54	45	9	21	33	47
Titer of ICA (JDF)	NE	NE	NE	20 (0–40)	30 (10–40)	40 (20–50)
Titer of GADA (units/ml)	NE	NE	NE	2.4 (0.7–29.8)	12.5 (0.8–50.1)	3.1 (0.9–8.1)
Titer of IA-2A (units/ml)	NE	NE	NE	0.4 (0.2–3.8)	0.7 (0.3–5.2)	4.3 (0.6–18.1)

Data are *n* (%), *n*, or median (interquartile range). The prevalence of circulating islet cell–specific autoantibodies was compared by Fisher's exact test. The titer of autoantibodies was compared by Mann-Whitney test. For statistical significance among the groups, see the text. NE, not evaluated.

high-risk alleles DQB1*0302 and DR4 were increased in both LADA and type 1 diabetic subjects compared with the control population. The frequencies of these alleles did not differ between the two diabetic groups. The prevalence of the risk allele DR3 was also increased in subjects with LADA and those with type 1 diabetes compared with the control subjects. There were no differences in the frequencies of DR3 and DQB1*0201 between LADA and type 1 diabetes. The frequency of the high-risk DR3/DR4 genotype was higher in the LADA group and in the type 1 diabetic patients compared with the control population. The prevalence of DR3/DR4 and DQB1*0201/DQB1*0302 did not differ between the two diabetic groups. Both LADA and type 1 diabetic groups had increased presence of the high-risk haplotype DR4-DQB1*0302 compared with the control subjects. No difference was observed between the patient groups. The proportion of subjects having DR3/DR4-DQB1*0302 was 16% in the LADA group, 14% in the type 1 diabetic group, and 2% in the control subjects. The frequency of the DR3/DR4-DQB1*0302 haplotype was not different between the two diabetic groups.

CONCLUSIONS — In this study, we compared the clinical features and the genetic background of the slowly (LADA) and the rapidly progressing autoimmune adult-onset type 1 diabetes. The clinical parameters of LADA were also compared with the data of type 2 diabetic patients. Patients with LADA had lower BMI, waist-to-hip ratios, and total cholesterol and triglyceride levels and higher HDL cholesterol levels than patients with type 2 diabetes. The presence of hypertension was also decreased in LADA compared with type 2 diabetes. There were no differences in these clinical parameters between LADA and adult-onset type 1 diabetes. Our results are similar to studies by Tuomi et al. (10) and Isomaa et al. (15). In contrast, Lohmann et al. (14) recently reported that diabetic patients with only multiple islet cell–specific autoantibodies or with high titer of GADA (LADA type 1) had lower BMI and lower frequency of

Table 3—*Prevalence of high-risk genotypes and haplotypes in patients with LADA, those with adult-onset type 1 diabetes, and control subjects*

HLA genotypes and haplotypes	LADA ($n = 50$)	LADA compared with control subjects		Adult-onset type 1 diabetes ($n = 50$)	Adult-onset type 1 diabetes compared with control subjects		Control subjects ($n = 336$)	LADA compared with adult-onset type 1 diabetes	
		P value	OR*		*P* value	OR		*P* value	OR
DQB1*0302	17 (34)	0.001	3.2 (1.6–6.4)	20 (40)	0.0007	3.2 (1.6–6.4)	49 (15)	0.93	0.9 (0.4–2.5)
DR4	19 (38)	0.0007	3.2 (1.6–6.3)	25 (50)	0.00006	3.8 (1.9–7.2)	60 (18)	0.70	1.2 (0.5–3.0)
DQB1*0201	21 (42)	ND		22 (44)	ND		ND	0.79	0.9 (0.4–2.2)
DR3	21 (42)	0.01	2.3 (1.2–4.4)	22 (44)	0.018	2.1 (1.1–4.0)	85 (25)	0.79	0.9 (0.4–2.2)
DR3/DR4	6 (12)	0.03	3.8 (1.2–13.0)	7 (14)	0.014	4.3 (1.3–13.6)	9 (3)	0.89	1.1 (0.2–5.1)
DQB1*0201/DQB1*0302	4 (8)	ND		7 (14)	ND		ND	0.89	1.1 (0.2–5.1)
DR4-DQB1*0302	17 (34)	0.0006	3.5 (1.7–6.9)	19 (38)	0.0006	3.3 (1.7–6.5)	47 (14)	0.93	0.9 (0.3–2.5)
DR3/DR4-DQB1*0302	8 (16)	0.04	4.4 (1.1–18.4)	7 (14)	0.0047	6.1 (1.7–21.4)	6 (2)	0.89	1.1 (0.2–5.1)

Data are *n* (%), unless otherwise indicated. Frequency differences were compared by logistic regression analyses, adjusted for age at diagnosis of diabetes and sex. *Odds ratio (95% CI). ND, not determined.

hypertension compared with autoantibody-negative type 2 diabetic patients. Patients with single antibodies of low titer (LADA type 2) had the same clinical and metabolic markers as autoantibody-negative type 2 diabetic patients (14). In our study, we did not observe different clinical phenotypes of patients with LADA; however, we did not make subgroups based on the islet cell–specific autoantibody titer. In agreement with former studies, we also observed that being overweight does not exclude diagnosis of LADA (11,19).

We compared the fasting serum C-peptide levels of subjects with LADA and those with adult-onset type 1 diabetes at onset and longer disease duration. As a new observation, fasting C-peptide levels did not differ between LADA and adult-onset type 1 diabetic subjects at the onset of diabetes. However, a significantly lower level of C-peptide was observed in type 1 diabetic subjects compared with those with LADA, with the longer disease duration conferring the more intensive β-cell destruction in the rapidly progressing type 1 diabetes.

The prevalence of single autoantibody positivity (ICA or GADA) was higher in subjects with LADA than in those with adult-onset type 1 diabetes. A similar difference in the autoantibody pattern was found in the newly diagnosed cases of LADA compared with new adult-onset or new childhood-onset type 1 diabetes cases. The titers of ICA and GADA were not different among the three groups. Based on this observation, we may conclude that the presence of single-autoantibody positivity rather than the low titer indicates the less aggressive destruction of islet-cells. We often detected single ICA positivity in our patients with LADA. Previously, Zimmet et al. (9) reported the important role of GADAs in the diagnosis of LADA. Later, the analysis of UKPDS showed that the presence of both ICAs and GADAs was a stronger predictor of insulin requirement than GADAs alone among patients older than 45 years of age (11). Because we often found single-ICA positivity among LADA patients, we suggest that both ICA and GADA tests may be used to identify LADA in patients with adult onset of diabetes. The ICA positivity documented at onset disappeared in six patients having LADA with longer disease course. This observation may suggest that the tendency of ICA to disappear with increasing disease duration is similar in LADA and type 1 diabetes (20,21). The presence of IA-2A was only seen in combination in the adult-onset autoimmune diabetes; therefore, screening of IA-2A does not seem to be worthwhile in adults.

We found an increased prevalence of predisposing HLA-DQB1*0302, -DR4, -DR3, and -DR3/DR4 genotypes and DR4-DQB1*0302 and DR3/DR4-DQB1*0302 haplotypes in the patients with LADA and adult-onset type 1 diabetes compared with the control population. There were no differences in the frequencies of these risk alleles and haplotypes between the two patient groups. The frequencies of DQB1*0201 and DQB1*0201/DQB1*0302 also did not differ between the two diabetic groups.

The prevalence of DR3/DR4 and DQB1*0201/DQB1*0302 seems to be age-related among childhood-onset type 1 diabetic patients (22,23). The data in adult-onset type 1 diabetes are controversial. Some studies have reported an age-related prevalence of high-risk genotypes, namely, a decreasing presence of DR3/DR4 (24) and DQB1*0201/DQB1*0302 in patients older than 20 years at onset of type 1 diabetes compared with the young-onset patients (23). Lohmann et al. observed relatively low frequency of the DQB1*0302 predisposing gene in adult patients >40 years at onset of type 1 diabetes (25). However, Tuomi et al. (10) did not find a difference in the genotype frequencies between the young-onset and the adult-onset type 1 diabetic patients. In the same study, the genetic background of the LADA patients differed from the type 1 diabetic patients in terms of the prevalence of DQB1*0302, DQB1*0201/0302, and DQB1*0602. They observed a lower frequency of DQB1*0302 (41%) and DQB1*0201/0302 (13%) in patients having GADA-positive type 2 diabetes (LADA) compared with the type 1 diabetic patients (10). In our study, we did find similar presence of the high-risk alleles DQB1*0302 (34%) and DQB1*0201/0302 (8%) in the patients with LADA, but the prevalence did not differ from type 1 diabetes. This diversity could be explained by the difference between the type 1 diabetic patients from the two studies. The onset of the type 1 diabetes might have been earlier in the patients in the study by Tuomi et al. compared with our type 1 diabetic patients. Among their 194 type 1 diabetic patients, they reported on 32 patients whose age was >20 years at the disease onset, and we might assume that the majority of their type 1 diabetic subjects had earlier onset. In our study, all of the type 1 diabetic patients had a "late" disease onset, >25 years of age. We agree with the conclusion by Tuomi et al. (10) that the prevalence of the DQB1*0201/DQB1*0302 (DR3/DR4) genotype may reflect age at onset of autoimmune diabetes, and thus the higher the prevalence at younger age at onset. The difference in genotype between LADA and type 1 diabetes they observed could be explained by the age-related phenomenon. On the other hand, a selection bias may exist in our study because our screening to find patients with LADA was not performed in an unselected group of patients with adult-onset diabetes. We usually tested the presence of islet cell autoantibodies in those patients in whom LADA was clinically suspected.

We found an increased prevalence of the high-risk haplotype DR4-DQB1*0302 in our LADA patients compared with control subjects, but the presence of DR3/DR4-DQB1*0302 was only weakly significant. Data from UKPDS confirmed our results, showing that both DR4-DQB1*0302 and DR3/DR4-DQB1*0302 provided major contributions to the antibody-positive type 2 diabetes (LADA), mainly in early adulthood, age <45 years (16). The prevalence of the DR3/DR4-DQB1*0302 haplotype decreased with later disease onset, age >45 years.

In our study, LADA appeared to have clinical characteristics similar to adult-onset type 1 diabetes. Although fasting C-peptide levels did not differ at onset between the two patient groups, they decreased less rapidly in LADA with longer disease duration. More patients with LADA had single islet cell–specific autoantibody positivity (ICA or GADA) compared with those with adult onset type 1 diabetes. The prevalence of HLA-DQB1*0302, -DR4, -DR3, and -DR3/DR4 risk alleles and DR4-DQB1*0302 and DR3/DR4-DQB1*0302 high-risk haplotypes did not show a difference between LADA and type 1 diabetes with adult onset. However, because of the limited number of patients in the present study, further investigations are necessary to draw a definite conclusion on the lack of genetic differences between the two forms of immune-mediated diabetes concerning

the predisposing HLA genes and to clarify the role of other genes or environmental factors that may modify the ongoing autoimmune process, resulting in rapid or slow β-cell destruction.

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