Latent Autoimmune Diabetes in Adults Differs Genetically From Classical Type 1 Diabetes Diagnosed After the Age of 35 Years

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OBJECTIVE — We studied differences between patients with latent autoimmune diabetes in adults (LADA), type 2 diabetes, and classical type 1 diabetes diagnosed after age 35 years.

RESEARCH DESIGN AND METHODS — Polymorphisms in *HLA-DQB1*, *INS*, *PTPN22*, and *CTLA4* were genotyped in patients with LADA ($n = 213$), type 1 diabetes diagnosed at >35 years of age ($T1D_{>35y}$; $n = 257$) or <20 years of age ($T1D_{<20y}$; $n = 158$), and type 2 diabetes.

RESULTS — Although patients with LADA had an increased frequency of *HLA-DQB1* and *PTPN22* risk genotypes and alleles compared with type 2 diabetic subjects, the frequency was significantly lower compared with $T1D_{>35y}$ patients. Genotype frequencies, measures of insulin secretion, and metabolic traits within LADA differed according to GAD antibody (GADA) quartiles, but even the highest quartile differed from type 1 diabetes. Having two or more risk genotypes was associated with lower C-peptide concentrations in LADA.

CONCLUSIONS — LADA patients differed genetically and phenotypically from both $T1D_{>35y}$ and type 2 diabetic patients in a manner dependent on GADA levels.

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Patients with latent autoimmune diabetes in adults (LADA) have a progressive insulin secretion defect, have less evidence of metabolic syndrome than type 2 patients, and share a genetic predisposition with both type 1 and type 2 diabetic patients (1–5). Whether LADA merely represents older-onset type 1 diabetes or is a distinct subgroup has been debated (6), but studies comparing LADA with classical type 1 diabetes in a comparative age-range are lacking. We investigated *1*) whether LADA differed genetically (with respect to genes associated with risk of type 1 diabetes: *HLA-DQB1*, *PTPN22*, *INS*, and *CTLA4*) and phenotypically (with respect to metabolic syndrome) from type 1 diabetes diagnosed after age 35 years; and *2*) whether the observed clinical heterogeneity within LADA depended on GAD antibody (GADA) levels and type 1 diabetes susceptibility genotypes.

RESEARCH DESIGN AND METHODS

— We included patients with age at onset >35 years with LADA (defined here as GADA-positive diabetes without insulin treatment for the first 6 months; $n = 213$) or with type 1 diabetes ($T1D_{>35y}$; $n = 35$), patients with type 1 diabetes diagnosed at <20 years of age ($T1D_{<20y}$; $n = 158$), and GADA-positive type 2 diabetic patients ($n = 648$) from the Botnia study (2) together with $T1D_{>35y}$ patients ($n = 222$) from the FinnDiane study (7). The non–insulin-treated subjects underwent an oral glucose tolerance test with blood samples drawn at −5, 0, 30, 60, and 120 min for measurement of plasma glucose, serum insulin, C-peptide, lipids, and GADA levels (8). The *HLA-DQB1**02, *0301, *0302, *0602, and *0603 alleles were genotyped (9) and classified as risk (*0302/*02, 0302/X), protective [*0602(3)/X, *0602(3)/*0301], or neutral (all other) (X = homozygous or other allele). The *INS* − Hph23 A/T (rs689), *CTLA4* CT60 (rs3087243), and *PTPN22* 1858C>T (rs2476601) variants were genotyped with TaqMan allelic discrimination (Applied Biosystems, Foster City, CA). Statistical analyses were performed with Statistical Package for Social Science software (version 13.0; SPSS, Chicago, IL) using the χ^2 test, the Kruskal-Wallis test, or a general linear model. Participants gave informed consent, and the study was approved by the local ethics committee.

RESULTS

— The *HLA-DQB1* risk genotypes were most frequent in $T1D_{<20y}$ patients (75.3%), followed by $T1D_{>35y}$ patients (50.2%) and LADA patients (32.2%) ($P < 0.00001$; Fig. 1), with the opposite trend for the protective genotypes. The type 1 diabetic patients also had a higher frequency of the *PTPN22* risk allele ($T1D_{<20y}$ 17.7% and $T1D_{>35y}$ 21.1%) than LADA patients (14.5%; $P = 0.008$). Compared with type 2 diabetes, the *HLA-DQB1* ($P < 0.00001$) and *PTPN22* risk genotypes and alleles ($P = 0.04–0.008$) were significantly more common in LADA. The *INS* and *CTLA4* risk genotypes were only associated with type 1 diabetes ($T1D_{>35y}$, LADA, type 2 diabetes: *INS* 77.4, 58.7, and 56.5%, re-

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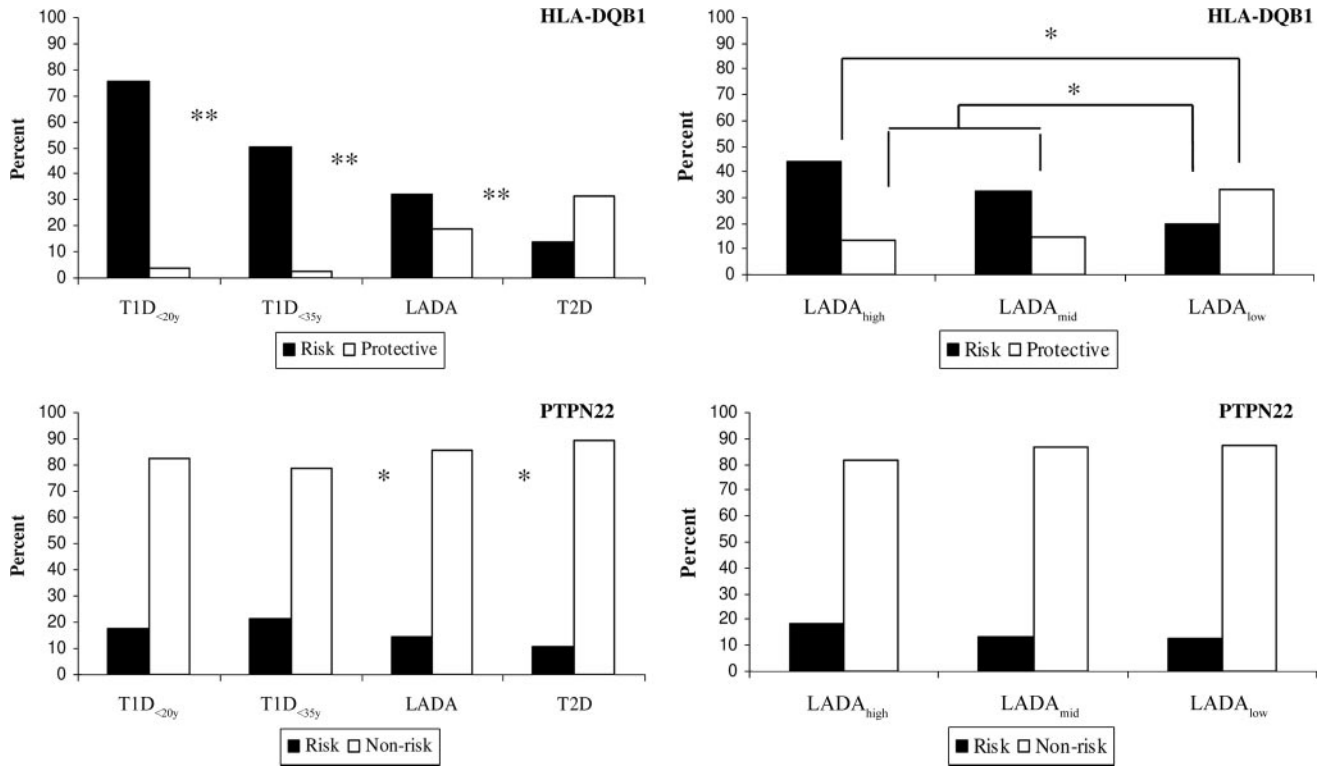

Figure 1—*On the left, distribution of* HLA-DQB1 *genotypes and* PTPN22 *(rs2476601) alleles in type 1 diabetic patients (T1D$_{<20y}$,* n = *158) or after the age of 35 years (T1D$_{>35y}$,* n = *257), LADA patients* (n = *213), and type 2 diabetic patients (T2D,* n = *648). On the right, distribution of* HLA-DQB1 *genotypes and* PTPN22 *(rs2476601) alleles in LADA patients stratified according to GADA quartiles: the highest, LADA$_{high}$* (n = *52); the two middle quartiles pooled, LADA$_{mid}$* (n = *109); and the lowest, LADA$_{low}$* (n = *52). Black columns represent risk genotypes and alleles, and white columns represent protective* (HLA-DQB1) *or nonrisk* (PTPN22) *genotypes and alleles. The χ^2 test was applied to test differences between diabetic groups. Significant differences are indicated with asterisks.* *P < *0.05.* **P < *0.0001.* HLA-DQB1: P = *0.01 across the GADA quartiles of LADA.*

spectively, and *CTLA4* 48.2, 45.0, and 41.6%, respectively).

All risk genotypes and alleles were most prevalent in patients in the highest GADA quartile (LADA$_{high}$, GADA >278 IU/ml) compared with the two middle (LADA$_{mid}$, 44–278 IU/ml) or the lowest quartiles (LADA$_{low}$, 33–43.9 IU/ml; Fig. 1), but the difference was significant only for *HLA-DQB1* ($P = 0.009$). However, even LADA$_{high}$ subjects differed from T1D$_{>35y}$ subjects ($P = 0.001$), and LADA$_{low}$ subjects had nonsignificantly more *HLA-DQB1* risk genotypes than type 2 diabetic subjects (19.6 vs. 14.0%). LADA$_{high}$ subjects also differed from the type 2 diabetic subjects with respect to the allele frequency of *PTPN22* ($P = 0.02$) and *CTLA4* ($P = 0.03$).

In a joint analysis of the four genes, two or more risk genotypes were most common in T1D$_{<20y}$ patients (82.1%), followed by T1D$_{>35y}$ patients (74.4%), LADA (54.1%), and type 2 diabetic (38.7%) patients ($P < 0.00001$). The frequency differed across the GADA quartiles ($P = 0.004$), and it was similar in LADA$_{high}$ and T1D$_{>35y}$ patients (72.5 vs. 74.4%), as well as in LADA$_{low}$ and type 2 diabetic patients (46.0 vs. 38.7%) (online appendix Table A3, available at http://care.diabetesjournals.org/cgi/content/full/dc09-2188/DC1). LADA patients with an increasing number of risk genotypes had decreasing fasting serum (fS)–C-peptide concentrations ($P = 0.015$).

The LADA patients had higher BMI and lipid concentrations than the T1D$_{>35y}$ patients (online appendix Table A1). Compared with type 2 diabetic patients, LADA patients had lower insulin secretion and BMI, as well as a better lipid profile. Going from LADA$_{low}$ to LADA$_{high}$, there was a significant trend toward lower insulin secretion, lipid levels, and BMI (online appendix Table A2). However, the fS–C-peptide concentration was higher in LADA$_{high}$ patients compared with T1D$_{>35y}$ patients and was somewhat lower in LADA$_{low}$ compared with type 2 diabetic patients. Despite the marked age difference, the T1D$_{>35y}$ and T1D$_{<20y}$ patients were metabolically similar.

CONCLUSIONS — We have shown a significant genetic difference between LADA and type 1 diabetes diagnosed after age 35 years. Although *HLA-DQB1* and *PTPN22* risk genotypes were increased in LADA, they were much less common than in type 1 diabetes. *INS* and *CTLA4* were only associated with type 1 diabetes.

As suggested previously (3,5,10), genetic differences and clinical phenotype were associated with GADA levels, explaining some of the observed heterogeneity within LADA. Of note, the LADA$_{high}$ group (GADA >278 IU/ml) roughly corresponds to the high GADA group (>300 IU/ml) in the Non Insulin Requiring Autoimmune Diabetes (NIRAD) Study, in which association with *PTPN22* was also found only in patients with high GADA (10).

Our study is the largest to date in adult-onset type 1 diabetic patients. In agreement with a smaller study (11), we clearly showed a lower frequency of *DQB1* risk genotypes in T1D$_{>35y}$ compared with T1D$_{<20y}$ patients. However, protective *HLA-DQB1* genotypes were as

rare in $T1D_{>35y}$ as in $T1D_{<20y}$ patients. The prevalence of *INS*, *PTPN22*, and *CTLA4* risk genotypes was similar in the two groups. As expected (2,4,10,12–14), LADA had an increased frequency of *HLA-DQB1* and *PTPN22* risk genotypes as well as a decreased frequency of *HLA-DQB1* protective genotypes compared with type 2 diabetic subjects. Contrary to previous reports (4,14,15), the *INS* variant was not associated with LADA in the Finnish subjects.

In conclusion, we have shown that patients with LADA differ genetically and phenotypically from type 1 diabetic patients diagnosed after age 35 years. The LADA group was heterogeneous, and both the genotype distribution and phenotypic characteristics were associated with GADA level. A significant trend was observed toward lower insulin secretion and metabolic trait values going from the lowest GADA quartile to the highest. Thus, LADA patients with high GADA concentrations were more similar, but not identical, to type 1 diabetic patients, and those with low GADA concentrations were more similar to type 2 diabetic patients.

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