Genetic Similarities Between Latent Autoimmune Diabetes in Adults, Type 1 Diabetes, and Type 2 Diabetes

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OBJECTIVE—Latent autoimmune diabetes in adults (LADA) is often considered a slowly progressing subtype of type 1 diabetes, although the clinical picture more resembles type 2 diabetes. One way to improve classification is to study whether LADA shares genetic features with type 1 and/or type 2 diabetes.

RESEARCH DESIGN AND METHODS—To accomplish this, we studied whether LADA shares variation in the HLA locus or *INS* VNTR and *PTPN22* genes with type 1 diabetes or the *TCF7L2* gene with type 2 diabetes in 361 LADA, 718 type 1 diabetic, and 1,676 type 2 diabetic patients, as well as 1,704 healthy control subjects from Sweden and Finland.

RESULTS—LADA subjects showed, compared with type 2 diabetic patients, increased frequency of risk for the HLA-DQB1 *0201/*0302 genotype (27 vs. 6.9%; $P < 1 \times 10^{-6}$), with similar frequency as with type 1 diabetes (36%). In addition, LADA subjects showed higher frequencies of protective HLA-DQB1 *0602(3)/X than type 1 diabetic patients (8.1 vs. 3.2%, $P = 0.003$). The AA genotype of rs689, referring to the class I allele in the *INS* VNTR, as well as the CT/TT genotypes of rs2476601 in the *PTPN22* gene, were increased both in type 1 diabetic ($P = 3 \times 10^{-14}$ and $P = 1 \times 10^{-10}$, respectively) and LADA ($P = 0.001$ and $P = 0.002$) subjects compared with control subjects. Notably, the frequency of the type 2 diabetes–associated CT/TT genotypes of rs7903146 in the *TCF7L2* were increased in LADA subjects (52.8%; $P = 0.03$), to the same extent as in type 2 diabetic subjects (54.1%, $P = 3 \times 10^{-7}$), compared with control subjects (44.8%) and type 1 diabetic subjects (43.3%).

CONCLUSIONS—LADA shares genetic features with both type 1 (HLA, *INS* VNTR, and *PTPN22*) and type 2 (*TCF7L2*) diabetes, which justifies considering LADA as an admixture of the two major types of diabetes. ***Diabetes*** **57:1433–1437, 2008**

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GADA, GAD autoantibody; LADA, latent autoimmune diabetes in adults.

See accompanying commentary on p. 1160.

Latent autoimmune diabetes in adults (LADA) has in the World Health Organization classification been considered as a subgroup of type 1 diabetes, i.e., slowly progressing type 1 diabetes. Although LADA patients often present with a clinical picture similar to type 2 diabetes, with an adult age at onset and insulin independence at diagnosis, they are characterized by circulating islet autoantibodies similar to those found in type 1 diabetes (1,2). One way to shed light on the classification of LADA would be to determine to what extent LADA shares genetic similarities with type 1 and type 2 diabetes.

There is some support for the view that LADA shares susceptibility genes with type 1 diabetes, but there are only a limited number of reports that have been large enough to address this issue (3–6). The HLA locus on the short arm of chromosome 6 that confers most of the genetic susceptibility to type 1 diabetes (7) has shown similar associations with LADA (3,5) but also distinct differences, e.g., DQB1 *0201/*0302 is a more common genotype in type 1 diabetes than in LADA, whereas the protective genotypes *0602/X and 0603/X are more common in LADA than in type 1 diabetes (6). The insulin gene variable number of tandem repeats (*INS* VNTR) on chromosome 11 falls into two general classes—class I (26–63 repeats) and class III (141–209 repeats)—and the short class I has shown strong susceptibility to type 1 diabetes (8) as well as to LADA, as shown in the U.K. Prospective Diabetes Study (4). However, no association was seen in the Finnish Botnia study (6). Other loci contributing to the genetic risk of type 1 diabetes include the protein tyrosine phosphatase nonreceptor type 22 (PTPN22) gene on chromosome 1 (9).

Previously, it was difficult to demonstrate genetic similarities between LADA and type 2 diabetes because no genes had been consistently associated with type 2 diabetes. However, recently, variation in the transcription factor 7-like 2 (*TCF7L2*) gene showed strong association with type 2 diabetes (10,11), making this gene a good candidate for genetic comparisons between LADA and type 2 diabetes. The *TCF7L2* gene showed no association with type 1 diabetes (12); however, a role in type 1 diabetes was suggested for another TCF gene, the *TCF7* (13). Mutations in yet other transcription factors cause monogenic forms of diabetes, e.g., *TCF1* (*HNF1α*; MODY3), *TCF2* (*HNF1β*; MODY5), and *TCF14* (*HNF4α*; MODY1).

The aim of this study was to elucidate whether LADA shares genetic similarities with type 1 and type 2 diabetes by comparing genetic variation within the HLA locus and the *INS* VNTR, *PTPN22*, and *TCF7L2* genes among patients with type 1 diabetes, LADA, or type 2 diabetes and healthy control subjects.

TABLE 1
Clinical characteristics of the subjects

	Swedish cohort				Finnish cohort		
	Type 1 diabetes	LADA	Type 2 diabetes	Control	LADA	Type 2 diabetes	Control
n (M/F)	394/324	73/91	553/447	553/447	83/113	361/315	338/366
GADA positive (%)	46	100	0	—	100	0	—
Insulin treatment (%)	99	58	16	0	47	42	0
Age at onset/visit (years)	17.9 ± 9.2	52.4 ± 11.4	54.3 ± 8.8	70.1 ± 2.9	54.6 ± 11.4	54.8 ± 9.3	53.7 ± 11.4
BMI (kg/m^2)	23.8 ± 3.3	25.9 ± 5.6	29.2 ± 4.8	27.6 ± 4.4	26.8 ± 5.0	29.1 ± 4.8	25.9 ± 3.7
Fasting plasma glucose (mmol/l)	12.4 ± 5.3	11.8 ± 4.6	11.0 ± 3.5	5.3 ± 0.4	10.3 ± 4.3	9.0 ± 3.0	5.3 ± 0.5
Fasting C-peptide (nmol/l)	0.04 ± 0.12	0.40 ± 0.45	1.04 ± 0.52	—	0.43 ± 0.40	0.66 ± 0.37	0.41 ± 0.22

Data are means ± SD.

RESEARCH DESIGN AND METHODS

Four groups of individuals from Sweden were included: 164 LADA patients (age at onset >35 years, GAD autoantibody [GADA] positive), 1,000 type 2 diabetic patients (age at onset >35 years, GADA negative), 718 type 1 diabetic patients (age at onset <35 years), and 1,000 nondiabetic control individuals (age at visit >40 years) (Table 1). The patients were recruited from the local diabetes registry (14), and diagnosis of diabetes was based on World Health Organization criteria (1). The control subjects were selected from the Malmö Preventive Project (15) without a family history of diabetes or treatment of hypertension. Type 2 diabetic patients and control subjects were matched for sex, BMI, and age, with the control subjects being at least 5 years older. Three groups of individuals from Finland, participating in the Botnia study (16), were also included: 197 LADA patients (defined by age at onset >35 years and as GADA positive), 676 unrelated GADA-negative type 2 diabetic patients (age at onset >35 years), and 704 unrelated control individuals (age at visit >35 years, no first- or second-degree relatives with diabetes) (Table 1). GADA levels >32 international units/ml (IU/ml) or 5 relative units were considered positive (see below for details). The prevalence of GADA-positive (LADA) patients among type 2 diabetic patients in the Botnia Study was 9% (6). All subjects gave their informed consent to the study, which was approved by the local ethics committee.

Measurements and assays. Plasma glucose was measured with a glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA), and insulin was measured with enzyme-linked immunosorbent assay (DAKO, Cambridgeshire, U.K.) with an interassay CV of 8.9%. Fasting serum C-peptide concentrations were determined by a radioimmunoassay with an interassay CV of 9% (Human C-peptide RIA; Linco, St. Charles, MO). GADAs were determined by a radiobinding assay using ^{35}S-labeled recombinant human GAD65 produced by coupled in vitro transcription-translation as described (17). Levels exceeding 32 IU/ml or 5 relative units were considered positive [relative units = (sample cpm − mean cpm of three negative control subjects)/(cpm of a positive internal reference − mean cpm of three negative control subjects) × 100] and according to the standardized IUs, 5 relative units equals 32 IU/ml. In the first Diabetes Antibody Standardization Programme (DASP) (2000), our GADA assay showed a sensitivity of 80% and a specificity of 96%; in the second DASP (2002), a sensitivity of 88% and a specificity of 87%; and in the third DASP (2003), a sensitivity of 82% and specificity of 93%.

Single nucleotide polymorphism genotyping. Genotyping of rs689 (*INS* VNTR), rs3842755 (*INS* VNTR), rs2476601 (*PTPN22*), rs7903146 (*TCF7L2*), and rs12255372 (*TCF7L2*) was performed either by allelic discrimination on an Applied Biosystems 7900HT system (Applied Biosystems, Foster City, CA) (Swedish cohort: rs689, rs3842755, rs2476601; Finnish cohort: rs7903146 and rs12255372), or by using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry with the resulting mass spectra analyzed by the SpectroTYPER RT 2.0 software (Sequenome, San Diego, CA). An error rate of <1% was determined with 5–10% re-genotyping. Primer sequences and additional genotyping details are available from the authors.

HLA genotyping. HLA genotyping was performed in the Swedish cohort as previously described (18,19). Briefly, the second exon of HLA-DQB1 was amplified and hybridized with lanthanide (III) chelate-labeled DNA probes specific for the HLA-DQB1 *0201, *0301, *0302, *0602, and *0603 alleles. Hybridization was evaluated by time-resolved fluorescence (Delfia Research Fluorometer, Wallac OY, Turku, Finland). The symbol X refers to a homozygous allele or any allele other than 0201, 0302, or 0602 (3). A Swedish control group was included in the table for comparison (20).

Statistical analysis. The χ^2 tests and multivariate logistic regression analyses adjusted for age (control subjects), age-at-onset (cases), BMI, and sex were performed to study association of single nucleotide polymorphisms with disease. The Mantel-Haenszel test was used to test for heterogeneity between the Swedish and Finnish cohorts. In addition, an extensive investigation of potential population stratification between these cohorts has recently been published (21). The prior hypothesis to be tested was whether LADA shares genetic features with type 1 or type 2 diabetes. Because this has previously been shown for the HLA and INS VNTR loci, we only applied a Bonferroni correction for the two new genes tested, i.e., *PTPN22* and *TCF7L2*, by multiplying the *P* value by 4 (2 genes × 2 cohorts). In pooled analyses of the two cohorts in regression analyses, adjustment was made for country-of-origin. A *P* value of <0.05 was considered statistically significant. Statistical analyses were performed using Number Cruncher Statistical Systems (NCSS), version 2000 (Kaysville, UT).

RESULTS

Genotype frequencies of studied single nucleotide polymorphisms are presented in Table 2 and 3. All genotypes were in the Hardy-Weinberg equilibrium. A Mantel-Haenzel test revealed no heterogeneity between the two cohorts regarding the tested polymorphisms. Therefore, the Finnish and Swedish cohorts were analyzed together.

HLA-DQB1. The frequencies of the HLA-DQB1 genotypes *0302/X and *0201/*0302 were higher in type 1 diabetes and LADA than in type 2 diabetic patients, whereas the

TABLE 2
Risk of different HLA genotypes for being associated with type 1 and type 2 diabetes compared with LADA

HLA genotype	LADA (%)	Type 1 diabetes				Type 2 diabetes				Control (%)
		%	OR	95% CI	*P*	%	OR	95% CI	*P*	
*0201/X	29	24	0.8	0.5–1.2	0.24	31	1.1	0.7–1.7	0.68	36*
*0302/X	29	33	1.2	0.8–1.9	0.32	21	0.6	0.4–1.0	0.05	16*
*0201/*0302	27	36	1.5	1.0–2.3	0.06	6.9	0.2	0.1–0.4	$<1 \times 10^{-6}$	6.0*
*0602/X*0603/X	8.1	3.2	0.3	0.1–0.7	0.003	25	3.5	1.8–6.9	0.0002	23*

X refers to a homozygous allele or any allele other than 0201, 0302, or 0602(3). The odds ratios (OR) refer to the risk of having type 1 and type 2 diabetes vs. LADA (OR = 1) if carrying the specific genotype, using logistic regression analysis adjusting for BMI, age, and sex. *Genotype frequencies of 216 Swedish control subjects (20).

TABLE 3
Frequency (%) of single nucleotide polymorphisms in the *INS* VNTR, *PTPN22*, and *TCF7L2* genes in the Swedish and Finnish cohorts

Gene/single nucleotide polymorphism	Genotype	Control subjects		Type 1 diabetic	LADA		Type 2 diabetic	
		Swedish	Finnish	Swedish	Swedish	Finnish	Swedish	Finnish
INS VNTR/rs689	AA	54.8		73.4	69.0			
	ATTT	45.2		26.6	31.0			
	$P =$			3×10^{-14}	0.001			
INS VNTR/rs3842755	CC	65.0		79.3	76.0			
	CAAA	35.0		20.7	24.0			
	$P =$			3×10^{-10}	0.008			
PTPN22/rs2476601	CC	82.9		68.0	71.3		76.9	
	CTTT	17.1		32.0	29.7		23.1	
	$P =$			1×10^{-10}*	0.002*		0.008*	
TCF7L2/rs7903146	CC	55.2	68.4	56.7	47.2	57.7	45.9	59.5
	CTTT	44.8	31.6	43.3	52.8	42.3	54.1	40.5
	$P =$			0.55	0.03*		3×10^{-7}*	
TCF7L2/rs12255372	GG	52.4	70.1	55.5	46.7	58.4	48.7	61.3
	GTTT	47.6	29.9	44.5	53.3	41.6	51.3	38.7
	$P =$			0.16	0.09*		0.003*	

Differences in genotype frequency were tested by χ^2 tests comparing diabetic groups with the control group. *P values corrected for multiple comparisons.

frequencies of the protective genotypes *0602/X and *0603/X were more than twice as high in LADA compared with type 1 diabetic patients (8.1 vs. 3.2%), but markedly lower than in type 2 diabetes (25%) (Table 2).

INS VNTR. The AA genotype of rs689 (class I) was increased in both type 1 diabetes (73.4%, $P = 3 \times 10^{-14}$) and LADA (69.0%, $P = 0.001$) (Table 3). The associations were also observed when using regression analysis adjusting for age, BMI, and sex (Table 4).

***PTPN22*.** The frequency of the CT/TT genotypes of rs2476601 in the *PTPN22* gene were increased in both type 1 diabetic (32.0%, $P = 1 \times 10^{-10}$) and LADA (29.7%, $P = 0.002$) compared with control (17.1%) subjects. In addition, the frequency of the CT/TT genotype was also increased in type 2 diabetic (23.1%, $P = 0.008$) compared with control subjects (Tables 3 and 4).

***TCF7L2*.** Importantly, the frequency of the CT/TT genotypes of rs7903146 in *TCF7L2* was increased to the same extent in LADA (52.8%; $p_{\text{pooled}} = 0.03$) as in type 2 diabetic (54.1%, $p_{\text{pooled}} = 3 \times 10^{-7}$) compared with control (44.8%) subjects (Table 3), with no difference between LADA and type 2 diabetes ($P = 0.59$). This was also seen when the data were analyzed using regression analysis adjusted for age, BMI, sex, and country of origin (Table 4). The results for single nucleotide polymorphism rs12255372 were virtually similar as those for rs7903146 (Tables 3 and 4). There was no difference in frequency of the CT/TT genotypes of rs7930146 between type 1 diabetic and control subjects (43.3 vs. 44.8%, $P = 0.55$).

DISCUSSION

The present study shows that LADA shares genetic features with both type 1 (HLA-DQB1, *INS* VNTR, *PTPN22*) and type 2 diabetes (*TCF7L2*), suggesting that LADA represents an admixture of the two major types of diabetes (Fig. 1).

The HLA and *INS* VNTR findings are in keeping with previous results. Several studies have shown increased frequencies of type 1 diabetes–associated high-risk HLA genotypes in patients with LADA, thereby concluding that LADA represents a subgroup of type 1 diabetes (3,4,22,23). However, it should be emphasized that LADA differs from type 1 diabetes with a smaller effect size of the associations with HLA, *INS* VNTR, and PTPN22, as was previously shown for both HLA (6,24) and *INS* VNTR (24). The association of *INS* VNTR with LADA from the U.K. Prospective Diabetes Study (24) was in contrast to the previous report from our laboratory in Finns (6). We cannot rule out whether this is due to population differences or power issues. The former is unlikely, since the frequency of the AA genotype of the *INS* VNTR was similar in the LADA patients from the U.K. as in our Scandinavian sample. The latter is more likely, since the current study

TABLE 4
Risk of type 1 diabetes, LADA, and type 2 diabetes, conferred by variants in the *INS* VNTR, *PTPN22*, and *TCF7L2* genes

Gene/single nucleotide polymorphism	Risk genotype	Type 1 diabetes			LADA			Type 2 diabetes		
		OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
INS VNTR/rs689	AA	2.3	1.8–2.9	$<1 \times 10^{-6}$	1.7	1.2–2.6	0.004			
INS VNTR/rs3842755	CC	2.2	1.7–2.8	1×10^{-6}	1.6	1.1–2.5	0.02			
PTPN22/rs2476601	CT/TT	2.3	1.8–3.1	$<4 \times 10^{-6}$*	1.7	1.1–2.7	0.05*	1.5	1.2–1.9	0.004*
TCF7L2/rs7903146	CT/TT				1.5	1.2–1.9	0.007*	1.4	1.4–1.7	$<4 \times 10^{-6}$*
TCF7L2/rs12255372	GT/TT				1.5	1.1–1.9	0.01*	1.3	1.1–1.5	0.01*

The odds ratios (OR) refer to risk of being diabetic, comparing diabetic groups with the control group, if carrying the particular genotype, using logistic regression analysis with adjustment of BMI, age, sex, and country of origin (where appropriate). *P values corrected for multiple comparisons.

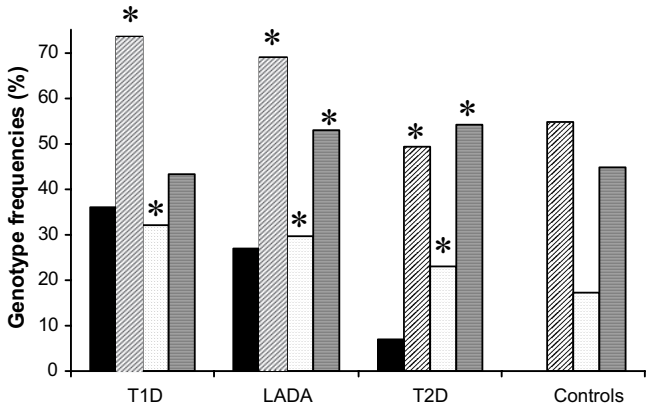

FIG. 1. Risk genotype frequencies of the four different susceptibility loci in type 1 diabetes, LADA, and type 2 diabetes. HLA-DQB1 was not genotyped in control subjects. *Significant association to diabetes ($P < 0.05$). ■, HLA-DQB1 (*0201/*0302); ▨, INS VNTR-rs689 (AA genotype); □, PTPN22-rs2476601 (CT/TT genotypes); ▤, TCF7L2-rs7903146 (CT/TT genotypes). T1D, type 1 diabetes; T2D, type 2 diabetes.

included almost twice as many LADA patients as the previous Finnish study.

The *PTPN22* gene on chromosome 1 encodes the lymphoid-specific tyrosine phosphatase LYP, involved in the suppression of T-cell activation and thereby T-dependent antibody production (25), and has been associated with increased susceptibility to type 1 diabetes (9) and other autoimmune diseases (26). In this study, we provide novel information that LADA also shows increased frequency of the CT/TT genotypes in the *PTPN22* gene compared with control subjects, although less than in type 1 diabetic subjects. Somewhat surprisingly, the same genotype(s) were also increased in patients with type 2 diabetes. Although this could be an interesting support for the role of inflammation in type 2 diabetes, we should note that this was a secondary analysis that would require much more stringent corrections for multiple testing. However, it is a finding that deserves replication efforts in other cohorts of type 2 diabetic patients. Taken together, the HLA, *INS* VNTR, and *PTPN22* data clearly point at a common genetic background between LADA and type 1 diabetes.

The identification of the *TCF7L2* gene as the strongest candidate gene for type 2 diabetes (10,11) has now opened for the possibility to test whether LADA also shares genetic features with type 2 diabetes. We provide compelling evidence that this is the case: LADA patients showed the same increased frequency of risk genotypes in the *TCF7L2* gene as type 2 diabetic patients. The association between LADA and TCF7L2 was not affected when the quartile with the lowest GADA levels were excluded, so the finding was not due to inclusion of false-positive type 2 diabetic patients in the LADA group (data not shown). In contrast, and in keeping with a previous report (12), the frequency of these risk genotypes did not differ between type 1 diabetic patients and control subjects.

There has been a striking increase in both type 1 and type 2 diabetes in Scandinavian countries during the past decades, with a convergence of the previously distinctive phenotypes, and it has been proposed that weight gain and insulin resistance could serve as a trigger for diabetes in both type 1 and type 2 diabetes ("accelerator hypothesis") (27). However, this was challenged by the fact that type 1 diabetes does not share a genetic background, like *TCF7L2*, with type 2 diabetes (28). Our data partially challenge this argument, since the late-onset form of autoimmune diabetes, LADA, clearly shares a genetic background with type 2 diabetes.

The mechanism by which variation in the *TCF7L2* gene contributes to diabetes is unclear; however, the intestinal proglucagon gene shows binding sites for TCF7L2 (29) and a potential mechanism could involve the incretin axis. We have recently shown that carriers of the risk T allele show impaired insulin secretion, impaired incretin effect, and enhanced expression of the *TCF7L2* gene in human islets (30). Also in previous studies, the *TCF7L2* variants have been associated with impaired insulin secretion (11,31). It is thus likely that similar nonautoimmune mechanisms are operative in islets from both patients with type 2 diabetes and LADA, causing impaired insulin secretion.

Some weaknesses of the study should be emphasized. Autoimmune diabetes has been subdivided into rapidly (type 1 diabetes) and slowly (LADA) progressing forms (World Health Organization criteria). In the diagnosis of LADA, lack of insulin treatment during the first 6 months after diagnosis is often used to distinguish LADA from adult-onset type 1 diabetes. However, the decision to initiate insulin therapy is very subjective and ketosis-prone type 1 diabetes in this adult age-group is very rare (<10% in our experience). We therefore decided only to use hard criteria like age at onset and presence of GAD antibodies for the diagnosis of LADA. Also the use of two different cohorts (Finnish and Swedish) in the analysis could introduce heterogeneity. However, these populations have successfully been used in a whole genome association study for type 2 diabetes with stratification biases excluded using Eigenstrat (21).

In conclusion, the data from this study positions LADA genetically as an admixture of type 1 and type 2 diabetes, rather than as a subgroup of type 1 diabetes.

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