

# Genetic Heterogeneity in Latent Autoimmune Diabetes Is Linked to Various Degrees of Autoimmune Activity

## Results From the Nord-Trøndelag Health Study

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**OBJECTIVE**—Previous studies have indicated that the latent autoimmune diabetes in adults (LADA) phenotype is heterogeneous and that LADA patients share features of type 1 and type 2 diabetes in various proportions. We tested for association of known type 1 and type 2 diabetes susceptibility genes in LADA subjects and analyzed relationships to a marker of autoimmune activity (titers of anti-GAD) and a phenotypic risk factor of type 2 diabetes (BMI).

**RESEARCH DESIGN AND METHODS**—Data were assembled from the Nord-Trøndelag Health Study (HUNT) study, which comprises the adult population of an entire county in Norway. We genotyped 60 single nucleotide polymorphisms (SNPs) known to be associated with type 1 or type 2 diabetes, including 14 tag SNPs used for HLA haplotyping in 120 type 1 diabetic, 126 LADA, and 1,090 type 2 diabetic patients and 1,503 age- and sex-matched nondiabetic subjects.

**RESULTS**—The majority of the strongly associated HLA haplotypes for type 1 diabetes were significantly associated with LADA in general, but mainly with high anti-GAD LADA patients. Two distinct HLA haplotypes were associated only with LADA and mainly in low anti-GAD LADA patients. There were no associations of non-HLA type 1 diabetes loci with LADA. Of type 2 diabetes-associated genes, the CC/CT genotypes of rs7961581 (*TSPAN8*) and the obesity-linked AA/AC genotypes of rs8050136 (*FTO*) were associated with LADA in general, but mainly in low anti-GAD LADA patients ( $P = 0.004$  and  $P = 0.004$ , respectively).

**CONCLUSIONS**—Genetic heterogeneity in LADA is linked to various degrees of autoimmune activity and may be partly distinct from both type 1 and type 2 diabetes. *Diabetes* 59: 302–310, 2010

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Latent autoimmune diabetes in adults (LADA) is a slowly progressive form of autoimmune-associated diabetes (1). It is a common form of diabetes; for example, in the second Nord-Trøndelag Health Study (HUNT2), 9% of diabetic patients were classified as LADA (2). However, the etiology of LADA remains less well understood than that of other forms of diabetes.

It has been discussed whether LADA is a mild form of type 1 diabetes or a distinct etiological entity (3–5). Previous studies reported that LADA shares genetic features with type 1 diabetes, including an increased frequency of the HLA-DQB1 allele of the *DQB1* gene (6,7). However, other results show that age, obesity, and physical inactivity are important risk factors for both LADA and type 2 diabetes (8). Furthermore, the type 2 diabetes-associated gene *TCF7L2* has been reported to be associated with LADA (9).

Thus, the etiology of LADA resembles partly type 1 and partly type 2 diabetes. However, the genetic participation of type 1 and type 2 diabetes susceptibility genes in the etiology of LADA needs further elucidation and is complicated by the phenotype of LADA being heterogeneous among and within populations (5). The diagnosis of LADA is usually based on the following: age older than 35 years, the presence of at least one circulating autoantibody against islet cell antigens (usually antibodies to GAD [anti-GAD]), and no apparent need for insulin for at least 6 months after diagnosis. These criteria leave room for heterogeneity. It has, for instance, been reported (10) that the phenotype of individual LADA patients is related to titer of anti-GAD, and in a recent study the need for insulin treatment over several years was more frequent in those with higher titers of anti-GAD (11).

In the present study, we first investigated the association of type 1 and type 2 diabetes candidate loci in LADA patients in general. Second, we tested for variability in genetic background in relation to a marker of autoimmune activity (anti-GAD) as well as a phenotypic risk factor for type 2 diabetes (BMI).

### RESEARCH DESIGN AND METHODS

**The HUNT2 study population.** The second HUNT study, a population-based study in Nord-Trøndelag County, was performed between 1995 and 1997. Nord-Trøndelag is located in the central part of Norway and is fairly demographically representative of Norway, making it suitable for epidemiological studies. All inhabitants  $\geq 20$  years ( $n = 92,936$ ) were invited to participate. The overall response rate was 71.3% ( $n = 65,258$ ). The survey included a clinical examination, blood sampling, and two general questionnaires that included more than 200 health-related items. In addition, a specific

TABLE 1  
Phenotypic characteristics for male and female subjects selected from the HUNT cohort

	LADA		Type 1 diabetes		Type 2 diabetes		Control subjects	
	Male	Female	Male	Female	Male	Female	Male	Female
<i>n</i>	68	58	72	48	534	556	740	763
Age when attended (years)	67 ± 12	70 ± 11	46 ± 16	52 ± 17	67 ± 11	69 ± 11	64 ± 15	68 ± 14
Age at diagnosis (years)	58 ± 11	60 ± 12	26 ± 16	34 ± 16	60 ± 11	61 ± 12		
BMI (kg/m <sup>2</sup> )	27.4 ± 3.8	29.4 ± 5.3	25.8 ± 3.4	26.6 ± 4.6	28.5 ± 3.8	30.8 ± 5.4	26.4 ± 3.4	27.1 ± 4.5
Waist-to-hip ratio	0.94 ± 0.06	0.85 ± 0.06	0.90 ± 0.06	0.80 ± 0.06	0.94 ± 0.06	0.86 ± 0.7	0.91 ± 0.06	0.82 ± 0.06
Cholesterol (mmol/l)	5.4 ± 1.1	6.3 ± 1.3	5.2 ± 1.0	6.0 ± 1.2	6.0 ± 1.2	6.5 ± 1.3	6.0 ± 1.1	6.6 ± 1.4
HDL cholesterol (mmol/l)	1.1 ± 0.4	1.4 ± 0.5	1.5 ± 0.4	1.7 ± 0.5	1.1 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.5 ± 0.4
Triglycerides (mmol/l)	2.2 ± 1.4	2.3 ± 1.3	1.4 ± 0.71	1.3 ± 0.73	2.6 ± 1.7	2.7 ± 1.4	1.9 ± 1.1	1.9 ± 1.1
Systolic blood pressure (mmHg)	154 ± 21	154 ± 26	140 ± 20	141 ± 25	152 ± 23	160 ± 24	146 ± 21	151 ± 26
Diastolic blood pressure (mmHg)	85 ± 12	80 ± 14	79 ± 10	78 ± 12	86 ± 13	86 ± 14	84 ± 12	83 ± 14
Nonfasting glucose (mmol/l)	11 ± 5.1	10.4 ± 4.6	11 ± 6.2	10.6 ± 5.5	9.8 ± 3.9	9.1 ± 4.0	5.6 ± 1.3	5.7 ± 1.9

Data are means ± SD.

questionnaire was administered to those who stated they had diabetes. Further details are described elsewhere (12).

**Identification of diabetic patients.** Diabetic patients were identified by self-reported answer of “yes” to the question “Do you have or have you had diabetes?” ( $n = 1972$ ). Subjects answering “yes” were invited to a follow-up examination where fasting C-peptide and anti-GAD were measured. In total, 73.6% participated ( $n = 1,451$ ). At follow-up, participants were also interviewed by the screening nurses to ensure year of diagnosis and details on start of different types of diabetes treatment (12).

**C-peptide and anti-GAD measurements.** Serum levels of C-peptide and anti-GAD were analyzed at the Hormone Laboratory of Aker University Hospital, Oslo, Norway. C-peptide was measured by radioimmunoassay (Diagnostic System Laboratories, Webster, Texas) and anti-GAD by immunoprecipitation using [<sup>3</sup>H] leucine translation labeled GAD65 (Novo Nordisk Pharma AS, Bagsvaerd, Denmark). The anti-GAD assay was based on a validated method (13). Anti-GAD levels were expressed as an index value relative to a standard serum, and a value  $\geq 0.08$  was considered positive. At the cutoff level of 0.08, the assay was previously shown (2) by the Diabetes Autoantibody Standardization Program (DASP) to have a sensitivity of 0.64 and specificity of 1.00.

**Classification of diabetes.** Patients starting insulin treatment within 6 months of diagnosis were classified as type 1 diabetes if, in addition, they were either anti-GAD positive or anti-GAD negative and had fasting C-peptide levels  $< 150$  pmol/l. Patients were classified as LADA if they were anti-GAD positive and had not been treated with insulin within 12 months of diagnosis. Type 2 diabetic case subjects were anti-GAD negative and without insulin treatment within 1 year of diagnosis. By these criteria, we included 120 type 1 diabetic, 126 LADA, and 1,090 type 2 diabetic patients in the present study. One hundred fifteen patients did not meet any of the criteria and were excluded.

**Classification of diabetic subjects not attending the follow-up examination.** Not all the identified diabetic case subjects in HUNT2 attended the follow-up examination ( $n = 519$ ). Nonattendees could therefore not be classified by the criteria given above. Blood and serum samples in nonfasting state were, however, available for 432 of these individuals in the HUNT Biobank. Measuring anti-GAD from the serum samples combined with information on age at diagnosis enabled us to classify these subjects by less stringent criteria (i.e., as type 1 diabetic if anti-GAD positive and age at diagnosis  $\leq 35$  years, as LADA if anti-GAD positive and age at diagnosis  $> 35$  years and as type 2 diabetic if anti-GAD negative and age of diagnosis  $> 35$  years). By these criteria, 16 subjects were classified as type 1 diabetic, 18 as LADA, and 255 as type 2 diabetic patients. One hundred forty-three patients did not meet any of these criteria and were therefore excluded.

**Control subject group.** Participants serving as control subjects ( $n = 1,503$ ) were drawn from the same study. They answered “no” to the question of having diabetes. They were frequency matched by sex and age (in years, by decade) to the diabetic patients.

**DNA extraction.** DNA for genotyping was extracted from peripheral blood leukocytes from EDTA whole blood or blood clots using the Gentra Puregene blood kit (QIAGEN Science, Germantown, MD). This was done manually or by automation with an Autopure LS (QIAGEN Science) mainly as described by the manufacturer.

**Genotyping.** The selected single nucleotide polymorphisms (SNPs) were based on publicly available results from type 1 (14–20) and type 2 (21–27)

diabetes studies. Genotyping was performed by applying TaqMan SNP allelic discrimination using ABI 7900HT and by SNPlex genotyping system (Applied Biosystems, Foster City, CA). Case and control subjects were equally distributed with four or more negative control subjects on each 384-plate. Criteria to pass the assay were 1) call rates  $> 90\%$ , 2) minor allele frequency  $> 1\%$  in the genotyped population, and 3) agreement with Hardy-Weinberg equilibrium in the whole population, and if  $P$  value was  $< 0.001$  it did not pass. Assays that did not pass quality control were excluded from further analysis.

**HLA haplotyping.** HLA haplotyping was performed as described by de Bakker et al. (28). They captured nearby single tag SNPs or haplotypes of combination of up to three SNPs as a predictor of known HLA alleles. The recommended tag SNPs or haplotypes for the HLA risk alleles for type 1 diabetes are shown in supplementary Table 1 in the online appendix, which is available at <http://diabetes.diabetesjournals.org/cgi/content/full/db09-0923/DC1>. We genotyped these tag SNPs using SNPlex genotyping system.

**Statistical analysis.** PLINK software (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>) was used to assess whether genotypes were in Hardy-Weinberg equilibrium and to test differences in genotype distribution between affected and unaffected subjects by logistic regression under additive, dominant, and recessive models. Odds ratios and 95% CIs were calculated. Adjustment for diabetes-specific risk factors such as age, sex, and BMI was applied when appropriate. Correction for multiple testing was done by max(T) permutation where 1,000 permutations were performed. The nonparametric Mann-Whitney  $U$  test was used to compare continuous variables between groups using Statistical Package for the Social Sciences, version 14.0 (SPSS, Chicago). A  $P$  value  $< 0.05$  was considered statistically significant. Phasing HLA haplotypes and testing for association within case and control subjects were carried out using PLINK.

**Ethics.** All participants gave written consent. The study was approved by the Regional Committee for Ethics in Medical Research and the Norwegian Data Inspectorate.

## RESULTS

**Clinical characteristics.** Age at onset was similar for LADA and type 2 diabetic patients and lower for type 1 diabetic patients (Table 1). Overweight status, measured by BMI and waist-to-hip ratios, was more marked in LADA and type 2 diabetic than in type 1 diabetic and control subjects. Relationships were similar for lipid parameters and blood pressure.

**Quality testing of SNPs.** Forty-two prioritized SNPs known to be associated with either type 1 or type 2 diabetes were genotyped on either TaqMan or SNPlex in 1,536 diabetic patients (106 additional patients were genotyped [TaqMan] for rs231775, rs689, rs2476601, and rs3118470) from whom genomic DNA was available and in 1,503 nondiabetic control subjects. All SNPs were in Hardy-Weinberg equilibrium ( $P \geq 0.001$ ) but two SNPs had genotype call frequency  $< 90\%$  and were therefore ex-

TABLE 2

Genotypes of known type 1 diabetes-associated loci in LADA and type 1 diabetic subjects compared with nondiabetic control subjects

Gene name, SNP	Genotype	LADA vs. control subjects				Type 1 diabetic vs. control subjects			
		OR (95% CI)	<i>P</i> <sup>*</sup>	<i>P</i> <sup>†</sup>	<i>P</i> <sup>‡</sup>	OR (95% CI)	<i>P</i> <sup>*</sup>	<i>P</i> <sup>†</sup>	<i>P</i> <sup>‡</sup>
PTPN22, rs2476601	AA/AG GG	1.40 (0.92–2.12)	0.117	0.157	1	2.82 (1.92–4.13)	1.17 × 10 <sup>-7</sup>	1.46 × 10 <sup>-5</sup>	0.001
PTPN22, rs2488457	CC/CG GG	1.39 (0.95–2.03)	0.088	0.134	1	1.70 (1.15–2.50)	0.008	0.034	0.851
IL2R, rs3118470	CC CT/TT	1.62 (1.03–2.54)	0.036	0.054	1	1.09 (0.66–1.82)	0.732	0.614	1
INS, rs689	TT AA/AT	1.10 (0.76–1.59)	0.626	0.598	1	2.44 (1.60–3.71)	3.54 × 10 <sup>-5</sup>	6.91 × 10 <sup>-5</sup>	0.005
INS, rs3842753	CC AA/AC	1.18 (0.80–1.74)	0.396	0.368	1	2.38 (1.54–3.66)	8.63 × 10 <sup>-5</sup>	1.89 × 10 <sup>-4</sup>	0.011

\**P* value from logistic regression assuming dominant or recessive model. †Adjusted *P* value from logistic regression for age, sex, and BMI. ‡Empirical *P* value corrected for multiple testing by 1,000 permutations.

cluded from further analysis. The average call frequency for the remaining SNPs was 98.6%.

A full statistical analysis including additive, dominant, and recessive models of all the genotyped type 1 and type 2 diabetes risk loci is shown in supplementary Table 2 for type 1 diabetic compared with control subjects, in supplementary Table 3 for LADA compared with control subjects, and in supplementary Table 4 for type 2 diabetic compared with control subjects.

**LADA in general: lack of association of non-HLA type 1 diabetes loci.** We analyzed 12 SNPs within eight candidate risk loci for type 1 diabetes for association with 126 LADA patients. The CC genotype of rs3118470 in the interleukin 2 receptor, alpha gene (*IL2Ralpha*) was associated with LADA (*P* = 0.036), but the association became less significant (*P* = 0.054) after adjustment for age, sex, and BMI (Table 2). None of the other studied type 1 diabetes loci was associated with LADA.

**LADA in general: association with type 2 diabetes loci.** For type 2 diabetes risk loci, we analyzed 28 SNPs within 25 loci for association with LADA. The CC/CT genotypes of rs7961581 upstream of the tetraspanin 8 gene (*TSPAN8*) and the obesity-linked AA/AC genotypes of rs8050136 in the fat mass and obesity-associated gene (*FTO*) were associated with LADA (*P* = 0.007 and *P* = 0.005, respectively) as well as type 2 diabetes (*P* = 0.009 and *P* = 0.005, respectively) (Table 3). Both loci remained significant after adjusting for age, sex, and BMI.

**Verification of associated SNPs.** To verify associations with LADA, we analyzed polymorphisms flanking the SNPs showing an association. We analyzed four additional SNPs in the *FTO* gene (rs4389136 and rs1861866, 68 kb and 12 kb downstream, respectively, and rs9931494 and rs2388405, 11 kb and 60 kb upstream, respectively) and two SNPs in *TSPAN8* (rs7306184, 45 kb downstream and rs7964431, 43

kb upstream) and in *IL2R* (rs9663421, 46 kb downstream and rs4747880, 51 kb upstream). We selected SNPs with essentially similar minor allele frequencies as the SNPs initially showing an association. Two SNPs (rs7306184 and rs4747880) were excluded from further analyses due to low genotype call rate (<90%).

The GG/GC genotypes of rs1861866 and TT/TC of rs9931491 in the *FTO* gene were associated with both LADA (*P* = 0.001 and *P* = 1.0 × 10<sup>-4</sup>, respectively) and type 2 diabetes (*P* = 0.047 and *P* = 0.004, respectively), and were still associated with LADA after adjustment for age, sex, and BMI (Table 3). The association of rs9931491 with type 2 diabetes remained strong after adjustment for age, sex, and BMI, however, the association of rs1861866 was lost. The SNP rs9931491 in the *FTO* gene was the only SNP that remained significantly associated with LADA after adjusting for multiple testing with 1,000 permutations (*P* = 0.016). None of the other SNPs tested was associated with LADA (supplementary Tables 2–4).

**Association of genes in relation to BMI.** We dichotomized patients and control subjects on the basis of BMI < (nonobese) or > (obese) 30 kg/m<sup>2</sup>. The AA/AC genotypes of rs8050136 in the *FTO* gene were significantly associated with obese LADA patients (*P* = 0.024) (supplementary Table 5). The two additional SNPs in the *FTO* gene, the TT/TC genotypes of rs1861866 and the GG/GC genotypes of rs9931494, were associated with both obese (*P* = 0.048 and *P* = 0.008, respectively) and nonobese (*P* = 0.024 and *P* = 0.017, respectively) LADA patients. However, the association of rs1861866 to obese LADA patients became less significant (*P* = 0.054) after adjusting for age, sex, and BMI.

The CC/CT genotypes of rs7961581 close to the *TSPAN8* gene were associated with nonobese LADA patients (*P* = 0.003).

TABLE 3

Genotypes of known type 2 diabetes-associated loci in LADA and type 2 diabetic subjects compared with nondiabetic control subjects

Gene name, SNP	Genotype	LADA vs. control subjects				Type 2 diabetic vs. control subjects			
		OR (95% CI)	<i>P</i> <sup>*</sup>	<i>P</i> <sup>†</sup>	<i>P</i> <sup>‡</sup>	OR (95% CI)	<i>P</i> <sup>*</sup>	<i>P</i> <sup>†</sup>	<i>P</i> <sup>‡</sup>
TCF7L2, rs7903146	CT/TT CC	1.25 (0.85–1.83)	0.252	0.141	1	1.75 (1.48–2.05)	1.89 × 10 <sup>-11</sup>	4.84 × 10 <sup>-14</sup>	0.001
TSPAN8/LGR5, rs7961581	CC/CT TT	1.68 (1.15–2.46)	0.007	0.01	0.443	1.24 (1.06–1.46)	0.009	0.033	0.869
FTO, rs8050136	AA/AC CC	1.94 (1.22–3.09)	0.005	0.005	0.255	1.28 (1.08–1.53)	0.005	0.005	0.291
FTO, rs1861866	CT/TT CC	2.55 (1.45–4.50)	0.001	0.003	0.149	1.21 (1.00–1.46)	0.047	0.091	0.997
FTO, rs9931494	CG/GG CC	2.72 (1.63–4.53)	1.27 × 10 <sup>-4</sup>	2.43 × 10 <sup>-4</sup>	0.016	1.29 (1.09–1.54)	0.004	0.008	0.396

\**P* value from logistic regression assuming dominant model. †Adjusted *P* value from logistic regression for age, sex, and BMI. ‡Empirical *P* value corrected for multiple testing by 1,000 permutations.



TABLE 4  
Clinical characteristics of LADA patients displayed as low and high titers of anti-GAD

Clinical characteristics	Patients compared ( <i>n</i> )		Median (25th–75th percentile)		<i>P</i> *
	Low anti-GAD	High anti-GAD	Low anti-GAD	High anti-GAD	
Age at diagnosis (years)	65	61	62 (56–71)	56 (46–66)	0.004
Years with insulin treatment	16	27	6.0 (1.3–9.8)	6.0 (4.0–11)	0.605
Time to insulin dependence (years)	16	26	6.8 (4.3–12)	4.0 (2.9–7.0)	0.048
BMI (kg/m <sup>2</sup> )	65	58	28 (26–31)	27 (25–31)	0.182
Waist-to-hip ratio	64	59	0.90 (0.85–0.96)	0.90 (0.85–0.93)	0.457
Cholesterol (mmol/l)	65	61	5.8 (5.2–6.7)	5.6 (4.8–6.6)	0.277
HDL cholesterol (mmol/l)	65	61	1.2 (0.9–1.5)	1.1 (0.9–1.5)	0.870
Triglycerides (mmol/l)	65	61	2.27 (1.22–2.98)	1.77 (1.12–2.66)	0.158
A1C	62	60	7.8 (6.4–9.4)	8.7 (7.1–10)	0.064
Systolic blood pressure (mmHg)	65	60	154 (143–174)	148 (132–170)	0.124
Diastolic blood pressure (mmHg)	65	60	82 (72–91)	81 (73–89)	0.861
Fasting glucose (mmol/l)	65	61	7.5 (6–8.9)	9.1 (6.9–10.6)	0.025
Fasting C-peptide (mmol/l)	65	61	697 (384–1,015)	334 (75–764.5)	0.0007

Data are median (25th–75th percentile) unless otherwise indicated. \**P* value from Mann-Whitney *U* test by comparing low and high anti-GAD LADA patients.

**Association of anti-GAD titers with genes and clinical characteristics.** We dichotomized LADA on the basis of anti-GAD levels below (low anti-GAD) or above (high anti-GAD) the median of anti-GAD. Regarding the *FTO* gene, the AA/AC genotypes of rs8050136, TT/TC genotypes of rs1861866, and GG/GC genotypes of rs9931494 were significantly associated with low anti-GAD LADA ( $P = 0.004$ ,  $P = 0.004$ , and  $P = 0.002$ , respectively) (supplementary Table 6). In addition, SNP rs9931494 was also associated with high anti-GAD LADA ( $P = 0.020$ , supplementary Table 7), but became less significant ( $P = 0.047$ ) after adjusting for age, sex, and BMI.

The genotypes CC/CT of rs7961581 close to the *TSPAN8* gene were associated with low anti-GAD LADA ( $P = 0.004$ ).

Among clinical characteristics (Table 4), LADA patients with high anti-GAD were younger at diagnosis ( $P = 0.004$ ) and displayed shorter time to insulin treatment ( $P = 0.048$ ). Further, fasting glucose was more elevated ( $P = 0.025$ ) and fasting C-peptide more depressed ( $P = 0.0007$ ) than in patients with low anti-GAD.

**HLA haplotyping.** Of 30 tag SNPs for HLA haplotyping, 18 were successfully genotyped. Of these, 14 could be used in the prediction of HLA haplotypes (supplementary Table 8). The localization of these SNPs in the major histocompatibility complex (MHC) is depicted in Figure 1. The majority of the strongly associated risk haplotypes for type 1 diabetes were also found to be significant risk haplotypes for LADA (supplementary Table 9). The frequency of ACCCG haplotype for DRB1\*0402-DQA1\*0301-DQB1\*0302 was increased in both LADA and type 1 diabetic patients compared with control subjects (0.16 and 0.32 vs. 0.12,  $P = 0.036$  and  $P = 3.9 \times 10^{-17}$ , respectively) (Table 5). The same was apparent with the GCCG haplotype for DRB1\*0401-DQA1\*0301-DQB1\*0302 (0.16 and 0.33 vs. 0.11,  $P = 0.028$  and  $P = 7.5 \times 10^{-21}$ ), CT haplotype for DRB1\*0901-DQA1\*0301-DQB1\*0303 (0.23 and 0.36 vs. 0.16,  $P = 0.009$  and  $P = 9.2 \times 10^{-14}$ ), AC haplotype for DRB1\*1,501-DQA1\*0102-DQB1\*0602 (0.41 and 0.5 vs. 0.33,  $P = 0.017$  and  $P = 5.3 \times 10^{-7}$ ), and TAT haplotype for DRB1\*0701-DQA1\*0201-DQB1\*0303 (0.12 and 0.25 vs. 0.07,  $P = 0.015$  and  $P = 2.3 \times 10^{-18}$ ). Interestingly, one distinct haplotype GCA for DRB1\*0401-DQA1\*0301-DQB1\*0301 was found to be associated with higher risk only for LADA (0.09 vs. 0.05,  $P = 0.013$ ).

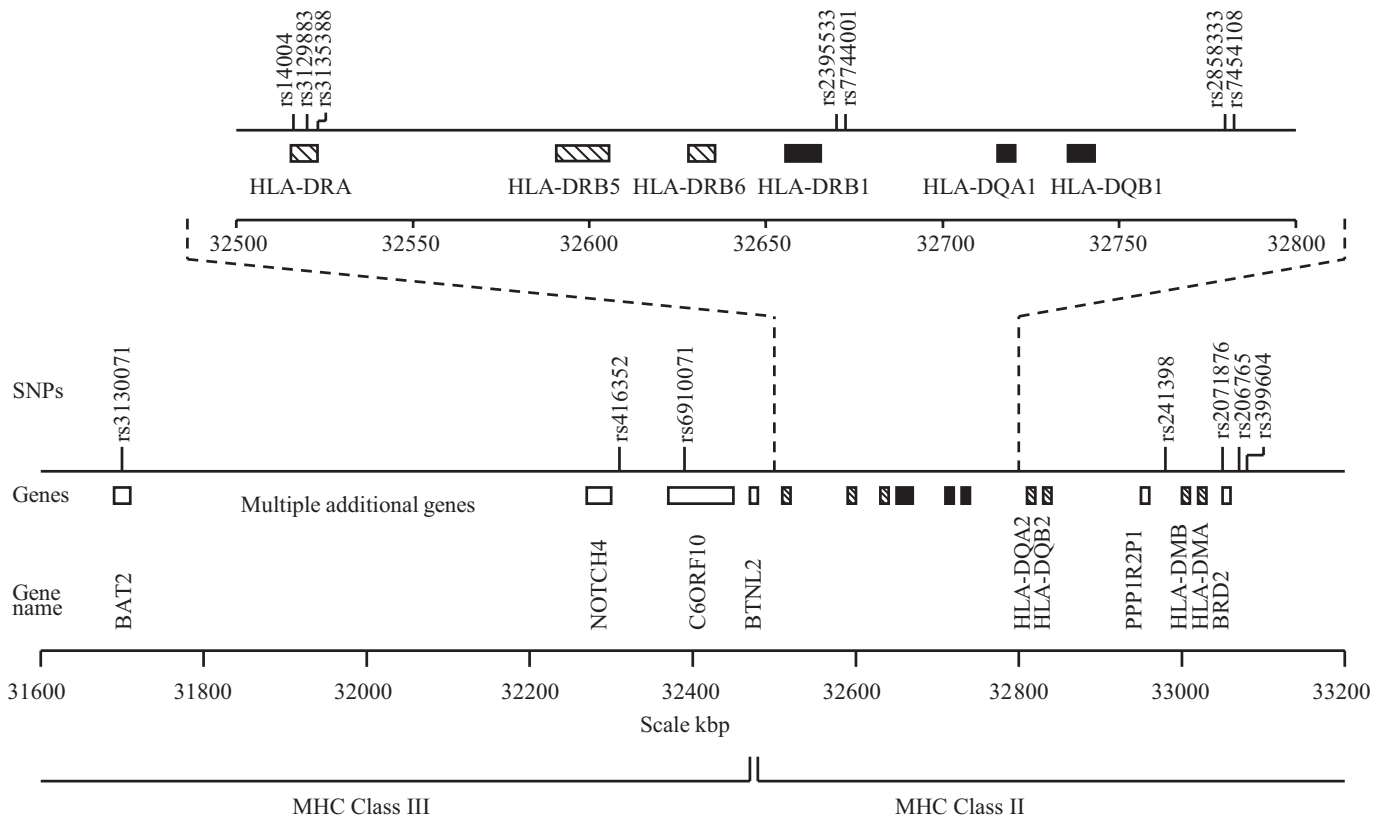
In general, the most strongly associated protective hap-

lotypes for type 1 diabetes were also found to be protective in LADA (supplementary Table 10). The frequency of ATTG haplotype for DRB1\*0401-DQA1\*0301-DQB1\*0302 was significantly lower in both LADA and type 1 diabetic patients compared with control subjects (0.18 and 0.06 vs. 0.25,  $P = 0.014$  and  $P = 2.9 \times 10^{-10}$ , respectively) (Table 6). The same was observed for the TC haplotype for DRB1\*0902-DQA1\*0301-DQB1\*0303 (0.15 and 0.06 vs. 0.24,  $P = 0.003$  and  $P = 1.5 \times 10^{-9}$ ), CT haplotype for DRB1\*1,501-DQA1\*0102-DQB1\*0602 (0.09 and 0.03 vs. 0.15,  $P = 0.025$  and  $P = 1.7 \times 10^{-6}$ ), and ATG haplotype for DRB1\*0401-DQA1\*0301-DQB1\*0301 (0.12 and 0.04 vs. 0.19,  $P = 0.005$  and  $P = 8.3 \times 10^{-9}$ ).

When dichotomizing LADA for high and low anti-GAD, both the risk and protective HLA haplotypes described above were now found to be associated with high anti-GAD LADA patients (Tables 5 and 6), except the GCA haplotype for DRB1\*0401-DQA1\*0301-DQB1\*0301, which was associated with higher risk in low anti-GAD LADA patients ( $P = 0.0003$ ). Surprisingly, one more distinct haplotype was found. The GCTA haplotype for DRB1\*0401-DQA1\*0301-DQB1\*0302 was associated with higher risk only in low anti-GAD LADA ( $P = 0.006$ ) and not with type 1 and type 2 diabetes.

Age at diagnosis of type 1 diabetes correlates with HLA haplotype frequencies (29). Dichotomizing the type 1 diabetic patients on the basis of age of diagnosis at older than or younger than 35 years enabled us to compare type 1 and LADA patients on a more equal age-at-onset basis. We selected the average frequency from the seven strongest associated risk HLA haplotypes and the average frequency from the seven most protective HLA haplotypes for type 1 diabetes to illustrate the differences in HLA frequencies in different types of diabetes (Fig. 2). Risk haplotype frequencies tended to decrease and protective haplotype frequencies to increase when comparing patients and control subjects in the following order: early-onset type 1 diabetic to late-onset type 1 diabetic through high anti-GAD LADA and low anti-GAD LADA to type 2 diabetic and control subjects. This suggests a continuous spectrum from strong to weak to nonexistent influence of autoimmunity.

**Including less stringently classified patients.** The clinical characteristics for the 18 patients who were classified as LADA by less stringent criteria were mainly the same as



**FIG. 1.** Schematic diagram of the tag SNPs used for HLA haplotyping in the MHC region on chromosome 6 including nearby located genes. The HLA genes that are predicted by the tag SNPs are shown as black boxes; other HLA genes are shown as hatched boxes. Additional genes are shown as white boxes.

patients characterized from the complete dataset (supplementary Table 11). After including them in the analysis, the type 1 diabetes-associated CC/CG genotypes of rs2488457 in the protein tyrosine phosphatase nonreceptor type 22 gene (*PTPN22*), CC genotype of rs2296336 in the inositol 1,4,5-triphosphate receptor type 3 gene (*ITPR3*), and the CC genotype of rs3118470 in the *IL2R* gene were associated with LADA ( $P = 0.039$ ,  $P = 0.008$ , and  $P = 0.008$ , respectively) (data not shown). The SNPs rs2296336 and rs3118470 remained significant after adjusting for age, sex, and BMI ( $P = 0.015$  and  $P = 0.019$ , respectively), however the rs2488457 showed only a trend ( $P = 0.073$ ) after adjustment. The associations to the type 2 diabetes loci *TSPAN8* and *FTO* remained significant, however, the associations were slightly weakened. There were no differences in the association of the *FTO* and *TSPAN8* genes to the dichotomized LADA patients. However, the type 1 diabetes-associated loci *PTPN22* (rs2476601), *ITPR3* (rs2296336), and *IL2R* (rs3118470) were now associated with nonobese LADA patients ( $P = 0.039$ ,  $P = 0.015$ , and  $P = 0.029$ , respectively) and high anti-GAD levels ( $P = 0.034$ ,  $P = 0.037$ , and  $P = 0.011$ , respectively). The associated HLA haplotypes with LADA became even stronger.

**DISCUSSION**

Our study indicates that in LADA patients 1) admixture of both type 1 and type 2 diabetes-associated genetic variants are present, 2) heterogeneity is related to autoimmune activity as assessed by anti-GAD and insulin resistance as assessed by BMI, and 3) there is suggestive evidence for genetic associations that are not found in either type 1 or type 2 diabetes.

Admixture of type 1 diabetes genes in LADA was apparent for HLA haplotypes that confer risk for or protection against type 1 diabetes. These findings are in agreement with previous studies (9,30). In general, the frequency of these haplotypes as well as significance levels were more pronounced for type 1 diabetes than for LADA, although differences were attenuated when comparing late age-at-onset type 1 diabetes and LADA (supplementary Tables 9 and 10). On the other hand, type 1 diabetes genes outside the HLA region were not in apparent association with LADA. We found, as reported (31), strong associations between type 1 diabetes and SNPs rs689 and rs3842753 in the insulin gene, whereas such associations were lacking in LADA. Similar difference between type 1 diabetes and LADA were also noted for the genes *PTPN22* and *CTLA4* (supplementary Tables 2 and 3).

In regards to admixture of genes associated with type 2 diabetes, there was correspondence with LADA for the *FTO* and *TSPAN8/LGR5* genes. The association with *FTO* and type 2 diabetes in the HUNT2 population was previously reported (32), whereas our finding in LADA patients is novel. Notably, both the associations in type 2 diabetes and in LADA patients remained after adjustment for BMI. Such adjustment did in most (21,33) but not all (32,34) other studies abolish the association with type 2 diabetes. The high expression of the *FTO* gene in hypothalamus and regulation by food intake suggests a role in controlling energy homeostasis (35,36). However, underlying mechanisms of influence in obesity and diabetes are not fully elucidated. *TSPAN8* is a member of the transmembrane 4 superfamily. Its role in the development of diabetes is still unknown.

TABLE 5  
Frequency of the HLA haplotypes associated with higher risk in LADA and type 1 diabetes

Risk HLA haplotypes	LADA							
	Type 1 diabetes ( <i>n</i> = 119)		Total ( <i>n</i> = 121)		Anti-GAD >0.11 ( <i>n</i> = 56)		Anti-GAD ≤0.11 ( <i>n</i> = 65)	
	Frequency ( <i>n</i> )	<i>P</i> *	Frequency ( <i>n</i> )	<i>P</i> *	Frequency ( <i>n</i> )	<i>P</i> *	Frequency ( <i>n</i> )	<i>P</i> *
DRB1*0402-DQA1*0301-DQB1*0302								
ACCCG	0.32 (38)	$3.90 \times 10^{-17}$	0.16 (20)	0.036	0.22 (13)	0.001	0.11 (7)	0.939
AACTG	0.34 (40)	0.023	0.33 (40)	0.065	0.37 (20)	0.037	0.3 (19)	0.527
DRB1*0401-DQA1*0301-DQB1*0302								
GCTA	0.02 (3)	0.847	0.04 (5)	0.107	0.01 (1)	0.341	0.07 (5)	0.002
GCCG	0.33 (40)	$7.48 \times 10^{-21}$	0.16 (19)	0.028	0.21 (12)	0.001	0.11 (7)	0.926
DRB1*0901-DQA1*0301-DQB1*0303								
CT	0.36 (43)	$9.23 \times 10^{-14}$	0.23 (27)	0.009	0.31 (17)	$4.81 \times 10^{-5}$	0.15 (10)	0.888
DRB1*1501-DQA1*0102-DQB1*0602								
AC	0.50 (60)	$5.31 \times 10^{-7}$	0.41 (50)	0.017	0.41 (23)	0.105	0.42 (27)	0.069
DRB1*0701-DQA1*0201-DQB1*0303								
TAT	0.25 (30)	$2.33 \times 10^{-18}$	0.12 (14)	0.015	0.17 (10)	0.0002	0.07 (5)	0.962
DRB1*0401-DQA1*0301-DQB1*0301								
GCA	0.04 (5)	0.571	0.09 (11)	0.013	0.04 (2)	0.543	0.14 (9)	$7.51 \times 10^{-5}$
GCG	0.36 (43)	$1.97 \times 10^{-10}$	0.20 (25)	0.483	0.23 (13)	0.305	0.18 (12)	0.986
DRB1*0403-DQA1*0301-DQB1*0302								
CCGTG	0.13 (15)	$7.57 \times 10^{-12}$	0.05 (6)	0.179	0.08 (4)	0.016	0.03 (2)	0.692

\**P* value corrected for age, sex, and BMI.

The association of *FTO* with LADA was found mainly in those with low titers of anti-GAD and in those with high BMI. An inverse relationship with autoimmune activity is consistent with the absence of these SNP associations in type 1 diabetes. Also, most of the HLA associations in LADA patients were attenuated at lower versus higher titers of anti-GAD. Relationships with BMI were generally the inverse of those with anti-GAD. Collectively, our data suggest a graded influence of the genetic predisposition from a more type 1 diabetes-like one in those who exhibit high autoimmune activity and lower BMI, toward a more

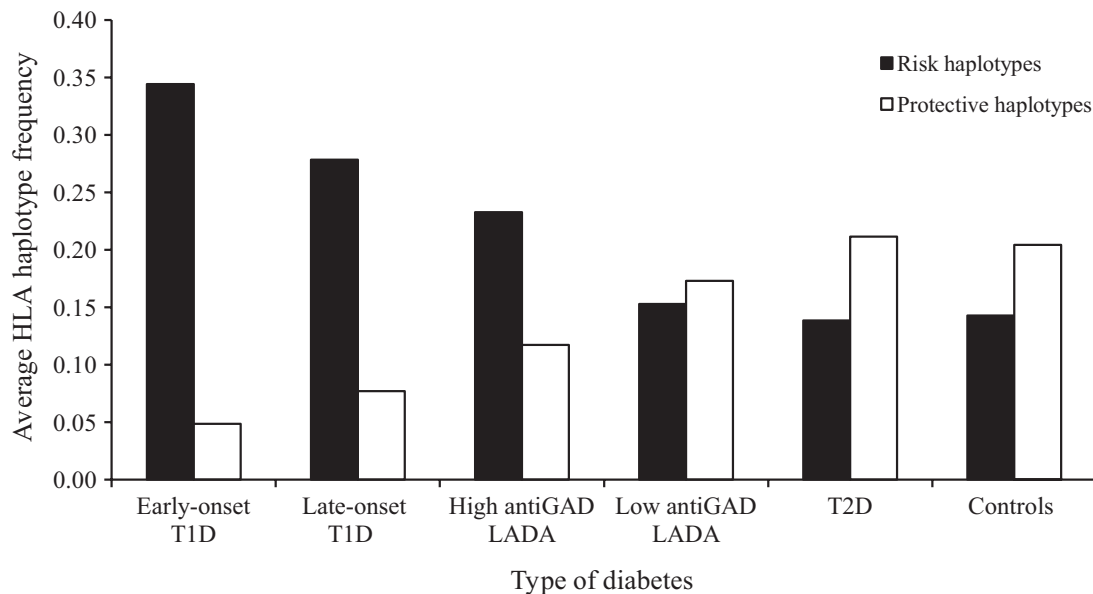
type 2 diabetes-like one in those that exhibit lower titers of anti-GAD and higher BMI.

We did not confirm an association between LADA and the *TCF7L2* gene (supplementary Table 3) that was reported by Cervin et al. (9). The definition of LADA in the study of Cervin et al. differs from ours, and there are differences in the anthropometric data. However, we have no clear-cut explanation for the discrepancy. One may argue that our study was underpowered to detect an impact of *TCF7L2* in LADA. Still the high level of significance for association in type 2 diabetes ( $P < 1.0 \times 10^{-10}$ ,

TABLE 6  
Frequency of the HLA haplotypes associated with protective effect in LADA and type 1 diabetes

Protective HLA haplotypes	LADA							
	Type 1 diabetes ( <i>n</i> = 119)		Total ( <i>n</i> = 121)		Anti-GAD >0.11 ( <i>n</i> = 56)		Anti-GAD ≤0.11 ( <i>n</i> = 65)	
	Frequency ( <i>n</i> )	<i>P</i> *	Frequency ( <i>n</i> )	<i>P</i> *	Frequency ( <i>n</i> )	<i>P</i> *	Frequency ( <i>n</i> )	<i>P</i> *
DRB1*0402-DQA1*0301-DQB1*0302, TCTTG	0.03 (3)	0.0003	0.07 (9)	0.092	0.04 (2)	0.031	0.1 (7)	0.731
DRB1*0401-DQA1*0301-DQB1*0302, ATTG	0.06 (7)	$2.94 \times 10^{-10}$	0.18 (21)	0.014	0.14 (8)	0.012	0.21 (14)	0.288
DRB1*0901-DQA1*0301-DQB1*0303, TC	0.06 (7)	$1.51 \times 10^{-9}$	0.15 (18)	0.003	0.11 (6)	0.003	0.19 (12)	0.203
DRB1*1501-DQA1*0102-DQB1*0602, CT	0.03 (4)	$1.73 \times 10^{-6}$	0.09 (11)	0.025	0.06 (3)	0.014	0.12 (8)	0.418
DRB1*0701-DQA1*0201-DQB1*0303, TGC	0.2 (24)	$7.67 \times 10^{-6}$	0.29 (35)	0.123	0.24 (13)	0.027	0.34 (22)	0.922
DRB1*0401-DQA1*0301-DQB1*0301, ATG	0.04 (4)	$8.28 \times 10^{-9}$	0.12 (14)	0.005	0.1 (6)	0.017	0.13 (9)	0.093
DRB1*0403-DQA1*0301-DQB1*0302, TTGTA	0.02 (2)	$1.46 \times 10^{-5}$	0.09 (11)	0.349	0.07 (4)	0.167	0.11 (7)	0.993

\**P* value corrected for age, sex, and BMI.



**FIG. 2.** Average HLA haplotype frequencies in different types of diabetes, calculated from the seven strongest associated risk HLA haplotypes and the seven strongest protective HLA haplotypes with type 1 diabetes. T1D, type 1 diabetes; T2D, type 2 diabetes.

supplementary Table 4) is in obvious contrast to the lack of significance in LADA. One could envisage that heterogeneity of LADA with regard to autoimmune activity assessed by anti-GAD could obscure an association with *TCF7L2* in a subgroup with lower autoimmune activity. However, we could not detect such an influence (supplementary Table 6).

Our results provide some suggestive evidence for genetic risk factors for LADA that are related to neither type 1 nor type 2 diabetes. The *IL2R* gene showed a trend toward association with LADA but not with type 1 or type 2 diabetes. Also, two distinct HLA haplotypes were associated only with LADA. These observations may serve as pointers for studies that rigorously test the notion of partly unique genetic predisposition for LADA.

A previous HUNT study (2) and a study in the U.K. (37), demonstrated inverse relationships between anti-GAD titer and the odds ratio for effect of family history of diabetes in LADA patients. This would suggest a strong effect of nonautoimmune genes (most of which have yet to be identified) in LADA patients.

Our study has limitations and strengths. An obvious limitation is the relatively low number of patients in the groups classified as type 1 diabetic and LADA. As a consequence, the inability to obtain formal levels of significance for some gene associations may reside in lack of power. Another potential weakness is the lack of complete data on all professed diabetic patients. After retrospectively analyzing anti-GAD in most of these individuals, we could classify them into different forms of diabetes by wider criteria than for those patients who were the main focus of this study. By including these patients in some of our analyses, we could estimate a possible bias occurring from their exclusion. The “new” LADA patients were in genetic terms perhaps more type 1 diabetes-like than the average LADA patients in whom we had access to complete data. However, including the new LADA patients in our analysis did not materially change the conclusions that were based on complete sets of data.

A technical limitation may pertain to anti-GAD measurements, which were not compared with an alternative

method. However, our assay has been used and validated previously (13). Furthermore, the outcome of a DASP investigation indicates very high specificity of the assay coupled with a lower sensitivity. The main concern here could then be that we have missed some diabetic subjects who, by other methods, could have been anti-GAD positive.

Strengths of our study pertain to the HUNT studies being all-population inclusive with a high attendance. Notably our population-based group of adult type 1 diabetes is rather unique because most other studies that include type 1 diabetic subjects are based on hospital records. Our data also indicate that the type 1 diabetic subjects are representative outside a regional perspective. Thus, data are in good agreement with published ones on type 1 diabetes with regard to specific risk and protective genes in white subjects and a diminished influence of diabetes-associated HLA haplotypes with increasing age of diagnosis (29). As to our type 2 diabetic and control groups, they have previously been part of a replication in a genome-wide association study (27) and other genetic studies (32,38,39). The results of the HUNT part of these previous studies showed in general good concordance with other patient materials. The evidence that our study is representative of both type 1 and type 2 diabetes should increase the validity of comparisons with LADA.

In summary, we find genetic resemblance to both type 1 and type 2 diabetes genes in LADA. Heterogeneity in susceptibility genes in LADA is related to various degrees of autoimmune activity and obesity. This highlights the concept that it is the combined burden of risk factors that brings on diabetes.

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