

AGT) and Lys4031 (Lys-Arg, AAG-AGG). The gene variants at Gly673 and Asp2674 and at Arg2828 and Leu4060 were in complete linkage disequilibrium with each other ($n=179$ and $n=95$ respectively). Gene variants at position Asp2678 and Asn3815 were not detected in our cohorts. Therefore we limited our studies to the gene variants at positions Asp2674, Arg2828 and Lys4031, which were subsequently studied in detail in the association studies (Table 1). Genotypes were determined by PCR-RFLP based methods in each individual after validation of the detection method by direct sequencing.

The observed frequencies were all in Hardy-Weinberg equilibrium (data not shown). Genotype and allele frequencies were not significantly different between patients and control subjects for either of the gene variants (All $p>0.2$, Table 1). Furthermore we did not observe significant associations with other parameters like BMI, glucose or insulin concentrations during OGTT (All $p>0.05$, Table 1). Insulin resistance as calculated by the HOMA insulin resistance index (HOMA IR) was also not significantly different between the different genotypes ($p >0.3$, Table 1). Trends observed in the initial cohort could not be replicated in an independent population-based sample from the Rotterdam study (Type 2 DM, $n=95$ and NGT, $n=188$, data not shown) [5]. A priori power calculations showed that we had an 80 percent power to detect differences in allele frequency around 10 percent. We have also reconstructed haplotype combinations of the different gene variants using the PHASE v1.0 program [6]. We observed six different haplotype combinations, however; they were not significantly different between the cases and controls ($p>0.2$, data not shown). Also the reconstructed haplotypes did not associate with diabetes related parameters (data not shown).

This report describes association studies with gene variants in the coding region of the *ALMS1* gene in Type 2 diabetes. The absence of significant associations suggest that the variants observed in our studies are not major factors in the pathogenesis of Type 2 diabetes mellitus or obesity. Furthermore we found no evidence for a disease-associated haplotype. We conclude that known gene variants in the coding regions of the *ALMS1* gene are not associated with Type 2 diabetes, BMI or other diabetes related parameters in a population based study in The Netherlands. Further studies in other (larger) cohorts and with gene variants in other parts of this large gene locus are necessary to fully investigate the role of this gene in the pathogenesis of Type 2 diabetes mellitus and/or obesity.

Autoantibodies in Type 1 and Type 2 diabetes in the Old Order Amish of Lancaster County, Pennsylvania

To the editor: Autoantibodies to IA-2 (IA-2A) and GAD (GADA) have been widely used to identify individuals with Type 1 diabetes [1]. Up to 90% of newly diagnosed Type 1 patients have IA-2A and/or GADA. The number of Type 2 patients

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L. M. 't Hart, J. A. Maassen
Department of Molecular Cell Biology,
Leiden University Medical Center, Leiden, The Netherlands

J. M. Dekker, R. J. Heine, J. A. Maassen
On behalf of the Hoorn study, VU University Medical Center,
Institute for Research in Extramural Medicine (EMGO),
Amsterdam, The Netherlands

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Corresponding author: Dr. J. A. Maassen, On behalf of the Hoorn study, VU University Medical Center, Institute for Research in Extramural Medicine (EMGO), Amsterdam, The Netherlands
E-mail: j.a.maassen@lumc.nl

with these autoantibodies is considerably lower, usually between 2 to 4% for IA-2 and 5 to 10% for GAD. Of non-diabetic control populations 1% or less have these autoantibodies.

Autoantibodies are of particular interest in genetically homogenous populations as in Finland [2] and Sardinia [3] since they could provide epidemiological and mechanistic insights into the role of these antibodies in the pathogenesis of diabetes. Such a genetically unique population also exists in the United States. The Old Order Amish emigrated from Western Europe to the United States at the beginning of the 18th century, approximately 200 families settled in Lancaster county, Pennsylvania [4]. The Old Order Amish are a genetically isolated and well-defined closed Caucasian population, with a high degree of consanguinity, and virtually no outsiders marrying into the community. Type 2 diabetes has been studied in this population [4], but autoimmune diabetes has not been examined.

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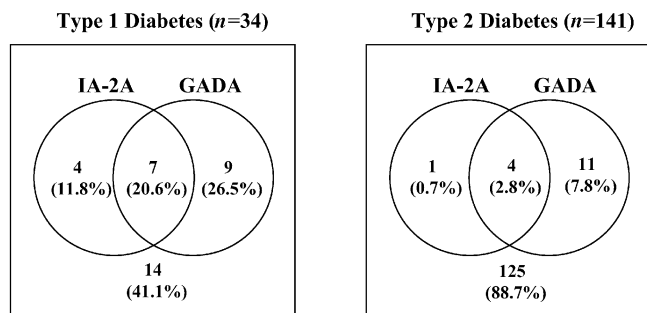


Fig. 1. Autoantibody distribution in 175 Type 1 and Type 2 diabetes patients

Table 1. Clinical characteristics of study subjects

	Diabetes	
	Type 1	Type 2
Total subjects (<i>n</i>)	34	141
Male (%)	50.0	32.1
Current age (yr)	29.3±2.5	64.8±1.1**
Diabetes duration (yr)	14.2±2.0	6.4±0.8**
BMI (kg/m ²)	22.0±0.8	28.9±0.5**
HbA _{1c} (%)	8.5±0.3	7.1±0.2*
Fasting glucose (mg/dl)	206±19.1	142±6.4*

* $p < 0.001$; ** $p < 0.0001$

We screened 422 subjects between 3 and 90 years of age from multiplex families of the Amish Family Diabetes Study [4] for IA-2A and GADA. Both IA-2A and GADA were measured by radioimmune precipitation assays. The sensitivity and specificity of our assay for IA-2A and GADA compared very favorably to the result of the 2002 Diabetes Autoantibody Standardization Program Workshop.

Of the patients, 175 with well-characterized diabetes were divided into two groups based on age of diagnosis of diabetes. In most of our cases, diabetes that developed before the age of 25 fit best into the category of Type 1 diabetes—all requiring insulin, whereas diabetes that developed after the age of 25 fit best into the category of Type 2 diabetes. Patients with Type 1 diabetes had significantly higher fasting blood glucose and HbA_{1c} levels and lower BMI and had been afflicted with diabetes for a longer time than patients with Type 2 diabetes (Table 1). About 60% of the patients with Type 1 diabetes had autoantibodies to IA-2, GAD or both (Fig. 1). In contrast, only 12% of the patients with Type 2 diabetes or latent autoimmune diabetes of adults had autoantibodies to IA-2, GAD or both.

Fewer than 3% of 247 non-diabetic subjects (75 of whom had abnormal glucose tolerance) had autoantibodies to IA-2 or GAD. It will be of interest to determine in the future which of these subjects develop diabetes.

Based on this well-characterized sample, the prevalence of autoantibodies in the closed Old Order Amish population falls within the range reported in a variety of studies on Type 1 and Type 2 diabetes in the general population [1, 5]. These findings suggest that the Old Order Amish in the United States might be a useful homogenous population for studying the genetic etiology of both Type 1 and Type 2 diabetes.

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J. Miura, T. I. Pollin, Y. Hu, S.-I. Harashima, A. L. Notkins, A. R. Shuldiner
Experimental Medicine Section, Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD, USA

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Corresponding author: Dr. A. L. Notkins, Experimental Medicine Section, Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bldg. 30 Rm. 121, 30 Convent Dr. MSC4322, 9000 Rockville Pike, Bethesda, MD, USA
E-mail: anotkins@mail.nih.gov