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Epidemiology and Immunogenetic Background of Islet Cell Antibody–Positive Nondiabetic Schoolchildren

Ulm-Frankfurt Population Study

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Islet cell antibodies (ICAs) were determined in a large cohort of white nondiabetic schoolchildren ($n = 4287$) from a homogenous population in southern Germany. The prevalence of ICA levels ≥5 Juvenile Diabetes Foundation (JDF) U was 1.05% (95% confidence interval 0.8–1.4%). Analysis of HLA-DRβ and -DQβ alleles revealed that the specificities found to be increased in insulin-dependent (type I) diabetic subjects with the same ethnic background were also associated with ICA positivity in the nondiabetic schoolchildren. HLA-DR3 ($P < 0.01$) and -DR4 ($P < 0.01$) phenotypes and absence of Asp residue ($P < 0.01$) at codon 57 of the HLA-DQ β-chain were significantly increased in ICA⁺ compared with control subjects. High levels of ICAs, which were categorized as either ≥17 or ≥30 JDF U, were found to be associated with amino acids other than Asp at position 57 of the HLA-DQ β-chain. No association of ICA level was found for HLA-DR phenotypes. *Diabetes* 40:1435–39, 1991

I slet cell antibodies (ICAs) are recognized as a major serological hallmark of insulin-dependent (type I) diabetes mellitus and prediabetic insulitis (1,2). Most of our knowledge on the natural course of prediabetic insulitis and type I diabetes is derived from studies of nondiabetic relatives of patients with type I diabetes (1,2). Therefore, a critical gap exists in our knowledge of the epidemiology of ICAs

and their corresponding risk factors in the general population (3). Thus far, only six population-based studies, from Japan, Spain, Netherlands, Italy, Finland, and the United States, have partly addressed this issue, each of them providing limited or no data on the correlation with risk factors (see ref. 3 for review).

Population and family studies suggest that type I diabetes develops on a particular genetic background that has been mapped to the major histocompatibility complex (MHC) (4). More than 95% of white type I diabetic subjects studied type as HLA-DR3 and/or -DR4. These alleles account for most of the immunogenetic background of the disease (4,5). Todd et al. (6) demonstrated that particular alleles at the HLA-DQB1 locus are more closely associated with susceptibility than other HLA-DRB1 alleles. As confirmed in further studies, genetic resistance to the disease in whites is correlated with the presence of Asp at amino acid position 57 of the HLA-DQ β-chain (7). In a case-control study, "dominant protection" was found with the HLA-DQw1.2 allele (Asp⁵⁷ DQβ allele) (8). Morel et al. (7) and Baisch et al. (8) also postulated that susceptibility is associated with all other amino acids (non-Asp) at codon 57, and "dominant susceptibility" was ascribed to the DQw8 allele (non-Asp). The molecular typing of the HLA-DQB1 chain provides a reliable molecular marker for type I diabetes, which in whites is superior to HLA-DR specificities.

We studied a large group of schoolchildren in a homogenous German population to establish the prevalences of ICA positivity. We provided age-related prevalances for ICAs. Immunogenetic risk markers were also analyzed. HLA-DR phenotypes were determined by HLA serology, and HLA-DQβ alleles were characterized by DNA typing. The aim of our study was to determine whether diabetogenic HLA risk factors are also associated with ICA positivity in the general population.

RESEARCH DESIGN AND METHODS

In a cross-sectional study between June 1988 and June 1989, 4287 schoolchildren from a homogenous white pop-

ulation in southern Germany (Alb-Donau County) were surveyed to determine ICAs. The total population of schoolchildren in Alb-Donau County was 8031 (3730 boys, 4301 girls). The average age was 13.9 yr with an effective range between 6 and 21 yr. No diabetic individuals took part in the study.

This study was performed in accordance with the principles of the Declaration of Helsinki. Written consent was given by the local ethical committee, the data protection council, individuals >18 yr of age, and the parents of the children studied. A letter describing the aim of the study was sent to all schools in Alb-Donau County. An appointment with teachers, parents, schoolchildren, and representatives of local newspapers was made, and the study protocol was discussed and drafted. All schools of Alb-Donau County showed a positive response, and 4287 of 8031 individuals volunteered for the study.

ICA testing. ICAs were determined by indirect immunofluorescence testing on unfixed cryostat sections of human pancreas (9,10). The lower limit of detection in our assay was 5 Juvenile Diabetes Foundation (JDF) U. In an international quality-control workshop (JDF ICA Workshop), our laboratory achieved values of 100% for consistency, specificity, sensitivity, and validity (2nd Juvenile Diabetes Foundation Workshop ICA Proficiency Program; laboratory no. 116). ICA tests were read in a blinded fashion by two independent observers (M.G. and J.S.), and the results were transferred to JDF units with the standard curve of the program mentioned above.

HLA serological typing. The HLA-DR phenotypes were determined by a two-color fluorescence technique according to van Rood et al. (11). Eight hundred eight randomly selected nondiabetic individuals from the same geographic area, 185 type I patients as disease control subjects and 36 ICA⁺ individuals were typed from June 1988 to June 1989. All individuals studied shared the same ethnic background. As an internal control of the HLA-typing procedure, 16 ICA⁺ individuals, 30 ICA⁻ control subjects, and 45 type I diabetic patients were typed twice. Samples were coded and the typing trays read in a blinded way by G.H., S.S., and P.K. The results of the two typings were identical.

HLA DNA typing. Purified genomic DNA (0.5–1 μg) isolated from peripheral blood leukocytes was subjected to 25–30 cycles of in vitro polymerase chain reaction amplification with the thermostable *Taq* polymerase (AmpliTaq, Perkin-Elmer/Cetus, Emeryville, CA) in a programmable thermal cycler (Perkin-Elmer/Cetus) (12). The two HLA-DQB1–specific primers used (5'-GATTTCGTGTACCAGTTTAAGG-3' and 5'-GCAGACACAACTACGAGGTGG-3') recognized the beginning and the end of the second exon that encodes the first domain of the HLA-DQ ß-chain. The amplified material was dot-blotted on Nytran-filters (Schleicher and Schüll, Braunschweig, Germany) and was then probed with a panel of T4 kinase radiolabeled sequence-specific probes with stringent conditions that allowed the discrimination of even single nucleotide mismatches (7). A panel of nine oligonucleotides was used to define eight HLA-DQB1 alleles. The filters were prehybridized at 42°C for 6 h and then probed at 42°C overnight with the oligonucleotides. The filters were washed six times in SSC (1 × SSC = 0.15 M NaCl/0.0015 M sodium citrate)/0.1% sodium dodecyl sulfate at a temperature based on Td [4(number of GC base pairs) + 2(number of AT base pairs)]. The appropriate temperatures required to wash off mismatched probes were 65°C for DQw3.2 (corresponding to amino acids [AA]54–59), 66°C for DQw3.1 (AA 54–59), 52°C for DQw3.1-26 (AA 23–29), 62°C for DQw1.1 (AA 54–59), 62°C for DQw1.9 (AA 54–59) and DQw2 (AA 52–58), and 60°C for DQw1.AZH (AA 54–59) and DQBlank (54–59).

HLA data are given as allele frequencies and relative risk for the disease (13). The χ^2 test was used to estimate statistical significance. $P = 0.05$ was used as the threshold for significance. Confidence intervals (CIs) were calculated with Poisson distribution.

RESULTS

Prevalence of ICAs. Forty-four (25 boys, 19 girls) of 4287 individuals were ICA⁺ with an ICA level ≥5 JDF U (Table 1). An overall prevalence of 1.05% (95% CI 0.8–1.4%) of ICA positivity was observed. None of these ICA⁺ individuals had a family history of type I diabetes. Age-specific rates were calculated for the prevalence of ICAs. Children ranging in age from 7 to 17 yr revealed the highest prevalence of ICA positivity. A clustering of ICA positivity between the ages of 12 and 15 yr was observed in boys (Table 1). Table 2 gives prevalence rates per 100 and 95% CI for ICA⁺ subjects.

HLA-DR phenotyping. Thirty-six ICA⁺ individuals (36 of 44, 82%), 808 local nondiabetic control subjects, and 185 type I diabetic patients (disease controls) were serologically typed for HLA-DR (Table 3). The phenotypic frequencies of the HLA-DR3 and -DR4 alleles were significantly increased in ICA⁺ schoolchildren compared with these control subjects. Relative risks for ICA positivity were 3.4 ($P < 0.01$) and 3.6 ($P < 0.001$), respectively. HLA-DR7 was significantly reduced ($P < 0.05$) in ICA⁺ schoolchildren compared with control subjects. A comparison of ICA⁺ with type I diabetic

TABLE 1
Islet cell antibody–positive (ICA⁺) subjects in study population according to age

	Female			Male		
	Tested			Tested		
Age (yr)	n	%	ICA⁺	n	%	ICA⁺
6	46	45	0	28	46	0
7	74	47	2 (2.7)	67	47	0
8	94	55	1 (1.0)	81	59	1 (1.2)
9	70	52	0	114	51	1 (0.9)
10	128	58	2 (1.6)	116	57	0
11	254	54	2 (0.8)	222	54	1 (0.5)
12	243	55	2 (0.8)	278	54	5 (1.8)
13	287	56	2 (0.7)	248	55	5 (2.0)
14	228	57	3 (1.3)	336	64	5 (1.5)
15	238	56	2 (0.8)	171	54	1 (0.6)
16	236	54	2 (0.8)	121	55	3 (2.5)
17	173	50	0	102	55	3 (2.9)
18	108	46	0	80	46	0
19	54	47	1 (1.8)	34	37	0
20	22	30	0	7	17	0
21	25	42	0	2	15	0
Total	2280	53	19 (0.8)	2007	54	25 (1.2)

Percentage ICA⁺ in parentheses.

TABLE 2
Prevalence rates of islet cell antibody (ICA) positivity

	Age (yr)					
	7–9	10–12	13–15	16–18	19–21	Total
Prevalence per 100 subjects	1.22	1.22	1.23	0.95	0.21	1.05
95% confidence interval	0.33–3.1	0.6–2.2	0.7–1.9	0.5–1.7	0.01–1.2	0.8–1.4

patients revealed a similar distribution of DQB1 phenotypes with no statistical significant differences.

HLA DNA typing. Data from HLA-DQB1 typing were summarized in Tables 4 and 5. HLA-DQB1 alleles were determined in 31 schoolchildren, 104 type I diabetic subjects, and 123 healthy control subjects. Twenty (64.5%) of the ICA$^+$ patients were non-Asp homozygous or non-Asp/Blank. Of the ICA$^-$ schoolchildren, 93.5% had a non-Asp$^-$ HLA haplotype. In contrast, a non-Asp haplotype was found in 71.5% of the local control subjects ($P < 0.01$). The results from the type I diabetic subjects and the control subjects compare well with the published data from Morel et al. (7) and Baisch et al. (8). When ICA$^+$ subjects were compared with the type I diabetic subjects, the number of non-Asp homozygous individuals was significantly increased in the latter group ($P < 0.02$).

ICA levels and HLA. The association of ICA level and HLA types is summarized in Table 6. No association of a high ICA level ≥17 or ≥30 JDF U with HLA-DR3 and/or HLA-DR4 was observed. When HLA-DQß alleles and ICA levels were compared, a clustering of high-level ICAs was observed in non-Asp$^+$ individuals. Fifty percent of the non-Asp$^+$ nondiabetic schoolchildren had ICA levels ≥30 JDF U compared with only 27% in Asp$^+$ individuals (Table 6).

DISCUSSION
This study shows that the presence of ICAs in a random population of whites 6–21 yr of age is associated with HLA-DRß alleles and the absence of Asp at codon 57 of the HLA-DQ ß-chain. This indicates that particular HLA-DQB1 alleles are not only genetic markers of type I diabetes (4,5), but they are also associated with an ongoing autoimmune process reflected by circulating ICAs. Only 2 of 31 (6.5%) ICA$^+$

nondiabetic individuals compared to 47 (38.2%) control subjects were homozygous for Asp at position 57 of the HLA-DQ ß-chain. The highest prevalence of non-Asp homozygotes was found in type I diabetic subjects. This genotype was significantly more frequent compared with control subjects and ICA$^+$ patients. Non-Asp homozygosity was described by Todd et al. (6) and Morel et al. (7) as the principle disease risk genotype in whites. Although this suggests that the immunogenetic background of our ICA$^-$ healthy children is not completely congruent with the high-risk HLA-markers found in type I diabetic subjects, the design of the study excluded ICA$^+$ non-Asp homozygous subjects if they were already diabetic. In this way, the healthy ICA$^+$ population may be slightly enriched with those ICA$^+$ individuals that will not develop diabetes.

Our study is the first that analyzed the association of polymorphisms at position 57 of the HLA-DQ ß-chain and ICA positivity. Six studies of different ethnic groups have examined the population prevalence of ICAs. Notsu et al. (14) reported a prevalence of 0.5% in 1242 rural Japanese villagers, Betterle et al. (15) found a 0.5% prevalence in 611 adults, and Riley et al. (16) observed 0.4% in 2500 control subjects. Later, Maclaren et al. (17) reported a 0.84% ICA positivity in 5003 healthy Florida schoolchildren. From Spain, Bergeau et al. (18) reported a prevalence of 0.35% in 2291 healthy teenagers, and Bruining et al. (19) reported 0.24% ICA$^+$ subjects from a group of 2805 Dutch children. In a report from Finland, 4.1% of 1212 children and young adults were ICA$^+$ (20). The prevalence of ICA positivity in our group was 1.05% with a CI of 0.8–1.4% and was comparable with the results of Maclaren et al. Until now, the only analysis of HLA markers in ICA$^+$ healthy children came from the Florida study (17). A significant increase of HLA phenotypes that

TABLE 3
Phenotypic frequencies of HLA-DR specificities in islet cell antibody–positive (ICA$^+$) individuals, control subjects, and insulin-dependent (type I) diabetic subjects

	ICA$^+$ ($n = 36$)			Control ($n = 808$)		Type I ($n = 185$)	
	n	Frequency (%)	Relative Risk	n	Frequency (%)	n	Frequency (%)
DR1	6	16.6*		150	18.5	39	21.1†
DR2	5	13.8*		222	27.3	13	7.0†
DR3	16	44.4‡	3.4	153	18.8	95	51.3†
DR4	19	52.7§	3.6	192	23.6	125	67.6†
DR5	7	19.4*		196	24.1	20	10.8†
DR6	7	19.4*		164	20.2	22	11.9†
DR7	3	8.3||	0.3	179	22.0	16	8.6†
DR8	2	5.5*		48	5.9	9	4.9†

*NS, ‡$P < 0.001$, §$P < 0.0001$, ||$P < 0.05$, vs. control.
†NS vs. ICA$^+$ individuals.

TABLE 4
HLA-DR serology, HLA-DQB1 alleles, codon 57, and islet cell antibody (ICA) levels in ICA⁺ schoolchildren

No.	Code	Gender/age (yr)	HLA-DRB1	HLA-DQB1	Codon 57 (DQ β-chain)	ICA level (JDF U)
1	8722012	M/17	3,-	2,3.1	NA/AA	90
2	8723913	M/13	3,11	2,3.1	NA/AA	9
3	8722050	F/15	4,6	3.2,1.2	NA/AA	17
4	8721415	F/19	2,2	1.1,1.1	NA/NA	30
5	8722062	F/15	1,2	1.2,1.2	AA/AA	17
6	8721733	M/14	6,11	1.2,1.2	AA/AA	9
7	8723944	M/13	3,4	2,3.1	NA/AA	17
8	8724196	M/12	4,7	3.1,2	AA/NA	9
9	9905031	M/14	1,2	1.1,1.AZH	NA/NA	54
10	8722436	F/11	4,11	3.2,1.1	NA/NA	5
11	9904972	F/07	3,6	2,-	NA/-	9
12	9904944	M/09	7,11	ND	ND	17
13	9905374	M/17	3,6	2,1.2	NA/AA	54
14	8722424	M/12	4,8	3.2,3.2	NA/NA	90
15	8722564	F/07	1,3	1.1,2	NA/NA	30
16	8721790	F/10	4,12	3.2,2	NA/NA	160
17	9904913	F/10	2,4	ND	ND	54
18	8723957	M/14	4,6	3.2,1.1	NA/NA	54
19	8724646	M/13	3,4	2,3.2	NA/NA	9
20	8722021	M/16	1,2	1.1,1.2	NA/AA	17
21	8723896	F/11	3,4	2,3.1	NA/AA	5
22	8723637	M/11	4,11	3.2,1.2	NA/AA	90
23	8722221	F/08	4,6	3.2,1,1	NA/NA	30
24	8724230	F/14	4,4	ND	ND	5
25	8724249	F/12	3,3	2,2	NA/NA	17
26	8721879	M/12	3,4	2,3.2	NA/NA	5
27	8726445	M/16	ND	ND	ND	9
28	8726605	F/14	3,-	2,-	NA/-	5
29	8722017	M/16	ND	1.1,1,1	NA/NA	30
30	8721398	F/16	1,3	1.1,2	NA/NA	90
31	9905184	M/17	3,-	2,2	NA/NA	17
32	9905277	F/16	ND	ND	ND	5
33	8721739	F/14	6,8	ND	ND	90
34	8723964	F/13	ND	ND	ND	17
35	8724882	F/13	3,4	2,3.2	NA/NA	90
36	8722263	M/08	ND	ND	ND	30
37	8727076	M/15	3,4	2,-	NA,-	90
38	8724239	M/13	ND	ND	ND	9
39	8724860	M/12	3,4	2,-	NA,-	9
40	8722179	M/14	4,-	3.2,3.2	NA/NA	9
41	8722482	M/13	ND	ND	ND	17
42	8724168	M/12	ND	ND	ND	9
43	8726644	M/14	1,7	ND	ND	9
44	8722414	F/12	4,11	ND	ND	9

JDF, Juvenile Diabetes Foundation; NA, amino acid other than aspartic acid at position 57 of the HLA-DQ β-chain; AA, aspartic acid at codon 57 of the HLA-DQ β-chain; ND, not determined; -, Blank.

included HLA-DR3 and/or -DR4 alleles was found when the comparison was made to healthy control subjects. This inital observation has recently been extended (21). In 31 of 49 ICA⁺ individuals, HLA-DR phenotypes were analyzed (20 subjects [64%] were DR4⁺ and 21 subjects [67%] were DR3⁺). The significant increase of these DR phenotypes compared with the control subjects was shared by the schoolchildren from Florida and our cohort of whites.

ICAs and genetic risk markers have been extensively studied in relatives of patients with type I diabetes (3). Family

TABLE 5
Relationship of presence or absence of aspartic acid to islet cell antibody (ICA) positivity

Haplotype at position 57	Controls (n = 123)	ICA⁺ (n = 31)	Type I (n = 104)
Asp/Asp or Asp/Blank	35 (28.4)	2 (6.5)*	0 (0)
Asp/non-Asp	47 (38.2)	9 (29.0)†‡	16 (15.4)§
Non-Asp/non-Asp or non-Asp/Blank	41 (33.4)	20 (64.5)‡	88 (84.6)§
Any haplotype including 1 non-Asp allele	88 (71.5)	29 (93.5)*‡	100 (100)§

Values in parentheses are percentages. Statistical analysis was performed within the control group, insulin-dependent (type I) diabetic patients, and ICA⁺ individuals.
*NS, §$P < 0.02$, vs. type I diabetic patients.
†NS, ‡$P < 0.01$, vs. control group.

TABLE 6
Islet cell antibody (ICA) levels and HLA-DRB1 and HLA-DQB1 alleles

ICA level	DR4⁺ (n = 19)	DRX* (n = 16)	DR3⁺ (n = 16)	DRX† (n = 19)	non-Asp/Asp or Asp/Asp (n = 11)	non-Asp/- or non-Asp/ non-Asp (n = 20)
≥17 JDF U	10 (53)	12 (75)	9 (56)	13 (68)	6 (55)	13 (65)
≥30 JDF U	8 (42)	7 (44)	6 (37)	9 (47)	3 (27)	10 (50)

Values in parentheses are percentages. JDF, Juvenile Diabetes Foundation; -, Blank.
*Denotes any HLA-DR phenotype other than HLA-DR4.
‡Denotes any HLA-DR phenotype other than HLA-DR3.

studies have shown that most first-degree relatives who became diabetic had ICAs detectable before diagnosis (1,2). However, only a portion of ICA⁺ individuals will develop the disease later on. ICAs can even occur in identical twins with only one of them developing type I diabetes (22). In this context, note that high levels of ICAs were described to be of high predictive value for future type I diabetes (1,2,23). Recently, Riley et al. (23) observed in a prospective study in relatives of type I patients that ICA levels ≥20 JDF U were associated with an increasing risk of the development of diabetes. High levels of ICAs, i.e., ≥17 or ≥30 JDF U were found more often in our population survey in non-Asp positives. Individuals with an Asp⁺ HLA haplotype had lower levels of ICAs. No association between particular HLA-DR phenotypes and high levels of ICAs could be detected in this study. This may indicate that the immune response to islet cell antigen, as reflected by the level of ICAs, is more strongly associated with HLA-DQB1 alleles compared with the HLA-DRß phenotypes.

After a follow-up of 976 proband yr, one individual in our cohort (no. 9905031) developed diabetes. This individual was DNA typed as non-Asp homozygous and revealed high levels of ICAs during the initial survey in 1988. We demonstrated that HLA-DR and -DQ alleles found in type I diabetic subjects were also associated with ICA positivity in a random population. We plan to follow the well-characterized population of ICA⁺ nondiabetic individuals described in this report to determine whether the combination of ICA testing and DQß determination will be a better predictor for type I diabetes susceptibility.

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