Original Articles

HLA-DQB1*0602 Is Associated With Dominant Protection From Diabetes Even Among Islet Cell Antibody–Positive First-Degree Relatives of Patients with IDDM

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HLA-DQB1 alleles confer susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM). We investigated whether the susceptibility alleles DQB1*0302 and DQB1*0201 affect progression to diabetes among islet cell antibody–positive (ICA^+) first-degree relatives of IDDM patients and whether the protective allele DQB1*0602 can be found and is still protective among such relatives. We human leukocyte antigen–typed and periodically tested β-cell function (first-phase insulin release [FPIR] during the intravenous glucose tolerance test) in 72 ICA^+ relatives, of whom 30 became diabetic on follow-up (longest follow-up 12 years); 54 (75%) relatives carried DQB1*0302 and/or DQB1*0201. The frequency of DQB1*0302 and DQB1*0201 and of the high-risk genotype DQB1*0302/DQB1*0201 did not differ significantly between diabetic relatives and those remaining nondiabetic. On follow-up, progression to IDDM was not statistically different for relatives with or without the DQB1*0302/DQB1*0201 genotype. However, those relatives with the DQB1*0302/DQB1*0201 genotype had a tendency to develop diabetes at an earlier age (log-rank $P = 0.02$). We found DQB1*0602 in 8 of 72 (11.1%) ICA^+ relatives. Relatives with DQB1*0602 did not develop diabetes or show any decline of FPIR versus 28 of 64 $DQB1*0602^-$ relatives who developed IDDM (log-rank $P = 0.006$; Wilcoxon's $P = 0.02$). The protective allele DQB1*0602 is found in ICA^+ relatives who have minimal risk of progression to IDDM. Therefore, DQB1*0602 is associated with protection from IDDM both in population studies and among relatives with evidence of autoimmunity who should not enter prevention trials. *Diabetes* 44:608–613, 1995

Our knowledge of the immunogenetics of insulin-dependent diabetes mellitus (IDDM) has significantly improved during the last decade, and it is now thought that IDDM is an autoimmune disease occurring in genetically susceptible individuals (1–3). A significant portion of IDDM genetic susceptibility appears to be encoded by the human leukocyte antigen (HLA)-DQB1 locus within the human histocompatibility complex and in particular by the alleles DQB1*0302 and DQB1*0201 found on DR4 and DR3 haplotypes, respectively (4–9). Individuals heterozygous for the above alleles have the highest risk of developing the disease. In contrast, DR2 haplotypes are rarely found in patients with IDDM, suggesting that they may carry protective alleles (9). Several studies indicate that the protection linked to DR2 haplotypes is closely associated with the DQB1 locus and in particular with the DQB1*0602 allele (4,7,8,10). Virtually all DR2 haplotypes found in Caucasian patients with IDDM carry the alleles DQB1*0601 or DQB1*0502 (AZH) (4,11–13) and not DQB1*0602. In addition, a few DR2 haplotypes with a recombinant DQB1 allele (i.e., DQB1*0402) have been recently reported among patients with IDDM (10,13). Several reports confirm that DQB1*0602 is very rare in patients with IDDM (6,8,14), and we have found it in only 1 of 182 patients with IDDM in contrast with approximately 20–25% of the general population. This one patient with IDDM and DQB1*0602 has polyendocrine type I autoimmune syndrome (15). Moreover, it has been suggested that the protective effect of DQB1*0602 is dominant over susceptibility encoded by high-risk DQB1 alleles (8).

At present, islet cell antibody (ICA) screening is widely used to identify individuals at increased risk for IDDM, especially among first-degree relatives of patients with IDDM, some of whom may then be enrolled in several ongoing preventive trials (16–18).

To investigate whether different HLA-DQB1 alleles are associated with different rates of progression to overt IDDM among ICA^+ relatives, we have analyzed HLA-DQB1 alleles

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BDC, Barbara Davis Center; FPIR, first-phase insulin release; GAD, glutamic acid decarboxylase; HLA, human leukocyte antigen; IAA, insulin autoantibody; ICA, islet cell antibody; ICARUS, Islet Cell Autoantibody Register User's Study; IDDM, insulin-dependent diabetes mellitus; IVGTT, intravenous glucose tolerance test; JDC, Joslin Diabetes Center; JDF, Juvenile Diabetes Foundation; PCR, polymerase chain reaction; SMS, stiff-man syndrome.

and progression to diabetes in 72 such relatives of patients with IDDM.

Our data indicate that the protective allele DQB1*0602 is unexpectedly carried by a significant portion of ICA$^+$ relatives, and in such relatives DQB1*0602 is associated with lack of progression to overt diabetes. Therefore, it may be important to identify ICA$^+$ relatives carrying DQB1*0602 to avoid their enrollment into prevention trials because they have little risk of developing IDDM despite their ICA positivity.

RESEARCH DESIGN AND METHODS

From our screening of first-degree relatives of patients with IDDM (19,20) for cytoplasmic ICA at the Joslin Diabetes Center (JDC) and at the Barbara Davis Center (BDC), we have analyzed HLA alleles, autoantibody status, and progression to diabetes in 72 ICA$^+$ relatives selected on the basis of sample availability for HLA typing. Of the first-degree relatives, 38 were siblings, 24 were offspring, 7 were parents, and 3 were identical twins of patients with IDDM; 42 relatives were male and 30 were female, with a mean age at the beginning of the follow-up of 15.9 ± 1.6 (mean ± SE) years. Also studied were 126 autoantibody-negative nondiabetic first-degree relatives (55 men and 71 women; age for 122 relatives was 34.5 ± 1.4 years; 43 were siblings, 21 were offspring, 56 were parents, and 6 were discordant identical twins of patients with IDDM).

Autoantibody testing. ICAs and insulin autoantibodies (IAAs) were measured as previously reported (21,22). ICA positivity was defined as ≥20 Juvenile Diabetes Foundation (JDF) U on at least two occasions. The upper limit of normal for the IAA assay is 39 nU/ml. The last Immunology Diabetes Workshop ICA proficiency test gave 100% sensitivity and specificity for the BDC ICA assay and 50% sensitivity and 100% specificity for the JDC ICA assay.

Intravenous glucose tolerance test (IVGTT). IVGTT was performed periodically (every 3–6 months) after detection of ICA positivity. Before the Islet Cell Autoantibody Register User's Study (ICARUS) recommendation, IVGTT was performed with a timing of infusion varying between 2 and 5 min. Since the ICARUS consensus was developed, IVGTT has been performed by this method with a timed 3-min infusion (23,24). The sums of one plus 3-min insulin levels (μU/ml) are reported. The JDC insulin assay compares with the Seattle insulin assay by the formula JDC = (1.7 μU/ml + 0.73) × (insulin levels [Seattle] μU/ml) (24).

HLA class II typing. Genomic DNA was extracted with standard procedures. DQB1 typing was performed with a dot-blot technique using two different protocols and reagents to ensure quality. The first protocol and reagents were from the XIth International HLA Workshop (25). In brief, 1 μg of DNA was amplified by polymerase chain reaction (PCR) with specific primers, and the PCR product was spotted onto pre-wet nylon membranes with a dot-blot apparatus. Membranes were hybridized with ^{32}P-labeled sequence-specific oligonucleotide (SSO) probes and then subjected to autoradiography. The second typing protocol used primers and horseradish peroxidase–labeled oligonucleotide probes developed by Perkin-Elmer/Cetus (Emeryville, CA) as previously described (26). Besides HLA-DQB1 typing, most relatives were also typed for HLA-DR alleles by serological methods as previously described (27).

Statistical analysis. The chi-squared (χ^2) test with Yates' correction and the Fisher's exact test were used for statistical comparisons. Only two-tailed P values are reported. The log-rank test, Wilcoxon's survival test, and the −2log(LR) test were used for the life table analysis.

RESULTS

In the analysis that follows, we will first describe the distribution of HLA-DQB1 alleles among 72 ICA$^+$ relatives (subdivided by the presence or absence of diabetes at the time of the study) and 126 nondiabetic autoantibody-negative relatives. We will then describe the results of the life table analysis by both chronological age and duration of follow-up after detection of ICA positivity.

Distribution of HLA-DQB1 alleles. Table 1 illustrates DQB1 genotype frequencies in 72 ICA$^+$ and 126 autoantibody-negative first-degree relatives. As expected, most of the 72 (75%, n = 54) ICA$^+$ first-degree relatives studied carried either one of the high-risk alleles DQB1*0302 (DR4) and/or DQB1*0201 (DR3). Of the ICA$^+$ relatives, 20% (15 of 72) were DQB1*0302/DQB1*0201 heterozygous vs. 5.5% (7 of 126) of the autoantibody-negative relatives (P = 0.002). Twenty-three ICA$^+$ relatives (31.9%) carried DQB1*0302 alone (21 without DQB1*0602) and 21 (29.1%) had only DQB1*0201 with DR3 (18 without DQB1*0602) (Table 1). The individual frequencies of DQB1*0302 and DQB1*0201 did not differ between ICA$^+$ and autoantibody-negative relatives. Only 13.8% (10 of 72) of ICA$^+$ relatives lacked DQB1*0302, DQB1*0201, and DQB1*0602 vs. 28.5% (36 of 126) of autoantibody-negative relatives (P = 0.02). Of the ICA$^+$ relatives, 11.1% (8 of 72) had the protective allele DQB1*0602 similar to 9.5% (12 of 126) of the autoantibody-negative relatives (P = 0.91).

TABLE 1
Frequency of DQB1 genotypes among 72 ICA$^+$ and 126 autoantibody-negative first-degree relatives

DQB1 genotype	ICA$^+$		Autoantibody-negative		
	n	F	n	F	P
0301/0201	15	0.208	7	0.055	0.002
0302/*X*	21	0.291	30	0.238	0.50
0201/*Y*	18	0.250	41	0.325	0.33
0602/*Z*	8	0.111	12	0.095	0.91
K/*K*	10	0.138	36	0.285	0.02

ICA$^+$, n = 72; autoantibody-negative, n = 126. X = any DQB1 allele but *0201 (DR3) or *0602; Y = any DQB1 allele but *0302 or *0602; Z = any DQB1 allele; K = any DQB1 allele but *0302, *0201 (DR3), or *0602.

Of 72 ICA$^+$ relatives studied, 30 (41.6%) developed IDDM on follow-up (longest follow-up was 12 years). Table 2 illustrates DQB1 genotype frequencies for diabetic (n = 30) and nondiabetic (n = 42) ICA$^+$ first-degree relatives. Although the frequency of the high-risk genotype DQB1*0302/DQB1*0201 was higher in those relatives who developed IDDM on follow-up than in those who did not (30% diabetic vs. 14.2% nondiabetic), the difference was not statistically significant (P = 0.18), nor was the frequency of DQB1*0302 or DQB1*0201 different among diabetic and nondiabetic ICA$^+$ relatives. We found no difference even if we excluded those relatives with DQB1*0602 from the analysis (data not shown). Although the presence of IAAs could accelerate the rate of progression to diabetes in ICA$^+$/DQB1*0602$^-$ relatives, IAAs were found in 23 of 29 (79.3%) diabetic relatives vs. 21 of 35 (60%) nondiabetic relatives (P = 0.16, NS).

Only the DQB1*0602/Z (Z = any DQB1 allele) genotype

TABLE 2
Frequency of DQB1 genotypes among 72 ICA$^+$ first-degree relatives with and without IDDM

DQB1 genotype	Diabetic		Nondiabetic		
	n	F	n	F	P
0302/0201	9	0.300	6	0.142	0.18
0302/*X*	8	0.266	13	0.309	0.89
0201/*Y*	8	0.266	11	0.238	0.95
0602/*Z*	0	0.000	8	0.190	0.01
K/*K*	5	0.166	5	0.119	0.73

Diabetic, n = 30; nondiabetic, n = 42. X = any DQB1 allele but *0201 (DR3) or *0602; Y = any DQB1 allele but *0302 or *0602; Z = any DQB1 allele; K = any DQB1 allele but *0302, *0201 (DR3), or *0602.

TABLE 3
DQB1 and DR genotypes, age range, ICA and IAA levels, duration of follow-up, and FPIR for eight ICA$^+$ relatives with the protective allele DQB1*0602

Patient number	DQB1 genotype	DR	Age range on follow-up (years)	ICA (JDF U)	IAA (nU/ml)	Years of follow-up	FPIR (μU/ml)	
							First test	Last test
9280	0602/0201	2/7	33–38.3	80	21	5.3	116	190
3401	0602/0201	2/3	38.7–46.9	1280	11	8.2	86	184
726	0602/0201	2/3	7–15.9	640	492	8.9	97	127
8974	0602/0302	2/4	4.2–8.5	320	424	4.3	56	53
8027	0602/0302	2/4	40.9–46.6	640	16	5.7	224	124
203253	0602/0301	2/4	10.8–13.2	40	66	2.4	66	117
1271	0602/0301	3/4*	42.9–52.3	640	19	9.4	124	242
2204	0602/0501	2/1	48.8–60.1	640	25	11.3	161	134

ICA level shown is the highest level observed for each individual. ICA positivity was defined as ≥20 JDF U on at least two occasions. IAA level shown is the mean level for each individual. Upper limit of normal for IAA is 39 nU/ml. First percentile of normal for FPIR is 48 μU/ml. *Patient 1271 has an unusual haplotype DR3, DQB1*0602.

was found with a significantly different frequency in diabetic and nondiabetic ICA$^+$ relatives. The protective allele DQB1*0602 was carried by none of the 30 ICA$^+$ relatives who developed IDDM on follow-up and by 8 of 42 (19%) nondiabetic ICA$^+$ relatives (P = 0.01). Table 3 shows the HLA-DQB1 and HLA-DR genotypes and clinical characteristics of the eight ICA$^+$ relatives with DQB1*0602. The protective effect of DQB1*0602 appears to be dominant over susceptibility encoded by DQB1*0302 and DQB1*0201, since two relatives carried DQB1*0302 and three relatives had DQB1*0201 on DR3 (n = 2) and DR7 (n = 1) haplotypes. Table 3 illustrates that only three of eight such relatives had mean IAA levels exceeding the upper limit of normal of 39 nU/ml (66, 424, and 492 nU/ml). Moreover, these relatives did not show any decline of first-phase insulin release (FPIR) as indicated by the lack of significant variation between the first and last IVGTT reported in Table 3. Four of eight ICA$^+$ relatives with DQB1*0602 had an initial FPIR of <100 μU/ml, and thus, depending on criteria for prevention trials, they might have been eligible for treatment. However, none of them had values of FPIR <48 μU/ml, our previously published first percentile (19).

Life table analysis. Figures 1–4 illustrate the survival analysis of ICA$^+$ relatives with different DQB1 genotypes by age and follow-up (starting from detection of ICA positivity).

The risk of IDDM in ICA$^+$ relatives carrying DQB1*0602 is very low, since none of eight such relatives developed diabetes on follow-up (longest follow-up for relatives with DQB1*0602 was 11.3 years) in contrast with most of the relatives lacking DQB1*0602 (Fig. 1*B*; log-rank test P = 0.006; Wilcoxon's P = 0.02; −2log[LR] test P = 0.0005). Fifty-one DQB1*0602$^-$ relatives were younger than 20 years old when follow-up began after detection of ICA positivity, and 21 relatives became diabetic within an average time of 3.6 ± 0.5 (mean ± SE) years. Of the eight relatives with DQB1*0602, seven relatives were followed for more than 4 years and six relatives for more than 5 years (Table 3). The three relatives younger than 20 years of age were followed for 8.9, 4.3, and 2.4 years. The oldest relative with DQB1*0602 is now 60.1 years old, and by life table analysis, 45% of ICA$^+$ relatives lacking DQB1*0602 became diabetic by age 25 (Fig. 1*A*; log-rank test P = 0.006; Wilcoxon's P = 0.03; −2log[LR] test P = 0.0003).

In order not to bias the following life table analysis of ICA$^+$ relatives with high-risk DQB1 alleles, we excluded the eight relatives with DQB1*0602. Of 64 ICA$^+$ relatives lacking DQB1*0602, 30 became diabetic on follow-up. Similar to studies by Deschamps et al. (28), we found that (Fig. 2*B*) among the 30 diabetic relatives 68% of those with the high-risk genotype DQB1*0302/DQB1*0201 developed IDDM

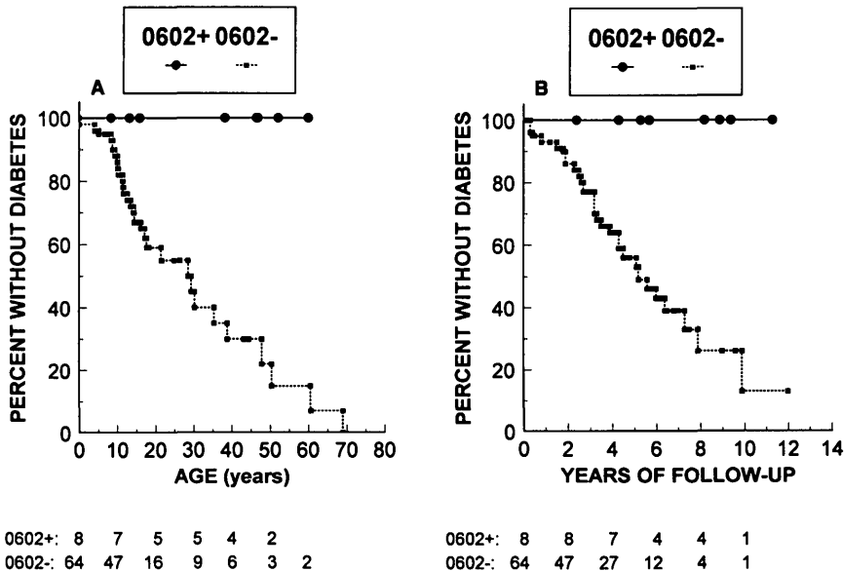

FIG. 1. Life table analysis of the risk of IDDM in 72 ICA$^+$ positive first-degree relatives with (n = 8) or without (n = 64) the protective allele DQB1*0602 by age (*A*) and follow-up (*B*).

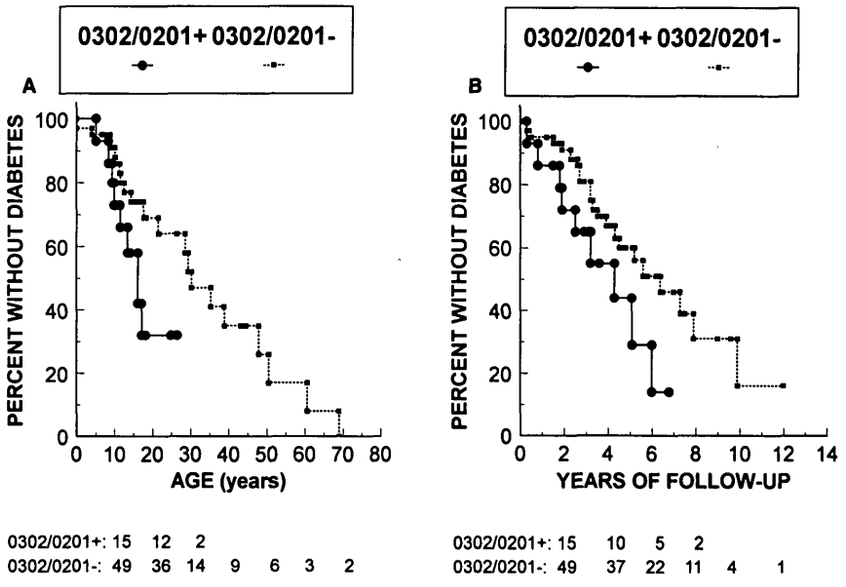

FIG. 2. Life table analysis of the risk of IDDM in 64 ICA$^+$/DQB1*0602$^-$ first-degree relatives with (n = 15) or without (n = 49) the high-risk genotype DQB1*0302/DQB1*0201 by age (A) and follow-up (B).

by age 25 in contrast with 36% of those without it and 14% of relatives with DQB1*0201 (DR3) only (DQB1*0302/DQB1*0201$^+$ vs. DQB1*0302/DQB1*0201$^-$: log-rank test P = 0.02, Wilcoxon's P = 0.057, −2log[LR] test P = 0.13; DQB1*0302/DQB1*0201$^+$ vs. DQB1*0201 only: log-rank test P = 0.006, Wilcoxon's P = 0.01, −2log[LR] test P = 0.055; data not shown). Although the analysis illustrated in Fig. 2B suggests a more rapid progression to diabetes by follow-up of those relatives with the DQB1*0302/DQB1*0201 genotype, the difference was not statistically significant versus those relatives without this genotype who could, however, carry either DQB1*0302 or DQB1*0201 (log-rank test P = 0.06, Wilcoxon's P = 0.09, −2log[LR] test P = 0.15). As shown in Fig. 3A, those ICA$^+$ relatives carrying DQB1*0201 on DR3 haplotypes (excluding those with the DQB1*0302/DQB1*0201 genotype and with DQB1*0602) developed IDDM at an older age than those without it (DQB1*0201$^+$ vs. DQB1*0201$^-$: log-rank test P = 0.01, Wilcoxon's P = 0.04, −2log[LR] test P = 0.20). However, we found no difference in progression to diabetes by follow-up of ICA$^+$ relatives between the two groups (log-rank test P = 0.64, Wilcoxon's P = 0.42, −2log[LR] test P = 0.83). Finally (Fig. 4), a statistically significant difference was found in age (log-rank test P = 0.04, Wilcoxon's P = 0.02, −2log[LR] test P = 0.34) but not in progression to IDDM (log-rank test P = 0.54, Wilcoxon's P = 0.72, −2log[LR] test P = 0.74) in relatives with or without the susceptibility allele DQB1*0302 (excluding those with DQB1*0602 and with the high-risk genotype DQB1*0302/DQB1*0201). Therefore, among ICA$^+$ first-degree relatives, DQB1*0602 was the major allele affecting progression to diabetes, with none of the relatives with DQB1*0602 developing diabetes on follow-up.

DISCUSSION

To evaluate the influence of HLA-DQB1 alleles on disease progression and whether such genotyping can be used to improve our ability to predict IDDM among at-risk relatives, we have typed HLA-DQB1 alleles and prospectively followed 72 ICA$^+$ first-degree relatives of patients with IDDM. As expected, the frequency of the highest-risk genotype DQB1*0302/DQB1*0201 was higher in the ICA$^+$ relatives than in 126 autoantibody-negative relatives (20.8% vs. 5.5%, P = 0.002; Table 1). In our study, the frequency of the high-risk

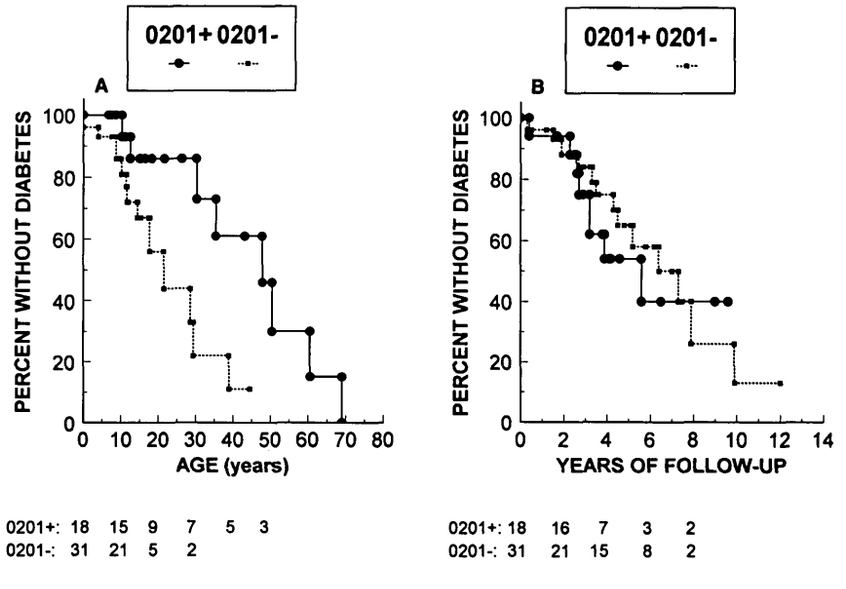

FIG. 3. Life table analysis of the risk of IDDM in 49 ICA$^+$ first-degree relatives with (n = 18) or without (n = 31) the susceptibility allele DQB1*0201 by age (A) and follow-up (B). Relatives with DQB1*0602 or with the DQB1*0302/DQB1*0201 genotype were excluded.

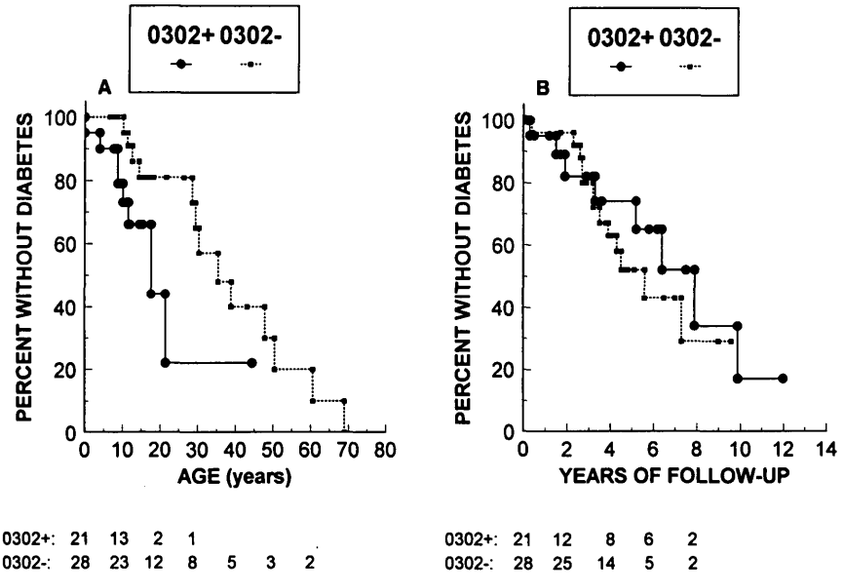

FIG. 4. Life table analysis of the risk of IDDM in 49 ICA$^+$ first-degree relatives with (n = 21) or without (n = 28) the susceptibility allele DQB1*0302 by age (A) and follow-up (B). Relatives with DQB1*0602 or with the DQB1*0302/DQB1*0201 genotype were excluded.

genotype DQB1*0302/DQB1*0201 was not statistically different between ICA$^+$ relatives who became diabetic on follow-up and those who have not developed IDDM. Moreover, we could find no difference in the frequency of DQB1*0302 or DQB1*0201 by itself among ICA$^+$ relatives followed to diabetes versus those who have not become diabetic. Also, the percentage of ICA$^+$ relatives with any one of the high-risk genotypes in Table 2 who became diabetic was not greater than that of those without DQB1*0302 or DQB1*0201. However, by life table analysis, diabetes onset was associated with an older age in the presence of DQB1*0201 alone in contrast with a younger age in the presence of DQB1*0302 only. These findings suggest a stronger role for DQB1*0302 and a more neutral role for DQB1*0201 in IDDM susceptibility, confirming previous case-control studies (8,9,14,25,29).

In this study, 11.1% (8 of 72) of our ICA$^+$ relatives carried the protective allele DQB1*0602, and the frequency of DQB1*0602 in ICA$^+$ relatives was not lower than that in autoantibody-negative relatives (11.1% vs. 9.5%, P = 0.91; Table 1). This unexpected finding suggests that autoimmunity to islet autoantigens may be triggered even in genetically protected individuals. Despite ICA positivity, none of the eight relatives with DQB1*0602 developed diabetes on follow-up (longest follow-up was 11.3 years), indicating a strong protective effect even after triggering of pancreatic autoimmunity. Among such relatives, first-phase insulin release also remained stable, consistent with their lack of progression to diabetes. Moreover, protection appears to be dominant over susceptibility encoded by the alleles DQB1*0302 and DQB1*0201. Although the mechanism of protection is still unknown, we have previously suggested an association between DQB1*0602 and high titers of autoantibodies against glutamic acid decarboxylase (GAD) (30,31), accounting for a particular ICA staining pattern on frozen islet sections termed "restricted" by us (32,33) or "selective" by Genovese et al. (34). Five of the eight ICA$^+$ sera from relatives with DQB1*0602 could be further characterized, and four had a restricted ICA pattern (32). A restricted ICA staining pattern is also found in patients with stiff-man syndrome (SMS), a rare neurological disorder of probable autoimmune origin often associated with IDDM (35,36). We have recently found also that patients with SMS are protected from IDDM in the presence of DQB1*0602 or sequence-related alleles (37,38), whose frequency in SMS patients is not lower than in the general population but is usually decreased in IDDM (25,39). An inverse correlation between humoral and cellular autoimmunity to GAD has been recently reported, suggesting the preponderance of a TH2 response in individuals with high levels of GAD autoantibodies (40). The prevalence of a TH2 (supporting humoral autoimmunity) over a TH1 response (supporting cellular autoimmunity) may be associated with the presence of DQB1*0602 or sequence-related alleles and at least in part genetically determined.

In conclusion, we find that the protective allele DQB1*0602 is found in a significant portion of ICA$^+$ relatives of diabetic patients and is associated with a very low risk for IDDM similar to a concurrent study (29). Because several prevention trials are being started worldwide, it is important to identify this subset of ICA$^+$ relatives. Such relatives should not be enrolled in prevention trials to avoid unnecessary treatment of individuals who are genetically protected from IDDM despite their ICA positivity.

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REFERENCES

1. Pugliese A, Eisenbarth GS: Type I diabetes: lessons for human autoimmunity. *J Lab Clin Med* 120:363–366, 1992

2. Nepom GT, Erlich HA: MHC class-II molecules and autoimmunity. *Annu Rev Immunol* 9:493–525, 1991
3. Atkinson MA, Maclaren NK: What causes diabetes? *Sci Am* 263:62–71, 1990
4. Todd JA, Bell JI, McDevitt HO: HLA-DQ beta gene contribution to susceptibility and resistance to insulin dependent diabetes mellitus. *Nature* 329:599–604, 1987
5. Horn GT, Bugawan TL, Long CM, Erlich HA: Allelic sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility. *Proc Natl Acad Sci USA* 85:6012–6016, 1988
6. Sheehy MJ, Scharf SJ, Rowe JR, Neme de Gimenez MH, Meske L, Erlich HA, Nepom BS: A diabetes susceptible HLA haplotype is best defined by a combination of HLA-DR and DQ alleles. *J Clin Invest* 83:830–835, 1989
7. Erlich HA, Bugawan TL, Scharf S, Nepom GT, Tait B, Griffith RL: HLA-DQB sequence polymorphism and genetic susceptibility to IDDM. *Diabetes* 39:96–103, 1990
8. Baisch JM, Weeks T, Giles R, Hoover M, Stastny P, Capra JD: Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. *N Engl J Med* 322:1836–1841, 1990
9. Thomson G, Robinson WP, Kuhner MK, Joe S, MacDonald MS, Gottschall JL, Barbosa J, Rich SS, Bertrams J, Baur MP, Partanena J, Tait BD, Schober E, Mayr WR, Ludvigsson J, Lindblom B, Farid NR, Thompson C, Deschamps I: Genetic heterogeneity, modes of inheritance, and risk estimates for a joint study of Caucasians with insulin-dependent diabetes mellitus. *Am J Hum Genet* 43:799–816, 1988
10. Carcassi C, Trucco G, Trucco M, Contu L: A new HLA-DR2 extended haplotype is involved in insulin-dependent diabetes mellitus susceptibility. *Hum Immunol* 31:159–164, 1991
11. Sorrentino R, De Grazia U, Buzzetti R, Nistico, L, Iannicola C, Costanzi S, Tosi R: An explanation for the neutral effect of DR2 on IDDM susceptibility in central Italy. *Diabetes* 41:904–908, 1992
12. Zeliszewski D, Tiercy J, Boitard C, Gu X, Loche M, Krishnamoorthy R, Simonney N, Elion J, Bach JF, Mach B, Starkers G: Extensive study of DRB, DQA, and DQB gene polymorphism in 23 DR2-positive, insulin-dependent diabetes mellitus patients. *Hum Immunol* 33:140–147, 1992
13. Erlich HA, Griffith RL, Bugawan TL, Ziegler R, Alper C, Eisenbarth GS: Implication of specific DQB1 alleles in genetic susceptibility and resistance by identification of IDDM siblings with novel HLA-DQB1 allele and unusual DR2 and DR1 haplotypes. *Diabetes* 40:478–481, 1991
14. Caillat-Zucman S, Garchon H-J, Timsit J, Assan R, Boitard C, Djitah-Saiah I, Bougneres P, Bach JF: Age-dependent HLA genetic heterogeneity of type I insulin-dependent diabetes mellitus. *J Clin Invest* 90:2242–2250, 1992
15. Eisenbarth GS, Jackson RA: The immunoendocrinopathy syndromes. In *Williams Textbook of Endocrinology*. 8th ed. Wilson JD, Foster DW, Eds. Philadelphia, Saunders, 1992, p. 1555–1566
16. Keller RJ, Eisenbarth GS, Jackson RA: Insulin prophylaxis in individuals at high risk of type I diabetes. *Lancet* 341:927–928, 1993
17. Chase HP, Butler-Simon N, Garg S, McDuffie M, Hoops SL, O'Brien D: A trial of nicotinamide in newly diagnosed patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 33:444–446, 1990
18. Elliot R, Chase H, Pilcher C, Edgar B: Effect of nicotinamide in preventing diabetes (IDDM) in children (Abstract). *J Autoimmun* 3:61A, 1990
19. Bleich D, Jackson RA, Soeldner JS, Eisenbarth GS: Analysis of metabolic progression to type I diabetes in islet cell antibody positive relatives of patients with type I diabetes. *Diabetes Care* 13:111–118, 1990
20. Srikanta S, Ganda OP, Soeldner JS, Eisenbarth GS: First degree relatives of patients with type I diabetes mellitus: islet cell antibodies and abnormal insulin secretion. *N Engl J Med* 313:461–464, 1985
21. Srikanta S, Rabizadeh A, Omar MAK, Eisenbarth GS: Assay for islet cell antibodies: protein A-monoclonal antibody method. *Diabetes* 34:300–305, 1985
22. Vardi P, Dib SA, Tuttleman M, Connelly JE, Grinbergs M, Rabizadeh A, Riley WJ, Maclaren NK, Eisenbarth GS, Soeldner JS: Competitive insulin autoantibody RIA: prospective evaluation of subjects at high risk for development of type I diabetes mellitus. *Diabetes* 36:1286–1291, 1987
23. Bingley PJ, Colman P, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EA: Standardization of IVGTT to predict IDDM. *Diabetes Care* 15:1313–1316, 1992
24. McCulloch DK, Bingley PJ, Colman PG, Jackson RA, Gale EA: Comparison of bolus and infusion protocols for determining acute insulin response to intravenous glucose in normal human: the ICARUS group: Islet Cell Autoantibody Register User's Study. *Diabetes Care* 16:911–915, 1993
25. Ronningen, KS, Spurkland A, Tait BD, Drummond B, Lopez-Larrea C, Baranda FS, Menendez-Diaz MJ, Caillat-Zucman S, Beaurain G, Garchon HJ, Ilonen J, Reijonen H, Knip M, Boehm BO, Rosak C, Loliger C, Kuhnl P, Ottenhoff T, Contu L, Carcassi C, Savi M, Zanelli P, Neri TM, Hamaguchi K, Kimura A, Dong RP, Chikuba N, Nagataki S, Gorodezky C, Debaz H, Robles C, Coimbra HB, Martinho A, Ruas MA, Sachs JA, Garcia-Pachedo M, Biro A, Nikaein A, Dombrausky L, Gonwa T, Zmijewski C, Monos D, Kamoun M, Layrisse Z, Magli MC, Balducci P, Thorsby E: HLA class II associations in insulin-dependent diabetes mellitus among blacks, Caucasoids, and Japanese. In *HLA 1991*. Tsuji K, Aizawa M, and Sasazuki T, Eds. New York, Oxford Univ. Press, 1991, p. 713–722
26. Bugawan L, Erlich HA: Rapid typing of HLA-DQB1 DNA polymorphism using nonradioactive oligonucleotide probes and amplified DNA. *Immunogenetics* 33:163–170, 1991
27. Johnson AH, Hurley CK, Hartzman RJ, Alper CA, Yunis EJ: HLA: the major histocompatibility complex of man. In *Clinical Diagnosis and Management by Laboratory Methods*. 18th ed. Henry JB, Ed. Philadelphia, Saunders, 1991, p. 761–794
28. Deschamps I, Boitard C, Hors J, Busson M, Marcelli-Barge A, Mogenet A, Robert JJ: Life table analysis of the risk of type I (insulin-dependent) diabetes mellitus in siblings according to islet cell antibodies and HLA markers. *Diabetologia* 35:951–957, 1992
29. Huang W, She J-X, Muir A, Laskowska D, Zorovich B, Schatz D, Maclaren NK: High risk HLA-DR/DQ genotypes for IDD confer susceptibility to autoantibodies but DQB1*0602 does not prevent them. *J Autoimmun* 7:889–897,1994
30. Baekkeskov S, Aanstoot H, Christgau S, Reetz A, Solimena MS, Cascalho M, Folli F, Richter-Olsen H, De Camilli P: Identification of the 64 k autoantigen in insulin dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151–156, 1990
31. Pugliese A, Gianani R, Alper CA, Awdeh Z, Jackson RA, Gordon MF, Kaufman M, Pfeiffer F, Eisenbarth GS: Class II determinants of restricted islet cell antibodies (stiff-man syndrome like anti-GAD ICA) and protection from type I diabetes (Abstract). *Clin Res* 40:299A, 1992
32. Gianani R, Pugliese A, Bonner-Weir S, Shiffrin AJ, Soeldner JS, Erlich H, Awdeh ZL, Alper CA, Jackson RA, Eisenbarth GS: Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type I diabetes. *Diabetes* 41:347–353, 1992
33. Gianani R, Jackson R, Eisenbarth GS: Evidence that the autoantigen of restricted ICA is GAD (Abstract). *Diabetes Res Clin Pract* 14(Suppl. 1):S13, 1991
34. Genovese S, Bonifacio E, McNally JM, Dean BM, Wagner R, Bosi E, Gale EAM, Bottazzo GF: Distinct cytoplasmic islet cell antibodies with different risks for type I (insulin-dependent) diabetes mellitus. *Diabetologia* 35:385–388, 1992
35. Solimena M, Folli F, Aparisi R, Pozza G, DeCamilli P: Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med* 322:1555–1560, 1990
36. McEvoy KM: Stiff-man syndrome. *Mayo Clin Proc* 66:300–304, 1991
37. Pugliese A, Solimena M, Awdeh ZL, Alper CA, Bugawan T, Erlich HA, De Camilli P, Eisenbarth GS: Association of HLA-DQB1*0201 with stiff-man syndrome. *J Clin Endocrinol Metab* 77:1550–1553, 1993
38. Pugliese A, Gianani R, Eisenbarth GS, De Camilli P, Solimena M: Genetics of susceptibility and resistance to insulin-dependent diabetes in stiff-man syndrome. *Lancet* 344:1027–1028, 1994
39. Mijovic CH, Todd JA, Barnett AH: Trans-racial-gene mapping studies. *Clin Endocrinol Metab* 5:321–340, 1991
40. Harrison LC, Honeyman MC, DeAizpurua HJ, Schimidli RS, Colman PG, Tait BD, Cram DS: Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. *Lancet* 341:1365–1369, 1993