Antibodies to glutamic acid decarboxylase are associated with HLA-DR genotypes in both Australians and Asians with Type 1 (insulin-dependent) diabetes mellitus

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Summary. Antibodies to glutamic acid decarboxylase, previously known as the 64 kD antigen, appear to be more predictive of Type 1 (insulin-dependent) diabetes mellitus in Caucasoids than other autoantibodies to islet cell antigens. However, seropositivity to glutamic acid decarboxylase is not universal at the onset of Type 1 diabetes and the prevalence in Asians is low compared to Caucasoid patients. This suggests the involvement of multiple pancreatic autoantigens in the Type 1 diabetes autoimmune process or, genetic differences within and between ethnic groups that contribute to the heterogeneous autoimmune response to glutamic acid decarboxylase or both. Alternatively some cases of Type 1 diabetes could have an aetiology unrelated to autoimmunity. This study examined the differential response to glutamic acid decarboxylase according to HLA-DR and -DQ genotypes, as determined by RFLP, in 49 white Australian and 44 Asian patients with Type 1 diabetes. Among Australians heterozygous for HLA-DR3, DR4, 85 % were positive for antibodies to glutamic acid decarboxylase, significantly dif-

ferent ($p = 0.039$) from the prevalence of 48 % in patients with at least one HLA-DR antigen other than DR3 or DR4. Also, among Australians, the presence of "low risk" HLA-DQ antigens, namely DQw5, DQw6 or DQw7, reduced the prevalence of antibodies to glutamic acid decarboxylase by 40 % ($p = 0.064$). Among Asians with Type 1 diabetes and with antibodies to glutamic acid decarboxylase, HLA-DR9 was significantly ($p = 0.037$) increased in frequency, at 63 % compared with 22 % in those without glutamic acid decarboxylase antibodies, and the presence of a "low risk" HLA-DQ allele reduced the antibody rates by 87 % ($p = 0.003$). These observations may reflect differential genetic/environmental interactions in Type 1 diabetes or differential persistence of glutamic acid decarboxylase antibodies in those with different genetic backgrounds.

Key words: Glutamic acid decarboxylase, Type 1 (insulin-dependent) diabetes mellitus, HLA-DR, HLA-DQ, Hong Kong, Korea, Japan

Autoantibodies to islet cell antigens, including insulin [1], cytoplasm [2] and glutamic acid decarboxylase (GAD) [3] have been described in patients with Type 1 (insulin-dependent) diabetes mellitus. GAD autoantibodies are present in most but not all newly-diagnosed Type 1 diabetic patients [4]. Recent attention has focussed on the possibility that autoantibodies to GAD, formerly known as the 64 kD islet cell antigen, would prove to be an early indicator of Type 1 diabetes. These autoantibodies appear to be even more predictive for diabetes than insulin or islet cell cytoplasmic antibodies [5].

Patients with GAD antibodies may also have a T-cell response to GAD [6], suggesting that autoimmunity to GAD has a central role in the onset of Type 1 diabetes, possibly through a mechanism of molecular mimicry [7]. Alternatively, antibodies to GAD may be secondary to destruction of the Beta-cell islets of the pancreas, as a consequence rather than a cause of the disease, although the

high frequency of antibodies to GAD relative to other potential pancreatic islet cell constituents makes this unlikely. Whether seropositivity to GAD is central or secondary to Type 1 diabetes, there are two unanswered questions: why do some patients with Type 1 diabetes show no evidence of autoimmunity to the GAD autoantigen at disease onset? [4] and why are there marked differences in the prevalence of GAD antibodies in Caucasoid Type 1 diabetic patients (63 %) compared with various Asian populations (5–31 %)? (P. Zimmet, unpublished observations).

Seropositivity to GAD in some patients, but not in others, could suggest that more than one pancreatic autoantigen is involved in the autoimmune process, such as the 65 kD or 67 kD isoforms of GAD [6]. However, even with a preparation of GAD that contains both isoforms, seropositivity by our present assay is no more than 70 % [4]. Alternatively, or additionally, genetic differences between

Table 1. Age at onset, duration of disease, and frequency of islet cell antibodies (ICA) and antibodies to glutamic acid decarboxylase (GAD) in Type 1 (insulin-dependent) diabetic subjects from the various ethnic groups studied

Ethnic group	No.	Age at onset (years) Median (range)	Disease duration (years) Median (range)	Antibodies to	
				ICA No. (%)	GAD No. (%)
Australian	49	22 (3–56)	13 (<1–42)	7 (14)	31 (63)
Cantonese	12	22 (4–36)	4 (<1–22)	0	4 (33)
Korean	19	24 (16–37)	4 (<1–16)	0	1 (5)
Japanese	13	24 (20–51)	5 (3–11)	2 (15)	3 (23)

individuals and between different ethnic groups could contribute to the heterogeneous autoimmune response to GAD.

In Caucasoids, HLA-DR3 and -DR4 [8] and their associated alleles HLA-DQw2 and -DQw8 [9], confer a particular risk for susceptibility to Type 1 diabetes, whereas in Chinese the high risk alleles are HLA-DR3 (DQw2) and -DR9 (DQw9) [10]. In Japanese, in whom HLA-DR3 is virtually absent, the high risk Type 1 diabetes alleles are HLA-DR4 (DQw4) and -DR9 (DQw9) [11]. The low incidence of Type 1 diabetes in Asian populations of about 0.5 cases per 100,000 per annum [12] compared with an excess of 30 cases per 100,000 per annum in Finland [13] may be attributable, at least in part, to the very low frequencies of HLA haplotypes DR3, DQw2 and DR4(Dw4), DQw8 in Asians [14].

This study has examined the HLA-DR and -DQ antigen associations with seropositivity to GAD in white Australian and Asian patients with Type 1 diabetes, to determine whether genetic factors contribute to differential autoimmune response to GAD within and between different ethnic groups.

Subjects and methods

Patients

The 49 white Australian patients with Type 1 diabetes attended the International Diabetes Institute, Caulfield, Victoria. Their median age of onset was 22 years, with median duration of diabetes of 13 years (Table 1). The 44 Type 1 diabetic Asian patients comprised 12 Hong Kong Cantonese, 19 Koreans and 13 Japanese; all were participants in the Third Asia-Oceania Histocompatibility Workshop [15]. Their median age of onset was 24.5 years with median duration of diabetes of 4 years (Table 1). All patients were unrelated and met the criteria for classification of insulin-dependent diabetes given by the National Diabetes Data Group [16]. Thus, the patients with Type 1 diabetes were non-obese and presented with symptoms such as polydipsia, polyuria and weight-loss; most patients had diabetes onset before 40 years of age, and all required daily insulin injections to control blood glucose concentrations.

Laboratory Methods

GAD antibody immunoprecipitation assay: soluble porcine GAD was purified from fresh pig brain and prepared for the GAD enzyme assay as previously described [4]. The purified and enzymatically active GAD showed both isoforms (67,000 and 65,000 M_r) by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This was confirmed by Western blot analysis with a GAD-6 antibody which reacts with GAD₆₅ (W. Knowles, unpublished observations) and by direct protein sequence analysis. For immunoprecipitation,

40 µl of ¹²⁵I-GAD (50,000 cpm) was added to 25 µl of test plasma diluted 1:2 in cold wash buffer (20 mmol/l Tris pH 7.4, 150 mmol/l NaCl, Triton X-100 0.5 % weight/volume). Samples were incubated overnight at 4 °C, further incubated with 50 µl of 50 % protein A-Sepharose (1 h, 4 °C), then centrifuged. The precipitate was washed three times in 750 µl of wash buffer, and radioactivity counted. A positive standard serum defined to contain 100 units of antibody was included in every assay, and the activity of test sera was expressed as a percentage of the counts precipitated by the reference serum. Sera from patients and controls for each ethnic group were tested in the same assay, and sera were classified as positive for antibodies to GAD when the reactivity was more than 3 SD above the mean reactivity of the control group.

Islet cell antibody tests: islet cell antibodies (ICA) were assessed by indirect immunofluorescence on cryostat sections of pancreas from a blood group 0 donor as previously described [17]. Results were expressed in Juvenile Diabetes Foundation (JDF) units, and a positive result was defined as 5 JDF U or above.

HLA typing: HLA-DR and -DQ types were assigned according to RFLP, as previously described [18]. DRB/TaqI RFLP discriminate the serologically-defined HLA-DR antigens. HLA-DQ types were assigned according to DQA/TaqI, DQB/TaqI and DQB/BamHI RFLP, which can discriminate the HLA-DQw1 subtypes, DQw5 and DQw6, and the HLA-DQw3 subtypes, DQw7 and DQw8.

The distributions of HLA-DR RFLP types in the Type 1 diabetic patients have been compared previously [15, 18] with those in healthy control subjects, matched for each ethnic group. In the Australian patients there was a significant increase in the antigen frequency of HLA-DR3 and -DR4 [18], while in Cantonese patients there was a significant increase in HLA-DR3. In the small Korean and Japanese series, the only significant difference in HLA-DR RFLPs between patients and controls was an increase in HLA-DRw13 RFLP in Japanese patients [15].

Statistical analysis

Differences in HLA antigen distributions and in the proportions of patients positive for antibodies to GAD were tested by Fisher's exact test. On the basis of an a priori assumption that HLA antigens that confer a high risk for Type 1 diabetes are likely to be associated with the presence of autoantibodies, one-tailed as well as two-tailed *p* values were calculated, but two-tailed *p* values are given here unless otherwise indicated.

Results

The frequencies of antibodies to GAD, and ICA, are shown in Table 1 for each ethnic group. Antibodies to GAD were present in 31 (63 %) of the Australians with Type 1 diabetes, in four (33 %) of the Hong Kong Cantonese, one (5 %) of the Koreans, and three (23 %) of the Japanese. ICA were present in seven (14 %) of the Australians, and two (15 %) of the Japanese, but were not found in

Fig. 1. Concentrations of antibodies to glutamic acid decarboxylase (GAD) according to time since diagnosis of Type 1 (insulin-dependent) diabetes in Australian (●) and Asian (○) patients

the Hong Kong Cantonese or the Koreans. With one exception in the Australian series, each of the patients who had ICA also had antibodies to GAD. The median level of antibodies to GAD for the positive sera was 60 (range 23–137) units for the Australians, and 76 (range 21–138) units for the Asians. Neither the levels of antibodies to GAD, nor the frequency of positive reactions could be related to the duration of disease (Fig. 1) or to sex.

HLA-DR antigen distributions are given in Table 2 for Australian and Asian Type 1 diabetic patients according to the presence or absence of circulating GAD antibodies. In Australian patients with detectable GAD antibodies, there was a slight increase in frequency of HLA-DR4, compensated mainly by a decrease in HLA-DR11. These differences were not statistically significant. In Asian pa-

tients with GAD antibodies, HLA-DR9 was significantly ($p = 0.037$) increased in frequency, at 62.5 % compared with 22.2 % in those without GAD antibodies. The increase in HLA-DR9 frequency was compensated by a decrease in all HLA-DR antigens except DR3 and DR4. This observation is not a consequence of population stratification arising from pooling data from Japan, Korea and Hong Kong, because the frequencies of HLA-DR9 in Type 1 diabetic patients in the three series were similar, at 38 %, 26 % and 25 % respectively.

Table 3 gives the prevalence of GAD antibodies according to HLA-DR genotypes in the Australian patients. When the patients were stratified according to age of onset of diabetes (Table 3), the prevalence of antibodies to GAD in those with onset after 20 years of age was 62.1 %, compared with 65.0 % in those with diabetes onset at 20 years or younger. An association between HLA-DR genotype and antibodies to GAD was observed in those with onset older than 20 years of age ($p = 0.048$); those with at least one antigen other than DR3 or DR4 had a prevalence of 36.4 % compared with 77.8 % in those with DR genotypes (DR3,4; DR4,4; DR3,3) known to confer particular susceptibility to Type 1 diabetes. The same association was observed in the total sample ($p = 0.043$), where patients with at least one antigen other than DR3 or DR4 had a prevalence of 47.8 % for antibodies to GAD compared with 76.9 % in those who did have high-risk HLA-DR genotypes. A similar trend was seen in those with age of onset at 20 years or younger. In the total sample, the highest prevalence of antibodies to GAD was observed in those heterozygous for DR3 and DR4 (84.6 %), significantly higher ($p = 0.039$) than the prevalence (47.8 %) in those with at least one low-risk HLA-DR antigen.

Eight Australian patients had duration of Type 1 diabetes of less than four years. When these eight patients, seven of whom had antibodies to GAD, were excluded from the analysis, the observed association between HLA-

Table 2. HLA-DR antigen frequencies in glutamic acid decarboxylase (GAD) antibody positive and negative Type 1 (insulin-dependent) diabetic patients from Australia and Asia

HLA-DR RFLP	Australian patients				Asian patients			
	GAD antibodies				GAD antibodies			
	Present		Absent		Present		Absent	
	No.	%	No.	%	No.	%	No.	%
<i>n</i>	31		18		8		36	
DR1	3	9.7	3	16.7	0	0.0	5	13.9
DR15	1	3.2	1	5.6	0	0.0	4	11.1
DR16	1	3.2	0	0.0	1	12.5	3	8.3
DR3	19	61.3	9	50.0	2	25.0	2	5.6
DR4	20	64.5	9	50.0	3	37.5	15	41.7
DR11	1	3.2	4	22.2	1	12.5	3	8.3
DR12	0	0.0	0	0.0	0	0.0	8	22.2
DR13	1	3.2	1	5.6	1	12.5	8	22.2
DR14	0	0.0	2	11.1	0	0.0	3	8.3
DR7	4	12.9	1	5.6	0	0.0	2	5.6
DR8	2	6.5	2	11.1	1	12.5	5	13.9
DR9	1	3.2	0	0.0	5	62.5 ^a	8	22.2
DR10	0	0.0	0	0.0	0	0.0	1	2.8

^a $p = 0.037$, when compared with the frequency of DR9 in Asian patients without GAD antibodies

Table 3. Prevalence of glutamic acid decarboxylase (GAD) antibodies according to age of onset and HLA-DR genotype in Australian Type 1 (insulin-dependent) diabetic patients

HLA-DR genotype	Age of onset						Total					
	< 20 years			> 20 years								
	No. tested		GAD antibody positive	No. tested		GAD antibody positive	No. tested		GAD antibody positive	No. tested		GAD antibody positive
	No.	%		No.	%		No.	%		No.	%	
DR3, DR4	4	4	100.0	9	7	77.8	13	11	84.6 ^c	75.0	4	80.0
DR4, DR4	1	1	100.0	4	3	75.0	5	4	80.0		5	62.5
DR3, DR3	3	1	33.3	5	4	80.0	8	5	62.5		5	45.5
DR4, DRX ^a	4	2	50.0	7	3	42.9	11	5	45.5	58.0	3	42.9
DR3, DRX	4	2	50.0	3	1	33.3	7	3	42.9		3	60.0
DRX, DRX	4	3	75.0	1	0	0.0	5	3	60.0		3	63.3
Total	20	13	65.0	29	18	62.1	49	31	63.3			

^a X is an HLA-DR antigen other than DR3 or DR4^b $p = 0.048$, when compared with the prevalence (36.4%) in patients with a non-DR3 or non-DR4 antigen, in those with age of onset > 20 years^c $p = 0.039$ and ^d $p = 0.043$, when compared with the prevalence (47.8%) in patients with a non-DR3 or non-DR4 antigen, in the total Australian series**Table 4.** Prevalence of glutamic acid decarboxylase (GAD) antibodies according to HLA-DR genotype in Asian Type 1 (insulin-dependent) diabetic patients

HLA-DR genotype	No. tested	GAD antibody positive	
		No.	%
DR3, DR9	1	1	100.0
DR3, DR3 or X ^a	3	1	33.3
DR9, DRX	12	4	33.3
DRX, DRX	28	2	7.1 ^b
Total	44	8	18.2

^a X is an HLA-DR antigen other than DR3 or DR9^b $p = 0.019$ when compared with the prevalence (37.5%) in Asian Type 1 diabetic patients with a DR3 or DR9 antigen**Table 5.** HLA-DQ antigen frequencies in glutamic acid decarboxylase (GAD) antibody positive and negative Type 1 (insulin-dependent) diabetic patients from Australia and Asia

HLA-DQB RFLP	Australian patients				Asian patients			
	GAD antibodies				GAD antibodies			
	Present		Absent		Present		Absent	
	No.	%	No.	%	No.	%	No.	%
<i>n</i>	31		18		8		36	
DQw2	23	74.2	10	55.6	2	25.0	4	11.1
DQw5	4	12.9	5	27.8	1	12.5	12	33.3
DQw6	2	6.5	2	11.1	2	25.0	16	44.4
DQw7	2	6.5	4	22.2	1	12.5	11	30.6
DQw4,8	21	67.7	11	61.1	3	37.5	15	41.7
DQw9	1	3.2	0	0.0	5	62.5 ^a	8	22.2

^a $p = 0.037$, when compared with the frequency of DQw9 in Asian patients without GAD antibodies

DR genotype and GAD antibodies was unchanged. Of the 22 patients with high risk genotypes (DR3,4; DR4,4; DR3,3), 72.7% had antibodies to GAD, compared with 42.1% in 19 patients with at least one antigen other than DR3 or DR4, with the marginal statistical significance ($p = 0.062$) a consequence of reduced sample size.

Table 4 gives the prevalence of GAD antibodies according to HLA-DR genotypes known to confer particular susceptibility to Type 1 diabetes in Asians. The small number of Asian patients with antibodies to GAD precluded strati-

fication of the sample by age of onset or duration of diabetes or by ethnic group. In Asian patients positive for HLA-DR3 or DR9 or both, 37.5% had GAD antibodies, a significantly higher ($p = 0.019$) prevalence than that of 7.1% observed in patients who lacked HLA-DR3 or DR9, as shown in Table 4. Among the three Asian study groups, HLA-DR3 is confined to Hong Kong Cantonese, but HLA-DR9 is considered a universal high risk specificity for Type 1 diabetes in Asians [15]. When the association between HLA-DR9 alone and antibodies to GAD was examined, the association remained significant ($p = 0.037$) as noted in the statistical analysis of data in Table 1.

HLA-DQ distributions are given in Table 5 for Australian and Asian Type 1 diabetic patients according to GAD antibody status. HLA-DQw4, 8 and 9 cannot be discriminated by DQB/TaqI and DQB/BamHI RFLP and HLA-DQw9 has been assigned on the basis of its known association with HLA-DR9 [19]. In Australians, there was a non-significant trend towards a higher rate of GAD antibodies in HLA-DQw2 positive patients; this was compensated by slightly lower rates in patients positive for HLA-DQw5, 6 or 7. In Asians, HLA-DQw9, as for HLA-DR9, was associated with a significantly ($p = 0.037$) higher prevalence of antibodies to GAD.

Certain HLA-DQ antigens are known to confer a reduced risk for Type 1 diabetes. HLA-DQw6 and DQw7 are considered protective against development of the disease, while HLA-DQw5, although "permissive" for Type 1 diabetes, does not carry the same high risks as HLA-DQw2 and -DQw8 in Caucasoids, and as HLA-DQw4 and -DQw9 in Asians [20]. Table 6 gives the prevalence of GAD antibodies in Australian Type 1 diabetes patients according to the presence or absence of the "low risk" alleles, HLA-DQw5, DQw6 and DQw7. There were marked differences between the two groups. In the Australian series, the prevalence of antibodies to GAD was higher (74.2%) in patients who lacked HLA-DQw5, DQw6 and DQw7 compared with 44.4% in those who had at least one "low-risk" HLA-DQ antigen ($p = 0.064$). The association was not a function of age of onset, as shown in Table 6.

Once again, exclusion of Type 1 diabetic patients with recent (less than four years) onset from the analysis did

Table 6. Prevalence of glutamic acid decarboxylase (GAD) antibodies according to age of onset and presence or absence of "low risk" HLA-DQ antigens in Australian Type 1 (insulin-dependent) diabetic patients

HLA-DQ DQw5, 6, 7 antigen	Age of onset						Total		
	< 20 years			> 20 years					
	No. tested	GAD Antibody positive		No. tested	GAD Antibody positive		No. tested	GAD Antibody positive	
		No.	%		No.	%		No.	%
Present	9	4	44.4	9	4	44.4	18	8	44.4 ^a
Absent	11	9	81.8	20	14	70.0	31	23	74.2
Total	20	13	65.0	29	18	62.1	49	31	63.3

^a One-tailed $p = 0.038$, two-tailed $p = 0.064$, when compared with the prevalence of antibodies to GAD in Type 1 diabetic patients lacking DQw5, 6 or 7

Table 7. Prevalence of glutamic acid decarboxylase (GAD) antibodies according to presence or absence of "low risk" HLA-DQ antigens in Asian Type 1 (insulin-dependent) diabetic patients

HLA-DQ DQw5,6,7 antigen	No. tested	GAD antibody positive	
		No.	%
Present	32	2	6.3 ^a
Absent	12	6	50.0
Total	44	8	18.2

^a One-tailed $p = 0.003$, two-tailed $p = 0.003$, when compared with the prevalence of antibodies to GAD in Type 1 diabetic patients lacking DQw5, 6 or 7

not alter the prevalence of GAD antibodies in the genotypic categories. When the eight recent-onset patients were excluded, among 16 patients positive for DQw5, DQw6 or DQw7, 43.8% had antibodies to GAD, compared with 68.0% in 25 patients lacking these HLA-DQ antigens. The comparison did not reach statistical significance ($p = 0.195$) in this smaller sample.

The association between prevalence of antibodies to GAD and HLA-DQ genotype in Australian patients was observed also in Asian Type 1 diabetic patients (Table 7). In patients positive for low risk antigens (DQw5, DQw6 or DQw7), 6.3% had GAD antibodies compared with 50.0% in those lacking these HLA-DQ antigens. The association was highly significant ($p = 0.003$).

Discussion

The levels and prevalence of ICA [21] and lymphocytotoxic antibodies [22] fall rapidly with increasing duration of Type 1 diabetes, but this is not seen with GAD antibodies [4, 23]. Thus, even though the incidence of Type 1 diabetes is low in Asians [13], as is the prevalence of GAD antibodies, it was possible to ascertain an adequate (albeit small) number of subjects with Type 1 diabetes and antibodies to GAD to demonstrate an association between HLA-DR9 (DQw9) and anti-GAD, noting that statistical significance can be attained in a small sample if the differences are marked.

The small number of seropositive Asian patients with Type 1 diabetes might be seen as a limitation to our study, but the Asian patients do serve as a useful, independent validation of the associations between HLA-DR,DQ al-

leles and antibodies to GAD seen in the Australian sample. Among Australians, there were very few patients who did not have at least one of the "high risk" combinations, DR3,DQw2 or DR4,DQw8, so the prevalence of antibodies to GAD in the non-DR3, non-DR4 group would be essentially untested. However, even one of the "low risk" antigens HLA-DQw5 (DR1, DRw10, DRw14, DRw16), DQw6 (DR2, DRw13) or DQw7 (DRw11, DRw12, some DR4) was sufficient to reduce the prevalence of GAD antibodies by as much as 40% in Australian and by 87% in Asian patients. Whether this suggests that certain HLA class II antigens protect against the rapidity and severity of disease onset remains to be tested in patients with disease of recent onset. It should be noted that patients included in the present study had an unusually late age of onset of Type 1 diabetes, and were studied well after their disease onset.

HLA specificities have been correlated with various measures of autoimmunity in Type 1 diabetes in other studies. HLA-B8, which is in linkage disequilibrium with HLA-DR3, is increased in frequency in diabetic patients with lymphocytotoxic antibodies [22] and with ICA [24], particularly in those with antibodies persisting for more than 5 years [25]. In middle-aged Caucasoid patients with slow-onset diabetes who are often considered Type 2 (non-insulin-dependent) but become insulin-requiring, complement-fixing ICA are associated with HLA-DR3, DR4 heterozygosity [26, 27]. In Japanese subjects with Type 1 diabetes, HLA-DR9 has been found at a significantly higher frequency in those with autoantibodies other than ICA [28].

Prevention of Type 1 diabetes will be dependent on the availability of tests to screen for pre-symptomatic disease before the Beta cells of the pancreatic islets are irreversibly destroyed [29]. Our study includes Type 1 diabetes of varying duration, but does suggest that Asians negative for HLA-DR9, DQw9 will rarely give positive results for anti-GAD in pre-clinical or clinical stages of Type 1 diabetes. This needs to be more rigorously tested in recent-onset Asian Type 1 diabetic patients. In contrast, in Caucasoids among whom nearly all DR3,DR4 and DR4,DR4 patients had antibodies to GAD, the assay has considerable potential. It has been suggested [6] that persistence of antibodies to GAD in patients with long-term Type 1 diabetes may reflect a response to GAD released from chronically damaged nerves. This seems unlikely, given the persistence of anti-GAD in most patients

with Type 1 diabetes and their virtual absence from patients with Type 2 diabetes [4].

In our study, exclusion of the small number of Australian patients with onset of diabetes within the past 3 years did not influence the association between antibodies to GAD and HLA-DR and -DQ genotypes. It appears that it is the persistence of GAD antibodies that correlates with high risk HLA-DR,DQ genotypes, at least in patients with a relatively late age of onset. Alternatively, since Type 1 diabetes may well be a clinically heterogeneous disease, different aetiological factors could operate in those with high risk and low risk HLA-DR,DQ genotypes.

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