HLA-DQβ Typing and Non-Asp^{57} Alleles in IDDM and Nondiabetic Subjects in New Zealand

LOUISA V. FORBES, BSC (HONS)
LAURIE J. BROWN, PHD
RUSSELL S. SCOTT, MD

OBJECTIVE — To determine the frequency of IDDM risk-associated HLA-DQβ alleles in New Zealanders with IDDM and nondiabetic control subjects, and to examine these as susceptibility markers in relation to IDDM incidence.

RESEARCH DESIGN AND METHODS — HLA-DQβ typing was conducted in 55 juvenile-onset IDDM subjects diagnosed between 1990 and 1992, and 53 nondiabetic control subjects. Allele typing was conducted by a polymerase chain reaction–restriction fragment-length polymorphism technique. All subjects were residents of Canterbury, New Zealand. IDDM incidence data were obtained from the Canterbury, New Zealand, Diabetes Registry.

RESULTS — The frequency of the susceptibility genotype DQβ*0201/0302 was 43.6 and 5.7% in the IDDM and control groups, respectively, reflecting the increased prevalence of allele 0302 in the IDDM group. Alleles 0301, 0501, and 0602,3 were more prevalent in the control group than the IDDM group. The frequency of non-Asp^{57} alleles was 90.9 and 61.3% in the IDDM and control groups, respectively. Overall, the HLA-DQβ allele distribution was similar to reports from other Caucasian populations. The 0- to 19-yr age-specific IDDM incidence rate over the period in which the diabetic subjects were diagnosed was 19.5/100,000 person-yr, the highest levels observed in Canterbury over the last decade. Our relatively high background prevalence of non-Asp^{57} alleles and high IDDM incidence rates were similar to results from some Scandinavian and other hotspot populations.

CONCLUSIONS — HLA-DQβ alleles are genetic susceptibility markers in New Zealand, and other Caucasian populations. Peak IDDM incidence levels observed in 1990–1992 in our population are in accordance with a high background population prevalence of non-Asp^{57} alleles. These results suggest that the high Canterbury incidence rates may be due to a large HLA-DQβ non-Asp^{57} at-risk population.

FROM THE LIPID AND DIABETES RESEARCH GROUP, CHRISTCHURCH, NEW ZEALAND.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO RUSSELL S. SCOTT, MD, LIPID AND DIABETES RESEARCH GROUP, SECOND FLOOR, HAGLEY, CHRISTCHURCH HOSPITAL, CHRISTCHURCH, NEW ZEALAND.

RECEIVED FOR PUBLICATION 14 JANUARY 1993 AND ACCEPTED IN REVISED FORM 11 FEBRUARY 1993.

IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; HLA, HUMAN LEUKOCYTE ANTIGEN; PCR, POLYMERASE CHAIN REACTION; RFLP, RESTRICTION FRAGMENT–LENGTH POLYMORPHISM; WHO, WORLD HEALTH ORGANIZATION; OR, ODDS RATIO; CI, CONFIDENCE INTERVAL.

Polymorphism at the HLA-DQ locus of chromosome 6 has been identified as a susceptibility marker for IDDM (1,2). This gene encodes a class II heterodimer, a cell surface molecule on antigen-presenting cells (3). Allelic variation may have a role in determining genetic susceptibility to IDDM by facilitating recognition and presentation of diabetogenic antigens to the immune system.

Studies comparing diabetic patients with control subjects have shown that Asp^{57} of the β-chain of the DQ molecule is associated with protection against IDDM, and conversely non-Asp^{57} encoding HLA-DQβ alleles are associated with predisposition to the disease (4–6). Different HLA-DQβ alleles and genotypes have different risk associations with IDDM (7,8). The allele 0302 and genotype 0201/0302 in particular have the strongest known risk associations with IDDM.

The non-Asp^{57} rule of susceptibility has been demonstrated in several Caucasian populations, including American, Norwegian, Finnish, French, Sardinian, and Algerian cohorts (5,8–13). The non-Asp^{57} rule does not hold in some reports of Japanese populations (14,15). It has been suggested that worldwide differences in population distribution of non-Asp^{57} alleles may contribute to the geographic variation in IDDM incidence (9).

The aim of this study was to determine whether HLA-DQβ alleles are genetic susceptibility markers for IDDM in New Zealanders as they are in other Caucasian populations. We compared the frequencies of risk alleles in a group of IDDM subjects and nondiabetic control subjects. We also examined the relationship between the non-Asp^{57} gene frequency and the observed IDDM incidence rates in Canterbury, New Zealand.

RESEARCH DESIGN AND METHODS

— The diabetic subjects were juvenile-onset IDDM cases diag-

nosed between 1 January 1990 and 31 August 1992 (n = 55). This group included all of the new IDDM cases diagnosed in Canterbury in the 0- to 19-yr age-group (n = 53), plus 1 patient living just outside the Canterbury Diabetes Registry catchment area and another was aged 20 at diagnosis of IDDM. There were 33 boys and 22 girls. Two sisters were in this study group, otherwise the subjects were unrelated.

The nondiabetic control subjects were unrelated healthy volunteers, either employees or students at the Christchurch Hospital. Mean ± SD age at the time of sampling was 33.2 ± 14.3 yr (range 4–68 yr). A random age control group was considered to best represent the background population. There were 29 males and 24 females.

A blood sample for DNA analysis was obtained from IDDM patients either at the time of diagnosis in Christchurch Hospital, or during attendance at the Christchurch Hospital Diabetes Clinic. Consent was obtained from all subjects, and the study was approved by the local ethics authority.

All control subjects were Caucasian. Of the IDDM subjects, 7.3% were nonCaucasian: two sisters were part Polynesian, one was part Melanesian, and one was Chinese. Overall, 90.6% of Canterbury's population (360,000) were European (as sole ethnic classification).

The incidence data were obtained from the Canterbury, New Zealand, Diabetes Registry (16,17). This population-based registry includes demographic data on all individuals who reside in Canterbury and are treated with insulin on a long-term basis for diabetes. New cases of IDDM have been prospectively added to the registry since 1982. Population denominators used to calculate incidence rates were obtained from the 1991 New Zealand national census of population and dwellings.

HLA-DQβ typing

Allelic typing of the HLA-DQβ locus was performed by a PCR and RFLP technique. The method was based on that developed in Pittsburgh (18), with some modifications.

Genomic DNA was extracted by a rapid preparation method (19) modified for EDTA whole blood using NH_4Cl precipitation and lysis of leukocytes. The amplified region was exon 2, the first extracellular domain of the HLA-DQβ gene. The primers for the first PCR (40 μM each) spanned codons 6–13 and 79–86 of the DQβ sequence, and for the second PCR on the amplification product, the primers spanned codons 14–20 and 79–86 (100 μM each). A hotstart PCR technique (20) was used, as well as the two-step procedure, to increase the specificity of amplification. Both of the PCR steps were 40 cycles, with 1 min at each temperature in the denaturation (94°C), annealing (62°C), and extension phases (72°C). Also, extra precautions such as DNase treatment of the PCR premix, use of aerosol-resistant pipette tips, site separation for conducting the different PCR procedures, and repeat controls and test samples for reproducibility checks, were included to eliminate contamination problems.

Allelic assignments were made by digestion of the final PCR product with restriction endonucleases. *Acy* I, *Cfo* I, *Hpa* II (Boehringer Mannheim, Mannheim, Germany) and *Tha* I/*Bst*UI (Bethesda Research Laboratories, Gaithersburg, MD/New England Biolabs, Beverly, MA) enzymes were used. The latter enzyme was used to confirm the assignment of allele 0604,5. In addition, SAU 961 (Boehringer Mannheim) was used to check assignments of the allele 0302. The digest products were run on Nusieve (FMC Bioproducts, Rockland, ME) agarose electrophoresis gels and stained with ethidium bromide. The fragment patterns were photographed with a Polaroid camera.

The series of nucleotide sequences used as the reference for allele assignments was from the WHO-HLA Nomenclature Committee Report 1990 (21,22). Fourteen of the 17 known DQβ alleles can be typed by this method, because three pairs of alleles cannot be distinguished (i.e., 0502 and 0504, 0602 and 0603, and 0604, and 0605). Occasionally, it is not possible to conclusively assign genotypes by the method used because of compromised enzyme digestion results. Where such uncertainty has occurred, samples have been reported as unsolved.

Statistical analysis

Differences in HLA-DQβ frequency were tested by χ^2 test and OR. Cornfield 95% CIs are given for ORs. Two-tailed P values were used for determining statistical significance. The 95% CIs for IDDM incidence rates were calculated from the Poisson distribution.

RESULTS — The frequencies of HLA-DQβ alleles typed in IDDM and control subjects are shown in Table 1. The most notable differences between the two groups were the increased frequency of allele 0302 ($P < 0.001$) and the decreased frequencies of 0301 ($P < 0.01$) and 0602,3 ($P < 0.001$) in the IDDM group compared with the control group.

Table 2 shows the observed genotype frequencies. There was a significantly higher prevalence of genotype 0201/0302 in the IDDM group than in the control group ($P < 0.001$). Also a higher frequency of genotype homozygous 0302 ($P < 0.01$) contributed to the overall increased prevalence of allele 0302 in the IDDM subjects. The IDDM subjects were predominantly typed as homozygous non-Asp^{57}, only 5 subjects were typed with at least one Asp^{57} encoding allele. The control subjects were more evenly distributed between the non-Asp^{57} and Asp^{57} classes. The total non-Asp^{57} gene frequencies were 90.9 and 61.3% for the IDDM and control groups, respectively, the non-Asp gene frequency conferring an OR of 6.7 (ex-

Table 1—*HLA-DQβ allele frequencies in IDDM subjects and nondiabetic control subjects in Canterbury, New Zealand*

HLA-DQβ Allele*	IDDM subjects (n = 55)	Control subjects (n = 53)	P†
0201	58.2	41.5	NS
0301	3.6	22.6	<0.01
0302	63.6	20.8	<0.001
03031	—	1.9	NS
03032	1.8	1.9	NS
0501	10.9	20.8	NS
05031	—	1.9	NS
0602,3	3.6	26.4	<0.001
0604,5	12.7	15.1	NS
Unsolved	1.8	5.7	NS

Frequencies expressed as the percentage of subjects presenting with a given allele.
Of the 17 reference alleles, only those shown were typed in this study.
†Calculated excluding unsolved cases.

cluding unsolved cases) (95% CI 2.8–16.9).

The IDDM incidence rate in Canterbury for IDDM diagnosed before 20 yr of age was 19.5/100,000 person-yr (95% CI 14.7–25.7/100,000 person-yr) between 1 January 1990 and 31 August 1992. This represents the highest incidence period in the Canterbury region over the last decade (17). Presentation of IDDM in Canterbury occurs with significant temporal variation such that incidence peaks and troughs occur, each spanning 2 or 3 yr (17). The average incidence rate over the preceding 8-yr period from 1 January 1982 to 31 December 1989 was 11.7/100,000 (95% CI 9.6–14.3/100,000). The incidence peak noted since 1 January 1990 was comprised of all but 2 of 55 IDDM subjects in this study. Hence, the study IDDM cohort is well-defined and corresponds to a specific incidence period. The high number of cases in 1990–1992 cannot be explained by familial multiplicity or denominator population characteristics, although the proportion of cases <10 yr of age was unusually high.

Figure 1 shows the Canterbury data in relation to results from some other studies. The depicted incidence rates are for the 0- to 14-yr age-group only, the Canterbury data point being 24.0/100,000 person-yr (95% CI 17.6–32.1/100,000 person-yr) for the 1990–1992 period. The non-Asp^{57} gene frequency was that of the nondiabetic control group. This model of IDDM incidence was not concerned with the distribution of non-Asp^{57} alleles in the diabetic population, except in supporting the non-Asp^{57} rule of IDDM susceptibility. The high IDDM incidence observed in the 1990–1992 period in Canterbury was in accordance with the relatively high population non-Asp^{57} gene frequency as proposed by Dorman et al.'s (9) model (Fig. 1). Canterbury's results were similar to those of Norway and Sardinia.

Table 2—*Frequencies of HLA-DQβ genotypes in IDDM subjects and nondiabetic control subjects in Canterbury, New Zealand*

	IDDM subjects		Control subjects	
HLA-DQβ Genotype	n	%	n	%
Homozygous non-Asp^{57}	49	89.1	25	47.1
0201/0201	5	9.1	5	9.4
0201/0302	24	43.6	3	5.7
0201/0501	1	1.8	5	9.4
0302/0302	8	14.5	—	—
0302/0501	1	1.8	—	—
0302/0604,5	2	3.6	2	3.8
0501/0501	3	5.5	4	7.5
0501/0604,5	1	1.8	2	3.8
0604,5/0604,5	4	7.3	4	7.5
Heterozygous non-Asp^{57}, Asp^{57}	2	3.6	15	28.3
0201/0302	2	3.6	4	7.5
0201/0602,3	—	—	5	9.4
0301/0302	—	—	3	5.7
0302/0502,3	—	—	3	5.7
Homozygous Asp^{57}	3	5.5	10	18.9
0301/0301	—	—	2	3.8
0301/03031	—	—	1	1.9
0301/03032	—	—	1	1.9
0301/0602,3	—	—	1	1.9
03032/03032	1	1.8	—	—
05031/0602,3	—	—	1	1.9
0602,3/0602,3	2	3.6	4	7.5
Unsolved	1	1.8	3	5.7
Total	55	99.8	53	100.0

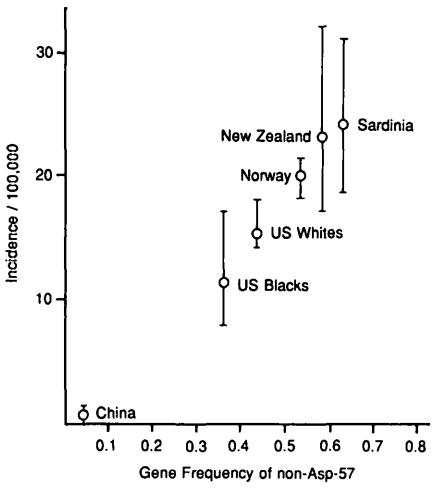

Figure 1—*Gene frequency of non-Asp^{57} in nondiabetic populations and 0- to 14-yr age-specific annual IDDM incidence rates (95% CI). From Dorman et al. (9). © by the Proceedings of the National Academy of Science USA.*

CONCLUSIONS — The results of this study indicate that the HLA-DQβ gene is a susceptibility marker in Canterbury, New Zealand. The increased proportion of the HLA-DQβ susceptibility allele 0302 and genotype 0201/0302 in the IDDM group is in agreement with other studies (8,10–12,23–25). Our results also show the disproportionately low prevalence of so-called nonsusceptibility alleles 0301, 0501, and 0602,3 in the IDDM group. Equally, the overall allele distribution in the nondiabetic control subjects, representing the background population, does not appear dissimilar to profiles of other Caucasian populations (8,10,11,25). Most Caucasian studies report a higher presence of the 0303 and 0503 alleles than our findings, and detect 0401 and 0402, which were not typed in our subjects. However, difficulties arise in comparing different studies because of varied specificities of different allele typing methods and sample size limitations. In summary, our results generally assimilate the susceptibility and nonsusceptibility associations of specific HLA-DQβ alleles and genotypes observed in other Caucasian populations.

Dorman et al. (9) showed that variation in IDDM incidence between five populations is associated with the background gene frequency of non-Asp^{57} HLA-DQβ alleles. Our results, as plotted, also fit the correlation (Fig. 1). The relatively high non-Asp^{57} gene frequency of 0.61 in Canterbury reflects the high proportion of control subjects with homozygous non-Asp^{57} genotypes. Only two other studies, in American (10) and Algerian (13) populations, have shown nondiabetic, non-Asp^{57} homozygosity of similar magnitude, rendering non-Asp^{57} frequencies of 0.58 and 0.57, respectively. All other Caucasian studies report a larger proportion of heterozygous non-Asp^{57}/Asp^{57} genotypes in the control population (5,8,9,11,12). Our results also differ from most, in that the non-Asp^{57}/Asp^{57} frequency in the IDDM cohort is very low. Therefore, a dose-response relationship between IDDM susceptibility and non-Asp^{57} as a genetic marker (9) is strongly implicated in this population. The only non-Asp^{57} allele that appears slightly more prevalent than in other studies is 0604,5. This result is unlikely to be an artifact because the assignment of this allele by our method involves an extra confirmatory enzyme digestion procedure. Hence, the non-Asp^{57} allele frequency in the Canterbury population is high and cannot be easily attributed to overrepresentation of specific alleles.

The scatter of points about the correlation line in Fig. 1 could realistically be quite wide. For example, results from a Finnish study (8) correspond to a non-Asp^{57} gene frequency of 0.47 (lower than Norway's 0.53), despite incidence rates of ~29/100,000 person-yr (9). Indeed, if the Canterbury incidence data were averaged over 1982–1992, the incidence rate would drop below the U.S. Whites rate, to 14.3/100,000 person-yr (95% CI 11.9–17.2/100,000 person-yr). An important assumption within this model is that the nondiabetic control subjects, defining the population non-Asp^{57} gene frequency, are representative of the background gene pool relating to the subjects integral to the IDDM incidence rate. Methodological differences in allele typing may also contribute to some inconsistencies. The Canterbury data for the high incidence period 1990–1992 is consistent with the association between IDDM incidence and population genetic risk observed in other countries.

Several factors may have influenced the peak IDDM incidence period observed in Canterbury, New Zealand, in 1990–1992. The presence of a specific environmental trigger or nonautoimmune etiology could be responsible for epidemic presentation. Alternatively, the high incidence may reflect an anomaly such that the number of genetically susceptible children in the population is unusually high at this time. The lack of other HLA genetic data in the New Zealand population and the random age control groups of this study do not allow this to be examined. Another possible explanation, induced by the results of this study, is that Canterbury has a high genetically at risk population, as defined by HLA-DQβ non-Asp^{57} gene frequency. The 1990–1992 period may reflect the IDDM attack rates reaching full potential as predefined by the background genetic risk of the population.

In conclusion, HLA-DQβ alleles and genotypes are genetic markers of susceptibility to IDDM in Canterbury, New Zealand. The results of this study suggest that the highest IDDM incidence observed in Canterbury may be attributable to a gene-environment interaction where the population gene characteristics primarily determine the upper incidence rate.

Acknowledgments — This study was supported by the R.G. Bell Charitable Trust, The Health Research Council of New Zealand, the Canterbury Medical Research Foundation, the New Zealand Lottery Board, and the National Diabetes Research and Training Centre Trust.

We appreciate the assistance of A. Fellowes and Dr. P. George, Christchurch School of

Medicine, in the development of allele typing technology. We would also like to thank Dr. J. Dorman, University of Pittsburgh.

References

1. Schreuder G, Tilanus M, Bontrop R, Bruining J, Giphart M, Van Rood J, DeVries R: HLA-DQ polymorphism associated with resistance to type 1 diabetes detected by monoclonal antibodies, isoelectric point differences, and restriction fragment length polymorphism. *J Exp Med* 164:938–43, 1986
2. Sterkers G, Zeliszewski D, Chaussee AM, Deschamps I, Font MP, Friedel C, Hors J, Betuel H, Dausset J, Levy JP: HLA-DQ rather than HLA-DR region might be involved in dominant non-susceptibility to diabetes. *Proc Natl Acad Sci USA* 84: 6473–77, 1988
3. Brown JH, Jardetzyk T, Saper MA, Samaouri B, Bjorkman PJ, Wiley DC: A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* (Lond) 332:845–50, 1988
4. Todd JA, Bell JI, McDevitt HO: HLA-DQβ gene contributes to susceptibility and resistance to insulin dependent diabetes mellitus. *Nature* (Lond) 329:599–604, 1987
5. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M: Aspartic acid at position 57 of the HLA-DQβ chain protects against type 1 diabetes: a family study. *Proc Natl Acad Sci USA* 85:8111–15, 1988
6. Ronningen KS, Iwe T, Halstensen TS, Sparkland Z, Thorsby E: The amino acid at position 57 of the HLA-DQβ chain and susceptibility to develop IDDM. *Human Immunol* 26:215–25, 1989
7. Nepom GT: A unified hypothesis for the complex genetics of HLA associations with IDDM. *Diabetes* 39:1153–57, 1990
8. Reijonen H, Ilonen J, Knip M, Akerblom HK: HLA-DQβ alleles and absence of Asp^{57} as susceptibility factors of IDDM in Finland. *Diabetes* 40:1640–44, 1991
9. Dorman JS, LaPorte RE, Stone RA, Trucco M: Worldwide differences in the incidence of type 1 diabetes are associated with amino acid variation at position 57 of the HLA-DQβ chain. *Proc Natl Acad Sci USA* 87:7370–74, 1990
10. 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* 322:1836–41, 1990
11. Ronningen KS, Spurkland A, Iwe T, Vartdal F, Thorsby E: Distribution of HLA-DRB1, -DQA1 and -DQβ1 alleles and DQA1-DQβ1 genotypes among Norwegian patients with insulin dependent diabetes mellitus. *Tissue Antigens* 37:105–111, 1991
12. Vallet-Colom I, Levy-Marchal C, Zarrouk D, Tichet J, Krishnamoorthy R, Czernichow P, Elion J: HLA-DQβ1 codon 57 and genetic susceptibility to Type 1 (insulin-dependent) diabetes mellitus in French children. *Diabetologia* 33:174–76, 1990
13. Fan Gu X, Larger E, Clauser E, Bessaoud K, Krishnamoorthy R, Elion J, Assan R: HLA-DQA1 and DQβ1 alleles in French and Algerian Type 1 diabetic subjects. *Diabetes Res* 17:189–97, 1991
14. Todd JA, Fukui Y, Kitagawa T, Sasazuki T: The A3 allele of the HLA-DQA1 locus is associated with susceptibility to type 1 diabetes in Japanese. *Proc Natl Acad Sci USA* 87:1094–98, 1990
15. Awata T, Kuzuya T, Matsuda A, Iwamoto Y, Kanazawa Y, Okuyama M, Juji T: High frequency of aspartic acid at position 57 of HLA-DQβ chain in Japanese IDDM patients and nondiabetic subjects. *Diabetes* 39:266–69, 1990
16. Scott RS, Brown LJ: Prevalence and incidence of insulin-treated diabetes mellitus in adults in Canterbury, New Zealand. *Diabetic Med* 8:443–47, 1991
17. Scott RS, Brown LJ, Darlow BA, Forbes LV, Moore MP: Temporal variation in incidence of IDDM in Canterbury, New Zealand. *Diabetes Care* 15:895–99, 1992
18. Trucco G, Fritsch R, Giorda R, Trucco M: Rapid detection of IDDM susceptibility with HLA-DQβ alleles as markers. *Diabetes* 38:1617–22, 1989
19. Lench N, Stanier P, Williamson R: Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 18:1356–58, 1988
20. Erlich HA, Gelfand D, Sninsky JF: Recent advances in the polymerase chain reaction. *Science* 252:1643–51, 1989
21. WHO-HLA Nomenclature Committee: Nomenclature for factors of the HLA system. *Tissue Antigens* 37:97–104, 1991
22. Marsh SGE, Bodmer JG: HLA class II nucleotide sequences. *Tissue Antigens* 37: 181–89, 1991
23. Owerbach D, Gunn S, Gabbay KH: Primary association of HLA-DQw8 with type 1 diabetes in DR4 patients. *Diabetes* 38:942–45, 1989
24. Sheehy MJ, Scharf SJ, Rowe JR, de Gimenez MH, Meske LM, Erlich HA, Nepom BS: A diabetes susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. *J Clin Invest* 83:830–35, 1989
25. Khalil I, Deschamps I, Lepage V, Al-Daccak R, Degos L, Hors J: Dose effects of cis- and trans-encoded HLA-DQAβ heterodimers in IDDM susceptibility. *Diabetes* 41:378–84, 1992