HLA-DQ Associations Distinguish Insulin-Resistant and Insulin-Sensitive Variants of NIDDM in Black Americans

MARY ANN BANERJI, MD
ALLEN J. NORIN, PHD
ROCHELLE L. CHAIKEN, MD
HAROLD E. LEBOVITZ, MD

OBJECTIVE — NIDDM in black Americans exists as two variants: one with a primary defect in insulin action (insulin-resistant variant) and the other with normal insulin action and a primary defect in insulin secretion (insulin-sensitive variant). The objective of this study was to determine whether these two variants were genetically distinct from each other and from normal control subjects as determined by HLA typing.

RESEARCH DESIGN AND METHODS — Insulin action was measured with the euglycemic insulin clamp with a 1 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin infusion with [3-^{3}H]glucose. A glucose disposal of <278 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ was considered insulin resistant, and a value greater than this was considered insulin sensitive. The study population consisted of 21 insulin-resistant and 25 insulin-sensitive black NIDDM patients and 89 normal, nondiabetic black control subjects from an urban hospital. HLA typing was performed with serological methods.

RESULTS — The frequency of HLA-DQW7 in the insulin-resistant population (76%) was significantly greater than that in the insulin-sensitive population (32%, corrected $P < 0.018$) and the normal control population (21%, corrected $P < 0.001$). The frequency of HLA-DQW6 was increased in the insulin-sensitive population (76%), corrected $P < 0.023$, as compared with the normal control subjects (33%). The relative risk of HLA-DQW7 in identifying insulin-resistant NIDDM patients compared with control subjects was 7.

CONCLUSIONS — At least one component that differentiates insulin-resistant and insulin-sensitive NIDDM in black Americans is under different genetic control. One or more loci responsible for insulin-resistant and insulin-sensitive NIDDM are likely to be in linkage disequilibrium with the DQ locus of the human MHC region of chromosome 6.

FROM THE DIVISIONS OF ENDOCRINOLOGY AND PULMONARY MEDICINE AND ANATOMY & CELL BIOLOGY, DEPARTMENT OF MEDICINE, SUNY HEALTH SCIENCE CENTER AT BROOKLYN, NEW YORK.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO M. BANERJI, MD, DIVISION OF ENDOCRINOLOGY, BOX 1205, SUNY HSC AT BROOKLYN, 450 CLARKSON AVENUE, BROOKLYN, NY 11203.

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HLA, HUMAN LEUKOCYTE ANTIGEN; NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; MHC, MAJOR HISTOCOMPATIBILITY COMPLEX; BMI, BODY MASS INDEX; GF, GENE FREQUENCY; AF, ANTIGEN FREQUENCY; FPG, FASTING PLASMA GLUCOSE.

NIDDM has a major genetic basis. Because NIDDM is a heterogenous disorder (1,2), the identification of specific genetic abnormalities is unlikely to be successful unless the specific subtypes of the disease can be recognized and appropriately analyzed. Indeed, studies of genes for insulin, its receptor, and glucose transporters in patients grouped as NIDDM have not identified any consistent abnormality (3).

Whether the primary defect in NIDDM is insulin resistance or impaired insulin secretion continues to be controversial (4,5). We have described that among black Americans, NIDDM exists as two variants (6). One variant has insulin resistance as its primary abnormality, and the other has normal insulin action with deficient insulin secretion (7–10). These two variants seemed unique and homogeneous enough to warrant a search for associated genetic abnormalities. We report our studies showing associations of HLA-DQw loci with insulin-resistant and insulin-sensitive NIDDM subtypes in black Americans.

RESEARCH DESIGN AND METHODS

The patient population was from the State University of New York (SUNY) Health Science Center and Kings County Hospital in Brooklyn and consisted of 46 black NIDDM patients and 89 black normal individuals without a personal or family history of diabetes mellitus. Informed consent was obtained from all subjects.

NIDDM was diagnosed on the basis of clinical characteristics, magnitude of residual insulin or C-peptide secretory responses, and lack of circulating anti-islet antibodies. Studies on many of these patients have been reported (10). Six patients were being treated with insulin, six with oral hypoglycemic agents, and the rest with diet alone. The patients were of normal weight or mildly obese with a BMI of 19.6–31.1 kg/m^2 and with only two exceeding 30 kg/m^2. Overall

glycemic control was good with measured mean GHb values of 15% above normal (mean ± SD of measured GHb value/upper limit of normal value for method 1.15 ± 0.34).

Euglycemic insulin clamp technique

Peripheral insulin action was measured with the euglycemic insulin (1.0 mU · kg^{-1} · min^{-1}) clamp technique with a constant [3-^{3}H]D-glucose infusion as described previously (6). Studies were performed at euglycemia (plasma glucose 5.5 mM) after an overnight fast at the clinical research center.

HLA typing

HLA phenotyping for class I and class II antigens were performed on T-cells and B-cells, respectively, obtained from 40 ml of peripheral blood and isolated by standard methods using Ficoll-Hypaque and nylon wool columns (11). Commercial typing trays were purchased from One Lambda (Los Angeles, CA) and Gentrak (Plymouth, PA). A standard eosin dye exclusion microcytotoxicity assay was used to test for serologically determined antigens (12). A minimum of two different positive sera were required for assigning an HLA antigen specificity. DQW6 and DQW5 are splits of DQW1; DQW7, DQW8, DQW9 are splits of DQW3. Individuals who were positive for DQW6 and DQW1 were assigned as DQW6; individuals positive for DQW1 but not for DQW6 were assigned DQW1 because sera for DQW5 were not available. Individuals assigned as DQW7 were positive for both DQW3 and DQW7, whereas those assigned as DQW3 were positive for DQW3 but not for DQW7. Sera for DQW8 and DQW9 were not available. DRw15 and DRw16 are splits of DR2; DRw13 and DRw14 are splits of DRw6. Subjects who were positive for DRw15 and DR2 were assigned DRw15. Because we had no sera for DRw16, we retained the parent antigen of DR2 for individuals who were positive for DR2 and negative for DRw15. Subjects who were positive for DRw13 and DRw6 were assigned DRw13. Subjects who were positive for DRw6 and negative for DRw13 were assigned the parent antigen of DRw6 because sera for DRW14 were unavailable.

Statistical analysis

Differences in HLA allele frequencies between normal control subjects and either the insulin-resistant or insulin-sensitive NIDDM populations as well as between the insulin-resistant and insulin-sensitive NIDDM populations were analyzed using the χ^2 or Fisher's exact test as appropriate. When comparing differences in HLA frequencies, the calculated P values were corrected by multiplying by the number of antigens that could be detected at each locus (6 for the DQ locus) (13). A corrected $P < 0.05$ was considered statistically significant. GF was calculated from the frequencies with which the antigens were observed using the formula $GF = 1\text{-}\sqrt{1-AP}$, where AF is the observed antigen frequency (14). No distinction was made between homozygosity for a particular allele and the presence of a blank. The correlation coefficient for associations between serological HLA-DR and -DQ types were calculated from the formula $r = (\chi^2/n)^{1/2}$. Odds ratios were calculated using Woolf's method and expressed as a relative risk (13). Data are means ± SE unless noted.

RESULTS

Insulin action

Figure 1 plots the frequency distribution of insulin-mediated (1 mU · kg^{-1} · min^{-1}) glucose disposal in our 46 black NIDDM patients. The striking feature of these data is that a bimodal distribution is observed: an insulin-sensitive (M > 278 μmol · kg^{-1} · min^{-1}, where 278 μmol · kg^{-1} · min^{-1} = 5 mg · kg^{-1} · min^{-1}) and an insulin-resistant subset (M < 278 μmol · kg^{-1} · min^{-1}). This distribution is bimodal based on the Z distribution of Haldane ($P < 0.0014$) (15). Table 1 characterizes these two discrete populations. Twenty-five insulin-sensitive patients had a mean ± SD insulin-mediated glucose disposal of 397 ± 59 μmol · kg^{-1} · min^{-1}, which is not different from that of normal, black control subjects (400 ± 30 μmol · kg^{-1} · min^{-1}, $n = 9$). The 21 insulin-resistant NIDDM patients had a mean ± SD insulin-mediated glucose disposal of 178 ± 46 μmol · kg^{-1} · min^{-1}, which differed significantly ($P < 0.001$) from both insulin-sensitive NIDDM and normal black patients. The ethnic backgrounds of the two NIDDM and control groups were similar.

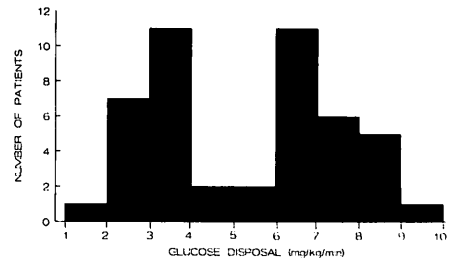

Figure 1—*Frequency distribution of insulin-mediated (1 mU/kg/min) glucose disposal in black NIDDM patients. Intervals are from $X_{1.01}$ to $X_{2.0}$. 1 mg · kg^{-1} · min^{-1} = 55.6 μmol · kg^{-1} · min^{-1}. The distribution is bimodal ($P < 0.0014$). Intervals on the Y-axis are in increments of 2.*

The two NIDDM populations differed slightly but significantly in their degree of obesity as defined by BMI, but neither group contained individuals that were more than mildly obese (Table 1). The insulin-sensitive group showed a male to female ratio of 1.8, whereas that of the insulin-resistant group was 0.6. The mean FPG, GHb, and age were not different between the two variants.

Frequencies of specific HLA phenotypes in normal black individuals and NIDDM patients

HLA typing of the 89 nondiabetic black individuals, the 25 insulin-sensitive, and the 21 insulin-resistant black NIDDM patients showed no differences in the frequencies of MHC class I antigens at the A, B, and C loci (data not shown).

Table 1—*Characteristics of patients with insulin-sensitive and insulin-resistant NIDDM*

	NIDDM patients	
	Insulin-sensitive	Insulin-resistant
N	25	21
Age (yr)	45.5 ± 9.3	46.9 ± 10.0
Sex (M/F)	16/9	8/13
BMI (kg/m^2)	25.6 ± 2.8	27.15 ± 2.04*
FPG (mM)	7.1 ± 2.7	7.2 ± 2.1
Glucose (umol · kg^{-1} · min^{-1}) disposal to insulin 1 mU · kg^{-1} · min^{-1}	397 ± 59	178 ± 46**

Data are means ± SD.
*$P < 0.013$.
†$P < 0.001$.

Among the MHC class II antigens, differences were noted in the DQW locus in both NIDDM variants when compared with each other and with the control population. DQW7 was present in 76% of the insulin-resistant population as compared with 32% of the insulin-sensitive population (Table 2). This difference was highly significant (corrected $P < 0.018$).

Analysis of the frequency of DQW phenotypes for the two NIDDM variants compared with our normal black population (Table 2) showed an increase in DQW7 (corrected $P < 0.001$) in patients with insulin-resistant NIDDM. The relative risk of insulin resistance being identified by DQW7 in the insulin-resistant group compared with normal control subjects was 7.0. In contrast, HLA-DQW6 is increased in the insulin-sensitive patients as compared with normal control subjects (corrected $P < 0.023$).

Table 3 compares the phenotypic and genotypic frequency of alleles from the HLA-DR loci in the two NIDDM variants and normal control subjects. They had no significant differences.

Analysis of the relationship between DQW and DR serological types in the control population showed significant associations between HLA-DQW6 and DR15 ($r = 0.45$, $P < 0.001$); between HLA-DQW7 and DR5 ($r = 0.37$, $P < 0.001$) and DRw4 ($r = 0.44$, $P < 0.001$). Significant associations also were noted between HLA-DQW1 and DR2 ($r = 0.38$, $P < 0.001$); between HLA-DQW2 and DR3 ($r = 0.24$, $P < 0.05$)

Table 2—*The association of specific HLA-DQ AFs and GFs with insulin-resistant and insulin-sensitive NIDDM in black Americans*

HLA-DQ antigen	Nondiabetic control subjects (*N* = 89)			Insulin-resistant NIDDM patients (*N* = 21)			Insulin-sensitive NIDDM patients (*N* = 25)		
	N	AF (%)	GF	*N*	AF (%)	GF	*N*	AF (%)	GF
DQW1	32	35.96	0.200	2	9.52	0.049	4	16	0.083
DQW2	31	34.83	0.193	5	23.81	0.127	8	32	0.175
DQW3	15	16.85	0.088	3	14.29	0.074	4	16	0.083
DQW4	4	4.49	0.023	3	14.29	0.074	2	8	0.042
DQW6	35	39.33	0.221	10	47.62	0.276	18	72	0.471
DQW7	28	31.46	0.172	16	76.19	0.512	8	32	0.175

		Statistical analyses					
		AF			GF		
Comparisons	HLA-DQ Antigen	χ^2	Uncorrected *P*	Corrected *P*	χ^2	Uncorrected *P*	Corrected *P*
Insulin-resistant vs. insulin-sensitive NIDDM	DQW 7	8.93	0.003	0.018	5.86	0.015	0.093
Control population vs. insulin-resistant NIDDM	DQW 7	14.2	0.00017	0.001	10.85	0.0009	0.006
Control population vs. insulin-sensitive NIDDM	DQW 6	8.38	0.0038	0.023	6.09	0.0136	0.082

Table 3—*The association of specific HLA-DR AF and GF with insulin-resistant and insulin-sensitive NIDDM in black Americans*

HLA-DR allele	Nondiabetic control subjects (N = 89)			Insulin-resistant NIDDM patients (N = 21)			Insulin-sensitive NIDDM patients (N = 25)		
	N	AF (%)	GF	N	AF (%)	GF	N	AF (%)	GF
DR 1	10	11.2	.058	2	9.5	.049	3	12	.062
DR 2	14	15.7	.082	3	14.3	.074	1	4	.02
DR 3	22	24.7	.132	4	19.0	.100	6	24	.128
DR 4	5	5.6	.028	3	14.3	.074	2	8	.041
DR 5	17	19.1	.101	7	33.3	.184	6	24	.128
DRw6	7	7.9	.040	3	14.3	.074	6	24	.128
DR 7	18	20.2	.107	2	9.5	.049	5	20	.106
DRw8	12	13.5	.07	5	23.8	.127	4	16	.084
DR 9	7	7.9	.040	2	9.5	.049	0	0	<.02
DRw10	1	1.1	.006	1	4.8	.024	0	0	<.02
DRw12	7	7.9	.040	2	9.5	.049	3	12	.062
DRw13	15	16.9	.088	2	9.5	.049	6	24	.128
DRw14	4	4.5	.023	0	0	<.02	0	0	<.02
DRw15	13	14.6	.076	2	9.5	.049	6	24	.128
DRw52	74	83.1		18	85.7		22	88	
DRw53	24	27.0		7	33.3		5	20.0	

and DR7 (r = 0.63, $P < 0.001$); and between HLA-DQW3 and DR3 (r = 0.41, $P < 0.001$). Neither NIDDM group had significant HLA-DQ-DR associations, presumably because of the relatively small numbers. Our normal control subjects showed HLA-DQ and DR GF and DQ-DR associations similar to that reported in blacks of both African-Caribbean and African-American origin (16–19).

Analysis of our data using GF (Table 2) showed significant differences of DQW7 frequencies between insulin-resistant NIDDM and normal control subjects. GF for HLA-DQW7 also were different between insulin-resistant and insulin-sensitive NIDDM and for DQW6 between insulin-sensitive NIDDM and control subjects.

CONCLUSIONS— The increased frequency of HLA-DQW7 observed in insulin-resistant black NIDDM patients as compared with either insulin-sensitive black NIDDM patients or nondiabetic black control subjects suggests that this MHC class II allele may be associated with one or more genes responsible for the insulin-resistant variant of NIDDM. The lack of association of HLA-DQW7 and the positive association of HLA-DQW6 with the insulin-sensitive variant are further evidence to support our previous data that these variants are uniquely different genetically and are probably not related to environmental factors, such as differing degrees of obesity or hyperglycemia.

It is well recognized that American blacks are genetically heterogeneous, and we must consider that any HLA differences observed between any two groups might represent differences in genetic admixture and not differences attributable to disease association.

Other investigators have failed to find any association between MHC class II antigens and NIDDM (19,20). The discrepancy is likely attributable to numerous factors, including the following: specific identification of DQW7 and DQW6 has been possible only recently; the associations are specific for each of the two variants of NIDDM, and, therefore, analysis of an unselected population of NIDDM patients would be less likely to show significant differences from a nondiabetic population; DQW associations observed in this study for black Americans might not occur or might be different in other racial groups.

We should emphasize the DQW associations we have observed may be directly related to or may be in linkage disequilibrium with the underlying genetic defect responsible for NIDDM. The HLA associations we have described may imply NIDDM has an autoimmune component, similar to that seen in IDDM (3); alternatively, the association may imply linkage with other genes that help determine insulin resistance and/or insulin secretion. NIDDM patients whose insulin resistance is secondary to environmental factors such as obesity or hyperglycemia (and presumably reversible) might be expected not to have an increased frequency of HLA-DQW7 but rather an increased frequency of HLA-DQW6. Most NIDDM patients who are hyperglycemic and have a BMI >30 kg/m^2 are likely to be insulin resistant, and separating those who have primary insulin resistance from those who have secondary insulin resistance may not be possible (8,9).

Previous studies have shown inconsistent associations of MHC class I phenotypes with NIDDM and have differed depending on race or ethnic background. They have not provided any further clues about the genetic bases of NIDDM (21–24).

Our data raise many questions and pose several directions for future research. It will be important to determine whether DQW7 is associated with insulin-resistant NIDDM in whites and other racial or ethnic populations as well as the syndrome of insulin resistance without NIDDM (25). Haplotype analyses for DQW7 and/or DQW6 in the appropriate NIDDM patients may provide insights

into the genetic basis of the various types of NIDDM.

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