Genes Within the Major Histocompatibility Complex Predict NIDDM in African-American Women in Alabama

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OBJECTIVE — To test the hypothesis that genes within the major histocompatibility complex (MHC) are associated with gestational diabetes mellitus (GDM) and, subsequently, non-insulin-dependent diabetes mellitus (NIDDM) in African-American women.

RESEARCH DESIGN AND METHODS — African-American women who presented with GDM were compared with pregnant African-American control subjects. Following pregnancy, GDM patients were assessed at various intervals of time (median = 6 years) to determine whether they had developed diabetes.

RESULTS — GDM patients who required insulin during pregnancy possessed a significantly higher frequency of A33, DR2, DR9, and BF-S phenotypes than control subjects. GDM patients who subsequently developed NIDDM had a significantly higher frequency of B41, DR2, and BF-S and a lower frequency of DR1 and DR6 phenotypes than control subjects. Even after controlling for age and body mass index, B41 and DR2 were independent predictors of developing insulin-requiring GDM and NIDDM in GDM subjects.

CONCLUSIONS — These results suggest that either one or more genes within the MHC are involved in the etiology of NIDDM.

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MHC, major histocompatibility complex; HLA, human leukocyte antigen; NIDDM, non-insulin-dependent diabetes mellitus; GDM, gestational diabetes mellitus; OR, odds ratio; CI, confidence interval; BMI, body mass index; GB, insulin-requiring gestational diabetes mellitus; TNF, tumor necrosis factor.

It is well established that genes within the major histocompatibility complex (MHC), i.e., human leukocyte antigens (HLAs) and Bf phenotypes, are markers for the development of insulin-dependent diabetes mellitus (1,2). Non-insulin-dependent diabetes mellitus (NIDDM) is felt to be a distinct disorder with different genetic and environmental risk factors (3). However, NIDDM may also be associated with various HLA phenotypes (4–11). Women with an abnormal glucose tolerance during pregnancy, but who had normal glucose tolerance before pregnancy, are classified as having gestational diabetes mellitus (GDM) (3). Although most of these women are usually normoglycemic after pregnancy, they are at high risk of developing subsequent NIDDM (12). HLA associations have also been reported in GDM subjects (13–15). We have been following a cohort of African-American women who presented with GDM. We now report the association of HLAs and Bf phenotypes with GDM and its subclasses and subsequent development of NIDDM in this cohort.

RESEARCH DESIGN AND METHODS

— Subjects were African-American women who had their first prenatal visit at any of the eight Jefferson County Alabama Health Department Clinics between 1981 and 1988. Participants were screened, diagnosed for GDM, and classified as insulin-requiring GDM (GB) patients, as previously described (3,16). Women with diabetes before pregnancy were excluded. Of the 789 GDM patients identified, 244 were HLA-typed, and of these, 211 (86.5%) have been followed up. Of these, 72 (34.1%) were classified as having NIDDM (3).

Control subjects (n = 274) consisted of African-American women who received prenatal care at one of the same eight Jefferson County prenatal clinics, who had no personal history of diabetes, and whose screening tests for GDM were negative. Women from this group delivered in the same hospital within 48 h after a GDM subject had delivered.

Table 1—*HLA phenotype frequencies deviating between GB or NIDDM patients and control subjects*

	Class GB	NIDDM	Control	GB vs. control		NIDDM vs. control	
HLA	% Positive	% Positive	% Positive	OR	95% CI	OR	95% CI
A33	18.6 (80)	12.5 (72)	8.0 (274)	2.6*	1.3–5.4	1.6	0.7–3.7
B41	5.1 (79)	7.0 (71)	1.8 (272)	2.8	0.7–10.9	4.0†	1.1–14.4
DR1	14.7 (75)	7.1 (70)	16.9 (237)	0.8	0.4–1.7	0.4†	0.1–1.0
DR2	36.0 (75)	44.3 (70)	21.9 (237)	2.0†	1.1–3.5	2.8*	1.6–5.0
DR6	14.7 (75)	12.9 (70)	24.9 (237)	0.5	0.3–1.0	0.4†	0.2–1.0
DR9	9.3 (75)	7.1 (70)	2.5 (237)	4.0†	1.3–12.2	3.0	0.9–10.0

*$P \leq 0.01$. †$P \leq 0.05$; (*n*), number tested.

All study subjects were informed of the study by a trained interviewer, and informed consent was obtained from those wishing to participate. Family history of disease of first- and second-degree relatives was obtained by interview at enrollment. Additional perinatal and postnatal clinical information was gathered from the obstetrical automated record, which is maintained for all patients seen in the prenatal clinics (16).

HLA and Bf typing

HLA-A and -B typing was performed between 1983 and 1988 by the microdroplet cytotoxicity procedure (17). For HLA class II typing, B-cells were purified from isolated lymphocytes by the nylon wool technique using plastic straw columns (17). Typing trays contained antisera that recognized the specificities defined at the 8th and 9th International Histocompatibility Workshops.

Plasma samples were collected in EDTA tubes, separated within 3 h of drawing, and stored at −70°C until Bf phenotyping was performed. Bf phenotyping was performed using agarose gel electrophoresis and immunoprecipitation as previously described (2).

Statistical analysis

Comparisons of the proportions between GDM patients and control subjects were made by χ^2 analyses or Fisher's exact tests where appropriate. The relative risks were estimated using Woolf's odds ratio (OR) method (18), and 95% confidence intervals (CIs) were calculated. Comparisons of means were conducted by using the Student's *t* test. Multivariable analyses were performed using logistic regression (19). All statistical analyses were performed using the SAS statistical package.

RESULTS — African-American women who developed GDM, as well as those who subsequently developed NIDDM, were significantly older, weighed more, had a higher body mass index (BMI), and had a greater prevalence of hypertension and diabetes in family members than control subjects. There were no significant differences in these characteristics between those followed up compared with those lost to follow-up. There were no significant differences in the frequencies of HLA-A, -B, or -DR phenotypes in all GDM subjects compared with control subjects. However, B8, B15, and B41 were increased (ORs = 2.0, 1.9, and 2.5, respectively). DR3 was only slightly increased (OR = 1.1) and DR4 decreased (OR = 0.9) in GDM patients compared with control subjects. Among those GDM patients classified as GB, A33, DR2, and DR9, frequencies were significantly increased in GB subjects (Table 1). In NIDDM subjects, the frequencies of B41 and DR2 were significantly increased; DR1 and DR6 were significantly decreased.

When age and BMI were included in a stepwise logistic regression analysis, no HLA allele was a significant determinant of these women developing GDM. However, B41 and DR2 were independent predictors of GB ($P = 0.034$, OR 8.6; $P = 0.003$, OR 4.1, respectively) and NIDDM ($P = 0.018$, OR 19.0; $P = 0.001$, OR 6.4, respectively).

The frequencies of Bf phenotypes were not observed to differ significantly in GDM subjects compared with control subjects. The frequency of Bf-F was decreased ($P = 0.03$, OR 0.5, 95% CI 0.2–0.9) and Bf-S increased ($P = 0.09$, OR 1.8, 95% CI 0.9–3.7) in class GB subjects compared with control subjects. The frequency of Bf-S also increased in NIDDM subjects compared with control subjects ($P = 0.06$, OR 2.0, 95% CI 1.0–4.4).

CONCLUSIONS — The role of genes within the MHC in the etiology of GDM has been suggested by several investigators. HLA-B8 was significantly decreased and B15 increased in GDM patients from Northern Ireland compared with blood donors (13). DR3 and DR4 were increased in a racially mixed sample from Chicago of GDM patients compared with randomly selected, glucose tolerance–tested control subjects (15). The increase of these phenotypes in African-American GDM patients reached statistical significance. However, a study of GDM racially mixed subjects from New York revealed no association with HLA phenotypes (14). In our African-American GDM subjects, there was also no significant association with any HLA phenotype. These results suggest that MHC genes are not a major influence in GDM etiology overall. However, the GB subjects, those most likely to develop NIDDM, had an increase of A33, DR2, DR9, and Bf-S. There have been no previous reports on this group.

Other investigators have also observed an association of HLA phenotypes with NIDDM (4–11). Our group previously reported an increase in A33 ($P = 0.122$, OR 7.3), B7 ($P = 0.006$, OR 2.2), and DR4 ($P = 0.019$, OR 2.4) in American Caucasians who were obese, not ke-

tosis-prone, and who developed diabetes after age 40 (20). One investigation revealed an association of B8 and B15 in GDM patients who subsequently developed NIDDM, while another found no significant differences (13,21). We observed that B41 and DR2 significantly increased in our African-American GDM subjects who subsequently developed NIDDM. One study of African-American children with an atypical form of diabetes, possibly maturity-onset diabetes of the young, also reported an increase in DR2 (22). Thus, these data from different racial and ethnic groups support the contention that one or more genes within the MHC are involved in the etiology of at least a subset of NIDDM. That B41 and DR2 are predictors, even with BMI in the logistic model, suggests that the gene or genes are not acting through obesity to cause NIDDM.

Given the wide variety of associations with various HLA phenotypes, it is more likely that the HLA loci are in linkage disequilibrium with the actual gene or genes. A possible candidate gene within the MHC region predisposing to NIDDM is tumor necrosis factor (TNF)-α. TNF-α has been reported to play a role in obesity, insulin resistance, and NIDDM in rats and mice (23). Moreover, the production of TNF-α is elevated in rodents with these syndromes. In these studies, TNF-α was found to downregulate GLUT4 mRNA, which suggests a role in glucose homeostasis. Recently, the glucagon-like peptide-1 receptor gene has been mapped to 6p21 (24). Variants of this gene, which has been proposed to contribute to impaired B-cell function, could be in linkage disequilibrium with genes within the MHC, especially in those NIDDM patients who have primarily B-cell deficiencies.

In conclusion, MHC associations do not appear to be related to the occurrence of GDM. However, one or more genes within or linked to the MHC may play an etiological role in the GB class of GDM and in NIDDM or its subtypes. We suggest that studies aimed at identifying genes involved in the etiology of NIDDM should consider those within the MHC region.

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