Evidence for Genetic Admixture as a Determinant in the Occurrence of Insulin-dependent Diabetes Mellitus in U.S. Blacks

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SUMMARY

In recent years, it has been proposed that genetic admixture may have played a role in the increased frequency of insulin-dependent diabetes mellitus (IDDM) in young U.S. blacks relative to African blacks. In support of this proposal, the similar associations of specific markers of the major histocompatibility complex (MHC) with IDDM in U.S. blacks with respect to U.S. whites have been cited. To determine whether racial admixture was a factor in the increased prevalence, we did three analyses of admixture. In the first we used nine genetic markers (ABO, Rh, Fy, Hp, Gc, PI, OR, Tfr, and Gm) and determined that there was significantly greater than zero genetic contribution from whites in our sample of U.S. black IDDM patients ($9.6 \pm 2.3\%$, $P < 0.01$) when a sample of U.S. blacks without IDDM was used as one "parental" population. In the next two analyses, we estimated the amounts of genetic contribution from whites in the U.S. blacks with and without IDDM using reported gene frequencies for West African blacks for four genetic markers (ABO, Rh, Fy, and Hp). The estimate of admixture ($21.4 \pm 2.8\%$) for the black IDDM sample was greater than that for the U.S. black controls ($17.9 \pm 2.3\%$), although the difference was not significant. Our estimate of genetic contribution from whites, 21.4% for black IDDM patients, supports the assumptions of 20% admixture which MacDonald and Rotter and Hodge used to test their respective models for the inheritance of IDDM. These results support the hypothesis that admixture with the white population is, in part, responsible for the increase in prevalence of IDDM seen in U.S. blacks. DIABETES 31:532–537, June 1982.

In the few population-based studies of the prevalence of IDDM in U.S. black children, a consistent finding has been that the frequency is less than that in white children.[1–3] Studies of clinical samples have also indicated that the prevalence of IDDM in blacks is less than whites in the United States.[4–6] Furthermore, IDDM in blacks from primitive and developing African areas is rare.[7–12] Thus, it appears that the prevalence of IDDM in U.S. blacks is higher than in African blacks but less than U.S. whites.

Two basic etiologic hypotheses can be considered to explain the increased prevalence of IDDM in U.S. versus African blacks. One hypothesis suggests that certain environmental factors found in the U.S. but not present in Africa may trigger IDDM in individuals genetically predisposed to this disease. This hypothesis would be analogous to complications that occur in those with glucose-6-phosphate dehydrogenase (G6PD) deficiency, who, on contact with the exogenous antimalarial agent primaquine, develop acute hemolysis.[13] The other hypothesis proposed by MacDonald[6,14] is that genetic contribution (admixture) from a more diabetes-susceptible population may have played a role in the increased rate of IDDM among young U.S. blacks relative to African blacks.

The association of IDDM with genes of the major histocompatibility complex (MHC) has provided a means to follow the genetics of this disease. Certain gene products (HLA) of the MHC have been found to be associated with IDDM in white populations of Europe, the British Isles, and North America. Of these, HLA-B8, B15, B18, Cw3, DR3, DR4, Dw3, and Dw4 are found to be significantly increased, while B7, B15, and DR2 are significantly decreased in white IDDM patients when compared with healthy white controls.[15–17] Some studies have suggested that the association of various HLA-A and -B specificities with IDDM may differ between various racial and ethnic groups.[17–20] The few studies of U.S. blacks have suggested that in general the pattern of HLA associations is similar to that found in whites. With respect to B locus antigens, three studies[21–23] have found an increase in HLA-B8 and B15 in black IDDM patients. Signifi-

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cant associations with D and DR locus specificities (Dw3, Dw4, DR3, and DR4) have been found in all U.S. black IDDM samples tested for these specificities.[22–25] These studies are consistent with the hypothesis that the increased prevalence of IDDM in the U.S. black population may be due to racial admixture.[14] The two reports testing the genetic hypothesis of admixture using a non-MHC-associated genetic marker, sickle cell hemoglobinopathy, have been inconsistent.[26,27] No estimates of genetic admixture based on frequencies of HLA or any other genetic markers in black IDDM patients in the U.S., however, have been documented.

We have set out to explore the hypothesis of admixture with respect to IDDM. For this study, it would be unwise to test the hypothesis of racial admixture in IDDM patients using genetic markers on the sixth chromosome, since HLA, which is on that chromosome, is known to be associated with the disease. We have utilized erythrocyte and serum genetic markers whose allelic frequencies differ between white and black populations, to determine (1) if the proportion of white genes in a sample of U.S. black IDDM patients is greater than in samples of U.S. black controls and (2) how our estimates of admixture relate to current hypotheses of the genetic etiology of IDDM.

MATERIALS AND METHODS

Study sample. All IDDM patients in the study were under the age of 40 at the onset of the disease. They were insulin-dependent, ketosis-prone, and showed an abrupt onset with indications of weight loss. The black and white control samples included U.S. Americans without any form of diabetes and a negative history of diabetes of any type in first-degree relatives. To minimize ascertainment error regarding admixture, all diabetic patients and controls in the study were at least third-generation U.S. Americans. In order to minimize any regional differences in the frequencies of the markers, we included only subjects from the southeastern region of the U.S.

Genetic markers. We chose only markers that had not been previously reported to be associated with IDDM in population studies. This was done to avoid the possibility that the estimate of admixture was influenced by IDDM population associations with the markers we examined. Furthermore, we compared the phenotypic frequencies of these markers between a white IDDM sample (N = 144) and the white controls (N = 97). With none of the nine markers were any significant population associations found (unpublished results).

Erythrocyte markers. All blood was collected in an anticoagulant (sodium heparin or acid-citrate dextrose). The red cell antigen markers tested according to standard blood banking techniques[28] with commercial antisera were ABO (anti-A, -B, -AB Ortho), Rhesus (Rh) (anti-C, -c, -D, -E, -e Gamma Biologicals, Houston, Texas), Duffy (Fy) (anti-Fy^a, -Fy^b Gamma Biologicals), MN (anti-M, -N Gamma Biologicals). Known positive and negative control cells from commercial sources were tested with each lot of antisera.

Serum and plasma markers. At least 5 ml of serum or plasma was collected from each subject and stored at −80°C until the assay was performed. Isoelectric focusing methods were used for the following markers: group-specific component (Gc),[29,30] alpha 1-antitrypsin (PI),[31] and transferrin (Tfr).[29] Haptoglobin (Hp) allelic types were determined by starch gel electrophoresis.[32] Orosomucoid (OR) was typed using agarose gel electrophoresis and immunofixation.[33] An indirect inhibition technique was utilized to detect immunoglobulin IgGl allotypes 1, 2, 4, 17 and IgG3 allotypes 5, 13, and 21 (Gm).[34]

Statistical analysis. Gene frequencies for the various markers along with their standard errors were determined by maximum likelihood methods.[35] For the Gm marker, comparisons were made based on differences in the sum of the Gm(4) and Gm(21) frequencies, where the frequencies in the white population were assumed to add up to 1.0.

The estimates of racial admixture were obtained by a maximum likelihood method using gene frequencies (MLGF) first formulated by Krieger in 1965[36] and expanded by Elston[37] to be general for dominance. The variance-covariance matrix of the estimates were obtained from the expected values of the second derivatives of the log likelihood. A Fortran IV program (ADMIXT) developed by Dr. Robert Elston was used. Estimates of admixture were determined from the gene frequencies estimated at loci in the designated hybrid and the frequencies given for the parental populations at the same loci. The black African estimates of gene frequency we used were derived from that used by Workman et al.[38] for their determination of white admixture in a U.S. black population from Claxton, Georgia, in the early 1960s.

RESULTS

Assuming that the gene(s) for susceptibility to IDDM in U.S. blacks was derived from the admixture of a U.S. white population with an essentially disease-free African black population, and given that not many generations of admixture have taken place and that the proportion of interracial mating is not high, then the genetic contribution from whites in the U.S. black IDDM population should be greater than in U.S. blacks without IDDM. This would occur since the probability of inheriting the white IDDM susceptibility gene would be greater for those with an increased proportion of white genes.

In order to test the hypothesis that U.S. blacks with IDDM have a greater proportion of white genes than already exist in U.S. black controls, we performed an analysis of admixture (MLGF) using marker allele frequencies from U.S. black and white controls as the "parental" populations and U.S. black IDDM patients as the "hybrid" population. If there were no differences in gene frequencies between our black IDDM sample and the U.S. black control sample for the markers tested, we would expect the estimate of admixture not to be significantly different from an expected value of zero. Table 1 lists for each population the maximum likelihood estimates of the gene frequencies ± SE for each of the alleles of the nine loci used in the analysis of admixture. The MN locus has been eliminated due to difficulty with the N antisera. It should be noted that different sample sizes were used for each marker estimate. Except for OR and PI, the estimate of gene frequency for the most prevalent allele at each marker for the U.S. black IDDM sample was between the estimated frequencies for the U.S. black controls and the U.S. white controls. Table 2 lists our computed estimates of the proportion of genetic contribution of white genes in the black IDDM sample for each individual marker and for all markers together. Our overall estimate of 9.6 ± 2.3% ge-

TABLE 1
Gene frequencies* ± SE for nine genetic markers in each population used in analyses of admixture

Marker locus	Alleles	U.S. black controls	Black IDDM patients	U.S. white controls
ABO		(N† = 183)	(N = 65)	(N = 97)
	A	0.128 ± 0.018	0.160 ± 0.034	0.248 ± 0.034
	B	0.151 ± 0.020	0.124 ± 0.030	0.075 ± 0.019
	O	0.721 ± 0.025	0.716 ± 0.042	0.677 ± 0.036
Fy		(N = 153)	(N = 62)	(N = 82)
	Fy^a	0.078 ± 0.016	0.075 ± 0.024	0.413 ± 0.043
	Fy^b	0.061 ± 0.014	0.075 ± 0.024	0.587 ± 0.043
	Fy^0	0.861 ± 0.020	0.850 ± 0.033	0.000
Rh		(N = 181)	(N = 64)	(N = 96)
	cde	0.221 ± 0.039	0.221 ± 0.061	0.404 ± 0.045
	Cde	0.012 ± 0.012	0.000	0.000
	cdE	0.000	0.000	0.013 ± 0.012
	cDe	0.539 ± 0.043	0.490 ± 0.067	0.023 ± 0.017
	CDe	0.142 ± 0.022	0.141 ± 0.031	0.417 ± 0.034
	cDE	0.086 ± 0.015	0.148 ± 0.031	0.143 ± 0.027
Gc		(N = 143)	(N = 66)	(N = 159)
	IF	0.738 ± 0.026	0.598 ± 0.043	0.151 ± 0.020
	IS	0.178 ± 0.023	0.205 ± 0.035	0.579 ± 0.028
	2	0.084 ± 0.016	0.197 ± 0.035	0.270 ± 0.025
PI		(N = 108)	(N = 66)	(N = 74)
	M1	0.861 ± 0.024	0.871 ± 0.029	0.685 ± 0.038
	M2	0.009 ± 0.006	0.046 ± 0.018	0.169 ± 0.031
	M3	0.074 ± 0.018	0.053 ± 0.020	0.106 ± 0.025
	S	0.014 ± 0.008	0.030 ± 0.015	0.020 ± 0.012
	Z	0.019 ± 0.009	0.000	0.020 ± 0.012
	rare	0.023 ± 0.010	0.000	0.000
Hp		(N = 151)	(N = 69)	(N = 103)
	Hp1	0.603 ± 0.028	0.573 ± 0.037	0.354 ± 0.033
	Hp2	0.397 ± 0.028	0.428 ± 0.037	0.646 ± 0.033
OR		(N = 119)	(N = 63)	(N = 101)
	F	0.538 ± 0.032	0.563 ± 0.044	0.465 ± 0.035
	S	0.462 ± 0.032	0.437 ± 0.044	0.535 ± 0.035
Gm‡		(N = 77)	(N = 54)	
	Gm 21	0.072 ± 0.021	0.074 ± 0.025	
	Gm 4	0.058 ± 0.019	0.139 ± 0.033	
	Gm other	0.870 ± 0.027	0.787 ± 0.039	
Tfr		(N = 106)	(N = 76)	(N = 104)
	C1	0.901 ± 0.021	0.896 ± 0.025	0.802 ± 0.028
	C2	0.079 ± 0.019	0.080 ± 0.022	0.197 ± 0.028
	D1	0.020 ± 0.010	0.024 ± 0.012	0.000

* Maximum likelihood estimates of gene frequencies.
† N = number of individuals tested.
‡ White frequencies for Gm(4) and Gm(21) assumed to sum to 1.00 for analysis of admixture.[43] Gm 21 includes haplotypes (1, 2, 17, 21) and (1, 17, 21); Gm 4 includes haplotype (4, 5, 13); Gm other includes haplotypes (1, 5, 13, 17), (1, 5, 17), and (1, 13, 17).

netic contribution of white genes in black IDDM patients using black controls as one of the "parental" populations was significantly different ($P < 0.01$) from the expected value of zero.

To compare directly the degree of genetic contribution of white genes in U.S. blacks with IDDM and U.S. black controls, we performed two additional analyses of admixture (Table 3). In analysis II, black IDDM patients were the "hybrid" with white controls and black Africans as "parental" groups. In analysis III, black controls were "hybrid" with white controls and black Africans the "parental" groups. Acceptable data for black Africans were available for four of the markers, which were also tested in our samples, ABO, Rh, Fy, and Hp. It was necessary to make the assumption that modern African black data on gene frequencies are nearly those of the ancestral populations from which U.S. blacks originated. For the four markers, overall, the proportion of genetic contribution of white genes was greater in U.S. black IDDM patients (21.4% ± 2.8%) than in U.S. black controls (17.9 ± 2.3%), although the difference was not significant. Both are within the range of estimates of genetic contribution of white genes in U.S. blacks of 10–30% compiled by Reed.[39]

TABLE 2
Maximum likelihood estimates of percent genetic contribution to black IDDM patients from U.S. black controls and U.S. white controls at each of the nine loci used and the overall estimate using all nine loci

	Black IDDM patients	
Genetic marker loci	Percentage of genes derived from U.S. blacks†	Percentage of genes derived from U.S. whites‡
ABO	70.9	29.1
Fy	98.3	1.7
Rh	92.6	7.4
Gc	76.3	23.7
PI	76.4	23.6
Hp	87.9	12.1
OR	100.0	0
Gm	90.4	9.6
Tfr	98.9	1.1
Overall	90.4 ± 2.3	9.6 ± 2.3*

* $P < 0.01$ for rejecting the hypothesis that the estimate of white genetic contribution equals zero.
† For black IDDM patients, the percentage of genes derived from U.S. blacks.
‡ For black IDDM patients, the percentage of genes derived from U.S. whites. This is in addition to the percentage of white genes found in U.S. blacks without IDDM, since they are one of the "parental" populations.

TABLE 3
Maximum likelihood estimates of percent genetic contribution to both black IDDM patients and black controls from West African blacks* and U.S. white controls for four loci

	Black controls†		Black IDDM patients‡	
Genetic marker loci	W. African blacks	U.S. white controls	W. African blacks	U.S. white controls
ABO	100.0	0	78.9	21.1
Rh	83.8	16.2	78.4	21.6
Fy	81.1	18.9	81.8	18.2
Hp	74.1	25.9	65.2	34.8
Overall	82.1 ± 2.3	17.9 ± 2.3	78.6 ± 2.8	21.4 ± 2.8

* Gene frequencies used in analysis for the West African black "parental" group were taken from the compilation by Workman et al.;[38] ABO-A 0.1470, B 0.1500, O 0.7030; Fy-Fy^a 0.0000, Fy other 1.00; Rh-cDe 0.5940, CDe 0.0690, cDE 0.0860, cde 0.2110, other 0.0400; Hp-Hp2 0.6900, Hp other 0.3100.
† For black controls, the percentage of genes derived from West African blacks and U.S. whites.
‡ For black IDDM patients, the percentage of genes derived from West African blacks and U.S. whites.

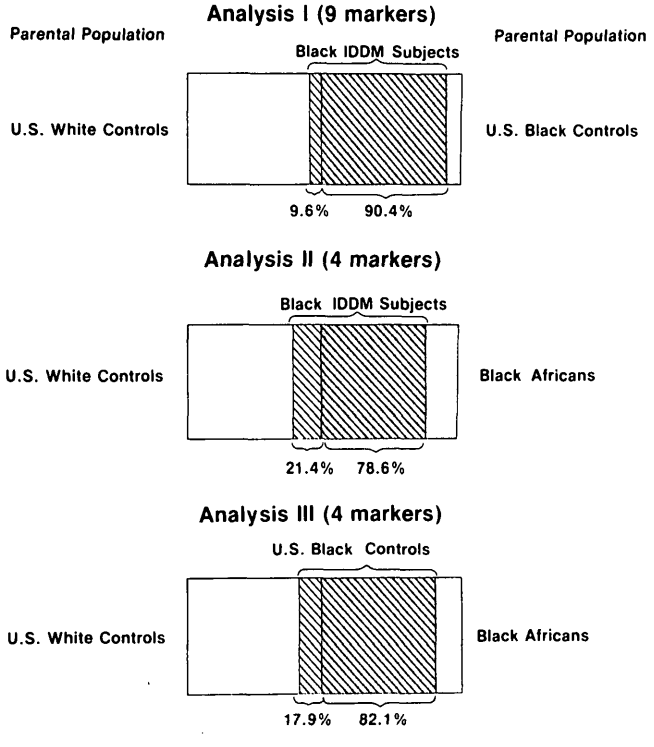
DISCUSSION

For ease of discussion, the three analyses with the parental populations and the results are presented in Figure 1. In all analyses, white controls were one "parental" population. It is important to note that the analysis in which the black controls were one "parental" population is on a different scale than are the two analyses where black Africans were the "parental" population. It is, therefore, not possible to directly compare the 9.6% difference between the blacks with IDDM and the black controls in the first analysis and the 3.5% difference in white admixture between the blacks with IDDM (21.4%) and black controls (17.9%) when black Africans were used as the parental population.

Our finding that the estimated genetic contribution from whites in U.S. black IDDM patients, using black controls as one of the "parental" populations, was significantly greater than zero ($P < 0.01$) supported the basic hypothesis that there is a slightly greater genetic contribution from whites in U.S. blacks with IDDM than in U.S. blacks without IDDM from the same area. The results of the analysis using black Africans as one of the "parental" populations, which indicate a greater degree of admixture in the U.S. black IDDM sample than in the U.S. black controls (although not significant), were also consistent with this hypothesis.

The direction of admixture was consistent for all markers except OR. OR, however, was the poorest discriminator of the black and white controls and, therefore, provided the least information for the overall admixture estimate. It should be noted that the gene frequencies used in the analyses were estimated, and improvement of the estimates of admixture could be achieved by increasing the sample sizes in each population and/or by increasing the number of markers examined. Efforts are currently underway to increase both.

FIGURE 1. A diagrammatical representation of the three analyses of admixture used to ascertain the contribution of "parental" populations to black IDDM or black control subjects. The values are percent of contribution to the hybrid gene pool.



The biologic significance of our findings can be seen by relating our results to the current hypotheses of the genetic etiology of IDDM. First, familial aggregation, twin studies, population genetic associations, and family linkage studies have shown that the occurrence of IDDM in man is under genetic influence. The results of this study provide independent evidence for this genetic influence.

Second, our results support the hypothesis that racial admixture is, in part, responsible for the increased prevalence of IDDM in U.S. blacks relative to the prevalence in African blacks. Whether the admixture that we have detected is responsible for the entire increase or whether there is also an environmental influence remains to be determined. We cannot be sure whether an overall admixture estimate as small as 21.4% could cause the increased prevalence seen. However, it is not possible to evaluate the influence of admixture on the prevalence until the precise mode of inheritance for IDDM is known. In assessing the size of the population estimates of admixture, it also must be recalled that on the individual level, if only the genes from a region of one chromosome are necessary to confer the susceptibility to IDDM (dominant mode of inheritance), then only that region from one of the 46 chromosomes of the U.S. black with IDDM needs to be of white origin. Therefore, it is theoretically possible for a U.S. black individual to have as small an individual admixture estimate as 0.0% and still have inherited the requisite genes from a white ancestor, particularly if no marker on that chromosome has been examined. Our estimates are based on population estimates of admixture. Although individual estimates of admixture are possible, they require many more markers to obtain a reliable estimate of admixture.

Third, there is considerable controversy concerning the mode of inheritance of IDDM. Some reports support simple dominant, some support recessive modes, and others, more complex modes of inheritance. The proponents of two different models of the inheritance of IDDM have assumed an admixture of 20% in the development of their models.[14,40] Our estimate of admixture of 21.4% provides support for this assumption. MacDonald[14] has proposed a model to test whether a trait is autosomal dominant or autosomal recessive using data from analyses of racial admixture. This model assumed unidirectional flow of genes from one population to the other, i.e., white genes into the black gene pool, and that the trait was rare in one of the parental populations (black population in this case). Under these conditions, a rigorous test of the two genetic transmission hypotheses—autosomal dominant and autosomal recessive—can be tested. If the trait was governed by an autosomal dominant gene, its frequency in the admixed black population would be proportional to the degree of admixed genes, i.e., the proportion of white genes in the black population. However, if the gene was recessive, then the frequency of the trait in the black population would be the square of the gene frequency and, hence, the prevalence of the trait in the black population would be far smaller than the degree of admixture (the percentage of genes received from the white population). As noted previously, the prevalence of IDDM in U.S. blacks was 20–50% of that observed in whites[1–6] and, given

our estimate of admixture of 21.4%, a dominant mode of inheritance appears more likely.

Finally, Rotter and Rimoin[41] have proposed that there is genetic heterogeneity within IDDM. In their model there are two different immunologic and clinical phenotypes, one in linkage disequilibrium with B8, the other, with B15. Rotter and Hodge[40] predict that because of differential frequencies and penetrances of the alleles in the parent white population, the two phenotypes should appear at different frequencies in the U.S. black population. A recent study by Neufeld et al.[42] has supported this model by finding that one of the phenotypes that associated with presence of pancreatic islet cell antibodies (B8) is significantly decreased in black IDDM cases. While our results do not directly apply to this model, a similar methodology of admixture can be used to examine it. A difference in estimates of admixture between U.S. black IDDM cases with the autoimmune phenotype and those without would provide support for the hypothesis that the two phenotypes are genetically different.

Our results have significance outside of the study of diabetes. There are a number of diseases similar to IDDM, where a genetic component is suspected and where differential prevalences are found between genetically admixing groups, and where a diseased versus disease-free genetic analysis of admixture similar to the one we employed is applicable. Such diseases include ankylosing spondylitis, acute lymphocytic leukemia, cirrhosis of the liver, and possibly essential hypertension. As first suggested by Reed[39] a decade ago, and as our results demonstrate, studies of admixture can be useful in determining the role of a genetic component in the occurrence of a disease.

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REFERENCES

[1] Bauer, M. L.: Characteristics of persons with diabetes. National Center for Health Statistics. Publication no. 1000, Series 10, 1967, pp. 1–44.

[2] Gorwitz, K., Howen, G. G., and Thompson, T. T.: Prevalence of diabetes in Michigan school-age children. Diabetes *25*:122–27, 1976.

[3] Bennett, P. H., Entmacher, P. S., Habicht, J-P., Knowles, H. C., and Whittlesey, L. M.: Diabetes Data—Compiled 1977. Washington, D.C., U.S. DHEW Publication no. (NIH) 78-1488, 1978.

[4] Emerson, H., and Larimore, L. D.: Diabetes mellitus: a contribution to its epidemiology based chiefly on mortality statistics. Arch. Intern. Med. *34*:585–630, 1924.

[5] Altschul, A., and Nathan, A.: Diabetes mellitus in Harlem Hospital outpatient department in New York. JAMA *119*:248–52, 1942.

[6] MacDonald, M. J.: Lower frequency of diabetes among hospitalized negro than white children: theoretical implications. Acta Genet. Med. Gemellol. *24*:119–26, 1975.

[7] Dodu, S. R. A.: The incidence of diabetes mellitus in Accra (Ghana). West Afr. Med. J. *7*:129–34.

[8] Seftel, H., and Schultz, E.: Diabetes mellitus in the urbanized Johannesburg African. S. Afr. Med. J. *35*:66–70, 1961.

[9] Kinnear, T. W.: The pattern of diabetes mellitus in a Nigerian teaching hospital. East Afr. Med. J. *40*:288–94, 1963.

[10] Seftel, H.: Diabetes in the Johannesburg African. The Leech *34*:82–86, 1964.

[11] Jackson, W., and Huskisson, J.: Diabetes. I. Inter-racial comparisons. S. Afr. Med. J. *39*:526–631, 1965.

[12] Osuntokun, B. O., Akinkugbe, F. M., Francis, T. I., Reddy, S., Osuntokun, O., and Taylor, G. O. L.: Diabetes mellitus in Nigerians. A study of 832 patients. West Afr. Med. J. *20*:295–312, 1971.

[13] Hockwald, R. S., Arnold, J., Clayman, C. B., and Alving, A. S.: Status of primaquine, IV. Toxicity of primaquine in negroes. JAMA *149*:1568, 1952.

[14] MacDonald, M. J.: Hypothesis: the frequencies of juvenile diabetes in American blacks and caucasians are consistent with dominant inheritance. Diabetes *29*:110–14, 1980.

[15] Christy, M., Green, A., Christau, B., Kromann, H., Nerup, J., Platz, P., Thomsen, M., Ryder, L. P., and Svejgaard, A.: Studies of the HLA system and insulin-dependent diabetes mellitus. Diabetes Care *2*:209–14, 1979.

[16] Acton, R., Barger, B., Boshell, B., Go, R., Murphy, C., Reitnauer, P., and Roseman, J.: Epidemiology and genetics of insulin dependent diabetes mellitus in the white and black population from the southeastern United States. *In* Frontiers in Immunogenetics. Hildemann, W. H., Ed. New York, Elsevier-North Holland, 1981, pp. 239–55.

[17] Svejgaard, A., Platz, P., and Ryder, L. P.: Insulin-dependent diabetes mellitus. *In* Histocompatibility Testing 1980. Terasaki, P. I., Ed. UCLA Tissue Typing Laboratory, 1980, pp. 638–56.

[18] Wakisaka, A., Aizawa, M., Matsukura, N., Nakagawa, S., Nakayama, E., Itakura, K., Okuno, A., and Wagatsuma, Y.: HLA and juvenile diabetes mellitus in the Japanese. Lancet *2*:970, 1976.

[19] Kawa, A., Nakazawa, M., Sakaguchi, S., Nakamura, S., Kono, Y., Hazeki, H., and Kanehisa, T.: HLA system in Japanese patients with diabetes mellitus. Diabetes *26*:591–95, 1977.

[20] Okimoto, K., Juji, T., Ishiba, S., Maruyama, H., Tohyama, H., and Kosaka, K.: HLA-Bw54 (Bw22-J, J-1) antigen in juvenile-onset diabetes mellitus in Japan. Tissue Antigens *11*:418–22, 1978.

[21] Patel, R., Ansari, A., and Covarrubias, C. L-P.: Leukocyte antigens and disease. III. Association of HLA-B8 and HLA-Bw15 with insulin-dependent diabetes in three different population groups. Metabolism *26*:487–92, 1977.

[22] Duquesnoy, R. J., MacDonald, M. J., Mullins, P., Hackbarth, S. A., Traisman, H. S., and Levitsky, L. L.: Increased frequency of HLA-Dw3 in North-American black patients with juvenile onset diabetes. Tissue Antigens *13*:369–72, 1979.

[23] Reitnauer, P. J., Roseman, J. M., Barger, B. O., Murphy, C. C., Kirk, K. A., and Acton, R. T.: HLA associations with insulin-dependent diabetes mellitus in a sample of the American black population. Tissue Antigens *17*:286–93, 1981.

[24] Rodey, G. E., White, N., Frazer, T. E., Duquesnoy, R. J., and Santiago, J. V.: HLA-DR specificities among black Americans with juvenile-onset diabetes. N. Engl. J. Med. *301*:810–12, 1979.

[25] Zeidler, A., Loon, J., Frasier, S. D., Kumar, D., Penny, R., and Terasaki, P.: HLA-DRw antigens in Mexican-American and Black-American diabetic patients. Diabetes *29*:247–50, 1980.

[26] Morrison, J. C., Schneider, J. M., Kraus, A. P., and Kitabchi, A. E.: The prevalence of diabetes mellitus in sickle cell hemoglobinapathies. J. Clin. Endocrinol. Metab. *48*:192–95, 1979.

[27] Triplett, G., and Eichold, S.: Concurrent diabetes mellitus and sickle cell disease (letter). Diabetes Care *2*:327, 1979.

[28] Miller, W. V. (Ed.): Technical Manual of the American Association of Blood Banks. 7th edit. Philadelphia, J. B. Lippincott Co., 1977.

[29] Hosie, B.: Group-specific component (Gc) and transferrin (Tf) subtypes ascertained by isoelectric focusing. Hum. Genet. *50*:75–79, 1979.

[30] Kueppers, F., and Harpel, B.: Group-specific component (Gc) "subtypes" of Gcl by isoelectric focusing in U.S. blacks and whites. Hum. Hered. *29*:242–49, 1979.

[31] Kueppers, F., and Christopherson, M. J.: Alpha l-antitrypsin. Further genetic heterogeneity revealed by isoelectric focusing. Am. J. Hum. Genet. *30*:359–65, 1978.

[32] Giblett, E. R.: Genetic markers in human blood. Oxford and Edinburgh; England, Blackwell Scientific Publications, 1969.

[33] Johnson, A. M., Schmid, K., and Alper, C. A.: Inheritance of human d_1-acid glycoprotein (Orosomucoid) variants. J. Clin. Invest. *48*:2293–99, 1969.

[34] Borel, H., Pryce, S., and Allen, F. H.: Gm typing with microtiter plates. Vox Sang. *12*:319–20, 1967.

[35] Elandt-Johnson, R. C.: *Probability Models and Statistical Methods in* Genetics. New York, John Wiley & Sons, 1971.

[36] Krieger, H., Morton, N. E., Mi, M. P., Azevedo, E., Freire-Maia, A., and Yasuda, N.: Racial admixture in north-eastern Brazil. Ann. Hum. Genet. *29*:113–25, 1965.

[37] Elston, R. C.: The estimation of admixture in racial hybrids. Ann. Hum. Genet. (Lond.) *35*:9–17, 1971.

[38] Workman, P. L., Blumberg, B. S., and Cooper, A. J.: Selection, gene migration and polymorphic stability in a U.S. white and negro population. Am. J. Hum. Genet. *15*:429–37, 1963.

[39] Reed, T. E.: Caucasian genes in American Negroes. Science *165*:762–68, 1969.

[40] Rotter, J. I., and Hodge, S. E.: Response: racial differences in juvenile-type diabetes are consistent with more than one mode of inheritance. Diabetes *29*:115–18, 1980.

[41] Rotter, J. I., and Rimoin, D. L.: Heterogeneity in diabetes mellitus—update 1978. Diabetes *27*:599–608, 1978.

[42] Neufeld, M., Maclaren, N. K., Riley, W. J., Lezotte, D., McLaughlin, J. V., Silverstein, J., and Rosenbloom, A. L.: Islet cell and other organ-specific antibodies in U.S. Caucasians and blacks with insulin-dependent diabetes mellitus. Diabetes *29*:589–92, 1980.

[43] Grubb, R.: The Genetic Markers of Human Immunoglobulins. New York, Springer-Verlag, 1970.