Association Between NIDDM, RH Blood Group, and Haptoglobin Phenotype

Results from the San Antonio Heart Study

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SUMMARY

We examined seven red cell antigen and 10 polymorphic protein phenotypes in 1237 Mexican Americans randomly selected from three San Antonio neighborhoods. Statistically significant associations were found between non-insulin-dependent diabetes mellitus (NIDDM) and RH blood type (X^2 = 32.87, df = 10, P = 0.0003) and haptoglobin phenotype (X^2 = 9.15, df = 2, P = 0.010). The haptoglobin association showed a dose effect with a single dose of the haptoglobin-1 allele associated with an approximately 50% increase and a double dose of the haptoglobin-1 allele associated with an approximately 100% increase in NIDDM prevalence. Multivariate analysis indicated statistically significant associations between NIDDM and age, sex, adiposity, and neighborhood of residence. However, even after taking these potential confounding variables into account, there was still a significant, independent association between NIDDM and haptoglobin phenotype. The results suggest that the haptoglobin gene may be in linkage disequilibrium with a major susceptibility gene for NIDDM. DIABETES 1986; 35:387–91.

Recently, considerable progress has been made in understanding the genetics of insulin-dependent diabetes mellitus (IDDM). It is now firmly established that this type of diabetes is strongly associated with HLA antigens Dw3 and Dw4.[1,2] Much less is known about the genetics of non-insulin-dependent diabetes mellitus (NIDDM) despite the fact that studies of monozygotic (MZ) twins indicate a stronger genetic component for this type of diabetes than for IDDM. Specifically, concordance for NIDDM among MZ twins approaches 100%,[3,4] whereas for IDDM, concordance is much lower.[1] Although sporadic associations between NIDDM and various HLA antigens have been reported,[5–9] no consistent pattern has emerged, and no HLA associations have been reported in Caucasian populations.

Mexican Americans have a high prevalence of NIDDM that is only partially explained by their high prevalence of obesity.[10] Moreover, we have recently reported that the prevalence of NIDDM in this population varies with the percent of genetic admixture from native American sources, suggesting a genetic component.[11] The present article reports the results of studies of associations between NIDDM and 17 genetic markers in Mexican Americans studied as part of the San Antonio Heart Study.

MATERIALS AND METHODS

The San Antonio Heart Study is a population-based study of Mexican American and non-Hispanic white adults, ages 25–64 yr. A detailed description of the study design and response rates has been published previously.[10–12] Briefly, households were selected at random from three San Antonio neighborhoods: a low-income barrio, a middle-income transitional neighborhood, and a high-income suburb. The response rates in the three neighborhoods were: barrio, 61.3%; transitional, 60.1%; and suburb, 69.5%. The present report concerns the 1237 Mexican Americans (out of a total of 1288) in whom a panel of 17 genetic markers was determined. Since not every marker was measured in every subject, the individual analyses for each marker are based on slightly different subsets of individuals. Rare phenotypes have been ignored in the present analyses.

Anticoagulated whole blood was typed for antigens of the ABO, RH, MNS, Duffy, Kell, and Diego red cell antigen systems and for protein phenotypes at the following 10 loci: adenylate kinase (AK), adenosine deaminase (ADA), acid phosphatase (ACP), esterase-D (ESD), glutamate pyruvate transaminase (GPT), glyoxalase-1 (GLO), haptoglobin (Hp), phosphoglucomutase-1 (PGM1), phosphogluconate dehy-

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drogenase (PGD), and phosphoglycolate phosphatase (PGP). Typing methods have been reported previously.[13]

Non-insulin-dependent diabetes mellitus (NIDDM) was diagnosed according to the National Diabetes Data Group (NDDG) criteria,[14] as modified for epidemiologic survey work.[11,12] Subjects who gave a history of diabetes but who failed to meet the NDDG plasma glucose criteria were considered to be diabetic only if they were currently under treatment with insulin or oral antidiabetic agents.[11,12] Of the 137 diabetic subjects identified, only 14 took insulin, 5 of whom were considered to have IDDM on the basis of age of onset <40 yr and/or body mass index <30.0 kg/m^2. The present analyses are thus based on the 132 cases who were thought to have NIDDM.

Associations between NIDDM and phenotypes were assessed by two methods. In the first, the prevalence of NIDDM according to phenotype was determined. Here the measure of association was the relative risk and the statistical significance of each association was assessed by the X^2 statistic. A limitation of this method is that the denominator for the prevalence rates was the entire population and therefore included, in addition to normals, individuals with impaired glucose tolerance, individuals with a family history of diabetes (parents or siblings), and individuals who gave a personal history of diabetes, although they failed to meet our study criteria (see above). To overcome this limitation, we also assessed associations using a case-control approach. We first defined a normal control group by excluding the categories of individuals just mentioned. The distribution of phenotypes in the 607 normal controls so defined was then compared with the phenotypic distribution in the 132 cases who met our study criteria for NIDDM. Here the measure of association was the odds ratio and the statistical significance of each association was again assessed by the X^2 statistic. Since the results of both types of analysis were nearly identical, only the first method is presented in detail.

Associations between phenotypes and NIDDM were adjusted for possible confounding variables using multiple logistic regression with backwards stepping to remove nonsignificant terms (BMDP Program LR).[15] (The P-value for removal was 0.30.) In these analyses, presence or absence of diabetes was the dependent variable and the following independent variables were examined: age, sex, adiposity, neighborhood, and phenotype. The measure of adiposity was the sum of the triceps and subscapular skinfold.[10] [Body mass index, which was highly correlated with sum of skinfolds ($r = 0.66$), was also given an opportunity to enter the model, but sum of skinfolds emerged as a more powerful predictor

TABLE 1
Prevalence of diabetes according to erythrocyte antigen phenotype

Marker	Phenotype	N	Number of diabetic subjects	Percent diabetic	X^2	df	P-value
ABO	O	694	68	9.8			
	A	368	48	13.0			
	AB	26	2	7.7			
	B	112	12	10.7	2.90	3	NS
RH	CDEe	30	6	20.0			
	CDe	270	31	11.5			
	CcDE	21	4	19.0			
	CcDEe	264	15	5.7			
	CcDe	277	26	9.4			
	cDE	63	12	19.0			
	cDEe	158	18	11.4			
	cDe	38	9	23.7			
	Ccde	3	1	33.3			
	cdE	1	1	100.0			
	cde	73	7	9.6	32.87	10	0.0003
MNS	MS	73	12	16.4			
	MSs	234	28	12.0			
	Ms	177	17	9.6			
	MNS	65	11	16.9			
	MNSs	257	20	7.8			
	MNs	244	26	10.7			
	NS	5	0	0			
	NSs	53	9	17.0			
	Ns	86	6	7.0	11.97	8	NS
Diego	$D_i^a(-)$	1131	125	11.1			
	$D_i^a(+)$	68	4	5.9	1.79	1	NS
Kell (K)	K(+)k(−)	1121	121	10.8			
	K(+)k(+)	49	4	8.2	0.34	1	NS
Kell (K_p)	$K_p^b(+)K_p^a(-)$	1151	123	10.7			
	$K_p^a(+)K_p^b(+)$	19	2	10.5	0.00	1	NS
Duffy	$F_y^a(+)F_y^b(-)$	371	45	12.1			
	$F_y^a(+)F_y^b(+)$	553	57	10.3			
	$F_y^a(-)F_y^b(+)$	252	27	10.7	0.78	2	NS

TABLE 2
Prevalence of diabetes according to protein phenotypes

Marker	Phenotype	N	Number of diabetic subjects	Percent diabetic	X^2	df	P-value
AK	1	1129	121	10.7			
	2–1	49	6	12.2			
	2	1	0	0	0.23	2	NS
ADA	1	1115	119	10.7			
	2–1	67	8	11.9	0.11	1	NS
ACP	A	81	10	12.3			
	AB	433	55	12.7			
	B	647	61	9.4			
	AC	4	1	25.0			
	BC	11	0	0	5.26	4	NS
ESD	1	862	92	10.7			
	2–1	298	33	11.1			
	2	19	2	10.5	0.04	2	NS
GPT	1	265	30	11.3			
	2–1	564	56	9.9			
	2	324	37	11.4	0.63	2	NS
GLO	1	176	20	11.4			
	2–1	513	44	8.6			
	2	494	64	13.0	5.06	2	0.080
Hp	1	258	39	15.1			
	2–1	584	63	10.8			
	2	339	25	7.4	9.15	2	0.010
PGM1	1	714	80	11.2			
	2–1	396	40	10.2			
	2	71	8	11.3	0.34	2	NS
PGD	A	1095	116	10.6			
	AC	70	9	12.9			
	C	1	0	0	0.47	2	NS
PGP	1	934	101	10.8			
	2–1	212	23	10.8			
	2	22	2	9.1	0.07	2	NS

variable in the present data set.] Neighborhood was included in the list of independent variables, since we have previously reported that NIDDM prevalence in Mexican Americans varied across neighborhoods.[11,12] Odds ratios can be calculated from the logistic regression coefficients as indicated in the footnote to Table 4.

RESULTS

Table 1 shows the prevalence of NIDDM according to phenotype for the red cell antigen markers. The only statistically significant association was with the RH blood group (X^2 = 32.87, df = 10, P = 0.0003). This association was confirmed using the case-control approach (X^2 = 22.38, df = 10, P = 0.013). The association appears to be due principally to a high prevalence of NIDDM among individuals with CDEe, CcDE, cDE, and cDe phenotypes and a low prevalence among individuals with CcDEe and CcDe phenotypes.

Table 2 shows the prevalence of NIDDM according to protein phenotypes. The only statistically significant association was with haptoglobin phenotype (X^2 = 9.15, df = 2, P = 0.010). This association shows a dose effect with the relative risk of the homozygous 1 allele being 2.04 times and the relative risk of the heterozygote being 1.46 times that of the homozygous 2 allele. When the case-control approach was used (Table 3), the association was still statistically significant (X^2 = 7.69, df = 2, P = 0.021) and a dose effect was again seen. Here the odds ratio for the homozygous 1 allele versus the homozygous 2 allele was 2.15, and the odds ratio for the heterozygote versus the homozygous 2 allele was 1.43.

The results of the mutiple logistic analysis are shown in Table 4. The Hosmer and Lemeshow X^2 for goodness of fit[16] was 7.04 (df = 8, P = 0.532), indicating a good fit of the model to the data. Age, sex, adiposity, and neighborhood are all significantly associated with NIDDM. However, even after taking these potentially confounding variables into account, there is still a significant, independent association between NIDDM and haptoglobin phenotype. The odds ratios calculated from the logistic regression coefficients are similar to those calculated from the data presented in Tables 2 and 3.

DISCUSSION

A possible explanation of the observed associations between phenotypes and NIDDM is that they are due merely to chance. Since we examined 17 potential associations, it is quite possible that one or two would be found to be statistically significant through chance alone. We believe, however, that these associations may have biologic significance for the following reasons. First, a dose effect is often taken as evidence that an observed association is causal. In our data,

TABLE 3
Distribution of haptoglobin phenotype in NIDDM cases and controls

Phenotype	NIDDM cases	Controls
11	39	121
21	63	294
22	25	167

$X^2 = 7.69$, df = 2, P = 0.021.

a single dose of the haptoglobin-1 allele increased the risk of NIDDM by approximately 50% and a double dose increased the relative risk by approximately 100%. Second, associations between RH blood group, haptoglobin phenotype, and NIDDM have been reported previously.

An association between RH blood group and diabetes has been reported from Oslo, Norway, by Berg et al.[17] and from Germany by Scholz et al.[18] Berg et al. noted a deficiency of phenotypes containing the R_1 (CDe) haplotype and an excess of phenotypes containing the R_2 (cDE) haplotype among diabetic subjects whereas Scholz et al. observed the opposite pattern. In a population-based study of the type described here, it is not possible to assign specific haplotypes based on individual phenotypes. In our data, a comparison of all phenotypes that could contain either the R_1 or R_2 haplotype did not reveal any significant differences between diabetic and control subjects for either haplotype.

The association between NIDDM and haptoglobin phenotype is intriguing because of the apparent dose effect. A similar association was observed by Berg in Norway,[17] where the odds ratio for the homozygous 1 allele versus the homozygous 2 allele was 1.97 and the odds ratio for the heterozygote versus the homozygous 2 allele was 1.30. This association was statistically significant ($X^2 = 8.97$, df = 2, $P < 0.02$) and the odds ratios are remarkably similar to ours. Three other studies, however, failed to observe an association between haptoglobin phenotype and diabetes.[18,20]

Of the five studies that report data on haptoglobin, ours is the only one that distinguished between NIDDM and IDDM and also the only one in which both the case series and the control group were population based. None of the earlier studies used a population-based case series. The Oslo study[17] and the two studies from Germany[18,20] used population-based control groups, but the study from Toronto[19] used blood donors as the controls. In addition, our study is the only one that excluded from the control group subjects with either impaired glucose tolerance or a family history of diabetes.

The frequency of the haptoglobin-1 allele is higher in native American (Amerindian) populations than in Caucasian populations (0.542 versus 0.430) (R. E. Ferrell, R. C. Chakraborty, M. P. Stern, S. M. Haffner, H. P. Hazuda, and M. Rosenthal, unpublished data). Also, there are differences between native Americans and Caucasians in the frequency of various RH haplotypes. Thus, it is possible that Mexican Americans having those phenotypes that we found to be associated with NIDDM also have a greater degree of native American genetic admixture. Since most native American populations have high rates of NIDDM,[21] a greater degree of native American admixture could confer increased susceptibility to NIDDM in a relatively nonspecific manner; i.e., the haptoglobin-1 allele or certain RH haplotypes might simply be a proxy for native American genetic admixture. In our own data, in fact, we have reported an association between native American admixture and NIDDM.[11] The observation of an association between haptoglobin phenotype and diabetes in a purely Caucasian population from Norway, however, weakens this argument.

It is possible that haptoglobin is directly involved in the pathogenesis of NIDDM. However, there is at present no evidence for this. Another possibility is that the haptoglobin locus is linked to a major susceptibility gene for NIDDM. The haptoglobin locus has been assigned to the long arm of chromosome 16 in the region of q_{21}–q_{22}, and our results suggest that other loci in this region should be examined for possible association or linkage with NIDDM. For example,. the gene for lecithin-cholesterol acyltransferase (LCAT) has been localized to the q_{21}–q_{23} region of chromosome 16.[22] We examined two markers on the short arm of chromosome 16 (PGP and GPT) and found no association with NIDDM.

Associations with NIDDM have also been reported for genetic markers assigned to chromosomes 6, 11, and 14. On chromosome 6, associations with NIDDM have been reported for HLA antigen A2 in African Blacks[5] and Pima Indians,[6] and for B22, Bw56, and Bw61 in various Pacific Island populations.[7-9] On chromosome 11, NIDDM has been associated with insertions in the 5′-flanking region of the insulin gene in a racially mixed U.S. population (Caucasians,. Blacks, and Pimas)[23] and in Caucasians from Denmark.[24] This association has not, however, been confirmed with more extensive data,[25,26] nor was it observed in Micronesians from Nauru.[8] Finally, on chromosome 14, an association between NIDDM and immunoglobulin (IgE) heavy-chain (Gm) polymorphism has been reported in Caucasians.[17,18] The diversity of findings raises the question of whether the genetic mechanism of

TABLE 4
Multiple logistic regression analysis of the association between NIDDM and haptoglobin phenotype adjusting for age, sex, adiposity, and neighborhood

Covariate*	Multiple logistic regression coefficient	P-value	Odds ratio†
Age (yr)	0.093	<0.001	
Sex	0.787	<0.001	2.20
Sum of skinfolds (mm)	0.038	<0.001	
Neighborhood			
Barrio versus suburbs	0.865	<0.010	2.37
Trans. versus suburbs	0.737	<0.030	2.09
Haptoglobin phenotype	0.365	<0.015	1.44, 2.07

*Sex was entered as an indicator variable (male = 1, female = 0), neighborhood was entered as two indicator variables with the suburbs as the comparison group, and haptoglobin phenotype was entered as an interval level variable (homozygous 2 allele = 0, heterozygote = 1, and homozygous 1 allele = 2). Interaction (product) terms between sex and haptoglobin and between sex and neighborhood were also considered, but were nonsignificant and so are not included in the final model.

†Odds ratios (OR) were calculated using the formula $OR = e^{\beta\Delta x}$, where β equals the logistic regression coefficient and Δx is 1 for the sex, neighborhood, and haptoglobin heterozygote versus homozygous 2 comparisons and is 2 for the homozygous 1 versus homozygous 2 comparison.

NIDDM might be different in different populations. In this regard it should be noted that, although Mexican Americans have significant native American genetic admixture, their gene pool is nevertheless >50% derived from Caucasian sources.[11,27]

Finally, it should be pointed out that all of the associations with genetic markers that have been reported thus far have been based on cross-sectional studies, and are thus subject to various types of bias (for example, differential survival). There is a need for prospective studies to assess whether any of the genetic markers thus far implicated, or any new markers, are predictive of the future development of diabetes.

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