Brief Genetics Report

Linkage Analysis of Diabetes Status Among Hypertensive Families

The Hypertension Genetic Epidemiology Network Study

Christy L. Avery,[1] Barry I. Freedman,[2] Gerardo Heiss,[1] Aldi Kraja,[3] Treva Rice,[3] Donna Arnett,[4] Michael B. Miller,[4] James S. Pankow,[4] Cora E. Lewis,[5] Richard H. Myers,[6] Steven C. Hunt,[7] Laura Almasy,[8] and Kari E. North[1]

Type 2 diabetes susceptibility is determined by multiple genetic and environmental factors. Genome-wide linkage scans have localized common regions, possibly harboring susceptibility genes on chromosomes 1, 2, 12, and 20. Variability in linkage findings underscores the probable genetic heterogeneity of type 2 diabetes. Thus, we conducted a genome scan of diabetes status using maximum likelihood methods that model affection status by a liability threshold model. Hypertensive sibships and their offspring and/or parents in the Hypertension Genetic Epidemiology Network study were recruited from five field centers. The diabetes phenotype was derived using the World Health Organization criteria and adjusted for race/study center, age, age^2, sex, and with and without percent body fat. In total, 567 diabetic participants were identified in 437 families. Variance component linkage analysis was performed among 1,545 Caucasians and 1,608 African Americans using race-specific marker allele frequencies. We detected a quantitative trait loci (QTLs) influencing diabetes variance (logarithm of odds = 3.4) on chromosome 22, which overlaps a positive type 2 diabetes finding among Canadian Oji-Cree Indians. We also observed suggestive evidence for linkage on chromosomes 1, 2, 5, 8, 14, 17, and 19. The identification and replication of type 2 diabetes QTLs will bring us closer to the detection of functional genes that influence diabetes susceptibility.
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From the [1]Department of Epidemiology, University of North Carolina Chapel Hill, Chapel Hill, North Carolina; the [2]Department of Internal Medicine, Wake Forest University, Winston Salem, North Carolina; the [3]Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri; the [4]Division of Epidemiology, University of Minnesota, Minneapolis, Minnesota; the [5]Department of Preventive Medicine, University of Alabama Birmingham, Birmingham, Alabama; the [6]Section of Neurogenetics, Boston University, Boston, Massachusetts; the [7]Cardiovascular Genetics Division, University of Utah, Salt Lake City, Utah; and the [8]Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas.

Address correspondence and reprint requests to Kari E. North, PhD, Department of Epidemiology, CB no. 8050, Suite 306, Bank of America, University of North Carolina Chapel Hill, Chapel Hill, NC 27514. E-mail: kari_north@unc.edu.

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HyperGEN, Hypertension Genetic Epidemiology Network; LOD, logarithm of odds; MERLIN, multipoint engine for rapid likelihood inference; PBF, percent body fat; QTL, quantitative trait locus.

Type 2 diabetes is a common disease with susceptibility determined by multiple genetic and environmental factors. Several rare mutations have been identified that influence diabetes susceptibility, yet only one common susceptibility gene has been identified, the *CAPN10* gene (1), and it is associated with diabetes in some study populations but not others (2). Genome-wide scans for linkage have been conducted in several populations, localizing a number of common chromosomal regions possibly harboring susceptibility genes on chromosomes 1q21–24 (3), 2q37 (1), 12q24 (4), and 20q (3). Such variability in linkage findings reported between populations underscores the probable genetic heterogeneity of type 2 diabetes. Thus, we conducted a genome scan of liability to diabetes in an attempt to localize new quantitative trait loci (QTLs) influencing diabetes susceptibility and/or to provide new evidence in support of previously identified QTLs.

This study examined 3,153 participants in the Hypertension Genetic Epidemiology Network (HyperGEN) of the Family Blood Pressure Program. HyperGEN methods, participant information, and exclusion criteria are detailed in the online appendix (available at http://care.diabetesjournals.org). The distribution of covariates by diabetes status for the combined sample is shown in Table 1. The prevalence of type 2 diabetes was 18% (n = 567), with a higher prevalence of female (64%) compared with male (36%) subjects. In African-American participants, 21% were classified as diabetic compared with 15% of the Caucasian sample. Overall, 385 of 437 HyperGEN families had at least 1 affected participant, with 98 families having 2 diabetic family members, 11 families having 3 diabetic members, 3 families having 4 diabetic members, and 1 family with 5 diabetic members.

The multipoint genome-wide logarithm of odds (LOD) scores for the combined sample by race, with and without percent body fat [PBF] adjustment for all peaks, with a LOD score ≥1.7 (suggestive evidence of linkage) (5) are given in Table 2. We detected a QTL influencing liability to diabetes status in the complete sample, with a corresponding LOD score of 3.4 on chromosome 22q12.1 at map

TABLE 1
Covariate distribution by diabetes status for 3,153 HyperGEN participants

	Diabetic participants	Nondiabetic participants
n	567	2586
Age in years	59.75 ± 10.7	51.09±13.7
Male	205 (36)	1065 (41)
Hypertensive	442 (78)	1909 (74)
PBF	40.36 ± 8.6	36.97 ± 9.7
Race/center		
Caucasians (North Carolina)	24 (04)	161 (06)
Caucasians (Minnesota)	51 (09)	357 (14)
Caucasians (Massechusetts)	59 (10)	376 (15)
Caucasians (Utah)	100 (18)	417 (16)
African Americans (North Carolina)	76 (13)	320 (12)
African Americans (Alabama)	257 (45)	955 (37)

Data are means ± SD or *n* (%).

location 28 cM (22q12.1, nearest marker GATA21F03) (Fig. 1), which overlaps three positive findings (Table 3). The robust *P* value that corresponds to our observed LOD score is 0.001, indicating that our nominal *P* value may have slightly overstated the evidence of linkage.

Hegele et al. (6) detected a QTL for type 2 diabetes ($P < 0.01$) among 88 Canadian Oji-Cree Indian kindred, and Sale et al. (7) observed linkage for type 2 diabetes in 1,276 affected African Americans (LOD = 1.3); both used affected sibpair linkage designs. Additionally, Pratley et al. (8) observed linkage for fasting plasma glucose in 109 nondiabetic Pima-Indian families (LOD = 1.8) using variance component methods.

A secondary significant genome-wide LOD score of 3.1 was detected on chromosome eight at map location 65 cM (8q11.23, nearest marker GATA8G10) for the combined sample after PBF adjustment (Fig. 1). This LOD score was only slightly attenuated (LOD = 2.5) in the absence of PBF adjustment. The robust *P* value that corresponds to our observed LOD score is 0.003, indicating that our nominal *P* value may have slightly overstated the evidence of linkage. This finding also supports three previous genome-wide scans. Elbein et al. (9) detected linkage to type 2 diabetes in 379 members of 19 multiplex Caucasian families using variance component methods (LOD = 1.4). Additionally, Mitchell et al. (10) and Chagnon et al. (11) both detected linkage to BMI measures in 10 large Mexican-American families (LOD = 3.2) and 364 Caucasian sibpairs (LOD = 2.0), employing variance component and affected sibpair linkage methods, respectively.

Evidence for linkage was also detected on chromosome 17 map location 53 cM (17q11.2, nearest marker GGAA9D03) for the combined (LOD = 3.0) and the Caucasian samples (LOD = 2.7). This signal appears to attenuate after PBF adjustment, which may indicate shared genetic effects between obesity and diabetes. The robust *P* value that corresponds to our observed LOD score is 0.003, indicating that our nominal *P* value may have slightly overstated the evidence of linkage. Likewise, this finding supports numerous genome-wide scans related to obesity measures, such as plasma leptin and adiponectin levels and BMI. Kissebah et al. (12), Wu et al. (13), and Comuzzie et al. (14) detected linkage to plasma leptin (LOD = 5.0), BMI (LOD = 2.5), and adiponectin (LOD = 1.7), respectively, using variance component linkage methods. In addition to the QTL on chromosomes 22, 8, and 17, we found several other regions demonstrating suggestive evidence for linkage on chromosomes 1, 2, 5, 14, and 19. Of these locations, all have been detected (based on various criteria) in other genome screens of diabetes-related phenotypes with the exception of the signal at 14q21.1.

Several candidate genes underlie the 1-cM LOD unit

TABLE 2
Estimated LOD scores suggestive of linkage (LOD ≥1.7)* combined, by race, and both with and without PBF adjustment from multipoint quantitative trait linkage analyses of derived diabetes status in 3,153 HyperGEN participants

	Chromosome	cM	Cytogenic location	LOD score
Sample without PBF adjustment				
Combined, Caucasians	2	128	2q14.1	2.0, 2.3
Combined	2	165	2q24.1	1.7
Combined	5	143	5q31.3	2.0
Caucasians	5	175	5q35.1	2.1
Caucasians	8	42	8p21.3	1.7
Caucasians	8	67	8q11.23	2.5
Combined, Caucasians	17	51, 53	17q11.2	2.7, 3.0
Caucasians	19	9	19p13.3	2.0
Combined, Caucasians	22	28	22q12.1	3.4, 2.0
Sample adjusted for PBF				
Combined, African Americans	1	68, 69	1p34.2	1.8
African Americans	1	226	1q32.2	1.9
Combined	1	250	1q42.2	1.9
Combined	2	168	2q24.1	1.9
Caucasians	8	49	8p21.3	1.9
Combined	8	65	8q11.23	3.1
Combined, Caucasians	8	124, 126	8q24.11	2.5, 2.1
Combined	14	44	14q21.1	2.2
Combined	22	29	22q12.1	2.0
Caucasians	22	46	22q11.21	2.0

*See ref. 5.

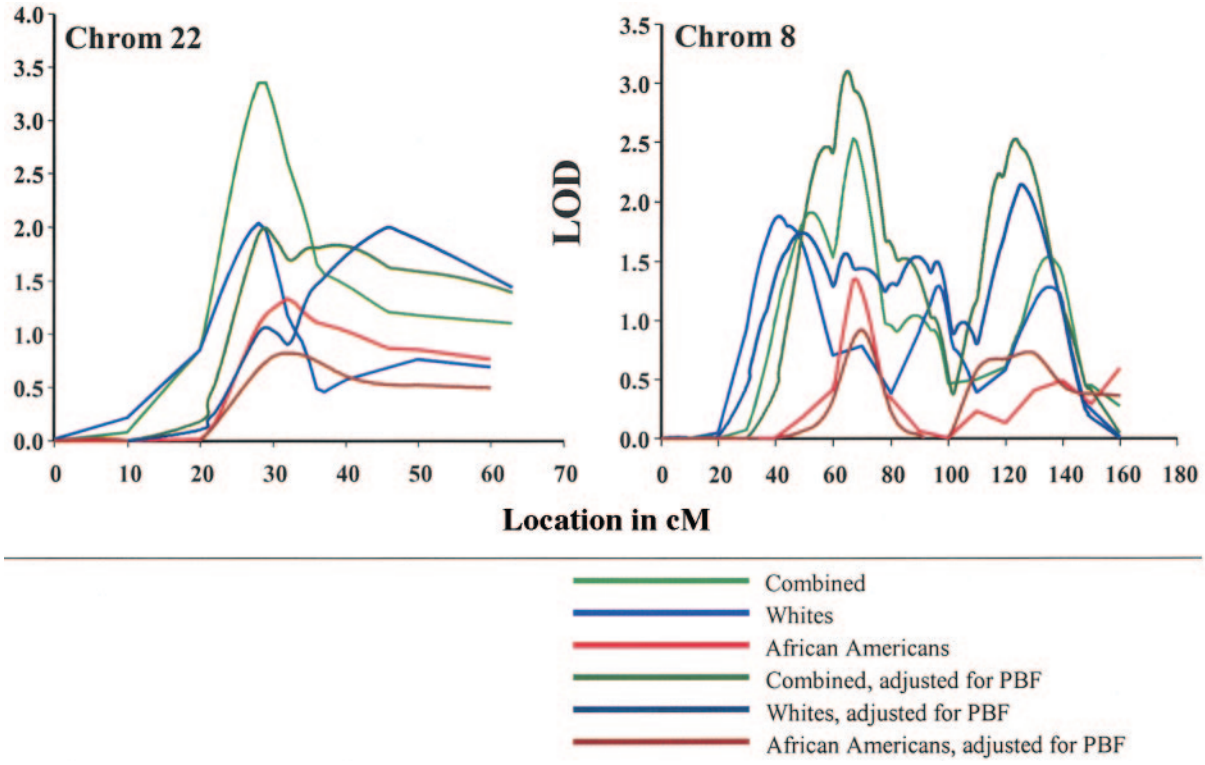

FIG. 1. Estimated LOD functions overall, by race, and adjusted for PBF obtained from multipoint quantitative trait linkage analyses of diabetes status for chromosomes 22 and 8.

drop support interval (9 cM, 8.3 megabases) corresponding to the linkage signal observed on 22q12.1, including the galanin receptor 3 (*GALR3*), leukemia inhibitory factor (*LIF*), and the apolipoprotein L1-L*4* (*APOL1-4*) cluster. Neuroanatomical distribution of *GALR3* mRNA suggests a mediation of galanin on food intake, fluid homeostasis, and cardiovascular function (15). *LIF* is a member of the interlukin 6 cytokine family and is a candidate for leptin

TABLE 3
QTLs from other genome-wide scans of type 2 diabetes, obesity, and related traits

Our findings	Supporting studies		
Cytogenic location	Phenotype	*P* value or LOD	Ref.
1p34.2	BMI, skinfold (Σ6), fat mass (Caucasians)	$0.009 < P < 0.02$	29
	Type 2 diabetes (Japanese)	1.5	30
1q32.2	Fasting glucose (Caucasians)	1.8	31
1q42.2	Mean glucose (Caucasians)	2.3	31
2q14.1	BMI and fat mass (Caucasians)	4.0, 2.0	32
2q24.1	Type 2 diabetes (Indigenous Australians)	2.6	33
	ASF (French Canadians)	$P = 0.005$	34
5q31.3	ASF, AVF (Caucasians)	$1.9 < \text{LOD} < 2.1$	35
5q35.1	ASF (French Canadians)	$P = 0.003$	34
8p21.3	Type 2 diabetes (Caucasians)	2.6	5
8p12_8q11.23	BMI (Caucasians)	1.4	9
	Type 2 diabetes (Caucasians)	3.2	10
	BMI (Mexican Americans)	2.0	11
8q24.11	Percentage fat mass (Caucasians)	1.5	32
17p12	Leptin (Caucasians)	5.0	12
	BMI (Caucasians)	2.5	13
	Adiponectin (Caucasians)	1.7	14
17q21.2	ASF (French Canadians)	2.2	34
19p13.3	Leptin (Caucasians)	$P = 0.0009$	11
	AVF (French Canadians)	$P = 0.01$	34
22q12.1	Fasting plasma glucose (Pima Indians)	1.8	8
22q12.3	Type 2 diabetes (Canadian Oji-Cree)	$P < 0.01$	6
	Type 2 diabetes (African Americans)	1.3	7
22q11.21	ASF (Caucasians)	2.0	35

ASF, abdominal subcutaneous fat; AVF, abdominal visceral fat.

circumvention and weight loss (16). *APOL1-4* represents a cluster of tandem gene duplications expressed in various human organs, including vascular tissue and endothelial cells. A lipid-related candidate gene may be relevant to diabetes susceptibility, as insulin resistance is associated with a lipid profile typified by increased triglyceride and LDL cholesterol levels and a predominance of small LDL particles. Moreover, North et al. (17) demonstrated common genetic effects on diabetes status and lipid values in American-Indian families participating in the Strong Heart Family Study.

Candidate genes within the 1-cM LOD unit drop support interval (24 cM, 33.3 megabases) surrounding the 8q11.23 QTL include the adrenergic β-3 receptor (*ADRB3*), corticotropin releasing hormone (*CRH*), and fibroblast growth factor receptor 1 (*FGFR1*). *ADRB3* is expressed in adipose tissue where it mediates lipolysis. Furthermore, Xiang et al. (18) detected an association between the *ADRB3* Trp64Arg mutation and BMI ($P = 0.019$) and waist circumference ($P = 0.045$) in type 2 diabetic Chinese subjects. *CRH* secretion by the paraventricular nucleus of the hypothalamus is one of the first steps in the mammalian stress response. Hart et al. (19) perturbed *FGFR1* dominant-negative signaling in the mouse pancreas and observed a phenotype typical of type 2 diabetes. They demonstrated that mice with attenuated *FGFR1* signaling developed diabetes with age, exhibited decreased numbers of β-cells, and had increased proinsulin content in β-cells.

Genetic heterogeneity of common complex traits is a long-standing problem for linkage studies. When genetic heterogeneity is present, there is reduced power for detection of QTLs. Genetic heterogeneity may be reduced by stratifying possible etiological subgroups, incorporating gene-environment and gene-gene interactions, and accounting for environmental influences of disease. Our study design may have reduced the problem of genetic heterogeneity, as we conducted a genome scan for diabetes in a largely hypertensive population.

This study may have been limited by our inability to adjust for potential disease confounders. Moreover, we lacked information on the age of onset of diabetes status for 99 participants who we classified as diabetic, which may have introduced type 1 diabetic participants into our sample. The population we examined was selected for hypertension, a phenotype correlated with diabetes; however, we have not used an ascertainment correction in these analyses. This is not likely a strong ascertainment effect because enriching our sample for hypertension-related susceptibility alleles would not be expected to strongly influence the distribution of diabetes QTLs. Neglecting a modest ascertainment effect would only result in some loss of information and a reduced power for our analysis, making the results presented here conservative, without any increase in the type I error rate (20). Nonetheless, these loci were detected in a largely hypertensive sample and may not be applicable to the general population.

We assumed that pleiotropic effects between type 2 diabetes and obesity QTLs were possible; yet we know that obesity is an important confounder, such that only by accounting for the variance due to obesity effects would we be able to identify type 2 diabetes QTLs. Indeed, the QTL-specific genetic signals varied substantially on chromosomes 22, 8, and 17, after adjustment for PBF. For example, the LOD score on chromosome 22q decreased from 3.4 to 2.0 in the combined-races sample after adjustment for PBF. Similarly, the LOD score on chromosome 17 decreased from 3.0 to 0.60 in the combined-races sample after adjustment for PBF, indicating the possibility of shared genetic effects between PBF and diabetes susceptibility. In contrast, upon adjustment for PBF, the QTL on chromosome 8q increased from 2.5 to 3.1 in the combined-races sample, suggesting that adjustment for PBF slightly improved our ability to detect this particular QTL. Further research is needed to test if there is a joint action of genes on diabetes status and PBF and whether modeling such an effect would improve our ability to localize diabetes susceptibility genes.

In conclusion, we found highly significant linkage results for diabetes susceptibility QTLs on chromosomes 22, 8, and 17. Specifically, the signal on 22q12.1 overlaps positive findings for diabetes status, abdominal subcutaneous fat, and plasma glucose and harbors several candidate genes, including *GALR3*, *LIF*, and the *APOL1-L4* cluster. The 8q11.23 signal supports previous findings for diabetes status and BMI and points to several candidate genes: *ADRB3*, *CRH*, and *FGFR1*. Additionally, all QTLs that demonstrated suggestive evidence of linkage with type 2 diabetes support earlier studies, with the exception of the QTL at 14q12.1. Ultimately, these findings suggest that multiple genes may regulate susceptibility to diabetes and that further research in this population may help to identify additional candidate genes that interact with the environment to influence diabetes susceptibility.

RESEARCH DESIGN AND METHODS

Informed consent was obtained from all participants, and this project was approved by the institutional review boards of all participating institutions. Serum glucose concentrations were estimated with the Elan glucose reagent (21). The diabetic phenotype was determined using World Health Organization recommendations, in which diabetic participants were classified as persons who received insulin treatment, an oral hypoglycemic agent, or had a fasting plasma glucose ≥126 mg/dl (22). PBF was derived from measured resistance and reactance using the Lukaski formula (online appendix). Genotyping was performed by the National Heart, Lung, and Blood Institute Mammalian Genotyping Service (Marshfield, WI) (available at http://research.marshfieldclinic.org/genetics) using screening set eight, which includes ~400 microsatellite markers equally spaced (~10 cM distance) throughout the genome. Average marker heterozygosity was 77.7%. Relationship status among the purportedly full sibs was analyzed using ASPEX (affected sibpair exclusion mapping) (23) and multipoint engine for rapid likelihood inference (MERLIN) (24). We used the University of California Santa Cruz (available at http://genome.ucsc.edu) and Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) websites to determine the cytogenic location of markers and search for candidate genes.

We conducted a genome scan of liability to diabetes using the variance component approach as implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines) (25). This approach is applicable to dichotomous traits under the assumption that an individual is classified as affected if an underlying genetically influenced liability, which is presumed to have a multivariate normal distribution, exceeds a certain threshold (online appendix).

We computed exact conditional probabilities using the Lander-Green algorithm in MERLIN (24). Allele frequencies from the random sample were calculated separately in African-American and Caucasian groups. The identical by descent probabilities computed by MERLIN were then combined into a single set of multipoint identical by descent files in the SOLAR format using the program Mer2sol (available at http://taxa.epi.umn.edu/mer2sol/), developed by Michael Miller at the University of Minnesota (online appendix).

We estimated the heritability of type 2 diabetes status using a minimum adjustment strategy with the covariates race/study center, age, age^2, and sex.

Although glycemic control deteriorates with diabetes progression, and some individuals become less obese (26) whereas others become more obese, models were also estimated with PBF. The heritability of type 2 diabetes status was also estimated with PBF, as previous research has demonstrated that obesity and body fat distribution are the strongest risk factors for the development of diabetes (27). Two participants with biologically implausible negative PBF values and 381 individuals with missing PBF measurements were excluded from these analyses. No additional covariate adjustments were made because we investigated prevalent diabetes status and did not know the true covariate values at diabetes onset.

As nonnormal trait distributions substantially increase type I error in variance component models, all continuously distributed covariates were examined for extreme observations outside 4 SDs (28). No covariates had observations that fell outside 4 SDs.

To verify our major linkage findings, we calculated the empirical distribution of the LOD scores under the assumption of multivariate normality, using 10,000 replicates and simulation methods incorporated into SOLAR (5). We used the empirical distribution of the simulated LOD scores to assign percentiles to each replicate and calculated an expected test statistic on the basis of the percentile. SOLAR produces a correction constant by regressing the expected LOD scores on the observed simulated LOD scores, which we used to determine the robust *P* value (36).

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