Brief Genetics Report

Genome-wide Linkage Scans for Fasting Glucose, Insulin, and Insulin Resistance in the National Heart, Lung, and Blood Institute Family Blood Pressure Program

Evidence of Linkages to Chromosome 7q36 and 19q13 From Meta-Analysis

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Genome-wide linkage analyses were performed using a multipoint variance components method in eight study groups from four multicenter networks (whites and blacks in GenNet; whites, blacks, and Mexican Americans in GENOA; whites and blacks in HyperGEN; and Asians in SAPPHIRe) that comprise the National Heart, Lung, and Blood Institute Family Blood Pressure Program (FBPP), in order to identify quantitative trait loci (QTLs) influencing fasting glucose, insulin, and homeostasis model assessment of insulin resistance (HOMA-IR). These study populations were enriched with subjects who had elevated blood pressure. Participants fasting <8 h, those with a history of type 2 diabetes, or those on antidiabetic medications were excluded from the current investigation. These three phenotypes were suitably transformed to approximate normal distributions. Each phenotype was adjusted for the effects of age, BMI, and field center separately by sex within each of the eight network ethnicity groups before genetic analysis. A total of 8,664 subjects comprising 5,923 sib-pairs from 4,043 families with 365 markers were available for conducting a meta-analysis using a modified Fisher's method of combining the *P* values from each of the eight scans. Evidence of linkages was found on chromosome 7q36 at 163 cM, with a logarithm of odds (LOD) score of 3.21 for HOMA-IR, and on chromosome 19q13 at 88 cM, with a LOD score of 3.33 for fasting glucose. We also found suggestive linkages (LOD score ≥2.2) on chromosome 7q36 at 163 cM, with LOD scores of 2.31 for fasting glucose and 2.26 for fasting insulin (versus the LOD score of 3.21 for HOMA-IR at this locus). In conclusion, QTLs were identified on chromosomes 7q36 and 19q13 for fasting glucose, insulin, and insulin resistance in large and multiple-ethnicity populations in the FBPP with good replications across several other independent studies for relevant traits. Follow-up dense mapping and association studies are warranted. *Diabetes* 54:909–914, 2005

Hyperinsulinemia and insulin resistance herald clinical hyperglycemia, the hallmark of type 2 diabetes development. Levels of fasting glucose, insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) (1) are known to be influenced in part by genes complicated by several interacting factors such as physical inactivity, obesity, and dyslipidemia. Previously, seven genome-wide linkage scans in nondiabetic subjects were carried out in Pima Indians (2), Finns (3), Mexican Americans (4), hypertensive Hispanics (5), whites from the Framingham Offspring Study (6,7), and whites and blacks from the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study (8) to identify quantitative trait loci (QTLs) for fasting glucose, insulin, and HOMA-IR. However, the

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Received for publication 1 November 2004 and accepted in revised form 9 December 2004.

FBPP, Family Blood Pressure Program; HERITAGE, Health, Risk Factors, Exercise Training and Genetics; HOMA-IR, homeostasis model assessment of insulin resistance; LOD, logarithm of odds; NHLBI, National Heart, Lung, and Blood Institute; QTL, quantitative trait locus.

TABLE 1
Basic characteristics of whites, blacks, Mexican Americans, and Asians

Network and race	*n* (%)*	Age (years)	BMI (kg/m^2)	Glucose (mg/dl)	Insulin (μU/ml)	HOMA-IR (uU/ml × mmol/l)
Male						
GenNet						
White	240 (13)	44.0 ± 14.7	28.9 ± 5.5	113.5 ± 28.8	6.7 ± 4.6	1.9 ± 1.5
Black	114 (1)	38.7 ± 10.4	27.9 ± 7.0	96.1 ± 15.1	6.7 ± 7.3	1.9 ± 3.6
GENOA						
White	608 (4)	54.8 ± 11.0	30.1 ± 5.0	98.0 ± 21.4	8.9 ± 6.0	2.3 ± 1.9
Black	362 (3)	58.3 ± 10.0	27.7 ± 4.7	96.5 ± 18.0	8.3 ± 7.1	2.1 ± 2.0
Mexican American	320 (17)	52.2 ± 12.2	30.2 ± 5.6	113.0 ± 46.9	13.5 ± 10.1	4.1 ± 4.6
HyperGEN						
White	747 (4)	56.7 ± 12.4	29.2 ± 4.5	98.6 ± 14.6	8.0 ± 5.8	2.0 ± 1.7
Black	569 (4)	46.2 ± 12.2	29.1 ± 5.9	97.6 ± 25.5	8.7 ± 8.7	2.2 ± 2.4
SAPPHIRe						
Asian	603 (3)	50.9 ± 8.9	26.2 ± 3.6	96.1 ± 18.9	8.1 ± 6.1	2.1 ± 1.7
Female						
GenNet						
White	280 (11)	44.6 ± 14.2	29.5 ± 7.0	107.0 ± 21.1	7.3 ± 5.3	2.1 ± 1.9
Black	199 (3)	41.5 ± 11.6	32.1 ± 8.1	93.7 ± 18.0	8.7 ± 9.0	2.1 ± 2.7
GENOA						
White	777 (3)	54.1 ± 10.9	30.3 ± 7.2	92.2 ± 15.5	8.4 ± 7.3	2.0 ± 2.3
Black	884 (6)	56.8 ± 10.5	31.8 ± 7.0	97.6 ± 23.1	10.2 ± 7.5	2.6 ± 2.3
Mexican American	410 (12)	50.7 ± 12.1	31.6 ± 6.0	103.1 ± 31.6	15.2 ± 14.6	4.2 ± 5.6
HyperGEN						
White	828 (4)	57.0 ± 11.6	29.2 ± 7.0	95.3 ± 15.9	7.4 ± 5.4	1.8 ± 1.6
Black	958 (4)	46.3 ± 12.7	33.0 ± 7.9	95.4 ± 20.0	10.5 ± 7.3	2.6 ± 2.0
SAPPHIRe						
Asian	765 (3)	51.4 ± 9.1	25.3 ± 4.0	92.8 ± 16.7	7.6 ± 5.2	1.8 ± 1.4

Data are means ± SD unless otherwise indicated. *Percentages of suspected diabetic subjects with fasting serum glucose concentrations ≥126 mg/dl.

linkage signals obtained from these studies on chromosome 1, 3, 7, 10, 11, 12, 17, 19, and 22 did not replicate across reports. Indeed, these traits are complex with probable genetic heterogeneity modified by a multitude of environmental influences. Aiming to identify real QTLs for these pre-diabetes traits, we took advantage of the large pooled database of the National Heart, Lung, and Blood Institute (NHLBI) Family Blood Pressure Program (FBPP) (9) to improve the power for detecting genes with small to moderate effects in specific populations.

In the present study, genome-wide linkage scans were performed using a multipoint variance components method in eight network ethnicity groups from four multicenter networks (whites and blacks in GenNet; whites, blacks, and Mexican Americans in GENOA; whites and blacks in HyperGEN; and Asians in SAPPHIRe) collectively known as the FBPP. We then pooled the FBPP-wide linkage evidence into a single genome-wide linkage scan using a modified Fisher's method (10,11) by combining the *P* values at every marker location throughout the genome. This combined analysis involved a total of 8,664 subjects comprising 5,923 sibpairs from 4,043 families in the FBPP. It represents the largest genome-wide linkage analysis effort designed to integrate and synthesize uniformly linked signals from individual scans in the FBPP and to locate QTLs that might harbor genes for these type 2 diabetes–associated traits.

Data characteristics of whites, blacks, Mexican Americans, and Asians separately by sex and FBPP network are presented in detail in Table 1. The multipoint genome-wide linkage scan results in each of the eight network ethnicity groups are depicted in Fig. 1*A* for fasting glucose, Fig. 1*B* for fasting insulin, and Fig. 1*C* for HOMA-IR. The meta-analysis results based on the eight individual linkage scans are given in Fig. 2, and suggestive linkages with logarithm of odds (LOD) scores of at least 2.2 (12) are summarized in Table 2. The strongest linkages were found on 7q36 at 163 cM (D7S3070, LOD score 3.21) for HOMA-IR (also a LOD score of 2.31 for fasting glucose and a LOD score of 2.26 for fasting insulin) and on 19q13 at 88 cM (D19S589, LOD score 3.33) for fasting glucose. In addition, two other suggestive linkages were found on 2p at 99–103 cM (D2S1777–D2S1790) for fasting insulin (LOD score 2.35) and HOMA-IR (LOD scores 2.27–2.62) and on 4q at 78–88 cM (D4S2367–D4S3243, LOD scores 2.33–2.48) for fasting insulin.

A modified Fisher's method of combining *P* values (10,11) was used to synthesize linkage information extracted from individual scans. Although each study was designed to have adequate power for capturing genes with moderate effect sizes, no single network alone can expect to localize all genes with small effects for any given complex traits. Fortunately, we are able to improve the power by combining the evidence across networks (13). One limitation of this meta-analysis of *P* values is that there is no way to disentangle parameters, such as the effect size and its SE. Therefore, this methodology cannot test for heterogeneity in the effect sizes (13). In the current study, the FBPP networks used a common set of anonymous markers genotyped at a central laboratory, which may have, to some degree, minimized sources of heterogeneity. In addition, the current study is highly enriched with hypertensive

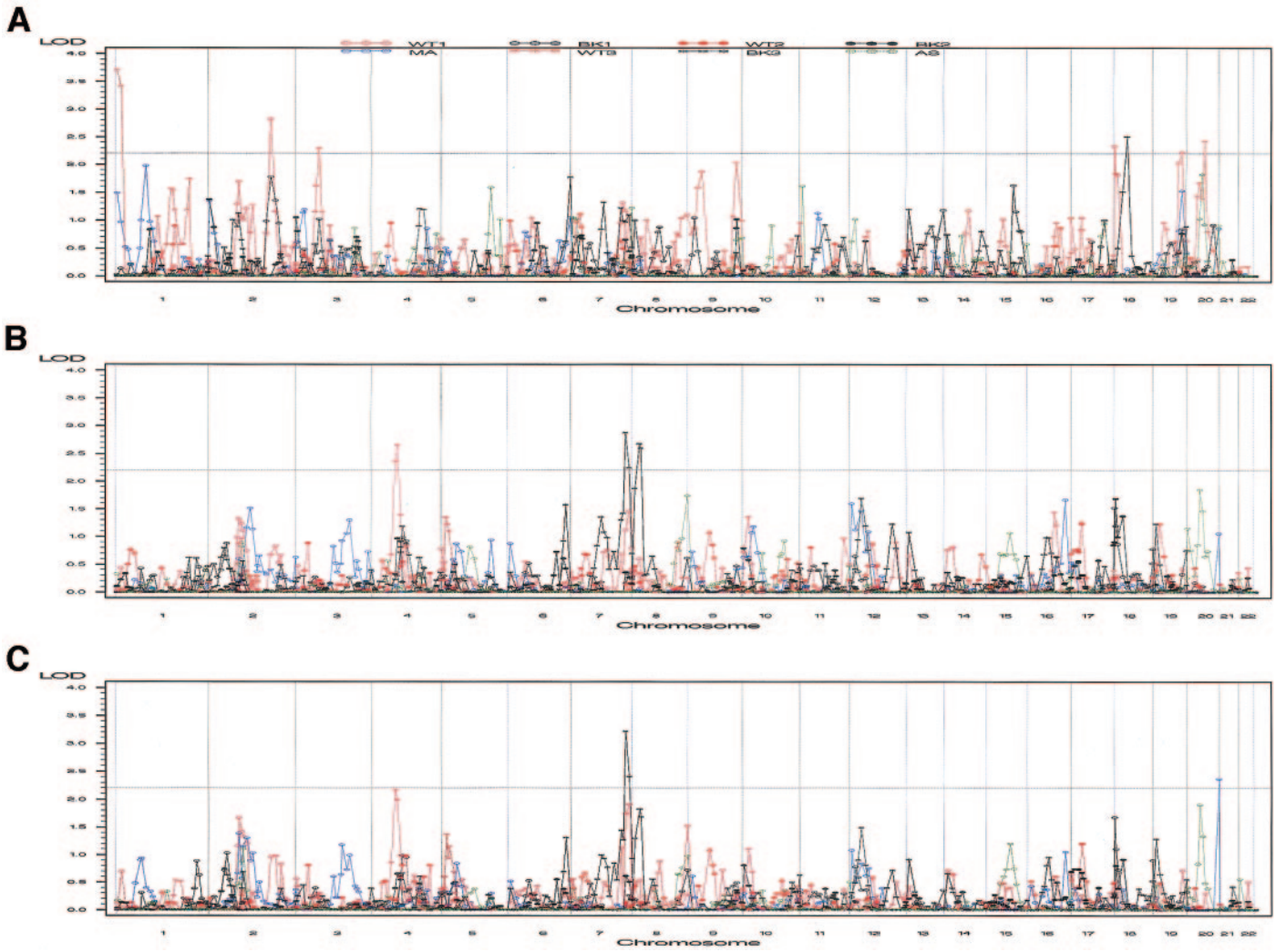

FIG. 1. Individual genome-wide linkage scan results in eight network ethnicity groups (whites and blacks in GenNet, GENOA, and HyperGEN; Mexican Americans in GENOA; and Asians in SAPPHIRe) in the FBPP for fasting glucose (*A*), fasting insulin (*B*), and HOMA-IR (*C*). LOD score of horizontal reference line is 2.2 for suggestive linkages. WT1, WT2, and WT3 denote whites in GenNet, GENOA, and HyperGEN, respectively. BK1, BK2, and BK3 denote blacks in GenNet, GENOA, and HyperGEN, respectively. MA and AS denote Mexican Americans in GENOA and Asians in SAPPHIRe, respectively.

subjects (29.6, 63.0, 83.8, and 66.6% in GenNet, GENOA, HyperGEN, and SAPPHIRe, respectively) (9), which might render the ability to detect linkages at certain loci but might also confound some type 2 diabetes–associated loci. We noted that the sample sizes differed largely across networks. However, it might be difficult to weight the studies precisely in this meta-analysis because other important interacting factors, including obesity and hypertension, are also accountable in complicated ways that have not been considered and assessed in the current report.

The present study represents our search for QTLs for type 2 diabetes–associated traits using the largest database on multiple ethnic groups. Two strong linkages (LOD scores ≥3) on 7q36 and 19q13 were found with not only the best LOD scores but also consistent linkage evidence at the same marker locations across all eight individual scans (and across all three nonindependent phenotypes on 7q). The QTL on 7q at marker D7S3070 (163 cM, 1-LOD support interval 155–175 cM) is most promising, with LOD scores of 2.31 for fasting glucose, 2.26 for fasting insulin, and 3.21 for HOMA-IR. QTLs on 7q for type 2 diabetes and its associated traits have been extensively studied previously. Whereas a broad genomic region of 90–134 cM was reported in a variety of populations (2,3,5,8,14–17 and P.A., M. Teran-Garcia, T. Rice, T. Rankinen, S.J. Weisnagel, R.N. Bergman, R.C. Boston, S. Mandel, D. Stefanovski, A.S. Leon, J.S. Skinner, D.C.R., C. Bouchard, unpublished observations), the current scan results for fasting glucose, insulin, and HOMA-IR inferred a different region at 163 cM. Our finding was consistent with that in the Framingham Study for an insulin resistance index (triglyceride–to–HDL cholesterol ratio) at D7S1805 (161 cM, LOD score 2.5) (18). In addition, two other nominal but compatible linkages were reported in the FUSION (Finland–United States Investigation of Non–Insulin-Dependent Diabetes Mellitus Genetics) study for type 2 diabetes at D7S3058 (174 cM) (19) and in Pima Indians for fasting glucose at D7S599 (183 cM) (2). There are two candidate genes around this QTL, including *PRKAG2* (AMP-activated protein kinase γ2 regulatory subunit, OMIM 602743) and *INSIG-1* (insulin-induced gene 1, OMIM 602055). AMP-activated protein

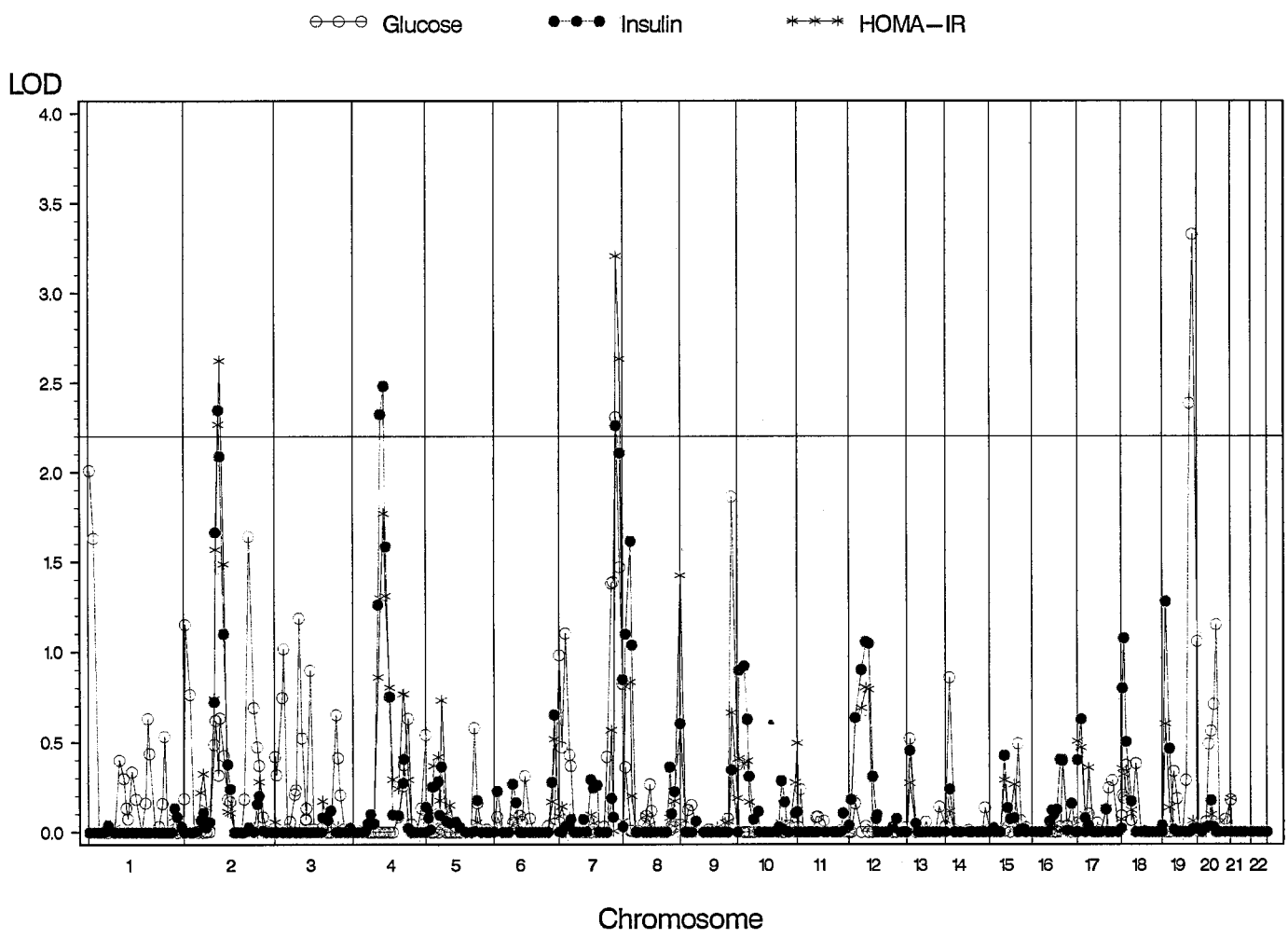

FIG. 2. Combined linkage analysis results from genome-wide linkage scans in the eight network ethnicity groups in the FBPP for fasting glucose, insulin, and HOMA-IR. LOD score of horizontal reference line is 2.2 for suggestive linkages.

kinase regulates vital cellular metabolic cascades and preserves cellular energy homeostasis through the regulation of lipid and glucose metabolic pathways (20,21). A previous study on exercise-stimulated transport in skeletal muscle suggested that AMP-activated protein kinase was involved in enhancing glucose transport by an insulin-independent signaling mechanism (22). *INSIG-1* is a central regulator in the processing of sterol regulatory element binding proteins. Two animal studies demonstrated an antilipogenic effect of *INSIG-1* in vitro and in vivo (23) and identified *INSIG-1* as a peroxisome proliferator–activated receptor γ target gene, providing a link between insulin sensitization and glucose homeostasis as well as lipid homeostasis (24).

The QTL on 19q at D19S589 (88 cM, 1-LOD support interval 75–95 cM, LOD score 3.33) was found solely for fasting glucose. The linkage peak was mainly attributed to appreciable and consistent linkages from whites of GenNet and Mexican Americans of GENOA. Both the samples were relatively richer with suspected diabetic subjects and

TABLE 2
Combined linkage analysis results of multipoint genome-wide linkage scans

Phenotype	Chromosome	Location (cM)	Marker	Heritability (±SE)	LOD score	*P*	Empirical *P*
Glucose	7q36.1	163.03	D7S3070	34 ± 7	2.31	0.000559	0.068063
	19q13.33	78.08	D19S246	28 ± 7	2.38	0.000462	0.068063
	19q13.41	87.66	D19S589	27 ± 7	3.33	0.000045	0.016545
Insulin	2p12	99.41	D2S1777	43 ± 20	2.35	0.000507	0.057268
	4q13.2	78.43	D4S2367	42 ± 11	2.33	0.000536	0.057268
	4q21.21	88.35	D4S3243	38 ± 16	2.48	0.000363	0.057268
	7q36.1	163.03	D7S3070	40 ± 15	2.26	0.000628	0.057268
HOMA-IR	2p12	99.41	D2S1777	39 ± 8	2.27	0.000671	0.056320
	2p13.3	103.16	D2S1790	38 ± 8	2.62	0.000255	0.031075
	7q36.1	163.03	D7S3070	33 ± 5	3.21	0.000061	0.022266
	7q36.2	173.71	D7S1823	35 ± 7	2.63	0.000249	0.031075

were characterized by significantly higher mean fasting glucose levels (Table 1). This locus might be speculated to harbor gene(s) for type 2 diabetes susceptibility and/or impaired glucose metabolism. The QTL is well replicated by some previous reports at 68–78 cM (D19S178–D19S867, LOD scores 1.8–2.8) for 2-h insulin in the Framingham Offspring Study (7) and empirical insulin resistance index in Finns (3), at 78–84 cM (D19S246-D19S571, LOD scores 1.3–1.7) for type 2 diabetes in Japanese (25) and Dutch (26), and at 88 cM (D19S589) for 2-h glucose (LOD score 1.1) in Pima Indians (2). It is also well replicated by fasting insulin (LOD score 1.6) in HERITAGE whites (17) and glucose effectiveness in response to endurance exercise training (LOD score 2.0) in HERITAGE blacks (P.A., M. Teran-Garcia, T. Rice, T. Rankinen, S.J. Weisnagel, R.N. Bergman, R.C. Boston, S. Mandel, D. Stefanovski, A.S. Leon, J.S. Skinner, D.C.R., C. Bouchard, unpublished observations). The human skeletal muscle glycogen synthase gene (*GYS1*, OMIM 138570) might be proposed as a candidate gene. The *GYS1* gene encodes glycogen synthase, which is a key enzyme in glucose storage without confirmed links to insulin resistance and type 2 diabetes (27,28). It has been reported that insulin stimulates *GYS1* mRNA expression and that such impaired insulin stimulation of the *GYS1* gene expression in type 2 diabetes is acquired and secondary to chronic hyperglycemia (29).

Two other suggestive linkages to 2p and 4q for fasting insulin and insulin resistance were also revealed in this study, but replications by other independent studies are needed. In conclusion, 7q36 and 19q13 for type 2 diabetes–associated phenotypes were found by combining genome-wide linkage scan results among large and multiple-ethnicity populations in the NHLBI FBPP. Good replication across other studies have been achieved with *PRKAG2*, *INSIG1*, and *GYS1* as plausible candidate genes. Eventual gene discoveries on 7q and 19q rely on follow-up dense mapping and association studies.

RESEARCH DESIGN AND METHODS

All subjects were selected from the four component networks of the FBPP. Details have been previously described in regard to study design (9) and linkage scans for hypertension in GenNet (30), GENOA (31), HyperGEN (32), and SAPPHIRe (33), as well as individual linkage scans for fasting glucose, insulin, and HOMA-IR using white and black data separately in HyperGEN (B.I.F., S.S. Rich, M.M. Sale, G. Heiss, L. Djousse, J.S. Pankow, M.A.P., D.C.R., C.E. Lewis, Y.-D.I.C., S.R. Beck, unpublished observations). Briefly, GenNet sampled white and black nuclear families through identification of young- to middle-aged probands with elevated blood pressure. Both GENOA and HyperGEN sampled white and black sibships containing sibpairs with essential hypertension. GENOA also sampled Mexican-American sibships containing sibpairs with type 2 diabetes. SAPPHIRe recruited three groups of Asian (Japanese and Chinese) sibpairs who were concordant for hypertension, concordant for hypotension, and extremely discordant for hypertension, respectively. Family relationships were confirmed using the marker data. Pedigree errors were corrected, and unrelated individuals were excluded from this analysis. Also excluded were those who fasted <8 h, those with diagnosed type 2 diabetes, and those being treated with insulin or oral hypoglycemic agents. A total of 8,664 subjects comprising 5,923 sibpairs from 4,043 families were available for this study (Table 1). The study protocol was approved by the Institutional Review Boards at each participating site, and written informed consent was obtained from each participant.

Raw fasting glucose, insulin, and HOMA-IR were skewed and therefore approximately normalized using a negative reciprocal of the square transformation for fasting glucose ($-x^{-2}$) and a log transformation for both fasting insulin and HOMA-IR [$\ln(x)$]. Before genetic analysis, each phenotype was adjusted for the effects of age, age^2, age^3, BMI, weight (in kilograms) divided by the square of height (in meters), and field center separately by sex within each of the eight network ethnicity groups in both the mean and variance using a stepwise multiple regression procedure. For each of the regressions, only terms significant at the 5% level were retained. Outliers were defined as at least four SD departures from the mean and at least one SD separated from the adjacent observation and set to missing in the current study. Finally, each of the adjusted variables was standardized (i.e., mean zero, SD 1) separately by sex within each of the eight network ethnicity groups.

Morning fasting serum samples from all study subjects were collected in a resting state. Briefly, these samples were measured in duplicate for fasting serum glucose concentrations using Elan Glucose reagent and, for fasting serum insulin concentrations, an automated immunoassay instrument with its ultra-sensitive insulin kit from Beckman Coulter (Fullerton, CA). Detailed measurement methods have been described elsewhere (B.I.F., S.S. Rich, M.M. Sale, G. Heiss, L. Djousse, J.S. Pankow, M.A.P., D.C.R., C.E. Lewis, Y.-D.I.C., S.R. Beck, unpublished observations). For the glucose assay, the sensitive range is 2–450 mg/dl, with the observed detection limit of 1.02 mg/dl. The sensitivity and dynamic range of the insulin assay are 0.03 and 0.03–300 mU/l, respectively, with zero cross-reactivity with proinsulin and C-peptide, 30% with bovine insulin, and 97% with porcine insulin (B.I.F., S.S. Rich, M.M. Sale, G. Heiss, L. Djousse, J.S. Pankow, M.A.P., D.C.R., C.E. Lewis, Y.-D.I.C., S.R. Beck, unpublished observations). HOMA-IR was calculated as the product of fasting insulin (in microunits per milliliter) and fasting glucose (in millimoles per liter) divided by 22.5 (1).

DNA was extracted from whole blood by standard methods at each of the four networks and was sent to the Mammalian Genotyping Service in Marshfield, Wisconsin (http://research.marshfieldclinic.org/genetics), for genotyping. In this study, the same set of 365 highly polymorphic microsatellite markers was uniformly available across all four networks, which has an average heterozygosity of 80%, an average intermarker spacing of 10 cM, and a 95% coverage of the entire human genome.

Multipoint linkage analyses were performed using the variance components model as implemented in the computer program SEGPATH (34,35). Under the variance components model, a phenotype is under the influence of the additive effects of a trait locus (g), a residual familial background modeled as a pseudo-polygenic component (G_R), and a residual nonfamilial component (r). The effects of the trait locus and the pseudo-polygenic component on the phenotype are quantified by the heritabilities h^2_g and h^2_r, respectively. Allele-sharing probabilities at each marker location for each sibpair were estimated using the multipoint approach in the computer program MAPMAKER/SIBS (12) and were input to the SEGPATH model. Other parameters in the model include spouse resemblance (u), additional sibling resemblance (b), and the phenotype means and variances. The linkage hypothesis is tested by restricting h^2_g ($h^2_g = 0$). A likelihood ratio test contrasting the null versus the alternative hypotheses is asymptotically distributed as a 50:50 mixture of a χ^2 with 1 degree of freedom and a point mass at zero (36), and the LOD score is computed as $\chi^2/(2 \times \log_e 10)$.

Multipoint genomic scans were separately performed in the eight network ethnicity groups using the same (and most recent) Marshfield map. Each network and ethnic group is testing the same hypothesis at each genomic location (i.e., the same trait gene is linked to the same marker locus). Fisher's approach combines P values across studies (10), and one can easily work on the LOD score for linkage analysis instead because there is a simple 1:1 correspondence between the two (37). However, as proposed by Province (11), a slight but significant modification of Fisher's method is needed to avoid bias in pooling nonparametric LOD scores that are exactly zero. Applying the modified Fisher's method of combining P values at each locus produces a meta-analysis genome scan. Here, the modified Fisher's method was used to combine a total of eight P values into a single P value, which was then converted back to the LOD score scale for result presentation in Table 2 and Figs. 1 and 2.

ACKNOWLEDGMENTS

The entire FBPP is supported by a series of cooperative agreements (U01) with the NHLBI. GenNet: HL45508 and HL47910; GENOA: R01 HL51021 and U10 HL54481, HL54464; HyperGEN: HL54473, HL54496, HL54472, HL54515, HL54495, HL54497, HL54471, and HL54509; SAPPHIRe: 2HHZ598.

The complete list of FBPP investigators and sources of support can be found at http://www.bloodpressuregenetics.org.

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