0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(12):5935–5943

doi: 10.1210/jc.2003-030553

Genome-Wide Linkage Scan for the Metabolic Syndrome in the HERITAGE Family Study

RUTH J. F. LOOS, PETER T. KATZMARZYK, D. C. RAO, TREVA RICE, ARTHUR S. LEON, JAMES S. SKINNER, JACK H. WILMORE, TUOMO RANKINEN, AND CLAUDE BOUCHARD

Human Genomics Laboratory, Pennington Biomedical Research Center (R.J.F.L., T.R., C.B.), Baton Rouge, Louisiana 70808; School of Physical and Health Education and Department of Community Health and Epidemiology, Queen's University (P.T.K.), Kingston, Ontario, Canada; Division of Biostatistics (D.C.R., T.R.), and Departments of Genetics and Psychiatry (D.C.R.), Washington University School of Medicine, St. Louis, Missouri 63110; School of Kinesiology University of Minnesota (A.S.L.), Minneapolis, Minnesota 55455; Department of Kinesiology, Indiana University (J.S.S.), Bloomington, Indiana 47405; and Department of Health and Kinesiology, Texas A&M University (J.H.W.), College Station, Texas 77845

The metabolic syndrome involves multiple and interactive effects of genes and environmental factors. To identify chromosomal regions encoding genes possibly predisposing to the metabolic syndrome, we performed a genome-wide scan with 456 white and 217 black participants from 204 nuclear families of the HERITAGE Family Study, using regression-based, single- and multipoint linkage analyses on 509 markers. A principal component analysis was performed on 7 metabolic syndrome-related phenotypes. Two principal components, PC1 and PC2 (55% of the variance), were used as metabolic syndrome phenotypes. ANOVA was used to quantify the familial aggregation of PC1 and PC2. Family membership contributed significantly ($P < 0.0023$) to the variance in PC1 ($r^2 = 0.38$ in whites; $r^2 = 0.55$ in blacks) and PC2 ($r^2 = 0.51$; $r^2 = 0.48$). In whites, promising evidence for linkage ($P < 0.0023$) was found for PC1 (2 markers on 10p11.2) and PC2 (a marker on 19q13.4). Suggestive evidence of linkage ($0.01 > P > 0.0023$) appeared for PC1 (1q41 and 9p13.1) and PC2 (2p22.3). In blacks, promising linkage was found for PC2 on 1p34.1, and suggestive linkage was found on 7q31.3 and 9q21.1. The genome-wide scan revealed evidence for quantitative trait loci on chromosomal regions that have been previously linked with individual cardiovascular disease and type 2 diabetes risk factors. Some of these chromosomal regions harbor promising potential candidate genes. (*J Clin Endocrinol Metab* 88: 5935–5943, 2003)

MULTIPLE METABOLIC ABNORMALITIES, including obesity, insulin resistance and hyperinsulinemia, dyslipidemia, hypertension, impaired glucose tolerance, type 2 diabetes mellitus, and other anomalies, tend to occur jointly in the same subjects more frequently than expected by chance alone (1). This clustering of several cardiovascular disease and diabetes risk factors has been referred to as the metabolic syndrome (2, 3).

The metabolic syndrome has recently been identified as a major public health problem in the United States. According to the National Cholesterol Education Program Adult Treatment Panel III, the age-adjusted prevalence is at approximately 24% of the adult population (4). This is cause for concern, as individuals with the metabolic syndrome are at an increased risk of morbidity and mortality from several metabolic and cardiovascular diseases (5).

The reasons for the joint occurrence are still unclear, but the clusters seem to be relatively stable traits that tend to track well from childhood into adulthood (6), perhaps even more so than the individual risk factors taken separately (7). Undoubtedly, the development of the metabolic syndrome involves multiple and interactive effects of genes and environmental factors, including physical inactivity and diet (8). There is evidence for significant familial aggregation for the individual components of the metabolic syndrome, including abdominal visceral fat (AVF) (9, 10), blood lipids (11, 12), blood pressure (13–15), and blood glucose/insulin levels (16).

Some studies have suggested that the components of the metabolic syndrome may share genetic determinants. For instance, data on 2508 adult male twins suggested the presence of a common underlying factor mediating the clustering of hypertension, diabetes, and obesity (17). This latent factor was influenced by both genetic (59%) and environmental (41%) effects. Furthermore the concordance rate for the clustering of all three conditions in the same individuals was 5-fold higher in monozygotic twins compared with dizygotic twins. In the San Antonio Family Heart Study, a common set of genes influenced insulin levels together with other metabolic syndrome-related traits (18). The results of the Swedish Adoption/Twin Study of Aging showed that all 5 principal components calculated from the measures of body mass index (BMI), insulin level, triglycerides, high density lipoprotein cholesterol (HDL-C) and systolic blood pressure were influenced by a single latent genetic factor (19). These studies indicate the presence of an underlying pleiotropic factor among the components of the metabolic syndrome.

The purpose of this paper is to report on a genome-wide linkage scan to identify genomic regions harboring genes that may influence the metabolic syndrome, using data from the HERITAGE Family Study. As no single measurement can adequately describe the metabolic syndrome, multivariate

Abbreviations: AVF, Abdominal visceral fat; %BF, percent body fat; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; IBD, identical by descent; LDL-C, low density lipoprotein cholesterol; MAP, mean arterial blood pressure; PC1, first principal component; PC2, second principal component; POMC, proopiomelanocortin; QTL, quantitative trait locus; TG, triglycerides.

phenotypes were derived using principal components analysis.

Subjects and Methods

Participants

The study cohort consisted of 456 white participants and (223 men and 233 women) from 99 nuclear families and 217 black participants (89 men and 128 women) from 105 families. The maximum numbers of sibling pairs used in the linkage analyses were 302 in whites and 60 in blacks.

The study design and inclusion criteria of the HERITAGE Family Study have been described previously (20). Briefly, to be eligible, individuals were required to be between the ages of 17–65 yr, healthy but sedentary (no regular physical activity over the previous 6 months), BMI under 40 kg/m^2, and systolic/diastolic blood pressures equal to or less than 159/99 mm Hg. Participants with BMIs slightly above 40 kg/m^2 (n = 6), who were considered by the supervising physician to be healthy and able to perform the requested exercise prescription, were included in the study. Further, individuals with confirmed or possible coronary heart disease, chronic or recurrent respiratory problems, and uncontrolled endocrine and metabolic disorders (including diabetes and the use of antihypertensive or lipid-lowering drugs) were excluded from the study. The study protocol had been approved by each of the institutional review boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from all participants. Although the HERITAGE Family Study involved a 20-wk aerobic exercise training program, only data from the initial baseline visit in the sedentary state are considered here.

Metabolic syndrome

In the present study the metabolic syndrome was represented by the following set of measurements: percent body fat (%BF), AVF, mean arterial blood pressure (MAP), and plasma HDL-C, triglycerides (TG), glucose, and insulin concentrations. All measurement and array protocols were standardized and carefully monitored using an extensive quality assurance and quality control program (21).

%BF was determined from measurements of body density from underwater weighing (22), with a correction for residual lung volume by the oxygen dilution technique (23), at three clinical centers and by the helium-dilution technique (24) at the fourth clinical center. Relative body fatness was estimated using the equation of Siri (25) for white men, Lohman (26) for white women, Schutte *et al.* (27) for black men, and Ortiz *et al.* (28) for black women. AVF areas were determined by computed tomography scans (29). Participants were in the supine position with their arms above their heads, and the abdominal scan was obtained at the L4–L5 vertebral level. The attenuation interval used in the quantification of the areas of adipose tissue was between −190 and −30 Hounsfield units. The AVF area was obtained by drawing a line within the muscle wall surrounding the abdominal cavity.

Resting systolic and diastolic blood pressures were measured twice on separate days in the morning after a 12-h fast. Measurements were made in a quiet room with the participant reclined at a 45° angle, with legs elevated. Blood pressure was determined after a 5-min rest period using a STBP-780 automated unit (Colin, San Antonio, TX) while a technician wore ear phones to confirm the values. The first measurements were discarded, and three valid measurements were made on each day. The average of six blood pressure measurements was used as the measure of systolic and diastolic blood pressures. MAP, calculated as [(2 × diastolic blood pressure) + systolic blood pressure]/3, was used in the present analyses.

Plasma concentrations of HDL-C, TG, glucose, and insulin were measured twice after a 12-h overnight fast. Blood samples were obtained from an antecubital vein and collected into Vacutainer tubes (BD Biosciences, Mountain View, CA) containing EDTA. For women, samples were collected in the early follicular phase of the menstrual cycle. Total cholesterol and TG levels were determined by enzymatic methods using the Technicon RA-500 analyzer (Bayer Corp, Inc., Tarrytown, NY), as previously described (30). Plasma very low density lipoprotein (density, <1.006 g/ml) was isolated by ultracentrifugation, and the HDL fraction was obtained after precipitation of low density lipoprotein cholesterol (LDL-C) in the infranatant (density, >1.006 g/ml) with heparin and $MgCl_2$ (31). The HDL-C and TG contents of the infranatant fraction were measured before and after the precipitation step.

Plasma glucose was enzymatically determined using a reagent kit distributed by Diagnostic Chemicals Ltd. (Oxford, CT), and plasma insulin levels were measured by RIA after polyethylene glycol separation, as described by Desbuquois and Aurback (32). Polyclonal antibodies that cross-react more than 90% with proinsulin (and presumably with its conversion intermediates) were used (33). Therefore, in this study insulin refers to immunoreactive insulin (defined as the sum of insulin, proinsulin, and split proinsulin). In the present sample, with normal fasting glucose levels and no history of diabetes, it is estimated that about 10% of the immunoreactive insulin is in the form of proinsulin and its conversion intermediates (34).

Standard principal component analysis was applied to the individual risk factors (%BF, AVF, HDL-C, TG, glucose, insulin, and MAP) for the purpose of deriving components that represent large fractions of the metabolic syndrome variance. Principal components derived from between-family covariance or within-family genetic covariance were not used, as it has been shown that standard principal components perform equally well or better in terms of both power or type I error in linkage analyses (35). The first two principal components (PC1 and PC2) were retained for further analysis. They both had eigen values of 1 or more. The factor loadings for PC1 and PC2 (eigen vectors) were plotted to obtain a visual representation of the profile of components (Fig. 1). We included MAP in the analysis, instead of systolic and diastolic blood pressures individually. Both methods resulted in two PCs that represented the metabolic syndrome equally well. Therefore, the most parsimonious method (MAP) was considered for further analyses.

Data adjustment

PC1 and PC2 were adjusted for the effects of age, sex, and generation in both mean and variance using stepwise multiple regression procedures (36). Briefly, PC1 and PC2 were regressed (mean regression) on up to a third degree polynomial in age (age, age^2, age^3) within each of the race by sex by generation subgroups. Only significant terms (5% level) were retained. The squared residuals from the mean regression were then regressed on up to a third degree polynomial in age to test for heteroscedasticity, and the predicted values were retained if significant. The residuals from the best regression were then standardized to zero mean and unit variance within each of the eight race by sex by generation groups and constituted the final phenotypes.

Familial aggregation analysis

To study whether PC1 and PC2 aggregate within families, we performed an ANOVA comparing the between-family to the within-family variances. The ANOVAs were conducted separately in blacks and whites using the age- and sex-adjusted values.

Genotyping

A total of 509 markers with an average spacing of 6.0 cM were used. PCR conditions and genotyping methods have been fully outlined previously (37). Automatic DNA sequencers from LICOR (Lincoln, NE) were used to detect the PCR products, and genotypes were scored using SAGA software. Incompatibilities with Mendelian inheritance were checked, and markers showing incompatibilities were regenotyped completely (<10% were retyped). Microsatellite markers were selected mainly from the Marshfield panel (version 8a), as were some candidate genes for obesity and comorbidities, including dyslipidemia, diabetes, and hypertension. Map locations were taken from the Genetic Location Database of Southampton, UK (which can be accessed online at http:/cedar.genetics.soton.ac.uk). The cytogenetic locations of the markers were obtained from the NCBI map viewer (which can be accessed online at http://www.ncbi.nlm.nih.gov).

Linkage analyses

Both single- and multipoint linkage analyses were performed with the sibling pair linkage procedure (38, 39) as implemented in the SIBPAL program of the S.A.G.E. 4.0 Statistical Package (40). Briefly, if there is

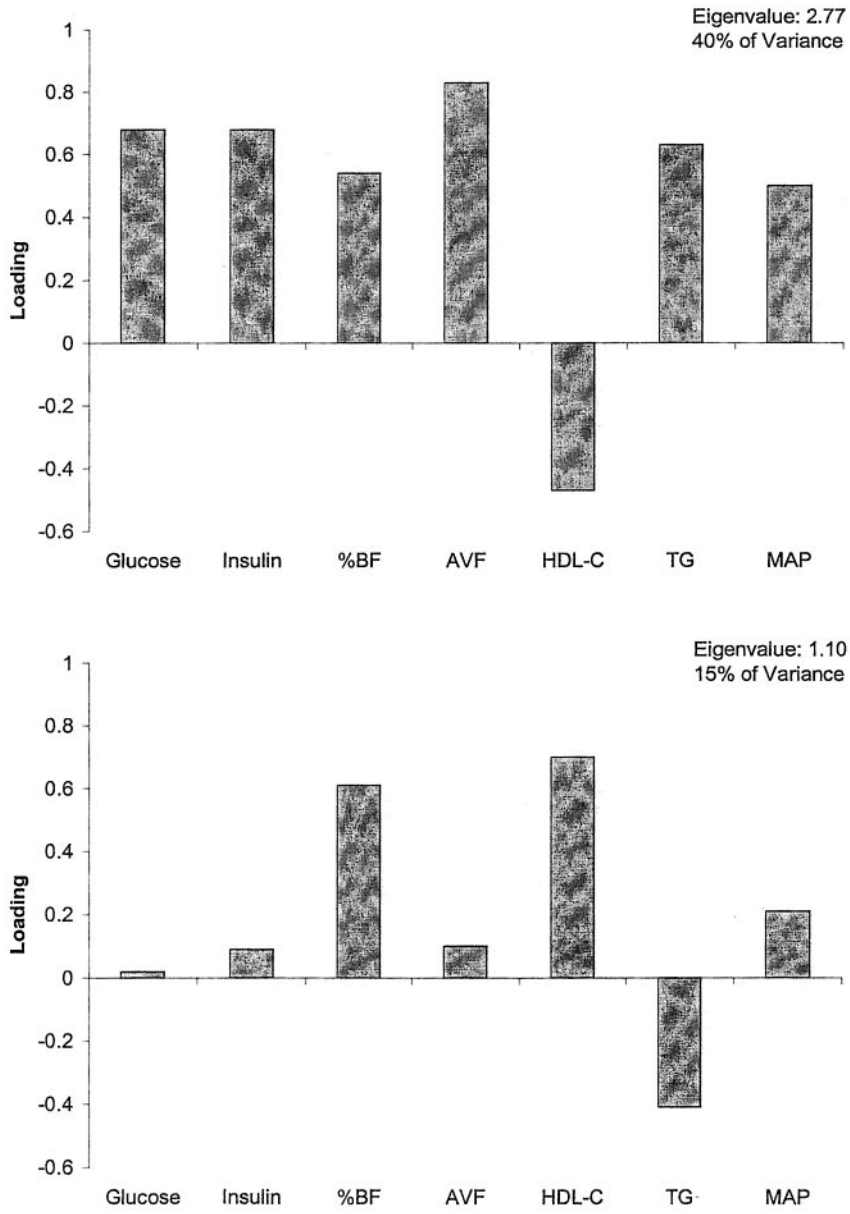

FIG. 1. Results of the principal components analysis of risk factors for the metabolic syndrome in the HERITAGE Family Study. PC1 and PC2 explained 55% of the variance in risk factors.

linkage between a marker locus and a putative gene influencing the phenotype, siblings sharing a greater proportion of alleles identical by descent (IBD) at the marker locus also will show a greater resemblance in the phenotype. Phenotypic resemblance of the siblings, modeled as the weighted combination of squared trait difference and squared mean corrected trait sum, is linearly regressed on the estimated proportion of alleles that the sibling pair shares IBD at each marker locus. Both single- and multipoint estimates of allele-sharing IBD were generated using the GENIBD program of the S.A.G.E. 4.0 package. Empirical *P* values (maximum of 100,000 replicates) were calculated for all markers with nominal $P \leq 0.01$. The α level used to identify promising results ($P < 0.0023$, corresponding to a LOD score of 1.75) represents, on the average, one false positive per scan for experiments involving approximately 400 markers (41). All analyses were conducted separately in blacks and whites.

Results

The descriptive characteristics of the participants are presented in Table 1, whereas Fig. 1 depicts the loadings for the first two principal components. PC1 explained 40% of the variance in the original variables and was characterized by positive loadings for glucose, insulin, %BF, AVF, TG, and MAP and a negative loading for HDL-C. PC2 explained an additional 15% of the variance and reflected a contrast between positive loadings for %BF and HDL-C concentration and a negative loading for TG concentration. Similar results were obtained when the factor analysis was performed for blacks and whites separately. Therefore, the PC1 and PC2 derived from the combined sample were used for further analyses.

The results of the familial aggregation analyses are presented in Table 2. In whites, family membership accounted for 38% ($P < 0.0001$) and 51% ($P < 0.0001$) of the variance in PC1 and PC2, respectively. In blacks, family membership accounted for 55% ($P < 0.0001$) and 48% ($P = 0.0026$) of the variance of PC1 and PC2, respectively. An examination of the F values indicates that there are 1.75–3.9 times more variance

TABLE 1. Descriptive characteristics of sample

Variable	Group	Blacks			Whites		
		n	Mean	SD	n	Mean	SD
Age (yr)							
	Men	89	31.5	11.1	223	36.1	14.8
	Women	128	32.1	11.0	233	34.1	14.0
BMI (kg/m^2)							
	Men	89	27.5	5.5	222	26.2	4.4
	Women	127	27.8	6.1	231	24.8	4.9
%BF							
	Men	89	23.0	8.4	223	22.4	8.8
	Women	128	35.8	8.6	233	29.7	9.9
AVF (cm^2)							
	Men	89	73.8	54.0	223	104.9	60.9
	Women	128	67.6	42.3	233	72.4	51.8
Resting diastolic blood pressure (mm Hg)							
	Men	89	72.3	7.7	223	68.2	9.2
	Women	128	71.8	9.1	233	63.5	7.1
Resting systolic blood pressure (mm Hg)							
	Men	89	125.0	9.5	223	120.2	10.8
	Women	128	122.3	13.3	233	112.4	10.1
MAP (mm Hg)							
	Men	89	89.9	7.4	223	85.5	8.8
	Women	128	88.6	9.8	233	79.8	7.4
Fasting plasma HDL-C (mmol/liter)							
	Men	89	0.97	0.20	223	0.94	0.19
	Women	128	1.12	0.26	233	1.15	0.26
Fasting plasma TG (mmol/liter)							
	Men	89	1.23	0.79	223	1.52	0.91
	Women	128	0.84	0.37	233	1.16	0.58
Fasting plasma glucose (mmol/liter)							
	Men	89	94.3	10.4	223	93.7	11.3
	Women	128	90.0	10.8	233	89.2	12.4
Fasting plasma insulin (pmol/liter)							
	Men	89	11.9	10.0	223	9.8	7.9
	Women	128	11.3	10.5	233	7.9	4.5

TABLE 2. Familial aggregation of principal components (PC1 and PC2) of indicators of the metabolic syndrome from the comparison of between-family to within-family variance components (by ANOVA)

	Blacks				Whites			
	n	r^2	F value	*P*	n	r^2	F value	*P*
PC1	217	0.55	2.29	<0.0001	456	0.38	2.35	<0.0001
PC2	217	0.48	1.75	0.0026	456	0.51	3.93	<0.0001

between families than within families for the two phenotypes. Thus, both PC1 and PC2 significantly aggregate within families.

Table 3 presents the suggestive ($P < 0.01$) and promising ($P < 0.0023$) linkage results detected in the genomic scan. Figures 2 and 3 depict the results for those chromosomes with promising linkages in whites (chromosomes 10 and 19) and blacks (chromosome 1). Overall, five chromosomal regions in whites and three chromosomal regions in blacks showed suggestive or promising linkages with either PC1 or PC2. In whites, markers on chromosomal regions 10p11.2 and 19q13.4 showed promising linkages with PC1 and PC2, respectively (Fig. 2). Markers in another three regions (1q41, 2p22.3, and 9p13.1) showed suggestive linkages with either PC1 or PC2 (Table 3). In blacks, two adjacent markers on chromosome 1p34 showed suggestive and promising linkage with PC2 (Fig. 3). In addition, suggestive evidence for a quantitative trait locus (QTL) for PC2 was found on chromosomes 7q31.3 and 9q21.1. There were no chromosomal regions harboring QTLs common to both blacks and whites.

Discussion

The results demonstrate that the metabolic syndrome, as defined by principle components of multiple risk factors, significantly aggregates within black and white families. Therefore, it is reasonable to undertake a search for QTLs and, ultimately, genes and mutations that may contribute to the clustering of risk factors seen in the metabolic syndrome. The results from the genomic scan revealed promising evidence for QTLs affecting the metabolic syndrome on chromosomes 10p and 19q in whites and on chromosome 1q in blacks.

Only a few studies (42, 43) have attempted a genome-wide scan for the metabolic syndrome. Differences in study population and in methods used to quantify the metabolic syndrome make comparisons among these studies difficult. Arya *et al.* (42) reported a genome-wide scan for the metabolic syndrome with 261 nondiabetic subjects from 27 Mexican-American families. As in our study, they performed a principal component analysis using 8 metabolic syndrome-related phenotypes: fasting glucose, fasting specific insulin, BMI, systolic blood pressure, diastolic blood pressure, fasting HDL-C, fasting TG, and fasting leptin. The factor analyses yielded 3 factors, factor 1 (adiposity-insulin factor: BMI, leptin, and fasting specific insulin), factor 2 (blood pressure

TABLE 3. Suggestive ($P < 0.01$) and promising ($P < 0.0023$) multipoint linkages and corresponding single-point linkages with principal components (PC1 and PC2) of risk factors for the metabolic syndrome in whites and blacks

Marker	Chromosome	Map position (cM)	Phenotype	Multipoint *P*	Single-point *P*
Whites					
D1S1602	1q41	233.055	PC1	0.0097[a]	0.0125
D2S390	2p22.3	45.422	PC2	0.0070[a]	0.0288
D2S2374	2p22.3	48.435	PC2	0.0045[a]	0.0036[a]
D9S1878	9p13.1	40.045	PC1	0.0095[a]	0.0092[a]
D10S208	10p11.2	27.469	PC1	0.0003[b]	0.0576
D10S1169	10p11.2	31.461	PC1	0.0084[a]	0.0674
D10S1768	10p11.2	38.415	PC1	0.0007[b]	0.0382
D19S589	19q13.4	59.573	PC2	0.0009[b]	0.0251
Blacks					
EDN2BSMA	1p34	54.888	PC2	0.0044[a]	0.7508
D1S193	1p34.1	56.093	PC2	0.0011[b]	0.4813
LEPNSPBI	7q31.3	134.312	PC2	0.0071[a]	0.0322
LEP_MSAT	7q31.3	134.313	PC2	0.0088[a]	0.0294
D9S301	9q21.1	60.036	PC2	0.0049[a]	0.0225

EDN2BSMA, Endothelin 2; LEP_MSAT, leptin microsatellite; LEPNSPBI, leptin.
[a] Suggestive multipoint linkage.
[b] Promising multipoint linkage.

factor: diastolic and systolic blood pressures), and factor 3 (lipid profile factor: HDL-C and TG). They found significant evidence of linkage for factor 1 to two regions on chromosome 6 near markers D6S403 (LOD = 4.2) and D6S264 (LOD = 4.9) and strong evidence of linkage for factor 3 on chromosome 7 between markers D7S479 and D7S471 (LOD = 3.2). None of these regions overlapped with those of our study. The 3 principal components from the report by Arya *et al.*, however, are substantially different from the components of the present study. De Andrade *et al.* (43) applied multivariate linkage analyses to 5 phenotypes related to the metabolic syndrome, taking 3 traits at a time, using 279 extended families from the Rochester Family Heart Study. Significant LOD scores were obtained on 5q and 6q for the combination of TG, fasting insulin, and fasting glucose. None of these regions coincided with our QTLs.

Other studies have reported genomic scans for individual components of the metabolic syndrome. Although the results of these prior studies are not directly comparable to those of the present study, there is some overlap between the chromosomal regions identified in our genomic scan and those reported in studies using univariate approaches.

In whites, for example, we observed promising evidence for linkage between 2 markers on 10p11.2 and PC1. For the same region, Pajukanta *et al.* (44) reported significant linkage for elevated TG levels in Finnish families with familial combined hyperlipidemia. This region also overlaps with a QTL for obesity reported by Hager *et al.* (45). This QTL reached maximal significance (maximum LOD scores = 4.85) at a marker (D10S197) less than 4 Mb from D10S208 and in the gene encoding glutamic acid decarboxylase 2, which is a major autoantigen in insulin-dependent diabetes mellitus (46). However, glutamic acid decarboxylase 2 is located outside our region of linkage on 10p11.2 and therefore cannot be considered as a candidate gene for the PC1. Furthermore, we recently reported promising linkage on 10p11.2 for training response of submaximal exercise stroke volume after a 20-wk endurance program (47).

Promising evidence for linkage was also found between a marker (D19S589) on chromosome 19q13.4 and PC2, which loaded primarily on %BF, and HDL-C and TG concentrations. Interestingly, the same marker and a marker close by (D19S927) showed suggestive linkage ($P = 0.01$) for familial combined hyperlipidemia in 35 extended Dutch families (782 individuals) (48).

Two markers on 2p22.3 showed suggestive linkage with PC2. Interestingly, the D2S1788 marker (<0.5 Mb from the D2S2374) showed suggestive linkage with serum TG levels (LOD = 1.7) in Pima Indians (49) and with systolic blood pressure ($P = 0.0089$) in the Genetic Epidemiology Network of Atherosclerosis (GENOA) cohort (50). In addition, markers in the 2p22.3 region have been shown to be significantly linked with serum leptin levels in Mexican-Americans (51, 52) and exhibited suggestive linkages in a French (45) and an African-American (53) population. The proopiomelanocortin gene (POMC) has been proposed as a potential candidate for these linkages with serum leptin concentrations. POMC is the precursor for several peptide hormones produced by posttranslational processing, some of which are involved in energy homeostasis, including αMSH, ACTH, and β-endorphin (54). POMC is highly expressed in neuronal cells of the arcuate nucleus, a region of the hypothalamus involved in the regulation of energy homeostasis (54). The POMC gene is located 4 Mb upstream of the D2S390 marker.

In blacks, the strongest evidence for linkage was found for PC2, with two adjacent markers on chromosome 1p34. In a French extended pedigree, two markers (D1S2892 and D1S2722) in the same region showed significant linkage (LOD score = 3.13) with autosomal dominant hypercholesterolemia, a disorder characterized by an isolated elevation of LDL-C that leads to premature mortality from cardiovascular complications (55). Potential candidate genes in this

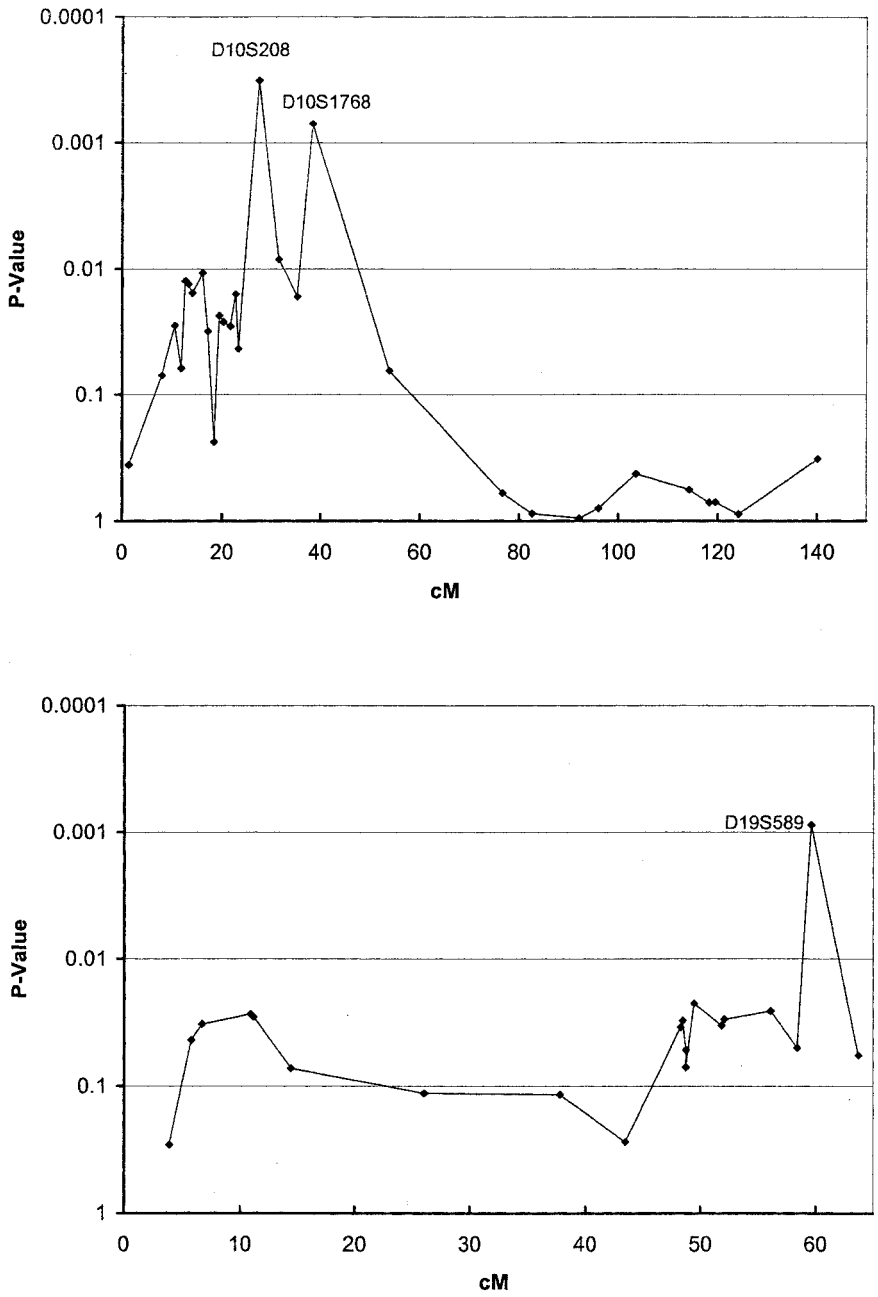

FIG. 2. Overview of the multipoint linkage results for chromosomes 10 (PC1) and 19 (PC2) in whites of the HERITAGE Family Study.

region for PC2 include the γ-subunit of nuclear factor Y, which may play a role in the regulation of both lipogenic and cholesterolegenic genes (56). The other candidates are sterol carrier protein 2, which encodes a lipid transport basic protein believed to facilitate the movement of cholesterol and phospholipids within the cell (57); fatty acid-binding protein 3, a protein transporting hydrophobic fatty acids through the cytoplasm (58); and the apolipoprotein E receptor 2, a member of the LDL-C receptor family, involved in the cellular recognition and internalization of LDL-C and most highly expressed in human brain tissue (59). Furthermore, one of the markers that showed evidence of linkage at 1p34 is located in the endothelin-2 gene, which encodes a potent vasoconstrictor peptide involved in the control of blood pressure (60). The endothelin-2 genotype was found to be associated with pretreatment diastolic blood pressure in hypertensive, but not in normotensive, patients (61). It was suggested that endothelin-2 influences the severity, rather than the initial development, of hypertension (61).

Suggestive evidence for linkage was found between markers on 7q31.3 and PC2. In the same sample we previously reported suggestive linkages with AVF (62) and systolic blood pressure at 80% maximal oxygen consumption (63) in the same region. The obvious candidate at 7q31.3 is the leptin gene. Leptin plays a role in the regulation of body weight and has been linked with various anthropometric measures in Caucasians (64–68), Mexican-Americans (69, 70), and African-Americans (71). In the Quebec Family Study (15), the

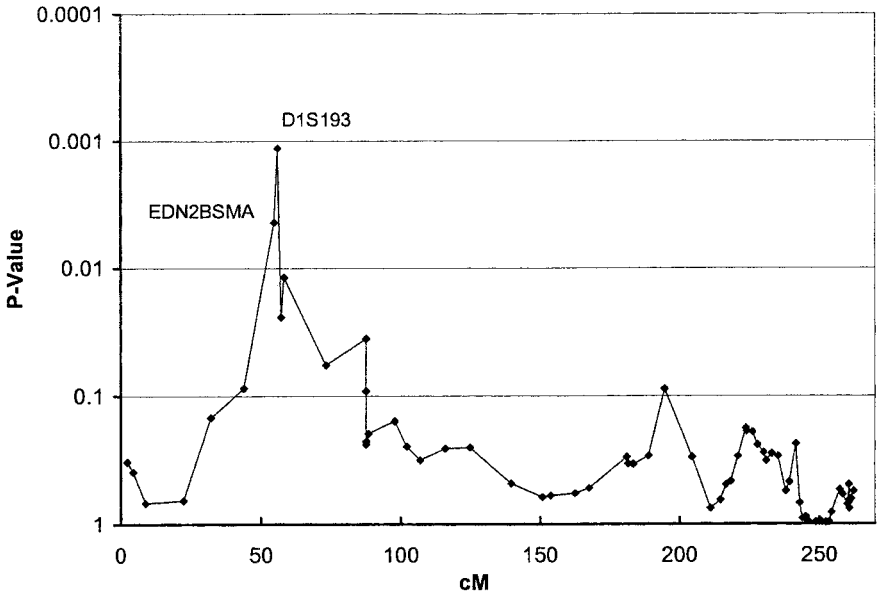

FIG. 3. Overview of the multipoint linkage results for chromosome 1 (PC2) in blacks of the HERITAGE Family Study.

same region (D7S530) was significantly linked with systolic blood pressure (LOD = 2.26; $P = 0.00063$) in 445 Caucasian subjects from 125 families. Moreover, Cheng *et al.* (72) found significant linkage between the D7S3061 marker and fasting insulin (LOD = 3.36; $P = 0.000085$) and suggestive linkage with systolic blood pressure (LOD = 2.06; $P = 0.0021$) in 390 Hispanic family members of 77 probands. Although both phenotypes (blood pressure and insulin resistance) are commonly viewed as parts of the metabolic syndrome, they did not load on PC2 in our study.

Blacks and whites had no QTLs in common for either PC1 or PC2. Although this may be due to the lack of power in the black sample, it is also possible that the loci for blacks and whites are truly distinct. A review of 101 linkage studies identified 2 factors that reduced the chance of finding consistent results: 1) small sample size, and 2) ethnic heterogeneity (73). Furthermore, although black Americans have about 7–20% white admixture, they are still genetically more similar to Africans (74).

In summary, we showed that two multivariate phenotypes representing some aspects of the metabolic syndrome significantly aggregate within families. Both phenotypes were found to be linked to several chromosomal regions in blacks and whites. Many of the markers showing suggestive ($P < 0.01$) or promising ($P < 0.0023$) linkages are in chromosomal regions that have been linked in other studies with cardiovascular disease and type 2 diabetes risk factors. The major QTLs warranting further studies with fine mapping were observed on chromosomes 10p11, 19q13, and 1p34. These chromosomal regions may encode genes that affect features of the metabolic syndrome that were captured in the first two principal components.

Acknowledgments

Thanks are expressed to Drs. Jean-Pierre Després and Jean Bergeron of the Laval University Medical Center, Lipid Research Unit, for the lipid and lipoprotein assays, and to Dr. André Nadeau from the Diabetes Research Unit for the glucose and insulin assays.

Received March 31, 2003. Accepted August 30, 2003.

Address all correspondence and requests for reprints to: Claude Bouchard, Ph.D., Pennington Biomedical Research Center, Human Genomics Laboratory, 6400 Perkins Road, Baton Rouge, Louisiana 70808. E-mail: bouchac@pbrc.edu.

The HERITAGE Family Study is supported by the NHLBI through Grants HL-45670 (to C.B.), HL-47323 (to A.S.L.), HL-47317 (to D.C.R.), HL-47327 (to J.S.S.), and HL-47321 (to J.H.W.). A.S.L. is partially supported by the Henry L. Taylor Endowed Professorship in Exercise and Health Enhancement. C.B. is partially supported by the George A. Bray Chair in Nutrition. R.J.S.L. is supported by a postdoctoral fellowship from the American Heart Association, Southeast affiliate (no. 0325355B). Blood drawing for lipids and iv glucose tolerance tests performed at the Clinical Center of the University of Minnesota were supported by NIH Grant MO1-RR-000400. The results of this paper were obtained using the program package S.A.G.E., which is supported by USPHS Resource Grant 1P41-RR-03655 from the National Center for Research Resources.

References

1. **Ferrannini E** 1993 Syndrome X. Horm Res 93(Suppl 3):107–111
2. **Reaven GM** 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595–1607
3. **Ukkola O, Bouchard C** 2001 Clustering of metabolic abnormalities in obese individuals: the role of genetic factors. Ann Med 33:79–90
4. **Ford ES, Giles WH, Dietz WH** 2002 Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 287:356–359
5. **Lakka H-M, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT** 2002 The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709–2716
6. **Katzmarzyk PT, Perusse L, Malina RM, Bergeron J, Despres JP, Bouchard C** 2001 Stability of indicators of the metabolic syndrome from childhood and adolescence to young adulthood: the Quebec Family Study. J Clin Epidemiol 54:190–195
7. **Bao W, Srinivasan MR, Wattigney W, Berenson GS** 1994 Persistence of multiple cardiovascular risk clustering related to syndrome X from childhood to young adulthood. The Bogalusa Heart Study. Arch Intern Med 154:1842–1847
8. **Bouchard C** 1995 Genetics and the metabolic syndrome. Int J Obes Relat Metab Disord 19(Suppl 1):S52–S59
9. **Rice T, Despres JP, Perusse L, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C** 1997 Segregation analysis of abdominal visceral fat: the HERITAGE Family Study. Obes Res 5:417–424
10. **Perusse L, Despres JP, Lemieux I, Rice T, Rao DC, Bouchard C** 1996 Familial aggregation of abdominal visceral fat level: results from the Quebec family study. Metabolism 45:378–382
11. **Perusse L, Despres JP, Tremblay A, Leblanc C, Talbot J, Allard C, Bouchard**

C 1989 Genetic and environmental determinants of serum lipids and lipoproteins in French Canadian families. Arteriosclerosis 9:308–318
12. **Perusse L, Rice T, Despres JP, Rao DC, Bouchard C** 1997 Cross-trait familial resemblance for body fat and blood lipids: familial correlations in the Quebec Family Study. Arterioscler Thromb Vasc Biol 17:3270–3277
13. **Katzmarzyk PT** 2001 Familial aggregation of seven-year changes in blood pressure in Canada. Can J Cardiol 17:1267–1274
14. **An P, Rice T, Gagnon J, Borecki IB, Perusse L, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC** 1999 Familial aggregation of resting blood pressure and heart rate in a sedentary population: the HERITAGE Family Study. Health, risk factors, exercise training, and genetics. Am J Hypertens 12:264–270
15. **Rice T, Rankinen T, Province MA, Chagnon YC, Perusse L, Borecki IB, Bouchard C, Rao DC** 2000 Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. Circulation 102:1956–1963
16. **Mayer EJ, Newman B, Austin MA, Zhang D, Quesenberry CP, Edwards K, Selby JV** 1996 Genetic and environmental influences on insulin levels and the insulin resistance syndrome: an analysis of women twins. Am J Epidemiol 143:323–332
17. **Carmelli D, Cardon LR, Fabitz R** 1994 Clustering of hypertension, diabetes, and obesity in adult male twins: same genes or same environments? Am J Hum Genet 55:566–573
18. **Mitchell BD, Kammerer CM, Mahaney MC, Blangero J, Comuzzie AG, Atwood LD, Haffner SM, Stern MP, MacCluer JW** 1996 Genetic analysis of the IRS: pleiotropic effects of genes influencing insulin levels on lipoprotein and obesity measures. Arterioscler Thromb Vasc Biol 16:281
19. **Hong Y, Pedersen NL, Brismar K, De Faire U** 1997 Genetic and environmental architecture of the features of the insulin-resistance syndrome. Am J Hum Genet 60:143–152
20. **Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, Gagnon J** 1995 The HERITAGE family study: aims, design, and measurement protocol. Med Sci Sports Exercise 27:721–729
21. **Gagnon J, Province MA, Bouchard C, Leon AS, Skinner JS, Wilmore JH, Rao DC** 1996 The HERITAGE Family Study: quality assurance and quality control. Ann Epidemiol 6:520–529
22. **Wilmore JH, Stanforth PR, Domenick MA, Gagnon J, Daw EW, Leon AS, Rao DC, Skinner JS, Bouchard C** 1997 Reproducibility of anthropometric and body composition measurements: the HERITAGE Family Study. Int J Obes Relat Metab Disord 21:297–303
23. **Wilmore JH** 1969 A simplified method for determination of residual lung volumes. J Appl Physiol 27:96–100
24. **Meneely GR, Kaltreider NL** 1949 The volume of the lung determined by helium dilution: description of the method and comparison with other procedures. J Clin Invest 28:129–139
25. **Siri WE** 1961 Body composition from fluid spaces and density, analysis of methods. In: Brozek J, Henschel A, eds. Techniques for measuring body composition. Washington DC: National Academy of Sciences; 223–244
26. **Lohman TG** 1986 Applicability of body composition techniques and constants for children and youths. Exerc Sport Sci Rev 14:325–357
27. **Schutte JE, Townsend EJ, Hugg J, Schoup RF, Malina RM, Blomqvist CG** 1984 Density of lean body mass is greater in blacks than in whites. J Appl Physiol 56:1647–1649
28. **Ortiz O, Russell M, Delay TL, Baumgartner RN, Waki M, Lichtman S, Wang J, Pierson Jr RN, Heymsfield SB** 1992 Differences in skeletal muscle and bone mineral mass between black and white females and their relevance to estimates of body composition. Am J Clin Nutr 55:8–13
29. **Sjostrom L, Kvist H, Cederblad A, Tylen U** 1986 Determination of total adipose tissue and body fat in women by computed tomography, 40K, and tritium. Am J Physiol 250:E736–E745
30. **Moorjani S, Dupont A, Labrie F, Lupien PJ, Brun D, Gagne C, Giguere M, Belanger A** 1987 Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. Metabolism 36:244–250
31. **Burstein M, Scholnick HR, Morfin R** 1970 Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 11:583–595
32. **Desbuquois B, Aurbach GD** 1971 Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 33:732–738
33. **Roder ME, Porte Jr D, Schwartz RS, Kahn SE** 1998 Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 83:604–608
34. **Kahn SE, Leonetti DL, Prigeon RL, Boyko EJ, Bergstrom RW, Fujimoto WY** 1995 Relationship of proinsulin and insulin with noninsulin-dependent diabetes mellitus and coronary heart disease in Japanese-American men: impact of obesity–clinical research center study. J Clin Endocrinol Metab 80:1399–1406
35. **Moser KL, Jedrey CM, Conti D, Schick JH, Gray-McGuire C, Nath SK, Daley D, Olson JM** 2001 Comparison of three methods for obtaining principal components from family data in genetic analysis of complex disease. Genet Epidemiol 21(Suppl 1):S726–S731
36. **Rice T, Borecki IB, Bouchard C, Rao DC** 1992 Commingling analysis of regional fat distribution measures: the Quebec family study. Int J Obes Relat Metab Disord 16:831–840
37. **Chagnon YC, Borecki IB, Perusse L, Roy S, Lacaille M, Chagnon M, Ho-Kim MA, Rice T, Province MA, Rao DC, Bouchard C** 2000 Genome-wide search for genes to the fat-free body mass in the Quebec Family Study. Metabolism 49:203–207
38. **Elston RC, Buxbaum S, Jacobs KB, Olson JM** 2000 Haseman and Elston revisited. Genet Epidemiol 19:1–17
39. **Haseman JK, Elston RC** 1970 The estimation of genetic variance from twin data. Behav Genet 1:11–19
40. **Department of Epidemiology and Biostatistics, Case Western Reserve University** 2002 Statistical analysis for genetic epidemiology (computer program package available from Statistical Solutions Ltd., Cork, Ireland). Cleveland: Case Western Reserve University
41. **Rao DC** 1998 CAT scans, PET scans, and genomic scans. Genet Epidemiol 15:1–18
42. **Arya R, Blangero J, Williams K, Almasy L, Dyer TD, Leach RJ, O'Connell P, Stern MP, Duggirala R** 2002 Factors of insulin resistance syndrome-related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic Mexican-Americans. Diabetes 51:841–847
43. **de Andrade M, Olswold C, Kardia SLR, Boerwinkle E, Turner Stephen T** 2002 Multivariate linkage analysis using phenotypes related to the insulin resistance-metabolic disorder. 11th Annual Meeting of the International Genetic Epidemiology Society. Genet Epidemiol 23:275
44. **Pajukanta P, Terwilliger JD, Perola M, Hiekkalinna T, Nuotio I, Ellonen P, Parkonnen M, Hartiala J, Ylitalo K, Pihlajamaki J, Porkka K, Laakso M, Ehnholm C, Taskinen MR, Peltonen L** 1999 Genomewide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. Am J Hum Genet 64:1453–1463
45. **Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V, Vaillant E, Lorentz N, Basdevant A, Clement K, Guy-Grand B, Froguel P** 1998 A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. Nat Genet 20:304–308
46. **Baekkeskov S, Aanstoot HJ, Christau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olsen H, De Camilli P, Camilli PD** 1992 Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151–156
47. **Rankinen T, An P, Perusse L, Rice T, Chagnon YC, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C** 2002 Genome-wide linkage scan for exercise stroke volume and cardiac output in the HERITAGE Family Study. Physiol Genomics 10:57–62
48. **Aouizerat BE, Allayee H, Cantor RM, Davis RC, Lanning CD, Wen PZ, Dallinga-Thie GM, de Bruin TWA, Rotter JI, Lusis AJ** 1999 A genome scan for familial combined hyperlipidemia reveals evidence of linkage with a locus on chromosome 11. Am J Hum Genet 65:397–412
49. **Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Fuller JH, Bennett PH, Hanson RL** 2000 A locus influencing total serum cholesterol on chromosome 19p : results from an autosomal genomic scan of serum lipid concentrations in Pima Indians. Arterioscler Thromb Vasc Biol 20:2651–2656
50. **Krushkal J, Ferrell R, Mockrin SC, Turner ST, Sing CF, Boerwinkle E** 1999 Genome-wide linkage analyses of systolic blood pressure using highly discordant siblings. Circulation 99:1407–1410
51. **Comuzzie AG, Hixson JE, Almasy L, Mitchell BD, Mahaney MC, Dyer TD, Stern MP, MacCluer JW, Blangero J** 1997 A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nat Genet 15:273–276
52. **Hixson JE, Almasy L, Cole S, Birnbaum S, Mitchell BD, Mahaney MC, Stern MP, MacCluer JW, Blangero J, Comuzzie AG** 1999 Normal variation in leptin levels in associated with polymorphisms in the proopiomelanocortin gene, POMC. J Clin Endocrinol Metab 84:3187–3191
53. **Rotimi CN, Comuzzie AG, Lowe WL, Luke A, Blangero J, Cooper RS** 1999 The quantitative trait locus on chromosome 2 for serum leptin levels is confirmed in African-Americans. Diabetes 48:643–644
54. **Pritchard LE, Turnbull AV, White A** 2002 Pro-opiomelanocortin (POMC) processing in the hypothalamus: impact on melanocortin signalling and obesity. J Endocrinol 172:411–421
55. **Varret M, Rabes JP, Collod-Beroud G, Junien C, Boileau C, Beroud C** 1997 Software and database for the analysis of mutations in the human LDL receptor gene. Nucleic Acids Res 25:172–180
56. **Teran-Garcia M, Rufo C, Nakamura MT, Osborne TF, Clarke SD** 2002 NF-Y involvement in the polyunsaturated fat inhibition of fatty acid synthase gene transcription. Biochem Biophys Res Commun 290:1295–1299
57. **Ohba T, Rennert H, Pfeifer SM, He Z, Yamamoto R, Holt JA, Billheimer JT, Strauss JF** 1994 The structure of the human sterol carrier protein X/sterol carrier protein 2 gene (SCP2). Genomics 24:370–374
58. **Phelan CM, Larsson C, Baird S, Futreal PA, Ruttledge MH, Morgan K, Tonin P, Hung H, Korneluk RG, Pollak MN, Narod SA** 1996 The human mammary-derived growth inhibitor (MDGI) gene: genomic structure and mutation analysis in human breast tumors. Genomics 34:63–68
59. **Kim D-H, Magoori K, Inoue TR, Mao CC, Kim H-J, Suzuki H, Fujita T, Endo Y, Saeki S, Yamamoto TT** 1997 Exon/intron organization, chromosome lo-

calization, alternative splicing, and transcription units of the human apolipoprotein E receptor 2 gene. J Biol Chem 272:8498–8504
60. **Deng AY, Dene H, Pravenec M, Rapp JP** 1994 Genetic mapping of two new blood pressure quantitative trait loci in the rat by genotyping endothelin system genes. J Clin Invest 93:2701–2709
61. **Sharma P, Hingorani A, Jia H, Hopper R, Brown MJ** 1999 Quantitative association between a newly identified molecular variant in the endothelin-2 gene and human essential hypertension. J Hypertens 17:1281–1287
62. **Rice T, Chagnon YC, Perusse L, Borecki IB, Ukkola O, Rankinen T, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC** 2002 A genome-wide linkage scan for abdominal subcutaneous and visceral fat in black and white families: The HERITAGE Family Study. Diabetes 51:848–855
63. **Rankinen T, An P, Rice T, Sun G, Chagnon YC, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C** 2001 Genomic scan for exercise blood pressure in the health, risk factors, exercise training and genetics (HERITAGE) Family Study. Hypertension 38:30–37
64. **Lapsys NM, Furler SM, Moore KR, Nguyen TV, Herzog H, Howard G, Samaras K, Carey DG, Morrison NA, Eisman JA, Chisholm DJ** 1997 Relationship of a novel polymorphic marker near the human obese (OB) gene to fat mass in healthy women. Obes Res 5:430–433
65. **Clement K, Garner C, Hager J, LeDuc C, Carey A, Harris TJ, Jury C, Cardon L, Basdevant A, Demenais F, Guy-Grand B, North M, Froguel P** 1996 Indication for linkage of the human OB gene region with extreme obesity. Diabetes 45:687–690
66. **Reed DR, Ding Y, Xu W, Cather C, Green ED, Price RA** 1996 Extreme obesity may be linked to markers flanking the human OB gene. Diabetes 45:691–694
67. **Roth H, Hinney A, Ziegler A, Barth N, Gerber G, Stein K, Bromel T, Mayer H, Siegfried W, Schafer H, Remschmidt H, Grzeschik KH, Hebebrand J** 1997 Further support for linkage of extreme obesity to the obese gene in a study group of obese children and adolescents. Exp Clin Endocrinol Diabetes 105: 341–344
68. **Feitosa MF, Borecki IB, Rich SS, Amett DK, Sholinsky P, Myers RH, Leppert M, Province MA** 2002 Quantitative-trait loci influencing body-mass index reside on chromosomes 7 and 13: the National Heart, Lung, and Blood Institute Family Heart Study. Am J Hum Genet 70:72–82
69. **Bray MS, Boerwinkle E, Hanis CL** 1999 Linkage analysis of candidate obesity genes among the Mexican-American population of Starr County, Texas. Genet Epidemiol 16:397–411
70. **Duggirala R, Stern MP, Mitchell BD, Reinhart LJ, Shipman PA, Uresandi OC, Chung WK, Leibel RL, Hales CN, O'Connell P, Blangero J** 1996 Quantitative variation in obesity-related traits and insulin precursors linked to the OB gene region on human chromosome 7. Am J Hum Genet 59:694–703
71. **Onions KL, Hunt SC, Rutkowski MP, Klanke CA, Su YR, Reif M, Menon AG** 1998 Genetic markers at the leptin (OB) locus are not significantly linked to hypertension in African Americans. Hypertension 31:1230–1234
72. **Cheng LSC, Davis RC, Raffel LJ, Xiang AH, Wang N, Quinones M, Wen PZ, Toscano E, Diaz J, Pressman S, Henderson PC, Azen SP, Hsueh WA, Buchanan TA, Rotter JI** 2001 Coincident linkage of fasting plasma insulin and blood pressure to chromosome 7q in hypertensive hispanic families. Circulation 104:1255–1260
73. **Altmuller J, Palmer LJ, Fischer G, Scherb H, Wjst M** 2001 Genomewide scans of complex human diseases: true linkage is hard to find. Am J Hum Genet 69:936–950
74. **Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Copper R, Forrester T, Allison DB, Deka R, Ferrell RE, Shriver MD** 1998 Estimating African American admixture proportions by use of population-specific alleles. Am J Hum Genet 63:1839–1851