Brief Genetics Report

A Quantitative Trait Locus on 7q31 for the Changes in Plasma Insulin in Response to Exercise Training

The HERITAGE Family Study

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Several genome-wide linkage scans have been carried out to identify quantitative trait loci for type 2 diabetes and related metabolic phenotypes. However, no previous linkage scans have focused on the response to exercise training of relevant metabolic traits. We performed a genome-wide linkage scan for baseline fasting glucose, insulin, and C-peptide and their responses to a 20-week exercise training program in nondiabetic white and black men and women from the HERITAGE Family Study. In SIBPAL linkage analyses, the maximum number of sibpairs available was 344 and 93 for baseline phenotypes and 300 and 72 for exercise training response phenotypes in whites and blacks, respectively. A total of 509 markers with an average spacing of 6.0 Mb were used. The strongest linkage was found for the changes in fasting insulin in response to exercise training with a marker in the leptin gene on 7q31 (empirical multipoint $P = 0.0004$) in whites. In blacks, the strongest linkage was observed for baseline fasting glucose on 12q13-q14 (empirical multipoint $P = 0.0006$). These regions harbor several potential candidate genes. The present findings may be important in identifying individuals at increased risk of developing type 2 diabetes and who are most likely to benefit from a physically active lifestyle. *Diabetes* 52:1583–1587, 2003

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IBD, identical by descent; PFKM, phosphofructokinase; PPP1R3, protein phosphatase 1 regulatory subunit 3; QTL, quantitative trait locus.

Type 2 diabetes is a multifactorial heterogeneous disease characterized by variable degrees of insulin resistance and pancreatic β-cell dysfunction, which together lead to glucose intolerance (1). Chronic hyperglycemia, even below the threshold diagnostic for diabetes, markedly increases the risk of cardiovascular diseases and premature mortality (1,2). Thus, the prevention of type 2 diabetes is a major challenge for clinicians and public health policy makers worldwide (2).

Type 2 diabetes results from the interactions between genetic predisposition and behavioral and environmental risk factors (1). Family and twin studies have demonstrated a strong genetic component for type 2 diabetes and related intermediate metabolic traits (3,4). Regular physical activity has been found to improve insulin sensitivity (5) and to reduce the risk of type 2 diabetes (6,7). Lifestyle intervention including regular exercise reduced the incidence of type 2 diabetes by 58% in individuals with impaired glucose tolerance (8,9). The HERITAGE Family Study indicates that physiological responses to regular physical activity vary considerably from person to person and that these individual differences are influenced by genetic factors (10).

A number of genome-wide linkage scans have been carried out to identify quantitative trait loci (QTLs) for type 2 diabetes and related intermediate metabolic phenotypes (11–17). There are no genome-wide scan reports for the changes in response to exercise training of intermediate metabolic traits of type 2 diabetes. Here, we report on a linkage scan for the clinically important fasting plasma glucose, insulin, and C-peptide levels, as well as their responses to a 20-week exercise training program in nondiabetic white and black men and women from the HERITAGE Family Study.

RESEARCH DESIGN AND METHODS

Subjects. The HERITAGE Family Study is a multicenter exercise training study, carried out by a consortium of five universities in the U.S. and Canada (18). The main objective is to assess the role of genetic factors in cardiovascular, metabolic, and hormonal responses to aerobic exercise training in sedentary families. The study design, sampling, and inclusion and exclusion criteria have been described in detail previously (18). In brief, the offspring

were required to be aged 17–40 years and the parents aged ≤65 years. The subjects were required to be sedentary, defined as not having engaged in regular physical activity over the previous 6 months, and free of diabetes, cardiovascular diseases, or other chronic diseases that would prevent their participation in a 20-week exercise training program. The exclusion criteria included severe obesity (BMI >40 kg/m^2), unless the subject could meet the demands of the exercise program, hypertension (resting blood pressure >159/99 mmHg), and the use of medication for hyperglycemia, hyperlipidemia, or hypertension. The study protocol was approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant. The present study cohort consists of 507 white subjects (247 men and 260 women) from 99 nuclear families and 283 black subjects (102 men and 181 women) from 105 nuclear families. The training response data were available for 459 whites (223 men and 236 women) and 211 blacks (75 men and 136 women). The maximum number of sibpairs available was 344 and 93 for baseline phenotypes and 300 and 72 for exercise training response phenotypes in whites and blacks, respectively.

Exercise training program. The exercise training program has been described in detail previously (18). Briefly, the exercise intensity of the 20-week program was customized for each participant based on the heart rate–oxygen uptake relationship measured at baseline. During the first 2 weeks, the subjects trained at a heart rate corresponding to 55% of the baseline $V_{O_{2max}}$ for 30 min per session. Duration was gradually increased to 50 min per session and intensity to the heart rate associated with 75% of the baseline $V_{O_{2max}}$, which were then sustained for the last 6 weeks. Training frequency was three times per week, and all training sessions were performed on cycle ergometers in the laboratory. Heart rate was monitored during all training sessions by a computerized cycle ergometer system (Universal FitNet System), which adjusted ergometer resistance to maintain the target heart rate.

Measurement of glucose, insulin, and C-peptide. Fasting plasma glucose, insulin, and C-peptide were determined at baseline and after the 20-week exercise training program. Glucose was enzymatically determined using a reagent kit distributed by Diagnostic Chemicals. A modification of the method of Heding requiring polyethylene glycol precipitation was used to measure C-peptide. For the measurement of glucose and C-peptide, the average of two values obtained 15 min apart was used. Insulin was determined using a radioimmunoassay kit. The intra- and interassay coefficients of variation for baseline insulin were 7.7 and 10.3%, respectively. Because of the skewness of the distributions of baseline glucose, insulin, and C-peptide, log-transformed values were used.

Molecular studies. A total of 509 markers with an average spacing of 6.0 Mb were used. PCR conditions and genotyping methods have been outlined previously (19). Automatic DNA sequencers from LI-COR were used to detect the PCR products, and genotypes were scored semiautomatically using the software SAGA. Mendelian inheritance was checked, and markers showing incompatibilities were regenotyped (<10% were retyped). Microsatellite markers were selected mainly from the Marshfield panel version 8a. The panel included also some candidate genes for glucose tolerance, insulin sensitivity, and insulin secretion. Map locations were taken from the Genetic Location Database of Southampton, U.K. (http://cedar.genetics.soton.ac.uk).

Data adjustment. Baseline fasting plasma glucose, insulin, and C-peptide were adjusted for age, sex, and BMI using stepwise multiple regressions (20). Training responses were also adjusted for their respective baseline values. In brief, baseline phenotypes were regressed on baseline BMI and up to a third-degree polynomial in age, separately within race-by-sex-by-generation subgroups. Training responses were additionally regressed on baseline values. Only significant terms (5% level) were retained (i.e., the model did not need to be saturated). The residuals (or the raw scores if no terms were significant) were then standardized to zero mean and unit variance within each subgroup and constituted the final phenotypes.

Linkage analyses. Both single- and multipoint linkage analyses were performed with the sibpair linkage procedure (21) as implemented in the SIBPAL program of SAGE 4 (22). Briefly, if there is a linkage between the marker locus and a putative gene influencing the phenotype, sibs sharing a greater proportion of alleles identical by descent (IBD) at the marker locus will also show a greater resemblance in the phenotype. Phenotypic resemblance of the sibs, modeled as a weighted combination of squared trait difference and squared mean-corrected trait sum, is linearly regressed on the estimated proportion of alleles that the sibpair shares IBD at each marker locus. Both single- and multipoint estimates of allele sharing IBD were generated using the GENIBD program of S.A.G.E. 4. Allele frequencies for the IBD calculations were derived from parents (biologically unrelated subjects). Empirical P values (max. 500,000 replicates) were calculated for all markers with nominal multipoint P values ≤0.01. Only empirical P values are presented. We regarded linkages with $P < 0.0023$ (LOD score >1.75) as promising, which represents one false positive per scan for experiments involving 400 markers (23). All analyses were conducted separately for blacks and whites.

TABLE 1
Unadjusted means and SDs for baseline fasting plasma glucose, insulin, and C-peptide and their responses to exercise training according to race, sex, and generation

	Whites			Blacks		
Variable Group	n	Mean	SD	n	Mean	SD
Baseline						
Glucose (mmol/l)						
Fathers	96	5.48	0.71	22	5.47	0.68
Mothers	86	5.18	0.64	46	5.27	0.90
Sons	154	5.02	0.46	77	5.17	0.58
Daughters	155	4.78	0.41	114	4.92	0.52
Insulin (pmol/l)						
Fathers	95	79.6	64.8	26	65.3	33.4
Mothers	92	63.1	30.2	51	85.9	77.7
Sons	152	66.3	40.6	76	76.7	54.1
Daughters	167	58.6	26.6	130	79.5	61.7
C-peptide (nmol/l)						
Fathers	96	0.84	0.32	22	0.71	0.31
Mothers	86	0.70	0.28	46	0.83	0.41
Sons	154	0.68	0.30	77	0.82	0.41
Daughters	155	0.66	0.22	114	0.74	0.32
Training response						
Glucose (mmol/l)						
Fathers	89	−0.04	0.47	16	−0.06	0.42
Mothers	76	0.06	0.36	30	0.23	0.71
Sons	137	0.04	0.39	59	0.14	0.58
Daughters	137	0.06	0.39	80	0.13*	0.41
Insulin (pmol/l)						
Fathers	90	−8.0*	33.3	21	−2.2	34.1
Mothers	83	−5.0*	20.8	38	−16.6	64.5
Sons	133	−5.5*	24.1	54	−11.2*	33.8
Daughters	152	−3.2	21.8	98	−10.3*	42.8
C-peptide (nmol/l)						
Fathers	89	−0.04	0.20	16	0.01	0.29
Mothers	76	0.00	0.18	30	−0.01	0.29
Sons	137	−0.01	0.24	59	0.00	0.39
Daughters	137	−0.02	0.18	80	0.03	0.19

*Statistically significant ($P < 0.05$) responses to exercise training.

RESULTS

At baseline, the mean age of fathers was 53.5 (44.4–64.3) and 50.0 (range 39.3–65.9) years, mothers 52.0 (42.4–65.2) and 46.6 (33.7–64.8) years, sons 25.2 (17.0–40.3) and 27.0 (15.9–45.8) years, and daughters 25.4 (17.2–40.9) and 27.6 (16.4–48.1) years in whites and blacks, respectively. The mean BMI of fathers was 28.4 and 27.5 kg/m^2, mothers 27.6 and 29.4, sons 25.6 and 27.4, and daughters 23.7 and 27.9. Baseline fasting plasma glucose, insulin, and C-peptide and their responses to exercise training are shown in Table 1. In response to exercise, insulin decreased in all race, sex, and generation groups. No significant training changes were found for glucose or C-peptide, and no linkage analyses were undertaken with these phenotypes.

The strongest evidence of linkage was found for fasting insulin response to exercise training with a marker in the leptin gene on chromosome 7q31 in whites (Fig. 1). In whites, linkages were also detected for baseline fasting glucose on chromosome 2p23, baseline fasting insulin on chromosomes 10q25 and 19q13, baseline fasting C-peptide on chromosome 12p13, and fasting insulin response to

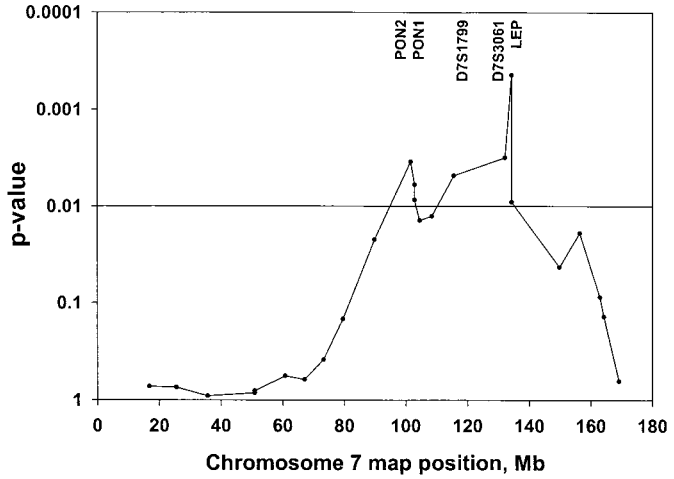

FIG. 1. Empirical multipoint linkages for fasting insulin response to exercise training on chromosome 7 in whites.

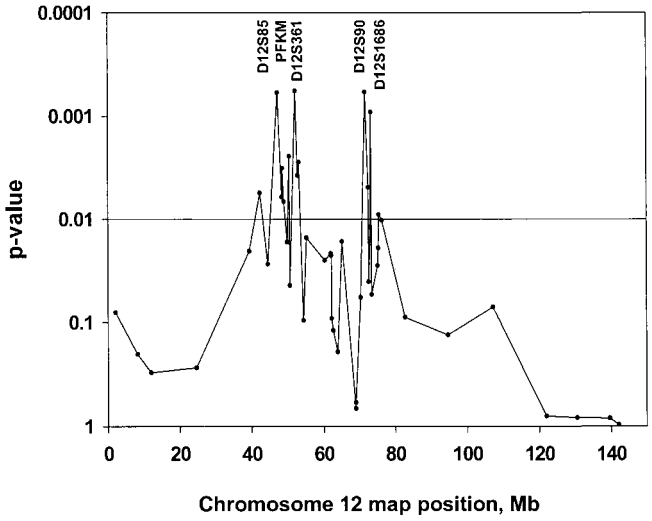

FIG. 2. Empirical multipoint linkages for baseline fasting glucose on chromosome 12 in blacks.

exercise training on chromosomes 1q21, 2q31, 7q21-q22, and 11q13 (Table 2). In blacks, the strongest multipoint linkage was observed for baseline fasting glucose on chromosome 12q13-q14 with markers D12S85 (47.24 Mb, $P = 0.0006$), D12S361 (52.17 Mb, $P = 0.0006$), D12S90 (71.63 Mb, $P = 0.0006$), and D12S1686 (73.251 Mb, $P = 0.0009$) (Fig. 2). Moreover, multipoint linkages in blacks were detected for baseline fasting glucose on chromosome 1p36 (marker D1S1612, 4.675 Mb, $P = 0.0023$) and 12q13 (marker PFKM, 48.425 Mb, $P = 0.0061$), for baseline fasting C-peptide on chromosomes 6p23 (marker D6S2434, 12.7 Mb, $P = 0.0003$), 6q25 (marker D6S2436, 158.466 Mb, $P = 0.0079$), and 12q13 (marker D12S1644, 68.96 Mb, $P = 0.0062$), as well as for fasting insulin response to exercise training on chromosome 15p11 (marker D15S63, 18.5 Mb, $P = 0.0059$).

DISCUSSION

The present study is unique in that it provides the first genome-wide linkage scan results for the changes in fasting plasma insulin levels in response to exercise training in nondiabetic individuals. The strongest evidence of linkage was found for fasting insulin response to exercise training on 7q21-q31 in whites. This linkage was with a marker in the leptin gene.

There is accumulating evidence for a linkage with diabetes-related phenotypes on 7q21-q31. A study in hypertensive Hispanic families demonstrated linkages with fasting insulin, homeostasis model assessment insulin, leptin, and blood pressure on 7q21-q31 (13). Fine mapping suggested that there may be a single locus on 7q31 contributing to these phenotypes (13). The same study had its strongest linkage for fasting insulin with marker D7S3061 (13). A study in Pima Indians suggested a potential diabetes QTL near marker D7S1799 among sibpairs affected before 45 years of age (16). Both of these markers were linked with the fasting insulin response to exercise training in our study. The 7q21-q31 region has also been found to harbor QTLs for insulin precursors and extremity skinfold thickness in Mexican Americans (14), BMI in Americans (24), abdominal subcutaneous fat in Québec

TABLE 2
Linkage results in whites

Chromosome	Marker	Map position (Mb)	Trait	Empirical *P* values from SIBPAL	
				Multipoint	Singlepoint
1q21	D1S394	150.991	Δ Ins	0.0086	0.0053
2p23	D2S405	43.463	Gluc	0.0046	0.4314
2q31	D2S1776	168.109	Δ Ins	0.0042	0.0059
2q31	D2S1391	193.234	Δ Ins	0.0064	0.0293
7q21	PON2	101.548	Δ Ins	0.0035	0.1342
7q21	PON1	102.837	Δ Ins	0.0060	0.0113
7q22	D7S1799	115.577	Δ Ins	0.0048	0.0292
7q31	D7S3061	132.182	Δ Ins	0.0031	0.4017
7q31	LEP	134.312	Δ Ins	0.0004	0.0024
10q25	ADRA2A	118.294	Ins	0.0039	0.0245
11q13	UCP3	92.071	Δ Ins	0.0097	0.0303
12p13	D12S372	2.129	C-pept	0.0054	0.0646
12p13	GNB3	8.216	C-pept	0.0055	0.0129
12p13	D12S391	11.989	C-pept	0.0053	0.0044
19q13	D19S589	59.573	Ins	0.0036	0.0429

All baseline phenotypes are adjusted for age, sex, and BMI. Exercise training response (Δ) of insulin is also adjusted for baseline insulin.

residents (25), BMI-adjusted leptin in Old Order Amish (17), and a lipid profile factor of the metabolic syndrome in Mexican Americans (26).

Chromosome 7q21-q31 harbors several genes potentially affecting glucose and insulin metabolism. Protein phosphatase 1 regulatory subunit 3 (PPP1R3) is a glycogen and sarcoplasmic reticulum–binding subunit of type 1 protein phosphatase that plays a major role in glycogen metabolism and glucose disposal in skeletal muscle (27). Common variants in the PPP1R3 gene have been associated with insulin resistance in Pima Indians (28) and Caucasians (29), insulin hypersecretion in Caucasians (29), and type 2 diabetes in Pima Indians (28). Leptin is crucial in the regulation of energy balance and body weight, but it also plays role in glucose and insulin metabolism (30). In the present study, a leptin marker showed promising linkage with fasting insulin response to exercise training. PON is an antioxidant enzyme that may be important in the etiology of type 2 diabetes (31). PON1 and PON2 genes are located very close to each other on 7q21. A common variant of the PON1 gene has been associated with glucose intolerance in nondiabetic individuals (31). We found suggestive linkage for fasting insulin response to exercise training with PON1 and PON2 markers.

In blacks, we detected the strongest evidence of linkage for baseline fasting glucose on 12q13-q14. The region coincides with the diabetes locus found among white families with early-onset type 2 diabetes (32). The locus is also close to a region where linkage was detected with 2-h insulin from an oral glucose tolerance test in Pima Indians (11) as well as with diabetes and impaired glucose homeostasis in whites (15). In the 12q13-q14 region, an obvious candidate gene is the muscle subtype of phosphofructokinase (PFKM). A deficiency of PFKM has been shown to cause peripheral insulin resistance and impair insulin secretion in response to glucose in an Ashkenazi Jewish family (33). Here, we observed a suggestive linkage for baseline fasting glucose with a PFKM marker.

The present study in nondiabetic individuals provides evidence that a genomic region close to the leptin locus may contribute to the fasting insulin response to exercise training. Fasting insulin levels have been inversely associated with insulin sensitivity, as estimated by the hyperinsulinemic-euglycemic clamp, and have predicted the incidence of diabetes in several studies (34). Thus, the observed changes in fasting insulin levels reflect the changes in insulin sensitivity in response to exercise training. The present findings may be important in the ongoing effort to identify individuals at increased risk of developing type 2 diabetes and who are most likely to benefit from regular physical activity in terms of prevention of diabetes.

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