An Autosomal Genome-wide Scan for Loci Linked to Pre-Diabetic Phenotypes in Nondiabetic Chinese Subjects From the Stanford Asia-Pacific Program of Hypertension and Insulin Resistance Family Study

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Type 2 diabetes is a complex disease involving both genetic and environmental components. Abnormalities in insulin secretion and insulin action usually precede the development of type 2 diabetes and can serve as good quantitative measures for genetic mapping. We therefore undertook an autosomal genomic search to locate the quantitative trait locus (QTL) linked to these traits in 1,365 nondiabetic Chinese subjects from 411 nuclear families. Residuals of these log-transformed quantitative traits were analyzed in multipoint linkage analysis using a variance-components approach. The most significant QTL for fasting insulin, which coincides with the QTL for homeostasis model assessment of insulin resistance, was located at 37 cM on chromosome 20, with a maximum empirical logarithm of odds (LOD) score of 3.01 (empirical $P = 0.00006$) when adjusted for age, sex, BMI, antihypertensive medications, recruitment centers, and environmental factors. In the same region, a QTL for fasting glucose was identified at 51 cM, with an empirical LOD score of 2.03 (empirical $P = 0.0012$). There were other loci with maximum empirical LOD scores ≥1.29 located on chromosomes 1q, 2p, 5q, 7p, 9q, 10p, 14q, 18q, and 19q for different diabetes-related traits. These loci may harbor genes that regulate glucose homeostasis either independently or via interactions of the genes within these regions. *Diabetes* 54: 1200–1206, 2005

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AUC, area under the curve; HOMA, homeostasis model assessment; HOMA-β, HOMA of β-cell function; HOMA-IR, HOMA of insulin resistance; LOD, logarithm of odds; QTL, quantitative trait locus; SAPPHIRe, Stanford Asia-Pacific Program of Hypertension and Insulin Resistance.

Type 2 diabetes is a complex disease that occurs because of a combination of genetic and environmental factors (1). Although the underlying pathogenesis of this disease is not well understood, insulin insensitivity and insulin secretion defects usually precede the development of disease (2). Many attempts, including both candidate gene and genome-wide linkage studies (3), have been taken to identify the possible genetic factors involved in the disease process of type 2 diabetes.

Up to now, >20 genome-wide scans have been performed to locate the loci with evidence of linkage to type 2 diabetes across chromosomal regions (4–26), and evidence for linkage with type 2 diabetes has been reported. However, the mapped regions vary from different studies, with the exception of chromosomes 1q (4,5,8,11,13,15,19, 22) and 20q (21,23–26). Factors contributing to the failure to replicate linkage results include the presence of different ethnic backgrounds, the complex nature of type 2 diabetes inheritance, different definitions of phenotypes, and the environmentally determined phenocopies of type 2 diabetes. In addition to genetic and allelic heterogeneity, epistasis (gene-to-gene interaction) and gene-to-environment interactions warrant further investigation.

Identification of the genes responsible for type 2 diabetes is complicated by the high degree of genetic heterogeneity and the involvement of multiple genes with small to moderate effects. Searching for quantitative trait loci (QTLs) that explain the variation in the "intermediate" phenotypes of type 2 diabetes has therefore been considered as a more powerful approach to dissect the genetic factors involved in different pathogenetic pathways that lead to diabetes (27). So far, a number of genome-wide linkage scans have been successfully carried out to identify the QTLs for the related intermediate phenotypes of type 2 diabetes (28–32). To our knowledge, this approach has not been reported for the Chinese population.

In previous studies, we have shown that the insulin resistance syndrome is familial in nature and highly hered-

itable in Chinese hypertensive families (33). We have demonstrated a strong genetic component, with a heritability ranging from 0.3 to 0.6, for most metabolic variables. In this study, we conducted a genome-wide scan to further identify the susceptibility QTLs for the intermediate phenotypes associated with insulin resistance syndrome from hypertensive families in the Stanford Asia-Pacific Program of Hypertension and Insulin Resistance (SAPPHIRe) study. The genome-wide scan was performed for plasma glucose and insulin levels, with or without adjustment for BMI, after accounting for the effects from recruitment centers, antihypertensive medications, physical activity, cigarette smoking, and alcohol consumption.

RESEARCH DESIGN AND METHODS

The SAPPHIRe study was designed to identify susceptibility genes for hypertension (34) and insulin resistance in selected Chinese and Japanese populations (34,35). The SAPPHIRe study design, recruitment, phenotyping, and genotyping have been detailed elsewhere (34–36). In this article, we only briefly review recruitment, genotyping, and phenotyping. The study design incorporated both concordant siblings (all siblings with hypertension) and discordant siblings (at least one hypertensive sibling). Index cases were ascertained as those with age at onset of 35–60 years or those >60 years of age with documentation of their hypertension status before age 60 years. We excluded from our analyses diabetic individuals defined by WHO (World Health Organization) criteria uncovered as a result of SAPPHIRe laboratory work and those subjects previously diagnosed with diabetes because diabetic individuals usually have abnormal trait measures. A total of 2,525 subjects of Japanese or Chinese descent were recruited from centers at San Francisco, Hawaii, and Taiwan. These subjects underwent a clinical and fasting laboratory examination, with written informed consent obtained before examination. We performed the genome-wide multipoint linkage analyses in 1,365 nondiabetic Chinese subjects (118 parents and 1,247 siblings) with genotyping data from 411 nuclear families. The numbers of families with one to eight siblings are 29, 138, 122, 63, 37, 16, 5, and 1, respectively.

Genotyping. Genomic DNA was extracted from peripheral lymphocytes using a Puregene kit (Minneapolis, MN). Genotyping was performed at the Marshfield Medical Research Foundation (Marshfield, WI) using a Weber screening set 9 (Research Genetics, Huntsville, AL). This procedure used 376 autosomal markers representing short tandem repeat polymorphisms and yielded an average map density of 10 cM. Genotyping quality was monitored by typing 30 samples in duplicate. We estimated an error rate of ~1% based on these duplicate samples. All markers were inspected for Mendelian errors, and genetic distances between markers were determined.

Phenotyping. The participants underwent anthropometric measurements at 8 A.M. after a 8- to 10-h overnight fast without wearing shoes and heavy clothes. Each subject was subjected to a 75-g oral glucose tolerance test after the anthropometric measurements. Fasting blood samples were collected for the measurements of plasma glucose and insulin. Then, 75 g glucose monohydrate (in 300 ml water) was administered to the subject to drink over 5 min. Blood samples were taken for plasma glucose and insulin 1 and 2 h after glucose loading. The patients were not allowed to eat or drink until the end of the test (37).

Plasma glucose was measured by a glucose oxidase method in a glucose analyzer (Elan Diagnostics, Smithfield, RI). Plasma insulin was measured by an enzymatic immunoassay (Access System; Beckman Coulter, Fullerton, CA). The sensitivity of the assay was 0.03 μU/ml. The precision was <10% coefficient of variation (CV) (37).

Homeostasis model assessment of insulin resistance (HOMA-IR) was derived as follows: fasting plasma insulin × fasting plasma glucose/22.5, with fasting plasma insulin in microunits per milliliter and fasting plasma glucose in millimoles per liter (37). HOMA of β-cell function (HOMA-β) was derived as follows: fasting insulin/(fasting plasma glucose − 3.5), with fasting insulin in microunits per milliliter and fasting plasma glucose in millimoles per liter. The area under the curve (AUC) for insulin was calculated as the AUC connecting the three points of fasting insulin and 1-h and 2-h insulin; $AUC_{glucose}$ was derived (37) analogously using fasting, 1-h, and 2-h glucose.

Covariates. BMI was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). Smoking was classified by four categories: nonsmokers, ex-smokers, current light smoker (taking at least one cigarette, but <15 cigarettes/day), and current heavy smoker (taking ≥15 cigarettes/day). Physical activity was an indicator variable categorizing whether the proportion of hours spent on sedentary activity relative to daily nonbasal activities (i.e., 24 h − hours of basal activity, including sleeping or lying down) was >0.5 or not. Alcohol consumption was categorized as "nondrinker," "light drinker," "modest drinker," and "heavy drinker" according to daily average consumption in ounces of alcohol (37). The recruitment centers included the Taiwan, San Francisco, and Hawaii centers. The antihypertensive medications possibly affecting plasma and glucose levels included ACE inhibitors and angiotensin receptor blockades (38). To make the adjustment for these factors, indicator variables were created for all of the categorical covariates.

Genome-wide multipoint linkage analyses. Quantitative demographic variables including age and BMI, as well as diabetes-related variables including fasting insulin, 1- and 2-h insulin, fasting glucose, 1- and 2-h glucose, HOMA-IR and -β, $AUC_{glucose}$, and $AUC_{insulin}$ were summarized in means ± SD separated by three recruitment centers. Percentages were provided for categorical environmental factors. Differences between these three centers were assessed using the generalized estimating equation method. Distributions of the quantitative diabetes-related traits were examined; Box-Cox transformations were applied to make their distributions more near-normal, as appropriate. As a result, a natural logarithm transformation was used for all of the traits, except that a transformation of (fasting glucose^2 − 1)/2 was used for fasting glucose.

Genome-wide scans on transformed diabetes-related quantitative traits as listed above were carried out for Chinese subjects, using a variance-components approach as implemented in the SOLAR (sequential oligogenic linkage analysis routines) software package, version 2.1.0. Adjustments were made for age, sex, recruitment centers, antihypertensive medications, and environmental factors of smoking, alcohol consumption, and physical activity, with or without BMI. The alleles that shared identity-by-descent between relative pairs were estimated using Genehunter (39) before being imported into SOLAR software for variance component analyses. The identity-by-descent between relative pairs was imputed every 1 cM across the genome based on the ~10-cM map. The possible QTLs were therefore located at every 1 cM. The variance-components model partitions the variability of a trait into components for a QTL, the residuals polygenic component, and the random environmental component (40). The likelihood ratio was derived by dividing the likelihood of the estimated variance component due to the QTL by the likelihood of this variance component being 0. Logarithm of odds (LOD) scores were calculated as the logarithm to base 10 of the likelihood ratios. One-unit LOD support intervals were obtained for maximum LOD scores ≥3. We performed computer simulations to assess pointwise statistical significance for regions with maximum LOD scores ≥1.5 to avoid inflated type I error rates caused by violation of the normality assumption. In the simulation analysis, a fully informative marker was simulated under the null hypothesis of no linkage, with the observed family structures and phenotypes. We generated 10,000 (or 100,000 for regions with maximum LOD scores ≥3) replicates for each interested region, and the resulting LOD score distribution was used to derive empirical (adjusted) LOD scores and empirical P values. A maximum empirical LOD score was reported as the local maximum of an adjusted LOD score curve for a chromosomal region. This procedure was implemented in the SOLAR program (40). The empirical P values (and LOD scores) were reported instead of the nominal LOD scores and P values.

Oligogenic scans (41) were performed by making multiple passes through the selected chromosomes: QTLs passing the criterion of maximum LOD scores ≥3 in the first genome scan were selected as QTLs 1; a second set of QTLs with maximum LOD scores ≥2 (QTLs 2) were selected by conditioning on the effect of QTLs 1; similarly, a third set of QTLs (QTLs 3) were identified after conditioning on the effect of the selected QTLs 1 and QTLs 2.

RESULTS

The descriptive statistics for demographic and trait values, grouped by recruitment centers, are displayed in Table 1. Of the subjects, ~85% were recruited from Taiwan. The mean age was statistically significantly higher for Chinese subjects from the Hawaii center when compared with Chinese subjects from the Taiwan center ($P < 0.0001$). In addition, the mean levels of the diabetes-related traits were compatible among the subjects recruited from the three regions, except for the mean levels of 2-h glucose and $AUC_{glucose}$: both were slightly higher for Chinese subjects from Taiwan than for those from San Francisco ($P \leq 0.0008$). As for the environmental factors (Table 2), the distributions of smoking and physical activity were similar among these three centers; however, the Hawaii and San Francisco centers tended to have higher percent-

TABLE 1
Demographic and metabolic characteristics of the subjects separated by center

Recruitment center	Taiwan	Hawaii	San Francisco
Families (*n*)	331	25	55
Subjects (*n*)	1,161	71	133
Male	512 (44.1)	27 (38.0)	69 (51.9)
Age (year)	50.1 ± 10.9 (1,161)*	56.8 ± 10.3 (71)	52.6 ± 10.3 (133)
BMI (kg/m^2)	25.0 ± 3.3 (887)	25.2 ± 3.7 (52)	25.3 ± 4.1 (119)
Fasting glucose (mmol/l)	4.9 ± 0.6 (1,156)	5.2 ± 0.5 (57)	5.0 ± 0.5 (123)
1-h glucose (mmol/l)	9.3 ± 2.2 (1,029)	9.0 ± 2.2 (34)	8.6 ± 2.2 (117)
2-h glucose (mmol/l)	7.2 ± 1.8 (1,002)†	6.9 ± 1.8 (38)	6.6 ± 1.8 (116)
Fasting insulin (pmol/l)	52.7 ± 35.0 (1,153)	51.4 ± 30.3 (57)	61.6 ± 68.0 (123)
1-h insulin (pmol/l)	548.2 ± 408.1 (1,023)	616.5 ± 529.3 (36)	582.4 ± 394.3 (118)
2-h insulin (pmol/l)	443.1 ± 410.1 (1,021)	465.8 ± 307.8 (38)	489.2 ± 414.1 (117)
HOMA-IR	1.7 ± 1.2 (1,152)	1.7 ± 1.0 (57)	2.0 ± 2.3 (123)
HOMA-β	108.0 ± 155.7 (1,152)	82.5 ± 44.6 (57)	112.8 ± 110.8 (123)
$AUC_{glucose}$ (mmol · h · l^{-1})	16.6 ± 3.1 (998)†	16.3 ± 3.1 (34)	15.6 ± 3.1 (116)
$AUC_{insulin}$ (pmol · h · l^{-1})	796.8 ± 572.5 (1,014)	896.0 ± 643.4 (35)	860.6 ± 564.4 (117)

Data are *n* (%) or means ± SD (*n*). *$P < 0.001$ vs. Hawaii; †$P < 0.001$ vs. San Francisco.

ages of light and heavy drinkers when compared with Chinese subjects from the Taiwan center ($P < 0.0001$).

All chromosomal regions with maximum empirical LOD scores ≥1.29 for the diabetes-related traits are summarized in Table 3. Two sets of covariates were adjusted for in the genome-wide scan: *1*) age, sex, recruitment center, antihypertensive medications, smoking, alcohol consumption, and physical activity; or *2*) age, sex, recruitment center, antihypertensive medications, smoking, alcohol consumption, physical activity, and BMI.

The most striking evidence for linkage was observed on chromosome 20 (Fig. 1 and Table 3). With adjustment for BMI, the maximum empirical (or adjusted) LOD score for fasting insulin was 3.01 ($P = 0.00006$), located at 37 cM between markers GATA81E09 and GGAA7E02, and had a 1-LOD support interval of 33–40 cM. By analyzing the subset of Chinese in Taiwan subjects, the same peak of maximum empirical LOD scores for fasting insulin increased to 3.19, with a support interval of 34–40 cM. As might be expected, because fasting insulin is a component of HOMA-IR and -β, this locus also showed evidence for linkage to HOMA-IR and -β levels with empirical LOD scores of 2.94 ($P = 0.00013$, support interval 33–41 cM) and 1.31 ($P = 0.0059$), respectively. Furthermore, the susceptibility QTL for BMI- and other covariate-adjusted fasting glucose was located at 51 cM, with an adjusted LOD score of 2.03 ($P = 0.0012$, near marker GATA42A03). The evidence for linkage to fasting glucose remained in this region (adjusted LOD 2.27), without the adjustment for BMI.

TABLE 2
Distribution of the environmental factors separated by center

Variable	Taiwan	Hawaii	San Francisco
Smoking			
Nonsmoker	662 (74.6)	30 (58.8)	86 (72.3)
Ex-smoker	57 (6.4)	16 (31.4)	26 (21.9)
Light smoker	70 (7.9)	2 (3.9)	4 (3.4)
Heavy smoker	99 (11.2)	3 (5.9)	3 (2.5)
Alcohol consumption*			
None	686 (78.9)	10 (32.3)	56 (53.3)
Light	64 (7.4)	12 (38.7)	24 (22.9)
Modest	31 (3.6)	3 (9.7)	3 (2.9)
Heavy	89 (10.2)	6 (19.4)	22 (21.0)
Physical activity			
Sedentary	557 (62.5)	36 (70.6)	90 (74.4)
Nonsedentary	334 (37.5)	15 (29.4)	31 (25.6)

Data are *n* (%). *$P < 0.0001$ for either Taiwan vs. San Francisco or Taiwan vs. Hawaii.

Two more chromosomal regions were identified with QTL for fasting insulin and HOMA-IR on chromosome 5 and 7. On chromosome 5, the QTLs for fasting insulin and HOMA-IR were identified at the same location of 85 cM, close to marker GATA52A12, with adjusted LOD scores of 2.21 ($P = 0.00070$) and 1.99 ($P = 0.0015$), respectively, with adjustment for BMI (Table 3). The maximum empirical LOD score was 2.18 at 153 cM (between markers ATA23A10 and GATA6E05) for the adjusted fasting glucose ($P = 0.0006$). This evidence was also observed when BMI was not adjusted for. Additionally, the susceptibility QTL for BMI-adjusted fasting insulin and HOMA-IR was found located at ~1 cM on chromosome 7 (near marker GATA24F03), with adjusted LOD scores of 1.29 ($P = 0.0077$) and 1.57 ($P = 0.0038$), respectively (Table 3).

In addition to the regions on chromosomes 5 and 20, the putative QTLs for fasting glucose were also found at other locations that showed weaker evidence of linkage, including a locus at ~140 cM on chromosome 9 (maximum empirical LOD score of 1.45) and a locus at 35 cM on chromosome 10 with maximum empirical LOD scores of 1.81 or 1.93, with or without adjustment for BMI, respectively.

It is noteworthy that the possible QTL for HOMA-β appeared to be located at 87 or 88 cM on chromosome 2, with maximum empirical LOD scores of 1.44 ($P = 0.0042$) or 2.28 ($P = 0.00060$), respectively, as well as at 44–45 cM on chromosome 14, with maximum LOD scores of 1.80 ($P = 0.0019$) or 2.41 ($P = 0.0004$) with or without BMI adjustment, respectively. The linkage evidence for 2-h insulin was observed at 75 cM on chromosome 9, near marker GATA89A11, with a maximum empirical LOD score of 2.22 ($P = 0.0002$) when BMI was adjusted for. The linkage QTL for 2-h glucose was found to be at ~246 cM on chromosome 1, with maximum empirical LOD scores of 1.56 ($P = 0.0035$) or 2.09 ($P = 0.0012$) with or without adjustment for BMI, respectively. Moreover, weak evi-

TABLE 3
Regions with maximum empirical LOD scores ≥1.29

Trait	Adjustment: Sex, age, center, medications, and environmental factors			Adjustment: Sex, age, center, medications, environmental factors, and BMI		
	cM	MELS	Empirical *P* value	cM	MELS	Empirical *P* value
Chromosome 1						
2-h glucose	248	2.09	0.0012	246	1.56	0.0035
Chromosome 2						
HOMA-β	87	2.28	0.00060	88	1.44	0.0042
Chromosome 5						
Fasting insulin	86	1.82	0.0033	85	2.21	0.00070
HOMA-IR	88	1.42	0.0056	85	1.99	0.0015
Fasting glucose	153	2.19	0.00050	153	2.18	0.00060
Chromosome 7						
HOMA-IR	—	—	—	0	1.57	0.0038
Fasting insulin	—	—	—	1	1.29	0.0077
Fasting glucose	9	1.38	0.0056	—	—	—
Chromosome 9						
Fasting glucose	140	1.45	0.0043	—	—	—
2-h insulin	—	—	—	75	2.22	0.00020
Chromosome 10						
Fasting glucose	35	1.93	0.00080	35	1.81	0.0022
Chromosome 14						
HOMA-β	44	2.41	0.00040	45	1.80	0.0019
Chromosome 18						
$AUC_{insulin}$	72	1.30	0.0065	—	—	—
Chromosome 19						
$AUC_{insulin}$	90	1.38	0.0049	—	—	—
Chromosome 20						
Fasting insulin	—	—	—	37	3.01	0.00006
HOMA-IR	—	—	—	37	2.94	0.00013
HOMA-β	—	—	—	37	1.31	0.0059
Fasting glucose	51	2.27	0.00020	51	2.03	0.0012

Empirical *P* values (and LOD scores) were derived from simulations of 10,000 (or 100,000) replicates. MELS, maximum empirical LOD score.

dence for linkage to $AUC_{insulin}$ was found at 72 cM on chromosome 18 (maximum empirical LOD score of 1.30, $P = 0.0065$) and at 90 cM on chromosome 19 (maximum empirical LOD score of 1.38, $P = 0.0049$) when BMI was not accounted for. None of susceptibility QTLs for 1-h insulin were identified in our analyses. HOMA-IR and fasting insulin essentially picked up the same signals because the correlation coefficient between these two traits is as high as 0.99. Note that the significance levels should be interpreted with caution because 10 correlated phenotypes were examined in this study and multiple comparisons were performed.

The results from the genome-wide scan for oligogenic loci are displayed in Table 4. No additional interesting QTLs were identified for the traits in this search. After conditioning on the effects of QTLs 1 on chromosome 20, the maximum empirical LOD scores for the QTL on chromosome 5 dropped from 2.21 to 1.87 ($P = 0.0010$) for fasting insulin, and rose from 1.57 to 2.11 ($P = 0.0007$) for the QTL on chromosome 7 for HOMA-IR. When conditioning on QTLs 1 and 2, the maximum empirical LOD scores increased from 1.29 to 2.59 ($P = 0.0045$) for the QTL on chromosome 7 for fasting insulin and increased from 1.99 to 2.16 ($P = 0.0042$) for the QTL on chromosome 5 for HOMA-IR. The presence of epistasis between these loci is worth further investigation.

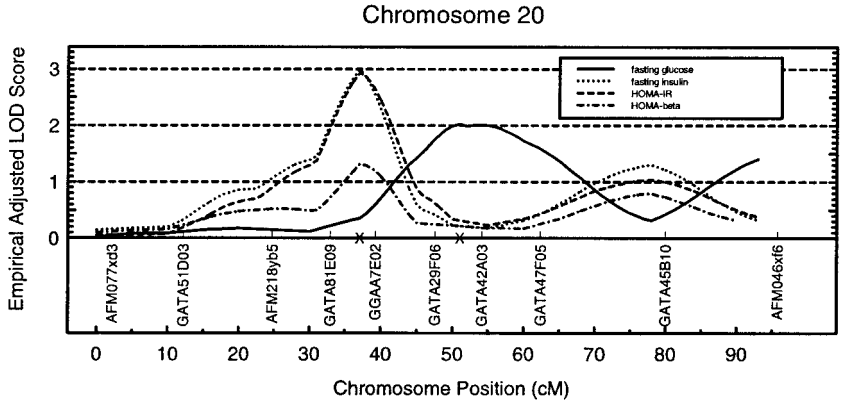

FIG. 1. LOD score curves for age-, sex-, center-, medication-, environmental factor–, and BMI-adjusted fasting glucose, fasting insulin, HOMA-IR and HOMA-β in nondiabetic subjects for chromosome 20.

TABLE 4
Genome-wide scans for oligogenic loci, adjusted for sex, age, drug, center, environmental factors, and BMI

	QTLs 1					QTLs 2					QTLs 3				
Trait	Chr	cM	MELS	Empirical *P*	H^2_{q1} (%)	Chr	cM	MELS	Empirical *P*	H^2_{q2} (%)	Chr	cM	MELS	Empirical *P*	H^2_{q3} (%)
Fasting insulin	20	37	3.01	0.00006	33.1	5	85	1.87	0.0010	24.9	7	0	2.59	0.0045	15.2
HOMA-IR	20	37	2.94	0.00013	31.5	7	0	2.11	0.0007	24.4	5	85	2.16	0.0042	15.5

QTLs 2 were identified conditional on effects of QTLs 1. QTLs 3 were identified conditional on effects of QTLs 1 and QTLs 2. Chr, chromosome; $H^2_{qi,}$ heritability attributed to QTLs i, with i = 1, 2, 3; MELS, maximum empirical LOD score.

DISCUSSION

The current study is unique in that it provides the first genome-wide linkage scan results for the QTLs for insulin resistance and β-cell function by HOMA as well as blood glucose and insulin levels in the nondiabetic individuals from a homogeneous population. Overall, there were 14 QTLs mapped on chromosomes 1q, 2p, 5q, 7p, 9q, 10p, 14q, 18q, 19q, 20p, and 20q, with weak or suggestive evidence for insulin/glucose homeostasis in Han Chinese subjects. The susceptibility QTLs for fasting glucose were located on chromosome 20q and were different from those on chromosome 20p for fasting insulin and HOMA-IR. Interestingly, the QTLs for fasting glucose were independent of BMI, whereas BMI was important for fasting insulin and HOMA-IR.

The strongest evidence of linkage was found for fasting insulin and HOMA-IR on 20q11-q12, indicating that the same gene(s) in this region play a similar role in plasma insulin and HOMA-IR. Because insulin level is also a component of HOMA-β, the identical region also conferred evidence of linkage to HOMA-β. These data indicate that both fasting insulin and HOMA-IR are largely genetically determined because the heritability attributed to the QTL on chromosome 20 is ~32–33% for both traits in the oligogenic models. This region has been confirmed in many genome scans from various studies to harbor susceptibility genes for type 2 diabetes (23–26) and has become the target of collaborative fine-mapping efforts (27). Importantly, there is another QTL (51 cM on chromosome 20) for fasting glucose close to this region, making this region rich in harboring genes for insulin resistance and resulting blood glucose regulation. In addition, the tentative QTLs for insulin, HOMA-IR, and fasting plasma glucose (10 cM on chromosome 7) are close to the reported loci for type 2 diabetes in Japanese individuals (18).

In the oligogenic search, the three-locus model, with a total adjusted LOD score (41) of 7.47 (corresponding to conditional adjusted LOD scores of 1.87 and 2.59, respectively, for the second [$P = 0.001$] and the third QTL [$P = 0.0045$]), accounted for 33, 25, and 15% of the variance resulting from the QTLs on chromosomes 20, 5, and 7, respectively, for fasting insulin. These three loci also explain 32, 24, and 15% of the variance resulting from the QTLs on chromosomes 20, 7, and 5, respectively, for HOMA-IR. In our genome-wide search, the susceptibility locus on chromosome 5 explains ~32–34% of the phenotypic variations marginally in fasting insulin levels and HOMA-IR (data not shown) when BMI was adjusted for. When conditioning on both QTLs on chromosomes 20p and 7p, however, the heritability attributable to this locus reduced dramatically, suggesting these three loci might not act additively. Possible interactions between this locus and the loci on chromosomes 20 (37 cM) and 7 (0–2 cM) are worth further investigation. Interestingly, in a large French pedigree, a locus on chromosome 5q showed evidence of linkage to type 2 diabetes susceptibility (42). The region on chromosome 5 harbors a number of candidate genes for obesity (43) that might contribute to the development of type 2 diabetes.

Previously, the regions on chromosome 1q21-q24 have shown significant linkage to type 2 diabetes or impaired glucose homeostasis in the Han Chinese population living in different regions of China (22,14,44). Our mapping for the QTLs responsible for the pre-diabetic phenotypes, including insulin and glucose homeostasis, however, resulted in different regions. These differences might be due to our use of nondiabetic subjects, adjusted for different environmental factors, and our comparatively large sample size. In the region on chromosome 1, a QTL for 2-h glucose level after an oral glucose tolerance test was mapped on 248 cM from pter, which is different from the locus on chromosome 1q21-q24 mapped for diabetes in the Chinese population (22). This region, however, is close to those reported from a genome scan for HbA_{1c} in a community-based sample of Caucasian pedigrees in the Framingham Offspring Study (45). This result raises the possibility that 2-h glucose levels might correlate better with HbA_{1c} in the nondiabetic population. Whether this can be extrapolated to explain the 2-h glucose levels as an independent risk, in addition to fasting glucose level, to the vascular complications in diabetic patients (46) remains to be further studied.

Our study demonstrates that distinct combinations of genetic loci are responsible for different physiological characteristics associated with the pre-diabetic phenotype. Identification of multiple QTLs suggests that there is a high degree of genetic heterogeneity for type 2 diabetes. This study also constitutes an important step for directing the search for the genetic factors involved in type 2 diabetes in the Chinese population.

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