

Linkage and Association Analyses of Type 2 Diabetes/Impaired Glucose Metabolism and Adiponectin Serum Levels in Japanese Americans From Hawaii

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Type 2 diabetes is a common disorder associated with obesity. Lower plasma levels of adiponectin were associated with type 2 diabetes. Candidate regions on chromosomes 1 (~70 cM) and 14 (~30 cM) were evaluated for replication of suggestive linkage results for type 2 diabetes/impaired glucose homeostasis in an independent sample of Japanese Americans. Replication of independent linkage evidence for serum levels of adiponectin on chromosome 14 was also evaluated. We investigated 529 subjects from 175 sibships who were originally part of the Honolulu Heart Program. Analyses included nonparametric linkage and association using SAGE (Statistical Analysis for Genetic Epidemiology) and FBAT (family-based test of association) programs and Monte Carlo simulation of Fisher's exact test in SAS. For type 2 diabetes/impaired glucose metabolism, nominal linkage evidence ($P < 0.02$) followed-up by genotypic association ($P = 0.016$) was found with marker D14S297 at 31.8 cM; linkage analyses using only diabetes phenotype were also nominally significant at this marker ($P < 0.02$). Nominal evidence for genotypic association to adiponectin serum level phenotype ($P = 0.04$) was found with the marker D14S1032 at 23.2 cM. The present study was limited by relatively small sample size. Nevertheless, these results corroborate earlier studies, suggesting that further research is warranted in the candidate region ~30 cM on chromosome 14. *Diabetes* 56:537–540, 2007

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Type 2 diabetes is a genetically influenced disorder associated with obesity, generally adult onset, affecting ~7% of the U.S. population (1–4). A GENNID (Genetics of NIDDM) genome scan (5) suggested linkage of diabetes and/or impaired glucose homeostasis in Japanese Americans to marker D14S608, at 28 cM (logarithm of odds [LOD] = 1.53). Evidence of linkage for serum levels of adiponectin, an adipocyte-derived protein, was reported in the same region at 29 cM, LOD = 3.2 (6). The adiponectin plasma level negatively correlates with total fat (7); lowered plasma levels of adiponectin were reported in type 2 diabetes (8). The GENNID Study also reported potential linkage in the 72.59 cM region on chromosome 1 at marker D1S3721 (LOD = 1.58). Other studies implicated other regions at chromosome 1, such as ~29.9 cM and particularly ~200 cM (9–12). We evaluated the GENNID candidate regions on chromosomes 1 and 14 in an independent sample of Japanese Americans.

RESEARCH DESIGN AND METHODS

The Honolulu Heart Program (HHP) is a prospective epidemiologic study of cardiovascular disease. Participants were men of Japanese ancestry living on the Oahu Island in 1965, born between 1900 and 1919 (age 45–68 years at first examination). A total of 8,006 men participated in the first examination in 1965–1968. Details of the selection process for the cohort were previously published (13). The entire cohort has undergone nine examinations so far. The study was approved by the institutional review committee of Kuakini Medical Center. The procedures followed were in accordance with institutional guidelines, and informed consent was obtained from all participants. The fourth exam (1991–1993) included a comprehensive 4-h examination with fasting glucose and 2-h glucose tolerance test. Using modified American Diabetes Association criteria, subjects were defined as having diabetes if they were taking medications for diabetes, if fasting glucose was ≥ 126 mg/dl, or if 2-h postload glucose was ≥ 200 mg/dl. This cohort has a high prevalence of diabetes (34% with diabetes and 37% with impaired fasting glucose or impaired glucose tolerance at exam four), despite being quite lean (14). BMI was defined as weight in kilograms divided by the square of height in meters, and mean BMI at exam four was 23.4 ± 3.2 .

This report is based on the Hawaii Family Diabetes Study (HFDS), which was performed in conjunction with the seventh examination of the HHP cohort in 1999–2000. HHP participants with diabetes at exam four were invited to participate in the HFDS if they also had a sibling who was willing to participate. The HFDS subjects were processed to obtain data for the present study, which includes 529 individuals in 175 sibships (not families) with a mean \pm SD size 2.5 ± 1.31 . This included 207 men from the HHP cohort (115 examined and 92 not examined but had DNA available from the fourth examination) and 322 siblings who were examined. There are 335 men and 194 women, and the mean age was 82.4 ± 8 years.

TABLE 1
Sibpair tests of mean allele sharing IBD at marker D14S297, conditional on type 2 diabetes

Index	Pair type	<i>n</i>	Estimate	SE	<i>T</i> value	<i>P</i> value
<i>p</i> _i	0	9	0.53	0.1	0.27	0.40
	1	107	0.46	0.02	-1.54	0.06
	2	420	0.51	0.01	0.51	0.31
<i>f</i> ₀	0	9	0.25	0.07	0.004	0.50
	1	107	0.26	0.03	0.41	0.34
	2	420	0.24	0.01	-0.43	0.34
<i>f</i> ₁	0	9	0.44	0.07	—	—
	1	107	0.55	0.03	—	—
	2	420	0.50	0.01	—	—
<i>f</i> ₂	0	9	0.31	0.14	0.40	0.35
	1	107	0.19	0.03	-2.08	0.02*
	2	420	0.26	0.02	0.43	0.33

Pair type: 0, concordant unaffected; 1, discordant; 2, concordant affected. *f*_{*j*}, proportion of sibpairs sharing *j* alleles IBD; *p*_{*i*}, mean proportion of alleles sharing IBD. **P* < 0.05.

Phenotype definition. The affected phenotype (*n* = 474) was type 2 diabetes/impaired glucose metabolism, defined as either diabetes or impaired glucose metabolism; linkage analyses were also performed using only diabetes as affected phenotype, excluding subjects with impaired glucose metabolism from analyses. Diabetes (*n* = 351) was based on the subject's report of physician's diagnosis of diabetes, the use of insulin (with no detectable C-peptide to eliminate type 1 diabetes), the use of oral hypoglycemic agents, and/or fasting glucose equal ≥126 mg/dl and/or a glucose level at 2 h (following 75 g oral glucose) ≥200 mg/dl. Impaired glucose metabolism (*n* = 123) was based on fasting glucose of 110–125 mg/dl (impaired fasting glucose) or 2-h glucose of 140–199 mg/dl (impaired glucose tolerance). There were 55 unaffected subjects. The quantitative adiponectin serum levels (in micrograms per milliliter) were assayed in duplicate using RIA (Linco Research catalog no. HADP-61HK). Serum levels of adiponectin were obtained for 437 subjects, with a mean level of 11.59 μg/ml. The mean adiponectin serum levels in 243 men and 194 women were 10.18 and 13.34 μg/ml, respectively.

Molecular genetics. DNA was extracted from whole blood using standard methodologies. Candidate regions included linked markers on chromosomes 1 and 14. The markers and their sex-averaged map positions on the Marshfield map were: chromosome 1: D1S2657 (70.4 cM), D1S3721 (72.6 cM), and D1S1616 (73.8 cM); chromosome 14: D14S1032 (23.2 cM), D14S1040 (31.8 cM), and D14S297 (31.8 cM). Respective numbers of detected alleles were 15, 14, 12, 13, 9, and 12. Primer sequences for each microsatellite marker were obtained from uniSTS (www.ncbi.nlm.nih.gov/genome/sts/). Each microsatellite was amplified individually; all amplifications were performed using True Allele PCR amplification mix (Applied Biosystems, Foster City, CA) per the manufacturer's instructions. In each reaction, 5 pmol forward and reverse primers (forward primer labeled with FAM, NED, or PET) were used. The resulting fluorescently labeled amplicons were pooled and analyzed on an ABI3700 automated sequencer. To ensure interassay reproducibility in allele calling, DNA from the CEPH (Centre d'Etude du Polymorphisme Humain) family member 1331-01 (<http://www.ceph.fr/>) was amplified and analyzed for each microsatellite with each run. Allele calling was performed using Genotyper Software (Applied Biosystems).

Inconsistencies with Mendelian inheritance were revealed in 1.4% of the total of 3,174 marker loci in 529 individuals. All inconsistent genotypes were removed from the subsequent statistical analyses, which did not remove all loci for any individual.

Statistical analysis. Model-independent sibpair linkage analyses for the type 2 diabetes/impaired glucose metabolism phenotype, and for diabetes phenotype, were performed with the SIBPAL program (15). The program performs mean tests in concordant affected, discordant (affected-unaffected), and concordant unaffected sibpairs, as well as regression analysis using all sibpairs. The covariates were age and sex. Tests of association were performed for the type 2 diabetes/impaired glucose metabolism phenotype with the FBAT (family-based test of association) program (16) only for a single marker that showed evidence of linkage to this phenotype. The FBAT evaluated marker genotype distributions in affected and unaffected individuals, and the allelic association with the trait assuming additive, dominant, or recessive genetic models for each allele separately.

Linkage analyses of the adiponectin serum level phenotype with markers on chromosome 14 were performed using a regression model in the SIBPAL program (17). The allelic association analyses of the adiponectin phenotype were performed with ASSOC program (17) under additive, dominant, and recessive models for each allele separately. The covariates in these linkage

and association analyses included age, sex, and BMI. The Monte Carlo simulation of Fisher's exact test (SAS 8.2) was used to compare genotype distributions for each marker on chromosome 14 in sparse contingency tables between affected and unaffected individuals for the type 2 diabetes/impaired glucose metabolism phenotype and between two extreme subgroups of subjects stratified by the adiponectin serum level. The Monte Carlo simulation generated 10,000 sample contingency tables to evaluate *P* values for the observed table. We report nominal *P* values for all statistical analyses because this is a replication study, rather than a report of new susceptibility loci.

RESULTS

There was no linkage evidence using the type 2 diabetes/impaired glucose metabolism phenotype, or using the diabetes phenotype, at chromosome 1. On chromosome 14, modest linkage evidence for the type 2 diabetes/impaired glucose metabolism phenotype was observed at the marker D14S297 (Table 1). The pattern of the estimated proportion of alleles sharing identical by descent (IBD) (0.53, 0.46, and 0.51) was consistent with that under linkage, although not significant. In addition, there was nominal evidence of a decreased proportion of sibpairs sharing two alleles IBD in 107 discordant sibpairs; the test for mean proportion of alleles sharing IBD in these 107 sibpairs was marginally significant.

In the linkage analysis of chromosome 14 markers using diabetes phenotype, nominal evidence of a decreased proportion of sibpairs sharing two alleles IBD persisted at this marker (*P* < 0.02, 73 discordant sibpairs), as did the allele-sharing pattern (0.53, 0.46, and 0.52). There was no evidence of linkage at any marker in regression analyses using either phenotype.

Tests of association were performed for the type 2 diabetes/impaired glucose metabolism phenotype only at the marker D14S297. There was no allelic association with phenotype for any of the 12 alleles. However, distribution of genotypes at this marker appeared to differ between 412 affected and 50 unaffected subjects ($\chi^2 = 94.9$, *P* < 0.00002). Because sparseness of the observed 36 × 2 genotypic contingency table can influence χ^2 statistics, we reanalyzed this table using Monte Carlo simulation of *P* values for the Fisher's exact test (10,000 samples, $\alpha = 0.01$). Genotypic distributions in affected and unaffected subjects were different at the 0.05 significance level (*P* = 0.016; 99% CI 0.013–0.019).

There was no linkage evidence for the adiponectin serum level phenotype with markers at chromosome 14. Allelic association analyses were unremarkable, showing a few nominally significant results from numerous tests at

these microsatellite markers. Similar results were obtained with and without BMI as a covariate, besides age and sex. Comparing the approximate lower and upper decile subgroups ($n = 46$ and $n = 48$, respectively) stratified by adiponectin serum levels, the Monte Carlo analysis of genotypic distributions (10,000 samples, $\alpha = 0.01$) for each of the three markers at chromosome 14 resulted in a marginal association ($P = 0.055$) at the marker D14S1032. The comparison of approximate lower quintile ($n = 76$, adiponectin $\leq 5 \mu\text{g/ml}$) and upper quintile ($n = 79$, adiponectin $> 17 \mu\text{g/ml}$) for this marker only provided a slightly more significant nominal result ($P = 0.041$; 99% CI 0.036–0.046).

DISCUSSION

The GENNID Study evidence was obtained using the type 2 diabetes and/or impaired glucose homeostasis phenotype, where the latter may be an intermediate stage in diabetes (5), potentially capturing genetically susceptible (but unaffected) individuals. The present replication study uses a similar phenotype of either type 2 diabetes or impaired glucose metabolism, as well as linkage analyses with only diabetes phenotype to address potential genetic heterogeneity. There was no linkage evidence for any phenotype from the candidate region at chromosome 1 (72.6 cM). However, evidence of linkage to type 2 diabetes was reported on chromosome 1 at the 73-cM position in a Chinese sample (nonparametric linkage = 2.409), supporting further interest in this region (18).

The LOD score of 1.53 for the type 2 diabetes/impaired glucose homeostasis phenotype in the GENNID Study was observed on chromosome 14, marker D14S608 at position 28 cM. For type 2 diabetes/impaired glucose metabolism, we observed nominal evidence of linkage, followed-up by evidence of genotypic association, to nearby marker D14S297 (31.8 cM). Our linkage analyses using only diabetes phenotype were also nominally significant at this marker. The GENNID Study also reported evidence of linkage for diabetes/impaired glucose homeostasis to marker D14S599 (40.7 cM), only in their phase 2 whites (LOD = 1.76). Multiple susceptibility loci for type 2 diabetes may exist on chromosome 14 (19,20).

Conventional linkage and association analyses of adiponectin serum level phenotype, a complex trait in its own right, did not provide noteworthy results with markers at chromosome 14. The Monte Carlo analysis of genotypic distributions provided nominal results consistent with a possible genetic factor(s) near marker D14S1032 at 23.2 cM, ~ 5.8 cM away from the site of independently reported linkage (6). The relatively small sample size may contribute to ambiguous results in the present study.

In conclusion, we report a degree of independent replication for statistical signal in the candidate region ~ 30 cM on chromosome 14 using type 2 diabetes/impaired glucose metabolism, type 2 diabetes, and adiponectin serum level phenotypes. Cumulative data suggest that further research is warranted to elucidate a potential etiological role of this region in type 2 diabetes, perhaps based on genetic regulation of adiponectin expression.

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