Original Article

Variations in Adiponectin Receptor Genes and Susceptibility to Type 2 Diabetes in Women

A Tagging–Single Nucleotide Polymorphism Haplotype Analysis

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Adiponectin has been associated with low diabetes risk. The metabolic effects of adiponectin are mediated by adiponectin receptors 1 (*ADIPOR1*) and 2 (*ADIPOR2*). We conducted a prospective, nested case-control study of 714 cases of type 2 diabetes and 1,120 control subjects. Six polymorphisms in *ADIPOR1* and 16 polymorphisms in *ADIPOR2* were determined. Haplotypes inferred from *ADIPOR1* polymorphisms were significantly associated with diabetes risk (overall test, −2log-likelihood = 15.1 on 5 df; *P* = 0.0098). A single copy of haplotype 001100 (0, common allele; and 1, minor allele) was associated with 24% decreased risk (odds ratio [OR] 0.76 [95% CI 0.61–0.96], *P* = 0.02) compared with the most common haplotype, 110000, adjusting for age, BMI, and other covariates. A 3′ untranslated region (UTR) polymorphism, rs1139646, showed the strongest and nominally significant association with greater diabetes risk (unadjusted OR 1.26 [1.03–1.53] and adjusted OR 1.36 [1.10–1.70]). However, such an association became marginal after controlling for multiple comparisons by permutation test (*P* = 0.08 on the basis of 10,000 permutations). There were not significant associations between *ADIPOR2* polymorphisms, individually or in haplotypes, and the risk of type 2 diabetes. In conclusion, our data indicate significant associations between *ADIPOR1* haplotypes and diabetes risk but do not support a relation between *ADIPOR2* variability and the disease. *Diabetes* 56:1586–1591, 2007

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Received for publication 14 October 2006 and accepted in revised form 17 February 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 6 April 2007. DOI: 10.2337/db06-1447.

LD, linkage disequilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism; UTR, untranslated region.

Many studies have documented that adiponectin, which is a hormonal cytokine secreted exclusively by adipose tissue, has strong insulin-sensitizing, anti-inflammatory, and antidiabetes effects (1,2). Adiponectin improves insulin sensitivity by affecting the metabolism of glucose and lipids in skeletal muscle and liver (3). In earlier analyses, we have found that blood adiponectin levels and variations in the adiponectin gene (*ADIPOQ*, also known as *APM1*) were associated with type 2 diabetes and cardiovascular risk (4–6).

Recent evidence indicates that the metabolic regulation of adiponectin is mainly mediated by two types of receptors, adiponectin receptors 1 (*ADIPOR1*) and 2 (*ADIPOR2*) (7,8). Downregulation of ADIPORs may blunt adiponectin-induced signaling (7). The expression of adiponectin receptor has been related to in vivo parameters of glucose and lipid metabolism (9) and was found to be lower in diabetic patients (10). The polymorphisms in the *ADIPOR1* gene were recently associated with adiposity, insulin resistance, and high liver fat (11–13). A few studies have investigated the associations between the variations in *ADIPOR1* and *ADIPOR2* genes and the risk of type 2 diabetes but generated mixed results (14–17). These studies, however, were largely limited by small sample size or a cross-sectional nature in design. The relation between *ADIPOR* genetic variability and diabetes risk has yet to be evaluated in prospective settings.

In this study, we comprehensively examined the variations in *ADIPOR1* and *ADIPOR2* genes and the risk of type 2 diabetes in a nested, case-control study from the Nurses' Health Study cohort. We selected linkage disequilibrium (LD) tagging single nucleotide polymorphisms (SNPs) that capture the maximum variance in both genes and also included polymorphisms that were previously associated with diabetes risk.

RESEARCH DESIGN AND METHODS

The Nurses' Health Study was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 large U.S. states completed a mailed questionnaire on medical history and lifestyle (18). The lifestyle factors, including smoking, menopausal status and postmenopausal hormone therapy, and body weight, have been updated by validated questionnaires every 2 years. Samples for the present case-control study were selected from a subcohort of 32,826 women who provided a blood sample between 1989 and 1990 and were free from diabetes, cardiovascular disease, stroke, or

TABLE 1
Polymorphisms in *ADIPOR1* and *ADIPOR2* genes

Polymorphisms	Allele	Position	Location	MAF
ADIPOR1				
rs2232853	G>A	−11760	5′ promoter	0.28
rs10494839	T>C	−1896	Intron 1	0.28
rs12733285	C>T	−1742	Intron 1	0.31
rs1342387	C>T	5843	Intron 4	0.46
rs1139646*	C>G	10225	Exon 8–3′ UTR	0.32
rs10920531	C>A	11363	3′ UTR	0.37
ADIPOR2				
rs11061925	C>T	−60276	Intron 1	0.29
rs7132033	G>C	−57414	Intron 1	0.27
rs7975600	T>A	−48258	Intron 1	0.13
rs11061937	T>C	−46897	Intron 1	0.30
rs12826079	C>T	−36954	Intron 1	0.07
rs11061946	G>A	−34983	Intron 1	0.07
rs10773983	C>T	−32264	Intron 1	0.32
rs11612383	C>T	−32155	Intron 1	0.32
rs1058322	C>T	−26531	Intron 1	0.31
rs12230440	T>G	−6557	Intron 1	0.15
rs929434	G>A	−1421	Intron 1	0.30
rs11061971	A>T	218	Intron 2	0.44
rs11061973	G>A	2426	Intron 2	0.14
rs2108642	C>A	3289	Intron 2	0.46
rs7316374	G>A	7621	Intron 2	0.14
rs1044471	C>T	33446	3′ UTR	0.48

Alleles are presented as common allele>minor allele. Location of polymorphism is relative to the translation start point. *rs1139646 is also known as rs7539542.

cancer at the time of blood collection. Incident cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire and diagnosed at least 1 year after blood collection through 2000. The supplementary questionnaire obtained information on symptoms, diagnostic tests, and hypoglycemic therapy used to define type 2 diabetic patients. Medical record review confirmed the diagnosis of type 2 diabetes with this questionnaire for 98% of cases, using the National Diabetes Data Group criteria (19). We used the American Diabetes Association diagnostic criteria for diagnosis of diabetes during the 1998 and 2000 cycles (20). In the present study, 714 incident cases of type 2 diabetes and 1,120 healthy control subjects were included. The selection of case and control subjects was described in detail elsewhere (21).

Assessment of covariates. Anthropometric data and lifestyle factors were derived from the 1990 questionnaire. BMI was calculated as weight in kilograms divided by the square of height in meters. Physical activity was expressed as MET (metabolic equivalent task) hours based on self-reported types and durations of activities over the previous year.

Tagging SNPs selection and genotype determination. DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA). Tagging SNPs for *ADIPOR1* were provided by A.D., who made the selection based on typing 28 common SNPs (minor allele frequency [MAF] ≥5%) covering the entire gene plus 5 Kb on each side (22). Tagging SNPs for *ADIPOR2* were selected from HapMap (HapMap Public Release no. 19) using multimarker (aggressive) tagging mode (23). We also included several polymorphisms that were previously associated with diabetes risk. Polymorphisms were genotyped using TaqMan SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). One polymorphism (rs4950894) had a relatively high missing value in genotyping (9.9%) and was not included in the analyses. The genotyped polymorphisms are presented in Table 1. Replicate quality control samples (10%) were included and genotyped with >99% concordance.

Statistical analyses. A χ^2 test was used to assess whether the genotypes were in Hardy-Weinberg equilibrium and to compare the genotype and allele frequencies between case and control subjects. Odds ratios (ORs) were calculated using unconditional logistic regression adjusting for type 2 diabetes risk factors, including age (continuous), BMI (<23, 23–24.9, 25–29.9, 30–34.9, or ≥35 kg/m^2), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9, and ≥21.0 MET h/week), smoking (never, past, and current), alcohol intake (nondrinker and drinker [0.1–4.9, 5–10, or >10 g/day]), family history of diabetes, and menopausal status (pre- or postmenopausal [never, past, or current hormone use]). As we simultaneously examined multiple gene polymorphisms, we used permutation testing to address the issue of multiple comparisons and to guide interpretation of nominally statistically significant associations. We estimated haplotype-specific ORs using an expectation-substitution approach to account for haplotype uncertainty given unphased genotype data (24,25). To test the global associations, we used a likelihood ratio test comparing a model with additive effects on the log odds scale for each common haplotype (treating the most common haplotype as the referent) to the intercept-only model. We considered haplotypes with greater than 5% frequency in at least one cohort or ethnic group to be "common." The SAS statistical package was used for the analyses (SAS, version 8.2 for UNIX). All *P* values are two sided.

RESULTS

The location and allele frequency of examined *ADIPOR1* and *ADIPOR2* polymorphisms is presented in Table 1. Among the study participants, all polymorphisms conformed to Hardy-Weinberg equilibrium ($P > 0.05$). The MAF of *ADIPOR1* polymorphisms ranges from 0.28 to 0.46, whereas the MAF of *ADIPOR2* polymorphisms ranges from 0.07 to 0.48. Figure 1 shows the LD matrix for *ADIPOR1* and *ADIPOR2* polymorphisms. The two 3′ UTR polymorphisms rs1139646 and rs10920531 are in strong LD ($D' = 0.93$; $r^2 = 0.68$) while the other four polymorphisms appeared to be in strong LD ($D' \geq 0.97$; r^2 ranges from 0.15 to 0.99). There were broadly three LD blocks in the *ADIPOR2* gene (Fig. 1). The polymorphisms in *ADIPOR1* and *ADIPOR2* genes were not associated with BMI.

Polymorphism rs1139646 at 3′ UTR of *ADIPOR1* showed nominally significant association with an increased risk of type 2 diabetes, especially in a dominant model (OR 1.26 [95% CI 1.03–1.53]) (Table 2). Adjustment for covariates strengthened the association. When multiple testing was controlled by permutation test, in which case-control status was randomly permuted 10,000 times for the polymorphisms examined, the association between rs1139646 became marginally significant ($P = 0.08$). Polymorphisms rs10920531 and rs1342387 also showed nor-

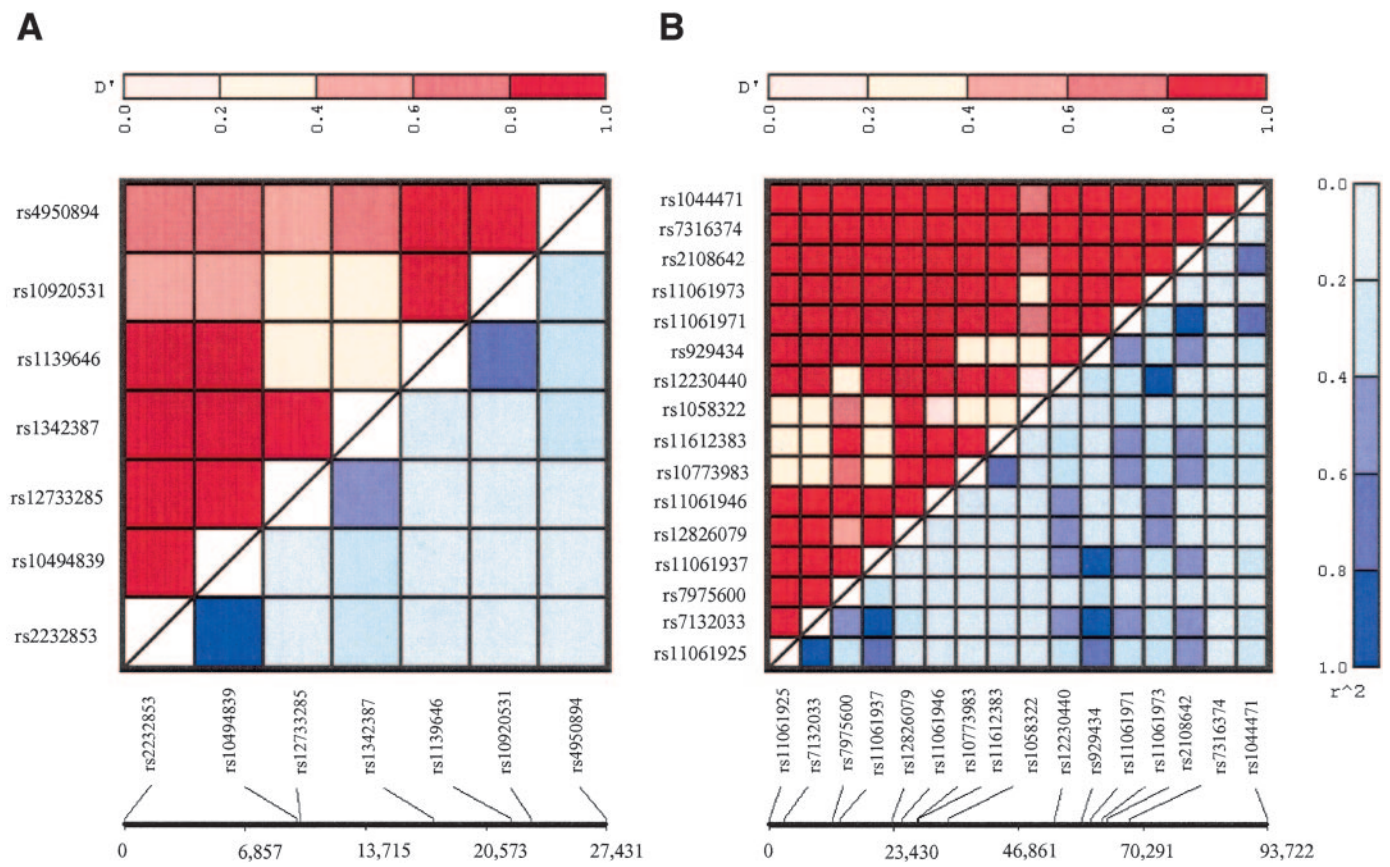

FIG. 1. Pairwise LD matrix for *ADIPOR1* (*A*) and *ADIPOR2* (*B*) genes. D′ is presented above the diagonal, and r^2 is presented below the diagonal.

TABLE 2
Genotype distributions of polymorphism in *ADIPOR1* and *ADIPOR2* genes in diabetic case and control subjects

Polymorphisms	Case (%)/control (%) by genotype			Odds ratio (95% CI)	
	Homozygote, major	Heterozygote	Homozygote, minor	Crude	Adjusted*
ADIPOR1					
rs2232853	341 (49.1)/565 (51.4)	297 (42.7)/440 (40.0)	57 (8.2)/94 (8.6)	1.10 (0.91–1.33)	1.16 (0.94–1.43)
rs10494839	340 (48.7)/557 (51.1)	296 (42.4)/438 (40.2)	62 (8.9)/95 (8.7)	1.11 (0.91–1.34)	1.17 (0.95–1.44)
rs12733285	344 (49.4)/511 (47.1)	293 (42.0)/485 (44.7)	60 (8.6)/88 (8.2)	0.91 (0.75–1.10)	0.87 (0.70–1.07)
rs1342387	210 (30.4)/297 (27.8)	347 (50.4)/558 (52.3)	132 (19.2)/212 (19.9)	0.88 (0.71–1.09)	0.77 (0.61–0.98)
rs1139646	290 (43.2)/513 (48.7)	306 (45.5)/433 (41.1)	76 (11.3)/108 (10.2)	1.26 (1.03–1.53)	1.36 (1.10–1.70)
rs10920531	266 (37.9)/464 (42.6)	329 (46.9)/470 (43.1)	107 (15.2)/156 (14.3)	1.21 (1.00–1.47)	1.33 (1.08–1.65)
ADIPOR2					
rs11061925	336 (48.4)/552 (50.7)	301 (43.3)/429 (39.4)	58 (8.3)/108 (9.9)	1.10 (0.90–1.32)	1.22 (0.99–1.51)
rs7132033	363 (51.5)/587 (53.2)	292 (41.4)/419 (37.9)	50 (7.1)/98 (8.9)	1.07 (0.88–1.29)	1.18 (0.96–1.46)
rs7975600	521 (75.7)/814 (75.7)	156 (22.7)/236 (22.0)	11 (1.6)/25 (2.3)	1.00 (0.80–1.25)	1.02 (0.80–1.31)
rs11061937	334 (48.0)/518 (48.1)	296 (42.5)/444 (41.3)	66 (9.5)/114 (10.6)	1.01 (0.83–1.22)	1.12 (0.90–1.38)
rs12826079	617 (87.6)/959 (86.7)	85 (12.1)/143 (12.9)	2 (0.3)/4 (0.4)	0.92 (0.69–1.22)	1.01 (0.74–1.38)
rs11061946	594 (84.4)/941 (85.0)	104 (14.8)/160 (14.5)	6 (0.8)/6 (0.5)	1.05 (0.81–1.36)	1.08 (0.81–1.44)
rs10773983	306 (44.5)/492 (45.5)	317 (46.1)/464 (42.9)	65 (9.4)/125 (11.6)	1.04 (0.86–1.26)	1.05 (0.85–1.30)
rs11612383	335 (47.6)/496 (45.9)	297 (42.2)/475 (44.0)	72 (10.2)/109 (10)	0.93 (0.77–1.13)	0.98 (0.80–1.21)
rs1058322	323 (46.6)/514 (48.0)	300 (43.3)/448 (41.8)	70 (10.1)/109 (10.2)	1.06 (0.87–1.28)	1.12 (0.91–1.38)
rs12230440	476 (70.7)/745 (70.8)	184 (27.4)/288 (27.4)	13 (1.9)/19 (1.8)	1.00 (0.81–1.24)	1.10 (0.87–1.39)
rs929434	321 (47.2)/496 (47.6)	295 (43.3)/435 (41.8)	65 (9.5)/110 (10.6)	1.02 (0.84–1.24)	1.13 (0.91–1.40)
rs11061971	216 (31.4)/335 (31.2)	337 (49.0)/510 (47.6)	135 (19.6)/227 (21.2)	0.99 (0.80–1.22)	1.09 (0.86–1.37)
rs11061973	501 (72.7)/783 (72.1)	173 (25.1)/284 (26.1)	15 (2.2)/19 (1.8)	0.97 (0.78–1.20)	1.02 (0.80–1.28)
rs2108642	195 (28.1)/313 (28.8)	343 (49.4)/516 (47.4)	156 (22.5)/259 (23.8)	1.03 (0.84–1.28)	1.16 (0.92–1.46)
rs7316374	542 (76.6)/818 (74.1)	156 (22.0)/267 (24.2)	10 (1.4)/19 (1.7)	0.88 (0.70–1.09)	0.89 (0.70–1.13)
rs1044471	185 (27.3)/319 (29.6)	329 (48.4)/514 (47.8)	165 (24.3)/243 (22.6)	1.12 (0.91–1.39)	1.11 (0.87–1.40)

rs1139646 is also known as rs7539542. *Adjusted for age, BMI, alcohol consumption, smoking, physical activity, family history of diabetes, and menopausal status—under dominant inheritance mode.

TABLE 3
Distributions of haplotypes* inferred from ADIPOR1 polymorphisms in diabetic case and control subjects

rs2232853	rs10494839	rs12733285	rs1342387	rs1139646	rs10920531	Frequency		OR (95% CI)†	*P*
						Cases	Controls		
1	1	0	0	0	0	0.255	0.243	1.0	—
0	0	1	1	0	0	0.188	0.208	0.76 (0.61–0.96)	0.02
0	0	0	0	1	1	0.206	0.189	1.00 (0.80–1.23)	0.94
0	0	0	1	0	0	0.113	0.120	0.85 (0.66–1.10)	0.22
0	0	1	1	1	1	0.080	0.063	1.28 (0.93–1.75)	0.13
Haplotypes <5%						0.157	0.176	0.78 (0.61–0.98)	0.03

Global test: −2log-likehood = 15.1 on 5 df, $P = 0.0098$. *0 codes the common allele, and 1 codes the minor allele. †Adjusted for age, BMI, alcohol consumption, smoking, physical activity, and menopausal status.

mally significant associations with diabetes risk in the multivariate analyses. However, these associations did not remain significant after adjustment for multiple testing.

In the haplotype analyses, five common haplotypes (frequency >0.05) possessing the tagging SNPs of *ADIPOR1* were identified that accounted for 84% allelic variance. *ADIPOR1* haplotypes showed significant associations with diabetes risk (overall test, −2log-likelihood = 15.1 on 5 df, $P = 0.0098$) (Table 3). A single copy of haplotype 001100 was associated with 24% decreased diabetes risk compared with the most common haplotype 110000 (OR 0.76 [95% CI 0.61–0.96], $P = 0.02$). Figure 2 presents a cladogram representing the inferred evolutionary relatedness of the haplotypes. Thus, no single polymorphism could distinguish haplotype 001100 from the reference haplotypes to suggest a functional variation.

None of the 16 *ADIPOR2* polymorphisms was significantly associated with diabetes risk. By using all the tagging polymorphisms, we inferred five common haplotypes (>5%) that account for 64.4% allelic variance. These haplotypes were not significantly associated with diabetes risk (data not shown). Haplotypes inferred from polymorphisms within each LD block (Fig. 1) were not significantly associated with the disease either. We further extensively examined the haplotype associations using a sliding window approach. Figure 3 shows the significance value $[-\log_{10}(P)]$ for the associations of haplotypes consisting of two to six markers that were fit as a sliding window across the *ADIPOR2* gene with diabetes risk. None of the haplotype windows showed nominally significant associations with diabetes risk nor did the haplotype windows covering more polymorphisms (more than six SNPs).

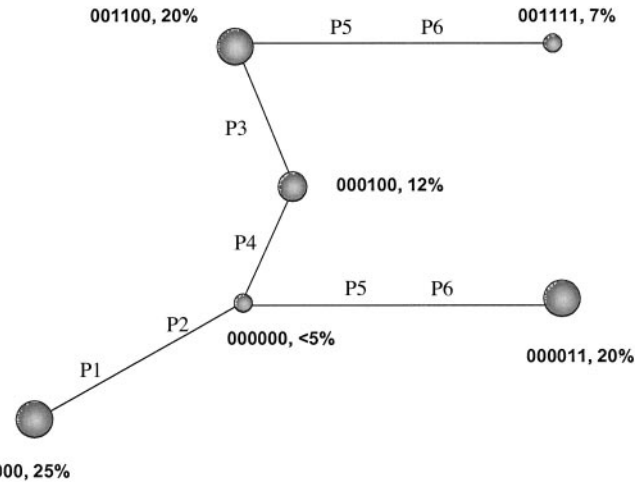

FIG. 2. Haplotype cladogram for the *ADIPOR1* gene. Gray circles represent the inferred haplotypes with their frequencies, which are proportional to the size (diameter) of the circles. P1–P6 represent the polymorphic changes (in the order of rs2232853, rs10494839, rs12733285, rs1342387, rs1139646, and rs10920531) separating one haplotype from the other.

DISCUSSION

In this prospective, nested, case-control study of 714 incident cases of type 2 diabetic and 1,120 matching control female subjects, we found that the haplotypes inferred from the LD tagging SNPs of the *ADIPOR1* gene were significantly associated with the risk of type 2 diabetes. *ADIPOR2* polymorphisms were not associated with the risk of type 2 diabetes.

Adiponectin is one of the most abundant adipose tissue–specific cytokines and has shown protective effects against insulin resistance, type 2 diabetes, and cardiovascular disease (26). It has been documented that adiponectin might increase insulin sensitivity by enhancing insulin's suppressive effect on gluconeogenesis and by promoting glucose utilization and fatty acid oxidation in liver and skeletal muscle (27,28). Recently, two isoforms of the cellular receptor for adiponectin, ADIPOR1 and ADIPOR2, were cloned and characterized. The metabolic and insulin-sensitizing effects of adiponectin are believed to be mediated by these receptors via activation of adenosine monophosphate–activated protein kinase and increasing peroxisome proliferator–activated receptor-α ligand activities (7,29). In mice, ADIPOR1 is ubiquitously expressed, whereas ADIPOR2 expression is more restricted to skeletal muscle and liver. Unlike in mice, both receptors are found to be highly expressed in skeletal muscle (30) and in pancreatic β-cells in humans (31). ADIPOR1 is a high-affinity receptor for globular adiponectin, while ADIPOR2 is an intermediate-affinity receptor for both globular and full-length adiponectin (7,29). Expression of *ADIPOR1* and *ADIPOR2* has been associated with insulin resistance (32) and was found to be lower in diabetic and normal glucose-tolerant subjects with family history of diabetes (10,30).

Several studies have documented that the variations in the *ADIPOR1* gene were related with adiposity, insulin resistance (11–13), and the risk of type 2 diabetes (14–17). Our findings that haplotypes possessing the tagging SNPs of the *ADIPOR1* gene were associated with diabetes risk lend further credibility for the potential roles of the variability of the *ADIPOR1* gene in the etiology of the disease. Earlier evidence indicates that the 3′ UTR polymorphism rs1139646 may affect the expression of the *ADIPOR1* gene (15). Although rs1139646 showed the strongest and nominally significantly association with diabetes risk in the present study, it is less likely to be a

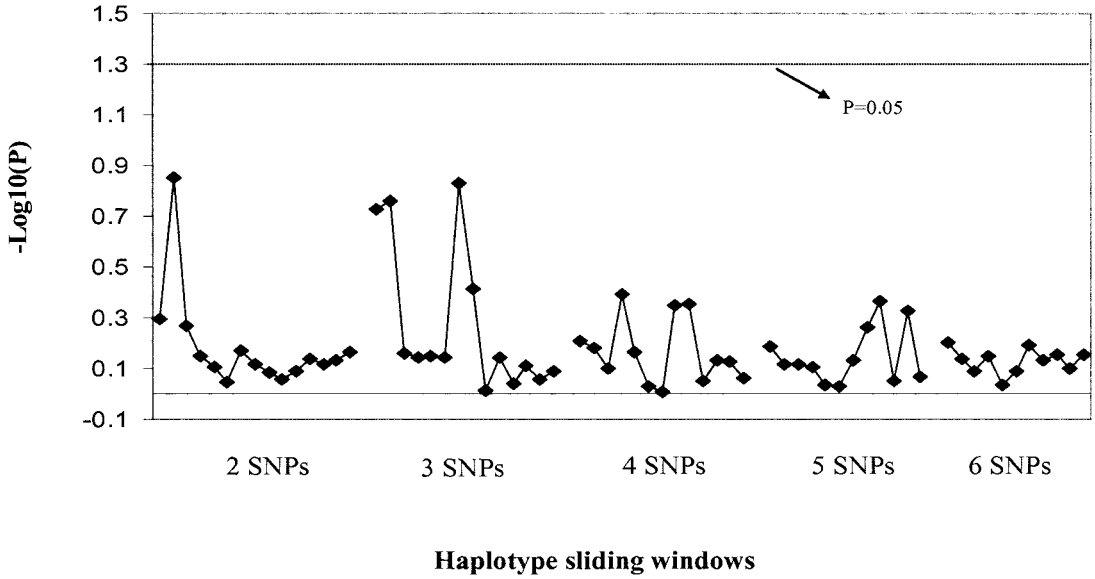

FIG. 3. Sliding window analyses of the associations between *ADIPOR2* haplotypes (possessing two to six SNPs) and the risk of type 2 diabetes. $-\log_{10} P$ values of global tests for the haplotype windows are presented as solid diamonds. The haplotype windows for a given number of SNPs (two to six) were generated by grouping the corresponding number of SNPs in the order of the position of polymorphisms and moving one SNP each time from 5′ to 3′ end.

causal variant because such an association was substantially attenuated after control of multiple testing. We assume the causal variants may be cosegregated with the risk haplotype identified. It is also possible that the observed associations were due to the effects of specific allele combination of several variations, which likely occur in the haplotype-driven selection (33).

Different haplotypes of the *ADIPOR2* gene were associated with the type 2 diabetes or combined type 2 diabetes/impaired glucose tolerance trait in two studies of Amish and French (14,17) but not in Asian Japanese (16) subjects. Of note, there is not a single genetic marker that has shown repeatable effect. We did not find significant associations between the variations in this gene, individually or in haplotypes, with diabetes risk in U.S. women. We have genotyped several previously reported polymorphisms including rs11061971, rs1044471, rs1342387 (14), and rs1139646 (15). Some other reported polymorphisms, rs767870, rs2286380 (tagged by rs7316374) (17), rs12342 (tagged by rs11061937), and rs2275737 (tagged by rs1342387) (14), could be well captured by the tagging SNPs used in our study (with $r^2 > 0.8$).

The cause of discrepancy among the studies is unknown but may be partly due to the diversity in ethnicity and population compositions, especially the sex profiles. In the present study, the genetic associations were assessed in women, while most previous studies included both men and women (14–17). The sex difference in the phenotypes of obesity and type 2 diabetes is well known. It has also been documented that adiponectin concentrations were higher in women than in men (34). A recent study suggests that the associations between circulating adiponectin levels and diabetes risk may be different in men and women (35). In addition, body fat may affect the expression of adiponectin and ADIPORs (29,36). The polymorphisms of *ADIPORs* have been inconsistently related to adiposity measurements in some studies (12,13,15). However, we did not find significant genetic associations with BMI in U.S. women. Again, we suspect the discrepant observations between various studies may be partly attributed to the difference in population compositions. Further adjustment for BMI did not materially change the associations between *ADIPOR* polymorphisms and diabetes risk.

Limited evidence suggests that the genetic variability of *ADIPOR1* may be more relevant to insulin resistance compared with *ADIPOR2* (12). *ADIPOR1* mRNA expression in human skeletal muscle cells was positively correlated with in vivo insulin secretion and plasma lipid concentrations, whereas the expression of *ADIPOR2* was not associated with most of these metabolic parameters (9). In addition, it was recently found that regulation of *ADIPOR1*, rather than *ADIPOR2*, might be involved in glucose and lipid metabolism in diabetic states (37).

The major strengths of this study include the prospective nature in design and large sample size. Most previous studies examined the associations using prevalent cases. The patient selection–inherent bias may skew the associations because the prevalence of diabetes is likely to be affected by life expectancy, which is subjected to the genetic effects on other conditions. In the analyses, we carefully controlled the potential influence of multiple comparisons using permutation tests. Attempts to address this issue can reduce the probability of false-positive results and help guide the interpretation of the results. As a limitation, population stratification may influence the observed associations. However, our populations are racially homogeneous, with the majority of the participants being white (~96%). Further adjustment for ethnicity or removing the minorities from the analyses did not appreciably change the results. In addition, our analyses were restricted to women and therefore may be not generalizable to men.

In conclusion, we found that the genetic variability in *ADIPOR1* was associated with the risk of type 2 diabetes. Our data did not support substantial associations between *ADIPOR2* variability and diabetes risk. Further analyses are warranted to replicate the associations in other populations, to identify the potential functional variants, and to elucidate the metabolic changes that may affect diabetes risk.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grants DK58845 and CA87969.

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