

Variations in adiponectin receptor genes and susceptibility to type 2 diabetes in women: A tagging-SNP haplotype analysis

Received for publication 19 September 2006 and accepted in revised form 27 February 2007.

Lu Qi^{1,3}, Alessandro Doria⁴, Elena Giorgi⁵, and Frank B. Hu^{1,2,3}

Affiliations:

- ¹: Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts
²: Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts
³: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts
⁴: Research Division, Joslin Diabetes Center, Department of Medicine, Harvard Medical School, Boston, Massachusetts
⁵: Department of Mathematics and Statistics, University of Massachusetts, Amherst, Massachusetts

Correspondence and requests for reprint:

Dr. Lu Qi
Department of Nutrition,
Harvard School of Public Health,
665 Huntington Ave,
Boston, MA 02115
E-mail address: nhlqi@channing.harvard.edu

Running Head: *ADIPOR1*, *ADIPOR2*, polymorphism, and type 2 diabetes

ABSTRACT

Adiponectin has been associated with low diabetes risk. The metabolic effects of adiponectin are mediated by adiponectin receptor 1 (*ADIPOR1*) and receptor 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 sixteen polymorphisms in *ADIPOR2* were determined. Haplotypes inferred from *ADIPOR1* polymorphisms were significantly associated with diabetes risk (overall test, $-2\log\text{-likelihood}=15.1$ on 5 DF, $P=0.0098$). A single copy of haplotype 001100 ('0', common allele; and '1', minor allele) was associated with 24% (OR=0.76, 95%CI 0.61-0.96, and $P=0.02$) decreased risk compared with the most common haplotype 110000, adjusting for age, BMI, and other covariates. A 3' UTR polymorphism, rs1139646, showed the strongest and nominally significant association with greater diabetic risk (unadjusted OR=1.26, 95%CI 1.03-1.53; and adjusted OR=1.36, 95%CI 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 permutation). 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.

INTRODUCTION

Many studies have documented that adiponectin, which is a hormonal cytokine secreted exclusively by adipose tissue, has strong insulin-sensitizing, anti-inflammatory, and anti-diabetic 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 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 receptor 1 (gene symbol *ADIPOR1*) and adiponectin receptor 2 (gene symbol *ADIPOR2*) (7; 8). Down-regulation 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 diabetes patients (10). The polymorphisms in *ADIPOR1* gene were recently associated with adiposity, insulin resistance, and high liver fat (11-13). 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, are largely limited by small sample size or 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 (NHS)

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.

MATERIALS and METHODS

Study population

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 their 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 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 diabetes cases. Medical record review confirmed the diagnosis of type 2 diabetes using 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 cases during the 1998 and 2000 cycles (20). 714 incident cases of type 2 diabetes and 1120 healthy control subjects were included in the present study. The selection of cases

and controls was described in detail elsewhere (21).

Assessment of covariates

Anthropometric data and lifestyle factors were derived from the 1990 questionnaire. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Physical activity was expressed as metabolic equivalent task (MET)-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 Dr. Doria (Joslin Diabetes Center, Boston, MA; personal communication) who made the selection based on typing twenty eight common SNPs (frequency of the minor allele, MAF $\geq 5\%$) covering the entire gene plus 5 Kb on each side (22). Tagging-SNPs for *ADIPOR2* were selected from HapMap (HapMap Public Release #19) using multi-marker (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 (9.9%) 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 chi-square test was used to assess whether the genotypes were in

Hardy-Weinberg equilibrium (HWE) 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/m²), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9, and ≥ 21.0 metabolic equivalent hours/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 odds ratios 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 (LRT) 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 HWE ($P>0.05$). 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 ($D'=0.93$ and $r^2=0.68$) while the other four polymorphisms appeared to be in strong LD ($D'\geq 0.97$ and r^2 ranges from 0.15 to 0.99). There were broadly three LD blocks in *ADIPOR2* gene (Figure 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 normally 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, $-2\log\text{-likelihood}=15.1$ on 5 DF, $P=0.0098$; Table 3). A single copy of haplotype 001100

was associated with 24% decreased diabetic risk compared with the most common haplotype 110000 (OR=0.76, 95%CI 0.61-0.96, and $P=0.02$). 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 sixteen *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 (Figure 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 [$-\text{Log}_{10}(P)$] for the associations of haplotypes consisting of 2 to 6 markers that were fit as a "sliding window" across *ADIPOR2* gene with diabetes risk. None of the haplotype windows showed nominally significant associations with diabetes risk, neither did the haplotype windows covering more polymorphisms (>6 SNPs).

DISCUSSION

In this prospective, nested case-control study of 714 incident cases of type 2 diabetes and 1120 matching control women, we found that the haplotypes inferred from the LD-tagging SNPs of *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 (AMPK) and increasing peroxisome-proliferator-activated receptor alpha ligand activities (7; 29). In mice, ADIPOR1 is ubiquitously expressed, whereas ADIPOR2 expression is more restricted to skeletal muscle and liver. Unlikely, both receptors are found to be highly expressed in skeletal muscle (30) and in pancreatic beta 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 diabetes and normal glucose-tolerant subjects with family history of diabetes (10; 30).

Several studies have documented that the variations in *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 *ADIPOR1* gene were associated with diabetes risk lend further credibility for the potential

roles of the variability of *ADIPOR1* gene in the etiology of the disease. An earlier evidence indicates that the 3' UTR polymorphism rs1139646 may affect the expression of *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 causal variant because such an association was substantially attenuated after control of multiple testing. We assume the causal variants may be co-segregated 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 *ADIPOR2* gene were associated with type 2 diabetes or combined type 2 diabetes/IGT trait in two studies of Amish and French (14; 17) but not in Asian Japanese (16). Of note, there is not any single genetic marker has shown repeatable effect. We did not find significant associations between the variations in this gene, individually or in haplotypes, with diabetes risk in US 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 discrepancy among the studies is unknown but may be partly due to the diversity in ethnicity and population compositions especially the gender profile. In the present study, the genetic associations were assessed in women while most previous studies included both men and women (14-17). The gender

difference in the phenotypes of obesity and type 2 diabetes is well known. It has been also 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 US 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 as compared to *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 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 that is subjected to the genetic effects on other conditions. In the analyses, we carefully controlled the potential influence of multiple comparisons using permutation test. 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.

Acknowledgements

This study was supported by NIH grant DK58845 and CA87969.

REFERENCE

1. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 360:57-58, 2002
2. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 361:226-228, 2003
3. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L: Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* 108:1875-1881, 2001
4. Qi L, Li T, Rimm E, Zhang C, Rifai N, Hunter D, Doria A, Hu FB: The +276 polymorphism of the APM1 gene, plasma adiponectin concentration, and cardiovascular risk in diabetic men. *Diabetes* 54:1607-1610, 2005
5. Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, Hunter D, Manson JE: Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. *Diabetes* 53:209-213, 2004
6. Qi L, Doria A, Manson JE, Meigs JB, Hunter D, Mantzoros CS, Hu FB: Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. *Diabetes* 55:1512-1516, 2006
7. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423:762-769, 2003
8. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K: Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 116:1784-1792, 2006
9. Staiger H, Kaltenbach S, Staiger K, Stefan N, Fritsche A, Guirguis A, Peterfi C, Weisser M, Machicao F, Stumvoll M, Haring HU: Expression of adiponectin receptor mRNA in human skeletal muscle cells is related to in vivo parameters of glucose and lipid metabolism. *Diabetes* 53:2195-2201, 2004
10. Debard C, Laville M, Berbe V, Loizon E, Guillet C, Morio-Liondore B, Boirie Y, Vidal H: Expression of key genes of fatty acid oxidation, including adiponectin receptors, in skeletal muscle of Type 2 diabetic patients. *Diabetologia* 47:917-925, 2004
11. Kantartzis K, Fritsche A, Machicao F, Haring HU, Stefan N: The -8503 g/a polymorphism of the adiponectin receptor 1 gene is associated with insulin sensitivity dependent on adiposity. *Diabetes Care* 29:464, 2006
12. Stefan N, Machicao F, Staiger H, Machann J, Schick F, Tschritter O, Spieth C, Weigert C, Fritsche A, Stumvoll M, Haring HU: Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. *Diabetologia* 48:2282-2291, 2005
13. Siitonen N, Pulkkinen L, Mager U, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Laakso M, Uusitupa M: Association of sequence variations in the gene encoding adiponectin receptor 1 (ADIPOR1) with body size and insulin levels. The Finnish Diabetes Prevention Study. *Diabetologia* 49:1795-1805, 2006

14. Duncanson CM, Ott SH, Pollin TI, Reinhart LJ, Wang J, O'Connell J R, Mitchell BD, Shuldiner AR: Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. *Diabetes* 54:2245-2250, 2005
15. Wang H, Zhang H, Jia Y, Zhang Z, Craig R, Wang X, Elbein SC: Adiponectin receptor 1 gene (ADIPOR1) as a candidate for type 2 diabetes and insulin resistance. *Diabetes* 53:2132-2136, 2004
16. Hara K, Horikoshi M, Kitazato H, Yamauchi T, Ito C, Noda M, Ohashi J, Froguel P, Tokunaga K, Nagai R, Kadowaki T: Absence of an association between the polymorphisms in the genes encoding adiponectin receptors and type 2 diabetes. *Diabetologia* 48:1307-1314, 2005
17. Vaxillaire M, Dechaume A, Vasseur-Delannoy V, Lahmidi S, Vatin V, Lepretre F, Boutin P, Hercberg S, Charpentier G, Dina C, Froguel P: Genetic analysis of ADIPOR1 and ADIPOR2 candidate polymorphisms for type 2 diabetes in the Caucasian population. *Diabetes* 55:856-861, 2006
18. Colditz GA, Manson JE, Hankinson SE: The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health* 6:49-62, 1997
19. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 28:1039-1057, 1979
20. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183-1197, 1997
21. Qi L, Meigs J, Manson JE, Ma J, Hunter D, Rifai N, Hu FB: HFE Genetic Variability, Body Iron Stores, and the Risk of Type 2 Diabetes in U.S. Women. *Diabetes* 54:3567-3572, 2005
22. Soccio T, Zhang YY, Bacci S, Mlynarski W, Placha G, Raggio G, Di Paola R, Marucci A, Johnstone MT, Gervino EV, Abumrad NA, Klein S, Trischitta V, Doria A: Common Haplotypes at the Adiponectin Receptor 1 (ADIPOR1) Locus Are Associated With Increased Risk of Coronary Artery Disease in Type 2 Diabetes. *Diabetes* 55:2763-2770, 2006
23. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D: Efficiency and power in genetic association studies. *Nat Genet* 37:1217-1223, 2005
24. Kraft P, Cox DG, Paynter RA, Hunter D, De Vivo I: Accounting for haplotype uncertainty in matched association studies: a comparison of simple and flexible techniques. *Genet Epidemiol* 28:261-272, 2005
25. Zaykin DV, Westfall PH, Young SS, Karnoub MA, Wagner MJ, Ehm MG: Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Hum Hered* 53:79-91, 2002
26. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930-1935, 2001
27. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947-953, 2001
28. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization

- and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288-1295, 2002
29. Kadowaki T, Yamauchi T: Adiponectin and adiponectin receptors. *Endocr Rev* 26:439-451, 2005
30. Civitarese AE, Jenkinson CP, Richardson D, Bajaj M, Cusi K, Kashyap S, Berria R, Belfort R, DeFronzo RA, Mandarino LJ, Ravussin E: Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. *Diabetologia* 47:816-820, 2004
31. Kharroubi I, Rasschaert J, Eizirik DL, Cnop M: Expression of adiponectin receptors in pancreatic beta cells. *Biochem Biophys Res Commun* 312:1118-1122, 2003
32. Bluher M, Bullen JW, Jr., Lee JH, Kralisch S, Fasshauer M, Kloting N, Niebauer J, Schon MR, Williams CJ, Mantzoros CS: Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: associations with metabolic parameters and insulin resistance and regulation by physical training. *J Clin Endocrinol Metab* 91:2310-2316, 2006
33. Guryev V, Smits BM, van de Belt J, Verheul M, Hubner N, Cuppen E: Haplotype block structure is conserved across mammals. *PLoS Genet* 2:e121, 2006
34. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE: Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 46:459-469, 2003
35. Snijder MB, Heine RJ, Seidell JC, Bouter LM, Stehouwer CD, Nijpels G, Funahashi T, Matsuzawa Y, Shimomura I, Dekker JM: Associations of adiponectin levels with incident impaired glucose metabolism and type 2 diabetes in older men and women: the hoorn study. *Diabetes Care* 29:2498-2503, 2006
36. Rasmussen MS, Lihn AS, Pedersen SB, Bruun JM, Rasmussen M, Richelsen B: Adiponectin receptors in human adipose tissue: effects of obesity, weight loss, and fat depots. *Obesity (Silver Spring)* 14:28-35, 2006
37. Inukai K, Nakashima Y, Watanabe M, Takata N, Sawa T, Kurihara S, Awata T, Katayama S: Regulation of adiponectin receptor gene expression in diabetic mice. *Am J Physiol Endocrinol Metab* 288:E876-882, 2005

Tables

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

Allele are presented as common allele>minor allele;

Location of polymorphism is relative to the translation start point; MAF: minor allele frequency

* : rs1139646 is also known as rs7539542

Table 2. Genotype distributions of polymorphism in ADIPOR1 and ADIPOR2 genes in diabetes cases and controls

Polymorphisms	Cases(%)/controls(%) by genotypes			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(8.1)	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(10.2)	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, body mass index, 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 diabetes cases and controls

rs2232853	rs10494839	rs12733285	rs1342387	rs1139646	rs10920531	Frequency		Odds Ratio† (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-likelihood=15.1 on 5d.f., 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

LEGENDS FOR FIGURES

FIGURE 1. Pairwise linkage disequilibrium matrix for (A) *ADIPOR1* and (B) *ADIPOR2* genes. D' is presented above the diagonal and r^2 is presented below the diagonal.

Figure 1

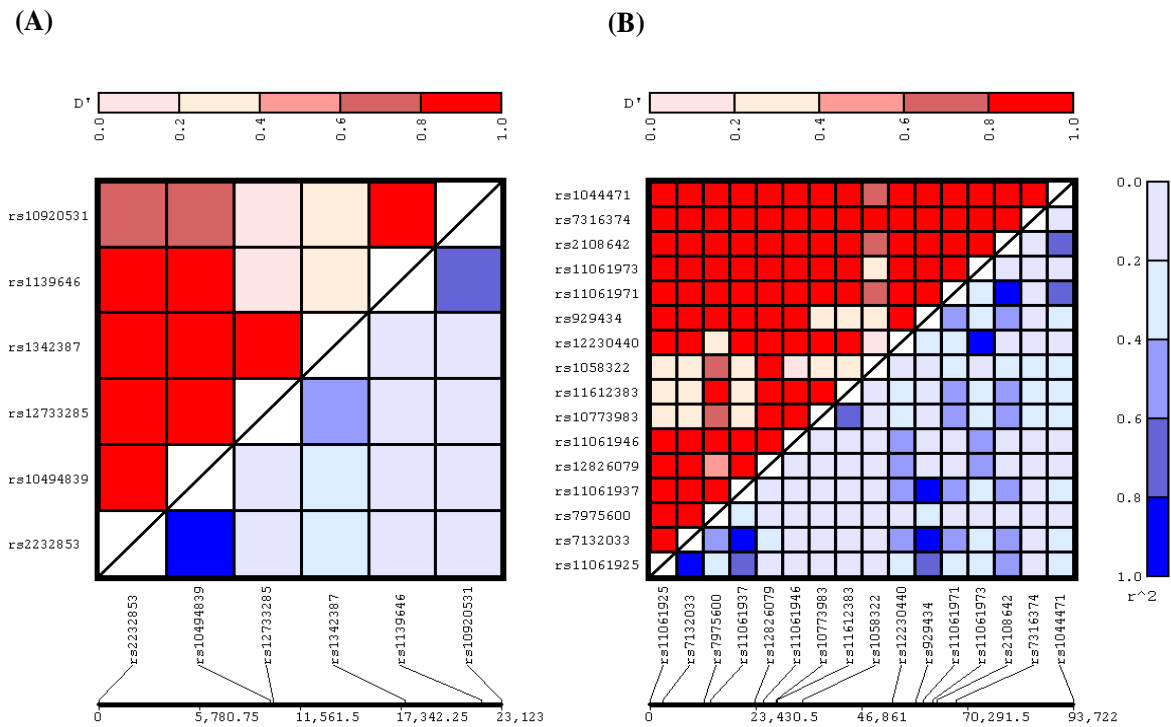


FIGURE 2. Haplotype cladogram for *ADIPOR1* gene. Gray circles represent the inferred haplotypes with their frequencies, which is proportional to the size (diameter) of the circles. P1 to P6 represents the polymorphic changes (in the order of rs2232853, rs10494839, rs12733285, rs1342387, rs1139646, and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

Figure 2

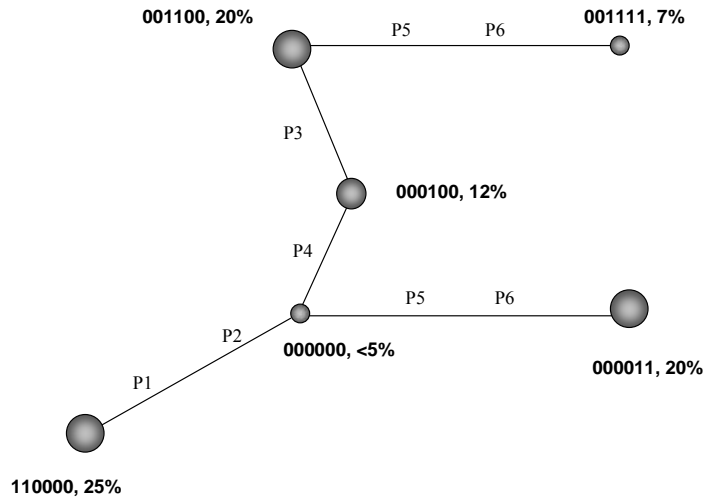


FIGURE 3. Sliding window analyses of the associations between *ADIPOR2* haplotypes (possessing 2-6 SNPs) and the risk of type 2 diabetes. $-\text{Log}_{10} P$ values of global tests for the haplotype windows are presented as solid diamonds. The haplotype windows for a given number of SNPs (2-6) were generated by grouping the corresponding number of SNPs in the order of the position of polymorphisms and move one SNP each time from 5' to 3' end.

Figure 3

