Brief Genetics Report

Genetic Variation in Adiponectin Receptor 1 and Adiponectin Receptor 2 Is Associated With Type 2 Diabetes in the Old Order Amish

Coleen M. Damcott,[1] Sandra H. Ott,[1] Toni I. Pollin,[1] Laurie J. Reinhart,[1] Jian Wang,[1] Jeffrey R. O'Connell,[1] Braxton D. Mitchell,[1] and Alan R. Shuldiner[1,2]

Adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2) are newly identified receptors for adiponectin, an adipocytokine with anti-inflammatory and insulin-sensitizing properties. We screened for polymorphisms by performing sequence analysis on all eight exons, splice junctions, and ~2 kb of the 5′ flanking regions of each receptor. We detected 5 single nucleotide polymorphisms (SNPs) in *ADIPOR1* and 16 SNPs in *ADIPOR2*. We genotyped these SNPs in Amish subjects with type 2 diabetes ($n = 137$), impaired glucose tolerance (IGT) ($n = 139$), and normal glucose tolerance ($n = 342$) to test for association with type 2 diabetes. Three intronic SNPs in *ADIPOR1* were significantly associated with type 2 diabetes ($P = 0.014$–0.007; odds ratio [OR] 1.61–1.65) and in high linkage disequilibrium ($r^2 = 0.97$–1.0). In *ADIPOR2*, we found that five SNPs delineated one large haplotype block ($r^2 = 0.9$–1.0) spanning >98 kb of the gene and promoter region, which was strongly associated with the combined type 2 diabetes/IGT trait ($P \leq 0.001$; OR 1.64–1.71). To our knowledge, these data provide the first evidence for association between variation in the adiponectin receptors and type 2 diabetes. *Diabetes* 54: 2245–2250, 2005

Adiponectin is an adipocytokine that is involved in lipid and glucose metabolism, energy homeostasis, and inflammatory pathways (1–5). Circulating concentrations of adiponectin are reduced in subjects with type 2 diabetes (6–9). Low plasma adiponectin levels are observed in rodent models of obesity and insulin resistance (2,10), and adiponectin-deficient mice exhibit diet-induced insulin resistance (11,12). The insulin-sensitizing effects of adiponectin appear to be mediated by increased fatty acid oxidation and glucose uptake (10,13) and suppression of gluconeogenesis (14,15) through activation of AMP-activated protein kinase (16,17) and peroxisome proliferator–activated receptor-α (18).

Recently, two receptors, adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2), were cloned, each consisting of eight exons that are located on chromosomes 1q32.1 and 12p13.33, respectively. In humans, ADIPOR1 is ubiquitously expressed, with highest levels in the skeletal muscle, while ADIPOR2 is predominantly expressed in skeletal muscle and liver (19). Yamauchi et al. (19) showed that activation of AMP-activated protein kinase and peroxisome proliferator–activated receptor-α by adiponectin is mediated by ADIPOR1 and ADIPOR2. In addition, normal glucose-tolerant Mexican-American subjects with a family history of type 2 diabetes exhibit significantly lower levels of ADIPOR1 and ADIPOR2 mRNA in skeletal muscle compared with those without a family history (20). Civitarese et al. (20) also observed that mRNA expression of both receptors was positively correlated with glucose disposal, while ADIPOR2 expression was positively correlated with plasma adiponectin levels. These studies suggest that downregulation or altered function of ADIPOR1 and ADIPOR2 may be responsible for development of insulin resistance in peripheral tissues. Therefore, we hypothesized that genetic variation in *ADIPOR1* and *ADIPOR2* may contribute to increased susceptibility to type 2 diabetes. We addressed this question by screening *ADIPOR1* and *ADIPOR2* for polymorphisms and testing for association between the observed sequence variation and type 2 diabetes in the Old Order Amish.

In *ADIPOR1*, DNA sequence analysis in 24 Amish indi-
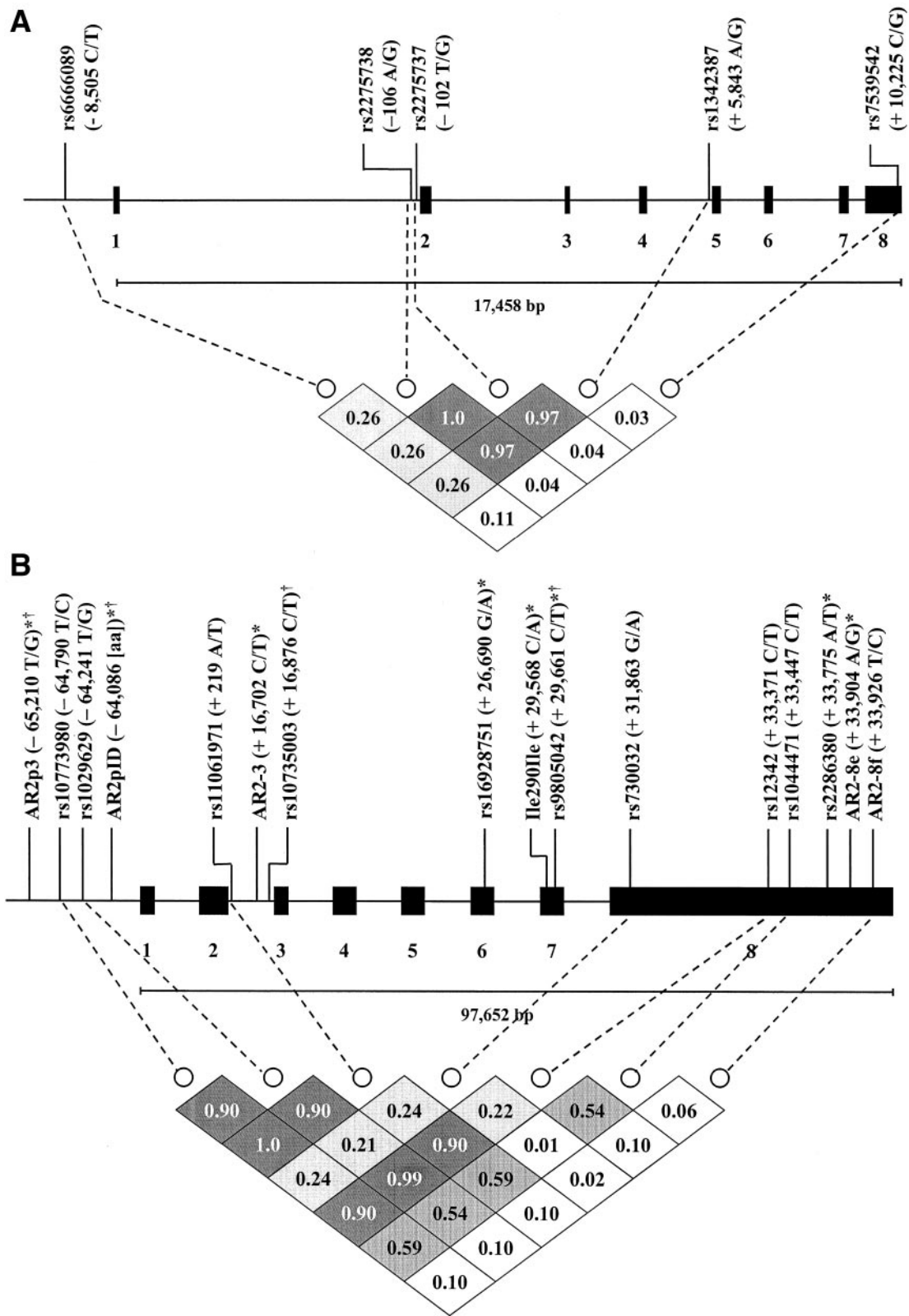
FIG. 1. Gene structure, location of polymorphic sites, and pairwise LD among SNPs in *ADIPOR1* (*A*) and *ADIPOR2* (*B*). The upper portion of the figures show the gene structure and location of polymorphisms identified by sequencing in the Amish. The base pair positions of the variants were determined from a sequence obtained from the May 2004 assembly of Genome Browser (University of California, Santa Cruz; http:// genome.ucsc.edu/) and based on numbering from the translation initiation site. The asterisks denote SNPs with MAFs <0.05 in the Amish. SNPs that were not genotyped in the full sample due to technical difficulties are marked with a dagger (†). The lower portion of the figures shows a schematic of the pairwise LD, calculated as r^2, among the common SNPs (MAF > 0.05). The dotted lines connect each SNP name and position with the corresponding cell in the LD matrix. Increasing level of LD is shown by darker grayscale.

TABLE 1
Allele frequencies and results of association analysis in type 2 diabetes and IGT cases and NGT control subjects for SNPs in *ADIPOR1*

SNP name	SNP location (bp)*	Major/minor allele	Minor allele frequency			Type 2 diabetes vs. NGT		Type 2 diabetes + IGT vs. NGT	
			Type 2 diabetes ($n = 137$)	IGT ($n = 139$)	NGT† ($n = 342$)	OR	P value‡	OR	P value‡
rs6666089	−8,505	C/T	0.135	0.128	0.126	1.38	0.172	1.25	0.509
rs2275738	−106	A/G	Perfect LD ($r^2 = 1$) w/rs2275737						
rs2275737	−102	T/G	0.405	0.336	0.307	1.65	**0.007**	1.40	0.051
rs1342387	+5,843	A/G	0.417	0.382	0.332	1.61	**0.014**	1.50	**0.021**
rs7539542	+10,225	G/C	0.482	0.442	0.433	1.21	0.354	1.13	0.408

*Base pair location from the ATG start site. †All SNPs fit the expectations of Hardy-Weinberg equilibrium in the NGT group. ‡P values are based on genotype frequencies, and the ORs were computed by comparing the odds of disease between subjects carrying one copy of the minor allele and subjects not carrying any copies of the minor allele. P values <0.05 are shown in bold.

viduals detected five SNPs previously reported in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Figure 1*A* shows the *ADIPOR1* gene structure, SNPs identified, and pairwise linkage disequilibrium (LD) (r^2). We genotyped all five SNPs in 618 individuals enrolled in the Amish Family Diabetes Study (AFDS) (137 subjects with type 2 diabetes, 139 subjects with impaired glucose tolerance [IGT], and 342 control subjects with normal glucose tolerance [NGT]). Two of the SNPs, rs2275738 and rs2275737, are located 4 bp apart in intron 1 and were in perfect LD ($r^2 = 1$); therefore, only one of them (rs2275737) was included in the analyses. Genotype frequencies of the two intronic SNPs, rs2275737 and rs1342387, differed significantly between subjects with type 2 diabetes and those with NGT ($P = 0.007$ and $P = 0.014$, respectively), with the minor allele being the risk allele for type 2 diabetes (odds ratio [OR] 1.65 and 1.61, respectively) (Table 1 and online appendix Fig. 1 [available at http://diabetes. diabetesjournals.org] for genotype frequencies in type 2 diabetes and IGT cases and NGT control subjects). The intronic SNPs span ~6 kb of the gene from intron 1 to intron 4 and were in high LD ($r^2 \geq 0.97$) (Fig. 1*A*). The two SNPs flanking the intronic SNP cluster, rs6666089 and rs7539542, were not significantly associated with type 2 diabetes ($P = 0.17$ and $P = 0.35$, respectively) nor were they in LD with each other or the intronic SNPs, suggesting that the intronic SNPs mark a type 2 diabetes susceptibility allele or haplotype. We used Haploscore to estimate four SNP haplotype frequencies and to identify haplotypes associated with type 2 diabetes. None of the haplotypes provided stronger evidence for association with type 2 diabetes than the single SNPs (online appendix Table 1). In addition to the case/control analysis, we examined the effects of *ADIPOR1* SNP genotype on BMI and plasma glucose and insulin during a 3-h oral glucose tolerance test (OGTT) in an expanded set of 698 nondiabetic AFDS subjects, but found no association (data not shown).

We previously reported evidence for linkage to type 2 diabetes on chromosome 1q21-q24 in the Amish (21), which raises the question of whether ADIPOR1 contributes to this linkage. While we cannot rule out that possibility, ADIPOR1 resides ~44 Mb downstream from the 1-LOD (logarithm of odds) support interval for our peak linkage to chromosome 1 and is thus unlikely to be the cause of the linkage.

We identified 16 SNPs by sequence analysis in *ADIPOR2*

(Fig. 1*B*) and genotyped 12 of the SNPs in 618 AFDS subjects. Four SNPs (AR2p3, AR2pID, rs10735003, and rs9805042) were eliminated due to difficulties with genotyping assays. AR2p3, AR2pID, and rs9805042 had minor allele frequencies (MAFs) <0.015, and rs10735003 had an $r^2 = 1$ with rs11061971 in the extended sequencing set ($n = 96$ alleles); thus, these SNPs were not pursued further. Three SNPs were in perfect LD ($r^2 = 1$), including rs10773980, rs11061971, and rs10735003 (estimated from 96 alleles), so only rs11061971 was included in the analyses. One large haplotype block spanning ~65 kb of the promoter, exon 1 through exon 7, and ~1.8 kb of exon 8 (>98 kb total) contains three common SNPs (rs1029629, rs11061971, and rs12342) that are in high LD ($r^2 \geq 0.90$) and show strong association with the combined type 2 diabetes/IGT trait ($P \leq 0.001$; OR 1.64–1.71), with the minor allele being the risk allele (Table 2 and Fig. 1*B*; see online appendix Fig. 2 for genotype frequencies in type 2 diabetes and IGT cases and NGT control subjects). This block is interrupted by one SNP (rs730032) that is neither in LD with the large haplotype block ($r^2 = 0.21–0.24$) nor associated with the type 2 diabetes/IGT trait ($P = 0.23$; OR 1.30). Two additional SNPs located 3′ of the large haplotype block (rs1044471 and AR2–8f) were significantly associated with type 2 diabetes/IGT ($P = 0.006$ and $P = 0.012$, respectively), but not in high LD with each other or the SNPs in the large haplotype block. The haplotype analysis revealed one haplotype (GTGTT) that was significantly associated with the combined type 2 diabetes/IGT trait ($P = 0.004$; global P value = 0.015) (Fig. 2). This haplotype contains the risk alleles for each of the single type 2 diabetes/IGT-associated SNPs. These results should be interpreted with caution since Haploscore does not account for relatedness between subjects.

Quantitative trait analysis of *ADIPOR2* SNPs in an expanded set of 698 nondiabetic subjects revealed significant associations between rs1029629 and mean plasma glucose levels and total glucose area under the curve during the OGTT ($P = 0.005–0.016$ and $P = 0.021$, respectively, for recessive model). Carriers of the G risk allele for rs1029629 exhibited significantly higher plasma glucose levels at each 30-min interval from 90 to 180 min and total glucose area under the curve during the OGTT (online appendix Fig. 3), providing additional evidence that the haplotype containing the G allele of rs1029629 influences glucose homeostasis and type 2 diabetes risk. There was

TABLE 2
Allele frequencies and results of association analysis in type 2 diabetes and IGT cases and NGT control subjects for SNPs in *ADIPOR2*

| SNP name | SNP location (bp)* | Major/minor allele | Minor allele frequency | | | Type 2 diabetes vs. NGT | | Type 2 diabetes + IGT vs. NGT | |
			Type 2 diabetes ($n = 137$)	IGT ($n = 139$)	NGT† ($n = 342$)	OR	P value‡	OR	P value‡
rs10773980	−64,790	T/C	Perfect LD ($r^2 = 1$) with rs11061971						
rs1029629	−64,241	T/G	0.379	0.392	0.306	1.39	0.051	1.71	**<0.001**
rs11061971	+219	A/T	0.420	0.398	0.327	1.47	**0.021**	1.71	**0.001**
AR2–3	+16,702	C/T	0.035	0.014	0.013	2.81	0.10	1.46	0.48
rs10735003	+16,876	T/C	Perfect LD ($r^2 = 1$) with rs11061971 (estimated from 96 alleles)						
rs16928751	+26,690	G/A	0.036	0.022	0.015	2.70	0.11	1.73	0.26
Ile290Ile	+29,568	C/A	0.035	0.018	0.015	2.61	0.12	1.48	0.44
rs730032	+31,863	G/A	0.320	0.284	0.330	1.00	0.919	1.30	0.227
rs12342	+33,371	C/T	0.378	0.398	0.310	1.38	0.055	1.64	**<0.001**
rs1044471	+33,447	C/T	0.526	0.507	0.457	1.43	**0.024**	1.47	**0.006**
rs2286380	+33,775	A/T	0.036	0.018	0.013	2.72	0.10	1.64	0.32
AR2–8e	+33,904	A/G	0.030	0.007	0.015	1.67	0.41	1.05	0.92
AR2–8f	+33,926	T/C	0.072	0.066	0.033	2.00	0.070	2.28	**0.012**

*Base pair location from the ATG start site. †All SNPs fit the expectations of Hardy-Weinberg equilibrium in the NGT group. ‡P values are based on genotype frequencies, and the ORs were computed by comparing the odds of disease between subjects carrying one copy of the minor allele and subjects not carrying any copies of the minor allele. P values <0.05 are shown in bold.

no association between SNPs in *ADIPOR2* and BMI or plasma insulin during the OGTT.

Although these type 2 diabetes/IGT-associated SNPs do not disrupt any obvious transcription factor–binding sites (22) or splice sites, these variants could possibly alter expression or mRNA stability. We explored the possibility of epistasis between polymorphisms in the receptors but found no evidence for interaction between SNPs in *ADIPOR1* and *ADIPOR2*.

In a recent report, Wang et al. (23) screened *ADIPOR1* (but not *ADIPOR2*) for sequence variation in Caucasian and African-American subjects but found no association between the SNPs they detected (including the five SNPs we examined) and type 2 diabetes, insulin sensitivity, or insulin secretion. The discrepancy between their study and ours may be a result of low power or underlying population differences. Alternatively, it is possible that we have detected a false-positive. We did not correct for multiple comparisons in our analyses. If the highly conservative Bonferroni correction were used in our case/control analysis, the significantly associated *ADIPOR1* SNPs would become borderline to nonsignificant whereas the *ADIPOR2* SNPs would remain significant at the $P = 0.01$ level. This observation strengthens the evidence that variation in *ADIPOR2* contributes to increased susceptibility to type 2 diabetes.

In summary, we have extensively screened the two adiponectin receptors to search for polymorphisms associated with type 2 diabetes in the Old Order Amish. We identified multiple polymorphisms in both *ADIPOR1* and *ADIPOR2* that were significantly associated with type 2 diabetes and related traits, providing evidence that genetic variation in the receptors, particularly *ADIPOR2*, plays a role in type 2 diabetes susceptibility. Alternatively, these SNPs may be in LD with an undetected functional SNP in the receptors or a neighboring gene. Further analysis in other populations, as well as functional studies, will be necessary to further elucidate the role of polymorphism in *ADIPOR1* and *ADIPOR2* in the pathogenesis of type 2 diabetes.

RESEARCH DESIGN AND METHODS

The AFDS was initiated in 1995 with the goal of identifying susceptibility genes for type 2 diabetes in the Old Order Amish. Details of the AFDS design, recruitment, phenotyping, and pedigree structure have been described previously (24). Briefly, probands with previously diagnosed type 2 diabetes (onset age 35–65 years) and, where possible, all first- and second-degree relatives of probands and spouses >18 years of age were recruited. Phenotypic characterization of study participants included medical and family history, anthropometry, and an OGTT with insulin levels. Total glucose and insulin areas under the curve during the OGTT were calculated using the trapezoid method. BMI was calculated as weight in kilograms divided by the square of height (in meters). This study includes additional subjects recruited subsequent to the report by Hsueh et al. (24) and excludes all subjects with NGT <38 years of

| rs1029629 | rs1106197 | rs730032 | rs12342 | rs1044471 | | Haplotype Frequency | | | |
		Alleles				T2DM + IGT	NGT	Hap-Score	p-value†
T	A	G	C	C		0.300	0.346	-1.58	0.114
T	A	A	C	T		0.102	0.126	-1.21	0.228
T	A	A	C	C		0.191	0.193	-0.10	0.920
T	T	G	C	T		0.023	0.021	0.11	0.915
G	T	G	T	T		0.384	0.301	2.87	**0.004**

FIG. 2. *ADIPOR2* haplotype frequencies and association analysis in cases with type 2 diabetes/IGT and NGT control subjects. *Haplotypes with estimated frequencies >0.01 in case and control subjects are shown. Redundant SNPs ($r^2 = 1$) and rare SNPs (MAF < 0.05) were removed from the haplotype analysis. Type 2 diabetes risk alleles are shaded. P values <0.05 are shown in bold. †Global P value = 0.015.

age in order to increase the probability of their capacity for diabetes resistance. Informed consent was obtained from all study subjects, and the Institutional Review Board at the University of Maryland School of Medicine approved the study protocol.

Sequence analysis and genotyping. Direct sequencing was used to screen the eight exons with splice junctions (~100 bp of the intronic flanking sequence) of both *ADIPOR1* and *ADIPOR2* and 1,712 bp and 1,976 bp of the region 5′ of the initiation codons of *ADIPOR1* and *ADIPOR2*, respectively, for genetic variation in 24 participants in the AFDS (eight subjects each with type 2 diabetes, IGT, and NGT). This sequencing set provides 91% power to detect at least one copy of the minor allele for SNPs with allele frequencies ≥0.05. Subsequent to PCR amplification both strands were sequenced on an ABI 3730 DNA sequencer and analyzed using Sequence Analysis 3.2 software (Applied Biosystems, Foster City, CA). All identified variants were genotyped in 618 individuals enrolled in the AFDS (137 subjects with type 2 diabetes, 139 with IGT, and 342 with NGT) with the exception of AR2p3, AR2pID, rs10735003, and rs9805042, which were not genotyped due to technical difficulties. To obtain better estimates of MAFs and LD for these four SNPs, an additional 24 subjects were sequenced. Genotyping for the other SNPs was completed using the SNPstream Ultra High Throughput genotyping platform (Beckman Coulter, Fullerton, CA) (25) and the Pyrosequencing PSQ HS 96A system (Pyrosequencing, Uppsula, Sweden). The error rate, based on blind replicates for the SNPs examined, was 0–2%.

Statistical analysis. Before analysis, genotypes were checked for Mendelian consistency using the PedCheck software program (26) in the extended Amish pedigree. Mendelian errors were resolved or removed before analysis. Allele frequencies were calculated by gene counting. All SNPs conformed to Hardy-Weinberg expectations. We evaluated the association between SNP genotype and disease status (type 2 diabetes versus NGT and type 2 diabetes/IGT versus NGT) under the additive genetic model using a variance component approach, in which we modeled the probability that the subject was a case or control as a function of the individual's age, sex, and genotype, conditional on the correlations in phenotype among relative pairs. Statistical testing was accomplished using the likelihood ratio test (27). The OR was computed by comparing the odds of disease between subjects carrying one copy of the minor allele and subjects not carrying any copies of the minor allele. The association analyses were carried out using the SOLAR software program (28).

In similar fashion, we also evaluated the effect of genotype on BMI and plasma glucose and insulin levels during an OGTT in an expanded set of 698 nondiabetic NGT ($n = 568$) and IGT ($n = 130$) AFDS subjects. We compared the likelihood of a model in which the trait values were allowed to vary by genotype (unconstrained model) to that in which the genotype effects were constrained to be zero using the likelihood ratio test. Within each model, we simultaneously estimated the effects of age, sex, and family relationship (27). Insulin values were transformed by their natural logarithms (ln) to reduce skewness. Quantitative trait analyses were conducted using the SOLAR program (28).

Pairwise LD (r^2) was computed using the ZAPLO software program (29). Haploscore was used to estimate and compare haplotype frequencies between case and control subjects (30).

REFERENCES

1. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K: cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 221:286–289, 1996

2. Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703, 1996

3. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 100:2473–2476, 1999

4. Havel PJ: Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* 53 (Suppl. 1):S143–S151, 2004

5. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I: Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 24:29–33, 2004

6. 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

7. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G: Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. *Diabetes* 52:1779–1785, 2003

8. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K, Saruta T: Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clin Sci (Lond)* 103:137–142, 2002

9. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000

10. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001

11. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002

12. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T: Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 277:25863–25866, 2002

13. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001

14. 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

15. 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

16. 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

17. Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang CC, Itani SI, Lodish HF, Ruderman NB: Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A* 99:16309–16313, 2002

18. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T: Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem* 278:2461–2468, 2003

19. 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

20. 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

21. Hsueh WC, St Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK, Shuldiner AR: Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21–q24. *Diabetes* 52:550–557, 2003

22. Wingender E, Chen X, Fricke E, Geffers R, Hehl R, Liebich I, Krull M, Matys V, Michael H, Ohnhauser R, Pruss M, Schacherer F, Thiele S, Urbach S: The TRANSFAC system on gene expression regulation. *Nucleic Acid Res* 29:281–283, 2001

23. 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

24. Hsueh WC, Mitchell BD, Aburomia R, Pollin T, Sakul H, Gelder EM, Michelsen BK, Wagner MJ, St Jean PL, Knowler WC, Burns DK, Bell CJ, Shuldiner AR: Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. *Diabetes Care* 23:595–601, 2000

25. Bell PA, Chaturvedi S, Gelfand CA, Huang CY, Kochersperger M, Kopla R, Modica F, Pohl M, Varde S, Zhao R, Zhao X, Boyce-Jacino MT, Yassen A: SNPstream UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. *Biotechniques* (Suppl.):70–77, 2002

26. O'Connell JR, Weeks DE: PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *AmJ Hum Genet* 63:259–266, 1998

27. Fu M, Damcott CM, Sabra M, Pollin TI, Ott SH, Wang J, Garant MJ, O'connell JR, Mitchell BD, Shuldiner AR: Polymorphism in the calsequestrin 1 (CASQ1) gene on chromosome 1q21 is associated with type 2 diabetes in the old order Amish. *Diabetes* 53:3292–3299, 2004

28. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211, 1998

29. O'Connell JR: Zero-recombinant haplotyping: applications to fine mapping using SNPs. *Genet Epidemiol* 19 (Suppl. 1):S64–S70, 2000

30. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA: Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 70:425–434, 2002