Genetic Analysis of Adiponectin Variation and its Association with Type 2 Diabetes in African Americans

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Abstract

Objective—Adiponectin is an adipocytokine that has been implicated in a variety of metabolic disorders, including T2D and cardiovascular disease. Studies evaluating genetic variants in *ADIPOQ* have been contradictory when testing association with T2D in different ethnic groups.

Design and Methods—In this study, 18 SNPs in *ADIPOQ* were tested for association with plasma adiponectin levels and diabetes status. SNPs were examined in two independent African-American cohorts (n_{max}=1116) from the Insulin Resistance Atherosclerosis Family Study (IRASFS) and the African American-Diabetes Heart Study (AA-DHS).

Results—Five polymorphisms were nominally associated with plasma adiponectin levels in the meta-analysis (p=0.035–1.02x10^{-6}) including a low frequency arginine to cysteine mutation

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(R55C) which reduced plasma adiponectin levels to <15% of the mean. Variants were then tested for association with T2D in a meta-analysis of these and the Wake Forest T2D Case-Control study (n=3233 T2D, 2645 non-T2D). Association with T2D was not observed (p 0.08), suggesting limited influence of *ADIPOQ* variants on T2D risk.

Conclusions—Despite identification of variants associated with adiponectin levels, a detailed genetic analysis of *ADIPOQ* revealed no association with T2D risk. This puts into question the role of adiponectin in T2D pathogenesis: whether low adiponectin levels are truly causal for or rather a consequence.

Introduction

Adiponectin, an adipocytokine secreted primarily by the adipose tissue, has been implicated in glucose homeostasis and fatty acid oxidation (1). Adiponectin was first identified by four independent groups and is also known as *Apm1* (adipose most abundant transcript), *Gbp28* (gelatin-binding protein), *Acrp30* (adipocyte complement-related protein 30), and *ADIPOQ*. Adiponectin is the most abundant adipocytokine found in the plasma, accounting for 0.01% of total plasma protein with levels ranging between 5–30μg/mL in the general population. Plasma adiponectin levels have been found to be decreased in a wide variety of conditions including obesity and type 2 diabetes (T2D). Multiple studies report a negative correlation between adiponectin levels and insulin sensitivity (2, 3).

The adiponectin protein is encoded by *ADIPOQ* located on chromosome 3q27. The gene spans approximately 17kb and contains 3 exons, encoding a 244 amino acid protein with a signal sequence, a non-homologous domain, a collagen-like domain, and a globular domain. The protein has structural homology with complement factor C1q. The protein self-assembles into trimers, hexamers, and higher molecular weight species through interactions in the collagen-like domain and disulfide bonds. The physiological actions of the active protein are still an active area of research.

Plasma adiponectin levels have been linked to several chromosomes in various populations. Family studies have shown plasma adiponectin levels to have heritability ranging from 40–70% (4–6). Additionally, studies have genotyped common SNPs in the *ADIPOQ* gene to find variants associated with plasma adiponectin levels. Common SNPs have cumulatively been reported to account for 6.7% of the variance in adiponectin levels (7), suggesting that much remains to be discovered in the search for genetic factors underlying adiponectin levels and its association with biomedical traits. The adiponectin gene itself is the strongest contributor to variation in circulating adiponectin (7–9) and the locus has been reported as a susceptibility locus for diabetes (10, 11). Numerous genetic analyses of *ADIPOQ* and T2D have been reported, with most studies being conducted in Caucasian and Asian populations. Many of these studies are characterized by limited sample sizes and frequently small numbers of polymorphisms, thus the true genetic relationship between adiponectin and T2D is unclear.

This report evaluated the contribution of genetic variation to adiponectin levels in the African American population with a focus on low frequency (minor allele frequency (MAF) <5%) variants. Direct sequencing and genotyping analysis was performed in order to

identify coding variations and test for association with plasma adiponectin levels in African American samples (n_{max}=1116). Additionally, in light of numerous published reports of adiponectin's association with T2D, we tested whether the variants identified as being associated with adiponectin levels were also associated with T2D status.

Methods and Procedures

Multiple African American samples were evaluated (Table 1). Written, informed consent was obtained from all study participants. Recruitment and sample collection procedures for all samples were approved by the Institutional Review Boards at Wake Forest School of Medicine and the local institutions.

Insulin Resistance Atherosclerosis Family Study (IRASFS)

The study design, recruitment, and phenotyping for IRASFS have been described in detail (12). Briefly, the IRASFS was designed to identify the genetic and environmental basis of insulin resistance and adiposity. The 566 subjects included in this report were recruited from a clinical center in Los Angeles, California. While a diagnosis of diabetes was not a requirement to participate, approximately 11.3% of the subjects had diabetes. Families were recruited to obtain an average of 22 members. The exam included a fasting blood draw and medical history interview. The clinical examination included an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) using the reduced sampling protocol (13). Glucose homeostasis parameters were computed with the MINMOD analysis program (14).

Insulin Resistance Atherosclerosis Study (IRAS)

The IRAS is the predecessor to the IRASFS. The study design, recruitment, and phenotyping in IRAS have been previously described (15). Briefly, US individuals of non-Hispanic White, Hispanic, and African American descent were recruited to reflect an equal representation of glucose tolerance status (normal, impaired glucose tolerance and diabetic), ethnicity, sex, and age (40–49, 50–59, and 60–69 years). This report includes 275 African Americans recruited from Los Angeles, California who provided consent for genetic studies and who had data from a frequently sampled intravenous glucose tolerance test.

Diabetes Heart Study (DHS)

The Diabetes Heart Study (DHS) is a family study of Caucasians and African Americans in which siblings concordant for type 2 diabetes, as well as unaffected family members, were recruited from internal medicine and endocrinology clinics in western North Carolina. The DHS design has been described in detail previously (16). To summarize, entry criteria required index case subjects to be diagnosed with T2D after the age of 34 years with no evidence of diabetic ketoacidosis. At least one sibling of an individual with T2D was recruited for each index case subject enrolled. Additional non-diabetic and diabetic family members also were enrolled, when possible. Only the African American participants (n=197) were included in this study.

African American-Diabetes Heart Study (AA-DHS)

Unrelated African American subjects with a diagnosis of T2D were recruited from internal medicine clinics and the community advertising in the parent Diabetes Heart Study (17) to participate in an ancillary study of the DHS with examination and clinical measures. Diabetes was diagnosed after the age of 30 years in the absence of evidence of ketoacidosis (18). For simplicity, we will refer to both the African American-Diabetes Heart Study (AA-DHS) and African American subjects in the parent Diabetes Heart Study described above as the AA-DHS cohort in the text.

Wake Forest African American T2D case-control study

A total of 2652 self-described African Americans with T2D or T2D with end stage renal disease (T2D-ESRD), and 1410 non-diabetic controls were evaluated. Only individuals with complete age data and proportions of African ancestry >0.50 were included. Ascertainment criteria and recruitment methods have been described (19). Subjects were unrelated, self-described African Americans born in North Carolina, South Carolina, Georgia, Virginia, or Tennessee. Subjects with T2D-ESRD were recruited from dialysis facilities. T2D was diagnosed as developing diabetes after the age of 25 years without prior diabetic ketoacidosis. Additionally, T2D-ESRD cases met at least one of the following criteria for inclusion: a) T2D diagnosed 5 years before initiating renal replacement therapy, b) background or greater diabetic retinopathy, and/or c) 100mg/dl proteinuria on urinalysis in the absence of other causes of nephropathy. Subjects with T2D without evidence of nephropathy were recruited from medical clinics, churches, health fairs, and community resources using the above criteria. African American controls without a current diagnosis of diabetes or renal disease were recruited from the community and internal medicine clinics. Samples were ascertained and recruited in identical fashion into two cohorts for the purposes of this study designated Cohort 1 and Cohort 2.

Laboratory Methods

Biomarkers—In the IRASFS and AA-DHS, total plasma adiponectin levels were measured by radioimmunoassay (RIA; Linco Research, St. Charles, MO). This RIA uses a polyclonal anti-adiponectin antibody which recognizes trimers and higher multimers of adiponectin and includes recognition of the globular domain. In addition, a subset of 200 samples from the IRASFS were measured with a monoclonal antibody-based ELISA, with good correlation (r=0.88) with the RIA (20).

DNA Isolation—Genomic DNA for all samples was purified using PUREGENE DNA isolation kits (Gentra Inc., Minneapolis, MN, USA). Total genomic DNA was quantified using a fluorometric assay by Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech Inc., San Francisco, CA, USA).

ADIPOQ Resequencing and SNP Genotyping—Direct sequencing of *ADIPOQ* was performed in individuals in the bottom 10% of adiponectin levels in both the IRASFS (n=60) and AA-DHS cohorts (n=55). The coding regions of *ADIPOQ* were sequenced, as well as the promoter region as defined by Kita *et al* (21). Sequences of *ADIPOQ* were PCR amplified and directly sequenced using Big Dye Ready Reaction Mix on an ABI3730xl

sequencer (Applied Biosystems, Foster City, CA). Sequence data was visualized using Sequencher Software version 4.9 (GeneCodes Corporation, Ann Arbor, MI). In addition, a priori SNPs identified in prior GWAS studies (3, 22), functional studies (23), previous work (24), and low frequency variants identified from DNA sequencing described above were also identified for genotyping. Finally, the surrounding +/−50kb region were selected using HapMap YRI and CEU populations (www.hapmap.org). Thirty-eight tag-SNPs were selected with an r^2 threshold of 0.8 and minor allele frequency (MAF) >5%., resulting in a total of 43 SNPs genotyped in the IRASFS African American sample (Supplementary Table 1). Following an initial association analysis for adiponectin levels in IRASFS African Americans, 18 associated, a priori and low frequency SNPs were selected for additional genotyping in the entire sample of African Americans (n=5878) to test for association with T2D risk. SNP genotyping was performed on the Sequenom MassARRAY Genotyping System (Sequenom, San Diego, CA). The genotyping efficiency was >90% and 134 blind duplicate samples included to evaluate genotyping accuracy were 100% concordant.

Statistical Analysis

All variants were examined for Mendelian inconsistencies using PedCheck (25) in the IRASFS and DHS cohorts, resulting in 4 of 29,779 genotypes or 0.01% converted to missing. There were no SNPs that violated Hardy-Weinberg equilibrium (p<0.05). Plasma adiponectin levels were log transformed to best approximate the distributional assumptions of the test and to minimize heterogeneity of the variance. Tests of association between the variants and plasma adiponectin were computed using a variance component model as implemented in Sequential Oligogenic Linkage Analysis Routines (SOLAR) (26). To test for association with T2D, a threshold model under a variance component framework was computed as implemented in SOLAR (IRASFS and AA-DHS) or using logistic regression (IRAS and WF T2D). For both adiponectin and T2D, inference was based on the additive genetic model adjusting for age, gender, BMI, and admixture. Admixture was calculated using 70 ancestry informative markers (AIMs) genotyped in 44 Yoruba Nigerians and 39 European Americans with individual ancestral proportions calculated using FRAPPE (27) under a two population model for all the cohorts except IRASFS. In the IRASFS, admixture was calculated with principal component analysis based on 36 AIMs selected in African Americans. SOLAR was also used to determine the contribution of genetic factors to adiponectin levels. A variance components analysis of family data decomposed the total variance of the phenotype into components that were due to genetic (polygenic) effects (additive genetic variance), measured covariates, and random environmental effects. Power analyses for the tests of association were computed using the software QUANTO (http://hydra.usc.edu/GxE) (28). Limited simulations suggest that for these pedigrees an effective sample size that is nearly equivalent to unrelated individuals for a quantitative trait is approximately 92%. Thus, the power calculations are based on a sample size of 92% of the number of individuals in these pedigrees. This approximation is helpful for providing a plausible estimate of the power for this study.

A meta-analysis for adiponectin was computed combining the IRASFS and the DHS cohorts using the software METAL (http://www.sph.umich.edu/csg/abecasis/metal/). Specifically, the variance component models described above were computed within each cohort and the

evidence of association from each cohort was combined using the weighted inverse normal method. Here let n_i and Z_i denote the sample size and standard normal random variant from the Wald test for cohort i. Then,

$$Z = \frac{\sum_i^k \sqrt{n_i} Z_i}{\sum_{i=1}^k \sqrt{n_i}} t$$

has a standard normal distribution under the null hypothesis of no association. The weighted inverse normal approach is a non-parametric meta-analysis method that allows for the difference in the scale of the trait. A test for heterogeneity of association between the cohorts was also computed. A meta-analysis for T2D was computed using the weighted inverse normal method as above, including IRASFS, IRAS, DHS, and Wake Forest African American T2D case-control cohorts.

Results

Characteristics of the study sample are summarized in Table 1. Samples consisted of a diverse group of genetically defined African Americans. Studies ranged from family studies to unrelated samples spanning the spectrum of metabolic disease from healthy to diabetics with increased CVD risk. The most extensively characterized sample with data for glucose homeostasis and adiponectin is the IRASFS. Thus, IRASFS was the focus of the initial analyses which were then extended to the other cohorts.

ADIPOQ variant identification in African Americans by direct sequencing

In the IRASFS, 566 African American individuals had plasma adiponectin measures. The bottom decile (n=60) of African Americans was selected for direct sequencing of the *ADIPOQ* gene. In addition, the individuals from the bottom decile (n=55) of AA-DHS were also sequenced for the *ADIPOQ* gene. From the sequencing, four variants were identified (Table 2), including two novel variants: a G38D mutation observed three times and an R55C mutation observed seven times. Two previously well documented variants, G15G (rs2241766) and Y111H (rs17366743) were also observed in the IRASFS samples.

ADIPOQ variant genotyping

Following direct sequencing, low frequency variants identified from direct sequencing (n=4) and the literature (3, 7, 23) (n=9) along with tagging SNPs were genotyped in the African Americans from IRASFS (Supplementary Table 1). Of the 44 SNPs genotyped, there were three monomorphic SNPs in African Americans that were excluded from further analysis. Seven variants were found to be associated with plasma adiponectin levels in the IRASFS African Americans (Supplementary Table 1). The low frequency variants and those associated with plasma adiponectin levels in IRASFS (n=18) were subsequently genotyped in additional African American samples for association with plasma adiponectin levels (IRASFS, n=555 and AA-DHS cohorts, n=384) and subsequently for association with T2D risk (IRASFS, IRAS, DHS, AA-DHS, WF T2D case-control cohorts, n=5878).

Association with adiponectin levels in IRASFS and AA-DHS

The SNPs were analyzed for association with plasma adiponectin levels adjusting for age, gender, body mass index (BMI), and admixture (Table 3). In the IRASFS African American cohort, seven SNPs were found to be associated with adiponectin (P<0.05), two of which were low frequency. Of all the SNPs associated with plasma adiponectin levels, the low frequency SNPs, rs17300539 and the novel SNP, R55C, were most strongly associated, with p-values of 0.0018 and 0.00030, respectively. rs17300539 is a low frequency variant (MAF 0.028) found −363bp 5′ of the *ADIPOQ* promoter. Individuals with the minor allele (A) for rs17300539 had higher plasma adiponectin levels with mean (SD) adiponectin levels of 8.98 (5.25) μg/mL and 11.16 (5.21) μg/mL with 0 and 1 copy of the minor allele, respectively, and consistent with previous publications in European-derived populations (29). The most strongly associated variant was the R55C coding variant (p=0.00030), initially identified through sequencing analysis in the IRASFS. It is located in exon 2 of the *ADIPOQ* gene in the collagen-like domain of the adiponectin protein. Individuals with the minor allele (T) had much lower levels of adiponectin with mean (SD) adiponectin levels of 9.06 (5.01) μg/mL and 1.20 (0.37) μg/mL with 0 and 1 copy of the minor allele, respectively. For both low frequency variants, there were no individuals homozygous for the minor allele. Notably, association analysis conditioned on the rs17300539 variant did not diminish association at R55C suggesting these signals are independent (Supplementary Table 2).

In the AA-DHS cohort, there were two variants associated with plasma adiponectin levels (Table 3), R55C and rs2241767, with p-values of 0.0010 and 0.035, respectively. Individuals with the R55C variant were again found to have significantly lower levels of plasma adiponectin with mean (SD) levels of 8.37 (6.9) μg/mL and 1.65 (0.7) μg/mL for those with 0 and 1 copy of the minor allele, respectively. The intronic variant rs2241767 had less dramatic differences in plasma adiponectin levels with mean (SD) levels of 8.51 (7.0) μg/mL and 6.63 (4.8) μg/mL for those with 0 and 1 copy of the minor allele, respectively.

In the meta-analysis of the IRASFS and AA-DHS cohorts (Table 3), five SNPs were associated with plasma adiponectin levels. These five SNPs included four SNPs that were found to be associated with plasma adiponectin in the IRASFS African American cohort and an additional SNP, rs822390 that was not associated in either IRASFS or AA-DHS alone, but was associated in the meta-analysis with a p-value of 0.035. Interestingly, the most strongly associated SNP was the novel SNP R55C with a p-value of 1.02×10^{-6}.

Association with T2D

The genotyped SNPs were initially analyzed for cross-sectional association with T2D status, following adjustment for age, gender, BMI, and admixture. In the IRASFS African American cohort (Table 4), two SNPs rs822391 and rs822394 were nominally associated. Both SNPs were low frequency non-coding variants (MAF<5%) in intron 1 and were observed in both the IRASFS and AA-DHS cohorts. When analysis was expanded to include meta-analysis of all of the African American cohorts encompassing over 5000 DNA samples (Table 4), no evidence of association with T2D was observed. A modest trend was observed for rs822391 (p=0.09), but if the number of comparisons is taken into account even this trend reflects little evidence of association. In addition, analyses were also performed to test

for an association with BMI however, there were no SNPs, even nominally, associated with BMI in individual cohorts or in the meta-analysis.

With little evidence of association to T2D, we evaluated the power to detect association with T2D in this cohort of approximately 5100 individuals (Supplementary Figures 1 and 2). The power analysis was calculated assuming a prevalence of T2D in African Americans of 18.7% (30) in an analysis of 20 variants for an $\alpha=0.0025$ (Bonferroni correction). For a p-value of 0.0025 there is substantial power to detect association with odds ratios of 1.2 or greater in variants with a MAF0.10. Additionally, Supplementary Figure 2 shows the power analysis repeated in order to adjust for a p-value of $5x10^{-8}$, or genome-wide significance. Notably, in either scenario, the current study sample size lacks sufficient power to detect association of the R55C variant (MAF=0.21%) with a nominal effect size (OR=1.09).

Discussion

There is an extensive literature examining the role of adiponectin in T2D pathogenesis. In this study we explored this connection between adiponectin and T2D by evaluating variants that were associated with adiponectin levels and whether they were also associated with T2D risk. Thus, it addresses the hypothesis that if adiponectin directly acts to contribute to T2D susceptibility, then SNPs that significantly alter adiponectin levels should in turn also be associated with T2D status if adiponectin is in fact an important component in T2D disease risk.

A set of over 40 tagging and low frequency (both coding and non-coding) variants was tested for association with adiponectin levels (Supplementary Table 1, Supplementary Figure 3). Seven of these SNPs were nominally associated with adiponectin (p<0.03) of which the low frequency coding variant R55C was the most strongly associated (p=0.00030). Based on the 19 SNPs genotyped in the follow-up study, only five were found to be associated with plasma adiponectin levels. Of these five SNPs, two were low frequency variants with a MAF<5%. The most strongly associated variant with the largest effect size is R55C (p=$1.02x10^{-6}$, β=−1.18), a novel low frequency variant initially identified from direct sequencing and confirmed with genotyping.

The R55C variant is located in exon 2 of the adiponectin gene in the collagen-like domain of the protein. The mutation results in an amino acid change from arginine to a cysteine. We observed that R55C heterozygous individuals had a >85% reduction in adiponectin levels compared to those who were homozygous for the major allele. Given the location of this mutation in the collagen-like domain, which is important for oligomerization (31), it could be speculated that the mutation could impair oligomerization of the protein and result in lower levels of high molecular weight plasma adiponectin. The importance of this variant to adiponectin variation is further emphasized with a beta value of −1.00 in the IRASFS cohort and −1.40 in the DHS cohort, and a beta of −1.18 in the meta-analysis (Table 3). Due to the low frequency of this variant, the current study lacks power to see association with T2D risk with this variant in the meta-analysis, in spite of an OR=1.09 (Table 4), the largest meta-analysis odds ratio of the SNPs analyzed. This odds ratio is quite nominal, however, and it should be noted that the mutation reduces circulating adiponectin levels to less than 20% of

the mean in the population. One would conjecture that such a reduction should have more than nominal influence on T2D risk. Notably, this SNP appears to be monomorphic in additional evaluation of Caucasians (n=1190) and Hispanic Americans (n=864). Also of interest is the observation that of the eight variants associated with plasma adiponectin levels, none were even nominally associated with T2D status in the meta-analysis of all African American cohorts. This lack of evidence of association with T2D in a sample of 5878 DNAs suggests that low adiponectin levels do not directly contribute to T2D pathogenesis; however, larger, well-powered studies are needed to validate this speculation. Several studies have found evidence of linkage for T2D on chromosome 3 over the adiponectin locus (10, 11). These have drawn attention to adiponectin as a candidate gene for T2D risk. However, studies evaluating SNPs found to be associated with plasma adiponectin levels have failed to find consistent associations with T2D. The results reported here were adequately powered to identify association of common variation with T2D (Supplementary Figure 1). In addition, Dastani *et al* (9) recently reported evidence for nominal association (P=0.004) between T2D and a collection of adiponectin-associated variants from throughout the genome in a large European-derived sample. Individual association with *ADIPOQ* variants was not reported however.

Along these lines, a recent study described an *in silico* meta-analysis of four widely studied *ADIPOQ* SNPs for association with T2D (but not adiponectin levels): rs2241766, rs1501299, rs17300539, and rs266729 (32). Han *et al* found evidence of association with T2D with rs266729 in their meta-analysis with 33 cohorts. Two of these four SNPs, rs2241766 and rs17300539, were evaluated in the African American cohorts in the study reported here. rs1501299 and rs266729 were not associated with adiponectin levels in the IRASFS African American cohort (*data not shown*). It is important to note that the majority of the studies included in Han *et al* were Caucasian cohorts, which have been shown to have a different genetic haplotype structure compared to African Americans (33). In a study in black South Africans (n=453), Olckers *et al* found evidence suggesting a protective role of the rs17300539 A allele for T2D (34), contrary to the results found in Caucasian populations which suggested an increased risk (35). In addition, Laumen *et al.* (36) used haplotype analysis of three *ADIPOQ* promoter SNPs, rs16861194, rs17300539, and rs266729, to show that the minor allele of all three SNPs led to a complete loss of promoter activity for the gene. They hypothesized that due to the haplotype leading to low levels of circulating adiponectin; there may be increased risk for T2D and coronary heart disease from this haplotype. In their study of 2340 Caucasian subjects, the haplotype with minor alleles for all three SNPs was not observed, supporting their claim that this promoter haplotype led to increased risk of T2D and coronary heart disease. Finally, in a recent report by Warren *et al.* of resequencing of *ADIPOQ* in a large (≈14,000 DNAs) primarily Caucasian samples, no evidence was found for association of *ADIPOQ* variants with T2D (37).

In this report, we failed to identify association between *ADIPOQ* SNPs and T2D in a well-powered and well-phenotyped sample that was comprehensively genotyped for both common and low frequency coding variants. As we have shown previously, low frequency coding variants can have a major influence on adiponectin both in individuals and in the population (20, 38). While many studies have found evidence of epidemiological association

between adiponectin levels and T2D in African Americans, including in the IRASFS (39, 40) and other populations, there are few comprehensive studies identifying genetic variants that influence plasma adiponectin levels and T2D risk. In the results reported here, we sought to fill this void in the literature. One of the major strengths of this report is the quality of the discovery cohort, IRASFS. The cohort has extensive, high quality phenotypes. This has allowed for the identification of novel variants associated with plasma adiponectin levels. These results are limited, however, in that not all cohorts had measures of plasma adiponectin levels.

In conclusion, we evaluated *ADIPOQ* SNPs for association with plasma adiponectin levels and T2D risk in African Americans. We identified five SNPs associated with plasma adiponectin levels, including a novel variant, R55C, located in the collagen-like domain that significantly reduced plasma adiponectin levels by >85%. However, when these genetic variants were evaluated for association with T2D, no variants were associated with T2D in a meta-analysis including over 5800 African Americans. This puts into question the role of adiponectin in T2D pathogenesis: whether low adiponectin levels are truly causal for T2D in African Americans or rather a consequence of T2D. This contrast is quite striking given the association of adiponectin with metabolic traits in clinical studies and suggests a deeper exploration of these relationships.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000; 20:1595–9. [PubMed: 10845877]

2. Vasseur F, Helbecque N, Lobbens S, et al. Hypoadiponectinaemia and high risk of type 2 diabetes are associated with adiponectin-encoding (ACDC) gene promoter variants in morbid obesity: evidence for a role of ACDC in diabesity. Diabetologia. 2005; 48:892–9. [PubMed: 15830179]

3. Hivert MF, Manning AK, McAteer JB, et al. Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes. 2008; 57:3353–9. [PubMed: 18776141]

4. Comuzzie AG, Funahashi T, Sonnenberg G, et al. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J Clin Endocrinol Metab. 2001; 86:4321–5. [PubMed: 11549668]

5. Lindsay RS, Funahashi T, Krakoff J, et al. Genome-wide linkage analysis of serum adiponectin in the Pima Indian population. Diabetes. 2003; 52:2419–25. [PubMed: 12941784]

6. Chuang LM, Chiu YF, Sheu WH, et al. Biethnic comparisons of autosomal genomic scan for loci linked to plasma adiponectin in populations of Chinese and Japanese origin. J Clin Endocrinol Metab. 2004; 89:5772–8. [PubMed: 15531541]

7. Heid IM, Henneman P, Hicks A, et al. Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals. Atherosclerosis. 2010; 208:412–20. [PubMed: 20018283]

8. Ling H, Waterworth DM, Stirnadel HA, et al. Genome-wide linkage and association analyses to identify genes influencing adiponectin levels: the GEMS Study. Obesity (Silver Spring). 2009; 17:737–44. [PubMed: 19165155]

9. Dastani Z, Hivert MF, Timpson N, et al. Novel Loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. PLoS Genet. 2012; 8:e1002607. [PubMed: 22479202]

10. Vionnet N, Hani EH, Dupont S, et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet. 2000; 67:1470–80. [PubMed: 11067779]

11. Kissebah AH, Sonnenberg GE, Myklebust J, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci U S A. 2000; 97:14478–83. [PubMed: 11121050]

12. Henkin L, Bergman RN, Bowden DW, et al. Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. Ann Epidemiol. 2003; 13:211–7. [PubMed: 12684185]

13. Steil GM, Volund A, Kahn SE, Bergman RN. Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model. Suitability for use in population studies. Diabetes. 1993; 42:250–6. [PubMed: 8425661]

14. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocr Rev. 1985; 6:45–86. [PubMed: 3884329]

15. Wagenknecht LE, Mayer EJ, Rewers M, et al. The insulin resistance atherosclerosis study (IRAS) objectives, design, and recruitment results. Ann Epidemiol. 1995; 5:464–72. [PubMed: 8680609]

16. Wagenknecht LE, Bowden DW, Carr JJ, Langefeld CD, Freedman BI, Rich SS. Familial aggregation of coronary artery calcium in families with type 2 diabetes. Diabetes. 2001; 50:861–6. [PubMed: 11289053]

17. Freedman BI, Hsu FC, Langefeld CD, et al. The impact of ethnicity and sex on subclinical cardiovascular disease: the Diabetes Heart Study. Diabetologia. 2005; 48:2511–8. [PubMed: 16261310]

18. Divers J, Wagenknecht LE, Bowden DW, et al. Ethnic differences in the relationship between albuminuria and calcified atherosclerotic plaque: the African American-diabetes heart study. Diabetes Care. 2010; 33:131–8. [PubMed: 19825824]

19. McDonough CW, Palmer ND, Hicks PJ, et al. A genome-wide association study for diabetic nephropathy genes in African Americans. Kidney Int. 2011; 79:563–72. [PubMed: 21150874]

20. Bowden DW, An SS, Palmer ND, et al. Molecular basis of a linkage peak: exome sequencing and family-based analysis identify a rare genetic variant in the ADIPOQ gene in the IRAS Family Study. Hum Mol Genet. 2010; 19:4112–20. [PubMed: 20688759]

21. Kita A, Yamasaki H, Kuwahara H, et al. Identification of the promoter region required for human adiponectin gene transcription: Association with CCAAT/enhancer binding protein-beta and tumor necrosis factor-alpha. Biochem Biophys Res Commun. 2005; 331:484–90. [PubMed: 15850785]

22. Heid IM, Henneman P, Hicks A, et al. Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals. Atherosclerosis. 208:412–20. [PubMed: 20018283]

23. Waki H, Yamauchi T, Kamon J, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem. 2003; 278:40352–63. [PubMed: 12878598]

24. Guo X, Saad MF, Langefeld CD, et al. Genome-wide linkage of plasma adiponectin reveals a major locus on chromosome 3q distinct from the adiponectin structural gene: the IRAS family study. Diabetes. 2006; 55:1723–30. [PubMed: 16731835]

25. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet. 1998; 63:259–66. [PubMed: 9634505]

26. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet. 1998; 62:1198–211. [PubMed: 9545414]

27. Tang H, Peng J, Wang P, Risch NJ. Estimation of individual admixture: analytical and study design considerations. Genet Epidemiol. 2005; 28:289–301. [PubMed: 15712363]

28. Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. Stat Med. 2002; 21:35–50. [PubMed: 11782049]

29. Gao H, Fall T, van Dam RM, et al. Evidence of a Causal Relationship Between Adiponectin Levels and Insulin Sensitivity: A Mendelian Randomization Study. Diabetes. 2012

30. [Accessed October 25 2011.] National Diabetes Fact Sheet: national estimates and general information on diabetes and prediabetes in the United States 2011. 2011. http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.aspx

31. Wang Y, Lam KS, Chan L, et al. Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. J Biol Chem. 2006; 281:16391–400. [PubMed: 16621799]

32. Han LY, Wu QH, Jiao ML, et al. Associations between single-nucleotide polymorphisms (+45T>G, +276G>T, −11377C>G, −11391G>A) of adiponectin gene and type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetologia. 2011; 54:2303–14. [PubMed: 21638131]

33. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science. 2002; 296:2225–9. [PubMed: 12029063]

34. Olckers A, Towers GW, van der Merwe A, Schwarz PE, Rheeder P, Schutte AE. Protective effect against type 2 diabetes mellitus identified within the ACDC gene in a black South African diabetic cohort. Metabolism. 2007; 56:587–92. [PubMed: 17445531]

35. Schwarz PE, Govindarajalu S, Towers W, et al. Haplotypes in the promoter region of the ADIPOQ gene are associated with increased diabetes risk in a German Caucasian population. Horm Metab Res. 2006; 38:447–51. [PubMed: 16933180]

36. Laumen H, Saningong AD, Heid IM, et al. Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. Diabetes. 2009; 58:984–91. [PubMed: 19074982]

37. Warren LL, Li L, Nelson MR, et al. Deep resequencing unveils genetic architecture of ADIPOQ and identifies a novel low-frequency variant strongly associated with adiponectin variation. Diabetes. 2012; 61:1297–301. [PubMed: 22403302]

38. An SS, Palmer ND, Hanley AJ, et al. Estimating the Contributions of Rare and Common Genetic Variations and Clinical Measures to a Model Trait: Adiponectin. Genetic Epidemiology. In Press

39. Hanley AJ, Wagenknecht LE, Norris JM, et al. Adiponectin and the incidence of type 2 diabetes in Hispanics and African Americans: the IRAS Family Study. Diabetes Care. 2011; 34:2231–6. [PubMed: 21816973]

40. Zhu N, Pankow JS, Ballantyne CM, et al. High-molecular-weight adiponectin and the risk of type 2 diabetes in the ARIC study. J Clin Endocrinol Metab. 2010; 95:5097–104. [PubMed: 20719834]

What is already known about this subject?

- Adiponectin is an important adipocytokine implicated in glucose homeostasis and fatty acid oxidation.

- Plasma adiponectin levels are decreased in a wide variety of conditions including obesity and type 2 diabetes (T2D).

What does this paper add?

- Identification of a novel variant, R55C, located in the collagen-like domain that significantly reduced plasma adiponectin levels by >85%.

- Evaluation of genetic variants associated with adiponectin failed to identify variants associated with T2D in a meta-analysis including over 5800 African Americans.

Table 1

Demographic information for study samples.

	IRASFS[a]	IRAS[b]	DHS[c]	AA-DHS[d]	WF T2D Cohort 1[e]	WF T2D Cohort 2[e]
N	566	275	197	550	1785	2505
Female (%)	59.5%	58.2%	67.0%	56.9%	58.2%	56.5%
Diabetes (%)	11.3%	33.1%	100.0%	100.0%	51.3%	73.3%
Age (years)[f]	42.8 ± 13.9	55.4 ± 8.3	58.9 ± 9.0	55.7 ± 9.7	55.6 ± 12.7	56.4 ± 12.9
BMI (kg/m²)[g]	30.1 ± 6.8	30.5 ± 6.0	34.1 ± 7.5	35.3 ± 8.6	29.8 ± 7.1	31.0 ± 7.6
Adiponectin (μg/mL)[h]	9.01 ± 5.24			8.30 ± 6.84		

[a] IRASFS, Insulin Resistance Atherosclerosis Family Study;

[b] IRAS, Insulin Resistance Atherosclerosis Study;

[c] DHS, Diabetes Heart Study;

[d] AA-DHS, African American Diabetes Heart Study;

[e] WF T2D Cohort, Wake Forest Type 2 Diabetes Case-Control Cohorts;

[f] mean age and standard deviation in years;

[g] mean body mass index (BMI) and standard deviation in kg/m²;

[h] mean adiponectin and standard deviation in ug/mL

Table 2

Sequence variants identified by DNA sequencing of *ADIPOQ*.

SNP	Position[a]	MAF[b]	location	amino acid change
rs2241766	188053586	0.05	exon 2	G15G
G38D	188053654	0.014	exon 2	G38D
R55C	188053704	0.032	exon 2	R55C
rs17366743	188054783	0.014	exon 3	Y111H

[a]Build NCBI36/hg18;

[b]MAF, minor allele frequency; African Americans from the bottom decile of IRASFS (n=60) and AA-DHS (n=55) were sequenced

Table 3

Meta-analysis of SNPs for association with plasma adiponectin levels.

SNP	Position[a]	location	coding change	Alleles[b]	IRASFS[c] MAF[e]	1/1[f]	1/2[f]	2/2[f]	p-value[h]	β	AA-DHS[d] MAF[e]	1/1[f]	1/2[f]	2/2[f]	p-value[h]	β	Meta-analysis p-value[h]	β
rs1648707	188034405	5′		A/C[g]	0.39	9.25 ± 4.73	9.19 ± 5.09	8.48 ± 5.10	0.017	−0.072	0.44	8.75 ± 9.3	8.20 ± 5.8	7.62 ± 4.3	0.40	0.042	0.01657	−0.02158
rs822387	188038731	5′		T/C	0.33	9.43 ± 5.10	8.88 ± 4.96	7.68 ± 4.95	0.23	−0.042	0.36	9.05 ± 8.0	7.40 ± 5.2	9.43 ± 7.8	0.98	0.0016	0.3445	−0.0236
rs17300539	188042154	5′		G/A	0.028	8.98 ± 5.25	11.16 ± 5.21	-	0.0018	0.32	0.013	7.00 ± 3.3	--	--	0.75	−0.060	0.02231	0.156023
rs822390	188045592	intron 1		T/G	0.071	8.94 ± 5.42	10.32 ± 5.06	8.13 ± 2.94	0.24	0.10	0.036	11.46 ± 10.2	--	--	0.071	0.26	0.03487	0.179431
rs16861209	188045808	intron 1		C/A	0.14	8.90 ± 5.20	9.26 ± 4.51	9.16 ± 5.61	0.034	0.086	0.18	8.39 ± 6.3	8.39 ± 6.3	6.10 ± 2.4	0.70	0.025	0.05942	0.058759
rs822391	188046497	intron 1		T/C	0.031	8.97 ± 5.11	9.49 ± 4.34	8.13 ± 2.94	0.67	−0.031	0.038	10.76 ± 10.0	10.76 ± 10.0	6.38 ± 0.6	0.24	0.13	0.6834	0.04213
rs822394	188049422	intron 1		C/A	0.029	8.93 ± 5.06	9.92 ± 4.62	5.87 ± 3.14	0.88	−0.010	0.034	11.08 ± 10.1	11.08 ± 10.1	--	0.11	0.22	0.3712	0.092191
rs2241766	188053586	exon 2	G15G	T/G	0.031	8.97 ± 5.04	9.29 ± 5.01	-	0.62	0.043	0.040	6.83 ± 4.7	6.83 ± 4.7	--	0.18	−0.18	0.6517	−0.05417
G38D	188053654	exon 2	G38D	G/A	0.019	9.04 ± 5.28	9.81 ± 4.33	-	0.76	−0.041	0.015	7.99 ± 6.02	7.99 ± 6.02	--	0.64	−0.10	0.604	−0.06676
R55C	188053704	exon 2	R55C	C/T	0.010	9.06 ± 5.01	1.20 ± 0.37	-	0.00030	−1.00	0.0046	1.65 ± 0.7	1.65 ± 0.7	--	0.0010	−1.40	1.016E-06	−1.17612
rs2241767	188053890	intron 2		A/G	0.028	9.06 ± 5.27	9.52 ± 5.26	-	0.31	0.087	0.044	6.63 ± 4.8	6.63 ± 4.8	2.51	0.035	−0.26	0.6792	−0.05886
rs3774262	188054508	intron 2		G/A	0.028	9.05 ± 5.26	9.52 ± 5.26	-	0.30	0.088	0.043	6.49 ± 4.5	6.49 ± 4.5	--	0.081	−0.22	0.8175	−0.04341
rs62625753	188054720	exon 3	G90S	G/A	0	9.01 ± 5.03	-	-	.	0	0.009	20.83	20.83	--	0.065	1.12	0.06498	0.495953
rs17366743	188054783	exon 3	Y111H	T/C	0.010	9.02 ± 5.04	7.28 ± 4.04	-	0.35	−0.20	0.0056	7.44 ± 4.3	7.44 ± 4.3	--	0.72	−0.10	0.3382	−0.15459
rs7639352	188061168	3′		C/T	0.33	8.62 ± 4.67	9.18 ± 5.76	10.20 ± 4.94	0.026	0.072	0.33	7.93 ± 6.0	7.93 ± 6.0	8.18 ± 5.6	0.77	−0.015	0.1059	0.034991
rs1865762	188062094	3′		A/G	0.010	8.95 ± 5.00	11.50 ± 5.69	-	0.17	0.19	0.013	6.41 ± 2.0	6.41 ± 2.0	9.98	0.93	0.017	0.2622	0.11473
rs6444175	188062438	3′		G/A	0.33	8.74 ± 4.64	8.97 ± 5.33	10.21 ± 4.98	0.024	0.076	0.33	7.96 ± 6.0	7.96 ± 6.0	8.60 ± 5.8	0.79	−0.015	0.09874	0.037586
rs17373414	188068221	3′		C/T	0.020	8.96 ± 4.99	11.05 ± 6.10	-	0.47	0.10	0.025	10.20 ± 9.4	10.20 ± 9.4	2.51	0.46	0.11	0.3032	0.100109

[a]Build NCBI36/hg18;

[b]major/minor alleles;

[c]IRASFS, Insulin Resistance Atherosclerosis Family Study;

[d]AA-DHS, African American Diabetes Heart Study;

[e]MAF, minor allele frequency;

[f]1 corresponds to the major allele and 2 corresponds to the minor allele;

g major and minor alleles are different for IRASF-FS and AA-DHS cohorts, IRASFS alleles are listed;

h a strict Bonferroni correction (n=18 tests) would set a significance threshold p-value<0.0028

Table 4

Meta-analysis of SNPs for association with T2D.

SNP	Position[a]	IRASFS[b]			IRAS[c]			Other Cohorts (AA-DHS[d], DHS[e], WF T2D[f] cohorts)			Meta-analysis		
		N	p-value	OR(CI)	N	p-value[g]	OR (CI)	N	p-value[g]	OR (CI)	N	p-value[g]	OR (CI)
rs1648707	188034405	496	0.25	0.97(0.94–1.00)	267	0.75	0.94(0.64–1.39)	4694	0.15	0.99(0.97–1.00)	5457	0.08	0.99(0.93–1.05)
rs822387	188038731	489	0.91	1.01(0.95–1.08)	225	0.66	0.91(0.59–1.39)	4526	0.40	0.99(0.98–1.01)	5240	0.40	0.98(0.92–1.05)
rs17300539	188042154	559	0.77	0.99(0.93–1.05)	272	0.89	0.88(0.15–5.07)	4690	0.74	0.98(0.92–1.06)	5521	0.67	0.97(0.74–1.27)
rs822390	188045592	521	0.027	0.92(0.87–0.98)	265	0.49	1.42(0.53–3.77)	4500	0.16	0.96(0.92–1.01)	5286	0.07	1.01(0.87–1.18)
rs16861209	188045808	494	0.36	0.98(0.93–1.03)	272	0.69	1.11(0.65–1.89)	4665	1.00	1.00(0.98–1.03)	5431	0.85	1.01(0.93–1.10)
rs822391	188046497	493	0.035	0.93(0.87–1.00)	272	0.41	1.40(0.63–3.11)	4671	0.19	0.96(0.92–1.01)	5436	0.09	1.01(0.89–1.15)
rs822394	188049422	497	0.025	0.93(0.87–0.99)	269	0.24	1.66(0.72–3.83)	4644	0.26	0.97(0.92–1.01)	5410	0.15	1.04(0.91–1.19)
rs2241766	188053586	495	0.46	0.96(0.92–1.00)	269	0.48	0.68(0.23–1.98)	4708	0.97	1.00(0.95–1.04)	5472	0.68	0.93(0.79–1.10)
G38D	188053654	560	0.42	0.97(0.90–1.04)	272	0.08	0.15(0.02–1.28)	4713	0.94	1.00(0.93–1.08)	5545	0.56	0.75(0.54–1.04)
R55C	188053704	494	0.17	1.36(1.29–1.43)	284[h]	.	.	4667	0.65	1.01(0.87–1.18)	5161	0.39	1.09(0.97–1.23)
rs2241767	188053890	557	0.72	1.02(0.92–1.13)	272	0.94	1.04(0.41–2.64)	4653	0.87	1.00(0.96–1.05)	5482	0.78	1.01(0.87–1.17)
rs3774262	188054508	559	0.70	1.02(0.92–1.13)	271	0.99	1.01(0.39–2.56)	4728	0.88	1.00(0.96–1.04)	5558	0.99	1.00(0.87–1.16)
rs62625753	188054720	493	1.00	1.00(1.00–1.00)	283[h]	.	.	4639	0.20	0.90(0.73–1.11)	5132	0.22	0.92(0.79–1.08)
rs17366743	188054783	494	0.36	0.89(0.78–1.01)	271	0.82	0.76(0.08–7.43)	4665	0.23	0.94(0.85–1.05)	5430	0.15	0.90(0.63–1.29)
rs7639352	188061168	559	0.58	1.00(0.96–1.04)	271	0.09	1.42(0.95–2.14)	4590	0.39	1.01(0.99–1.03)	5420	0.18	1.06(0.99–1.13)
rs1865762	188062094	493	0.24	1.13(1.02–1.25)	271	0.20	0.34(0.06–1.80)	4618	0.43	1.03(0.95–1.11)	5382	0.42	0.88(0.68–1.15)
rs6444175	188062438	492	0.98	0.99(0.95–1.03)	272	0.11	1.40(0.93–2.09)	4526	0.47	1.01(0.99–1.02)	5290	0.31	1.06(0.99–1.13)
rs17373414	188068221	497	0.60	1.00(0.93–1.08)	272	0.20	0.41(0.1–1.62)	4700	0.74	0.98(0.92–1.05)	5469	0.67	0.86(0.70–1.07)

[a] Build NCBI36/hg18;

[b] IRASFS, Insulin Resistance Atherosclerosis Family Study;

[c] IRAS, Insulin Resistance Atherosclerosis Study;

[d] AA-DHS, African American Diabetes Heart Study;

[e] DHS, Diabetes Heart Study;

[f] WF T2D, Wake Forest Type 2 Diabetes;

g a strict Bonferroni correction (n=18 tests) would set a significance threshold p-value<0.0028;

h These SNPs were not included in the IRAS sample association with T2D because they were either monomorphic or contained only 1 individual with the variant