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Novel Loci for Adiponectin Levels and Their Influence on Type 2 Diabetes and Metabolic Traits: A Multi-Ethnic Meta-Analysis of 45,891 Individuals


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Circulating levels of adiponectin, a hormone produced predominantly by adipocytes, are highly heritable and are inversely associated with type 2 diabetes mellitus (T2D) and other metabolic traits. We conducted a meta-analysis of genome-wide association studies in 39,883 individuals of European ancestry to identify genes associated with metabolic disease. We identified 8 novel loci associated with adiponectin levels and confirmed 2 previously reported loci (P = 4.5 × 10⁻⁸, 1.2 × 10⁻⁴). Using a novel method to combine data across ethnicities (N = 4,232 African Americans, N = 1,776 Asians, and N = 29,347 Europeans), we identified two additional novel loci. Expression analyses of 436 human adipocyte samples revealed that mRNA levels of 18 genes at candidate regions were associated with adiponectin concentrations after accounting for multiple testing (p < 3 × 10⁻⁶). We next developed a multi-SNP genotypic risk score to test the association of adiponectin decreasing risk alleles on metabolic traits and diseases using consortium-level meta-analytic data. This risk score was associated with increased risk of T2D (p = 4.3 × 10⁻⁶, n = 22,044), increased triglycerides (p = 2.6 × 10⁻¹⁴, n = 93,440), increased waist-to-hip ratio (p = 1.8 × 10⁻⁶, n = 77,167), increased glucose two hours post oral glucose tolerance testing (p = 4.4 × 10⁻³, n = 15,234), increased fasting insulin (p = 0.015, n = 48,238), but with lower in HDL-cholesterol concentrations (p = 4.5 × 10⁻¹³, n = 96,748) and decreased BMI (p = 1.4 × 10⁻⁴, n = 121,335). These findings identify novel genetic determinants of adiponectin levels, which, taken together, influence risk of T2D and markers of insulin resistance.


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Introduction

Adiponectin is a highly abundant adipocyte-derived plasma protein whose levels correlate inversely with a range of important clinical parameters including blood glucose, indices of insulin resistance, proatherogenic dyslipidemia, and risk of type 2 diabetes (T2D), stroke and coronary artery disease [1,2,3,4]. Collectively these conditions account for most of the burgeoning pandemic of...
Adiponectin: Multiethnic Genome-Wide Meta-Analysis

Author Summary

Serum adiponectin levels are highly heritable and are inversely correlated with the risk of type 2 diabetes (T2D), coronary artery disease, stroke, and several metabolic traits. To identify common genetic variants associated with adiponectin levels and risk of T2D and metabolic traits, we conducted a meta-analysis of genome-wide association studies of 45,891 multi-ethnic individuals. In addition to confirming that variants at the ADIPOQ and CDH13 loci influence adiponectin levels, our analyses revealed that 10 new loci also affecting circulating adiponectin levels. We demonstrated that expression levels of several genes in these candidate regions are associated with serum adiponectin levels. Using a powerful novel method to assess the contribution of the identified variants with other traits using summary-level results from large-scale GWAS consortia, we provide evidence that the risk alleles for adiponectin play a mediating role in at least some of these obesity-related morbidity and mortality that poses a severe and highly prevalent human disease.[6,7]

Twins and family studies have revealed moderate to high estimates of heritability (30–70%) for plasma adiponectin levels [8,9,10,11]. However, until recently, few genes associated with adiponectin levels have been identified. Candidate and genome-wide association studies (GWAS) have shown pronounced associations between common polymorphisms in the adiponectin gene (ADIPOQ) and adiponectin levels [12,13,14,15]. A recent meta-analysis of three GWAS for adiponectin levels identified variants in a novel candidate gene, ARL5, that was associated with adiponectin levels, coronary heart disease (CHD), T2D and other metabolic traits [16]. Furthermore, CDH13 and KNG1 genes were found to be associated with adiponectin levels in two studies involving East Asian populations [17,18]. Although part of the variance explained by the ADIPOQ locus, most of the heritability of adiponectin levels remains unaccounted for. Therefore, we sought to identify novel common variants influencing adiponectin levels and test their association with risk of T2D and related metabolic traits within the framework of a large multi-ethnic consortium of GWAS.

We combined genome-wide association results of 35,355 individuals from three different ethnicities (white Europeans (n = 29,347), African American (n = 4,232) and East Asians (n = 1,776), applying a novel meta-analytic method to allow for heterogeneity in allelic effects between populations of different ethnic backgrounds. We next examined whether identified genome-wide significant single nucleotide polymorphisms (SNPs) were also associated with expression of their nearest gene in human adipocytes, the main source of adiponectin. Since adiponectin has also associated with expression of their nearest gene in human genome-wide significant single nucleotide polymorphisms (SNPs) at 16q23.2 (rs12922394[T]; β = 0.1, p-value = 3.60 x 10^-41), KNG1 (rs2062632[T]; β = 0.05, p-value = 2.52 x 10^-15) on 3q27.3, and CDH13 (rs12922394[T]; β = 0.1, p-value = 3.60 x 10^-41) on 16q23.3 (Table 1). Furthermore, we identified variants that showed genome-wide significant association in eight novel independent loci including rs9853056 (within the STAB1 gene, rs4282054 (within the NT5DC2 gene), rs13083798 (within the PRAM1 gene), rs1108842 (within the GNL3 gene), rs11235 (within the NEK1 gene), rs2710323 (within the ITIH1 gene), rs3617 (within the ITIH3 gene), and rs2535627 (within 200 Kb of ITIH4 gene) at 3p21.1; rs1597466 (within 1 Mb of TSC22D2 gene) at 3q25.1; rs2980879 (within 1 Mb of TRIB1 gene) at 8q24.13; rs7955516 (within 1.3 Mb of PDE3A gene) at 12p12.2; rs601339 (within the GPR109A gene) at 12q24.31; rs6488986 (within the ATP6V0D2 gene) at 7q13.37；and rs7978610 (within the ZNF664 gene) at 12q24.31, which is 1.3 Mb away from GPR109A; rs2952979 (within the CMIP gene at 16q25.22 gene); and rs731839 (within the PEPD gene) at 19q13.11 (Figure 2A–2E, Table 1).

In our analysis a common variant (rs601339, MAF = 0.18, allele G) downstream of the GPR109A gene (the putative nicotinic receptor) was associated with adiponectin (β = 0.04, p = 7.94 x 10^-10), and HDL-C (β = 0.03, p = 5.59 x 10^-7) in the global lipid analysis. In a coincident candidate gene analysis 11 SNPs were typed in GPR109A/B in CoLaus and LOLIPOP cohorts, containing individuals of European descent. A single nominally significant coding SNP R311C (rs7311976, MAF = 0.14) within the GPR109A gene was taken forward for replication and found to be consistently associated with adiponectin in the three cohorts (CoLaus, Fenland and MRC Ely), n = 8285, p = 4.6 x 10^-7) and HDL-cholesterol (HDL-C) in four cohorts (CoLaus, Fenland, Ely study and Lolipop, n = 18425, p = 2.9 x 10^-6) (Figure S2A, S2B). However R311C and rs601339 were not in linkage disequilibrium with each other (r2 ≤ 0.04). Therefore the two variants represent two independent signals from the same locus but with similar effects on HDL-cholesterol and adiponectin.

In silico follow-up phase. In the *in-silico* follow-up phase 468 SNPs demonstrating genome-wide significant (p<5 x 10^-8) or suggestive (p<5 x 10^-6) association with adiponectin in the discovery phase were tested for association in additional European cohorts. (Table S3). These SNPs were tested in seven additional GWAS datasets (n = 6,623) from NHS, HPFS, HABC, ERF2, LLS, GARP and ARIC studies and the combined meta-
analysis of the discovery and follow-up in-silico GWAS datasets detected additional loci on chromosomes 1q41 near the LPLA1 gene (rs3001032, p = 3.6 × 10^{-8}) and chromosome 6q21.1 near the VEGFA gene (rs9983894, p = 5.8 × 10^{-12}) that reached genome-wide significance. While we confirmed seven loci that had reached genome-wide significance at the discovery stage (Table 1, Figure 2F and 2G, Table S2), two identified loci (3q25.1 and 12q24.3) did not remain genome-wide significant in the joint analysis of discovery and follow-up results.

**De novo follow-up phase.** Next, in the de-novo genotyping follow-up phase, we genotyped 10 SNPs with suggestive evidence of association (5 × 10^{-6} < p < 5 × 10^{-5}) from the meta-analysis of the discovery and in-silico follow-up phases in an additional 3,913 individuals. Meta-analyzing the discovery and 2 follow-up stages identified a SNP in ARL15 (rs6450176 [G]; B = 0.026, p = 5.8 × 10^{-8}), which was initially described in a previous GWAS for adiponectin levels (Table S3) [16].

**Multi-ethnic meta-analysis.** To identify loci influencing adiponectin levels in non-European individuals we performed an additional analysis in 4,232 individuals from an African American population and 1,776 individuals from an East Asian population. In the African American populations, only associations at the ADIPOQ locus reached genome-wide significance, while in the East Asian population there was evidence of association at the ADIPOQ and CDH13 loci (Table S4). Subsequently, we performed a meta-analysis that combined all available GWAS including those of white European origin, African American and East Asian ancestry using novel method MANTRA [24]. This analysis identified two additional loci in or near IRS1 gene on 2q36.3 and at 6q24.1 within a gene desert (Table 2, Figure 1B).

**Secondary GWAS analyses.** We next performed meta-analysis of the GWAS data in women (n = 16,685) and men (n = 12,662) separately (Figure S2A, S2B, Tables S5 and S6). Although no novel loci reached genome-wide significance in women or men separately, three loci (chromosome 3p, 8 and 12) associated with adiponectin levels in the sex-combined analysis showed evidence of association (p value < 5 × 10^{-8}) in women (Figure S3). Since different assays were used to measure adiponectin levels, we next tested whether stratification by assay rendered similar results and found the results were highly concordant with the combined analysis. GWAS for high molecular weight adiponectin in the CHS study (n = 2,718) identified 2 SNPs in ADIPOQ (rs17300539, p = 3.0 × 10^{-16}) and CMIP (rs2927307, p = 2.7 × 10^{-8}). These two genes are located within the loci identified in our discovery meta-analysis of adiponectin levels.

**Gene Expression Studies.** Through gene expression studies we sought to address two questions: First, are any of the SNPs that were genome-wide significant for adiponectin levels associated with expression of their nearest transcripts (cis-eQTLs) and second, whether mRNA levels of loci identified through the GWAS for adiponectin levels are associated with circulating adiponectin levels. To address the first question, we examined whether SNPs within 1 Mb of the SNPs achieving genome-wide significance in the discovery stage were associated with the expression levels of nearby genes in human adipocytes from 776 participants of the MuTHER Consortium [25]. We identified 74 SNPs in three eQTLs to be associated with the expression of five genes in adipocytes, using an array-wide level of statistical significance for eQTLs (P < 5.1 × 10^{-5}). See Materials and Methods for details). These genes included NT5DC2 on chromosome 3; CCDC92, GPR109A, and ZNF664 on chromosome 12; and PEPE on chromosome 19 (Table 3). The cis-eQTL

<table>
<thead>
<tr>
<th>Table 1. Lead SNP per Locus for Genome-Wide Significant SNPs Arising from the Sex-Combined Meta-Analysis in European Populations.</th>
<th>Nearest**</th>
<th>Gene</th>
<th>Lead SNP†</th>
<th>Region</th>
<th>Chr/position¶</th>
<th>EA/NEA*</th>
<th>EAF‡</th>
<th>Beta§</th>
<th>SE</th>
<th>P</th>
<th>I2</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discovery Phase Results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPLA1</td>
<td>rs3001032</td>
<td>1q41</td>
<td>1/21779402</td>
<td>T/C</td>
<td>0.7</td>
<td>−0.02</td>
<td>0.005</td>
<td>1.98E-06</td>
<td>0</td>
<td>29,321</td>
<td>−0.02</td>
<td>0.004</td>
</tr>
<tr>
<td>GNL3</td>
<td>rs1108842</td>
<td>3p21.1</td>
<td>3/52695120</td>
<td>C/A</td>
<td>0.50</td>
<td>0.03</td>
<td>0.004</td>
<td>3.66E-11</td>
<td>0.33</td>
<td>29,338</td>
<td>0.03</td>
<td>0.004</td>
</tr>
<tr>
<td>TSC22D2</td>
<td>rs1597466</td>
<td>3q25.1</td>
<td>3/151538251</td>
<td>T/G</td>
<td>0.1</td>
<td>−0.04</td>
<td>0.008</td>
<td>1.88E-08</td>
<td>0</td>
<td>29,319</td>
<td>−0.03</td>
<td>0.007</td>
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<td>ADIPOQ</td>
<td>rs5810075</td>
<td>3q27.3</td>
<td>3/188031259</td>
<td>T/C</td>
<td>0.6</td>
<td>0.06</td>
<td>0.005</td>
<td>3.60E-41</td>
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<td>29,140</td>
<td>0.06</td>
<td>0.004</td>
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<tr>
<td>VEGFA</td>
<td>rs985884</td>
<td>6q21.1</td>
<td>6/43865874</td>
<td>T/G</td>
<td>0.5</td>
<td>0.03</td>
<td>0.005</td>
<td>5.84E-08</td>
<td>0.3</td>
<td>28,167</td>
<td>0.03</td>
<td>0.005</td>
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<tr>
<td>TRIB1</td>
<td>rs2980879</td>
<td>8q24.13</td>
<td>8/126550657</td>
<td>T/A</td>
<td>0.7</td>
<td>0.03</td>
<td>0.005</td>
<td>1.08E-08</td>
<td>0</td>
<td>24,084</td>
<td>0.03</td>
<td>0.005</td>
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<tr>
<td>PDE4A</td>
<td>rs7955516</td>
<td>12q12.2</td>
<td>12/20389303</td>
<td>C/A</td>
<td>0.4</td>
<td>0.03</td>
<td>0.005</td>
<td>2.43E-08</td>
<td>0.1</td>
<td>29,178</td>
<td>0.02</td>
<td>0.004</td>
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<tr>
<td>GPR109A</td>
<td>rs601339</td>
<td>12q24.31</td>
<td>12/121740696</td>
<td>G/A</td>
<td>0.2</td>
<td>0.04</td>
<td>0.006</td>
<td>3.87E-11</td>
<td>0</td>
<td>29,325</td>
<td>0.03</td>
<td>0.005</td>
</tr>
<tr>
<td>DNAH10</td>
<td>rs7133378</td>
<td>12q24.31</td>
<td>12/122975455</td>
<td>T/G</td>
<td>0.7</td>
<td>−0.03</td>
<td>0.005</td>
<td>1.29E-09</td>
<td>0</td>
<td>29,223</td>
<td>−0.02</td>
<td>0.004</td>
</tr>
<tr>
<td>CMIP</td>
<td>rs2925979</td>
<td>16q23.2</td>
<td>16/80922911</td>
<td>G/A</td>
<td>0.3</td>
<td>−0.04</td>
<td>0.005</td>
<td>1.87E-18</td>
<td>0</td>
<td>29,347</td>
<td>−0.04</td>
<td>0.005</td>
</tr>
<tr>
<td>CDH13</td>
<td>rs12292394</td>
<td>16q23.3</td>
<td>16/81229828</td>
<td>T/C</td>
<td>0.1</td>
<td>−0.10</td>
<td>0.011</td>
<td>3.16E-18</td>
<td>0.3</td>
<td>24,466</td>
<td>−0.08</td>
<td>0.010</td>
</tr>
<tr>
<td>PEPE</td>
<td>rs7318389</td>
<td>19q13.11</td>
<td>19/38590905</td>
<td>G/A</td>
<td>0.3</td>
<td>−0.04</td>
<td>0.005</td>
<td>2.20E-03</td>
<td>0.03</td>
<td>29,166</td>
<td>−0.03</td>
<td>0.004</td>
</tr>
</tbody>
</table>

All SNPs achieving genome-wide significance in the joint analysis phase are marked in italics.

*Joint analysis indicates results from the meta-analysis of discovery and follow-up in-silico and de-novo phases.
**When possible, plausible biological candidate genes have been listed; otherwise, the closest gene is designated.
†Lead SNP is the SNP with the lowest p-value for each locus.
¶Betas are estimated from models using the natural log transformed adiponectin.
*EA: Effect allele, NEA: Non-effect allele.
‡EAF: Effect allele frequency.
§P-values.
SNPs often are proxies for the lead SNPs from the GWAS, however, this relationship may also be influenced through mechanisms that are independent from gene expression, such as gene function.

We next identified that mRNA levels of 18 genes arising from six candidate loci were correlated with circulating adiponectin levels (Table 4). Since circulating adiponectin levels may be associated with a surplus of adipocyte transcripts we next tested for enrichment of signal from the candidate loci. There were 133 transcripts in the identified candidate regions, of which 8.2% (11/133) were associated with adiponectin levels at an array-wide level of significance ($p = 5 \times 10^{-6}$), while 7.5% of the 24k probes on the entire array exceeded the same $p$-value threshold, indicating there was therefore no additional enrichment of signal at these candidate loci.

**T2D and Metabolic Traits**

Using data from several large-scale GWAS consortia, some of the significantly associated variants identified here demonstrated associations with T2D and its related traits (Table S7A, S7B, S7C, and S7D). Several individual SNPs showed evidence for association with T2D and various metabolic traits after accounting for the number of statistically independent SNPs ($p$-value threshold of $5 \times 10^{-4}$) among the SNPs that were genome-wide significant for adiponectin. These include associations with HDL-C ($n = 104$ SNPs), triglycerides (TG) ($n = 65$ SNPs), total cholesterol (TC, $n = 12$ SNPs), LDL-cholesterol (LDL–C, $n = 11$ SNPs), and waist-hip ratio (WHR) ($n = 65$ SNPs) [26]. (However, we note that since sample sizes are different across different consortia power to identify associations is not consistent.) Among these, coding and intronic variants in *STAB1* and *NT5DC2* genes were associated with WHR and HDL-C, while the variants 1 Mb near *TRIB1* were associated with all lipid traits. The coding and intronic variants ariants in the locus on chromosome 12 harboring ZNF664, CCDC92, and DNAH10 showed evidence of association with WHR, HDL-C, and TG. Finally, variants in the *PEPD* gene were associated with TG.

We next calculated a multi-SNP genotypic risk score based genome-wide significant SNPs from the discovery phase. This multi-SNP genotypic risk score explained 5% of the variance of natural log-transformed adiponectin levels. We then tested the association of this risk score with risk of T2D and metabolic related traits. The multi-SNP genotypic risk score was associated with increased risk for T2D ($\beta = 0.3$, $p = 4.3 \times 10^{-5}$), where $\beta$ is the average additive effect of adiponectin-decreasing risk alleles on the
Figure 2. Regional plots of eight newly discovered genome-wide significant chromosomal regions associated with adiponectin concentrations in European populations. A) chromosome 16q23.2, B) chromosome 19 q13.11 C) Chromosome 3p21.1, D) two loci on chromosome 12q24.31, E) chromosome 8q24.13, F) chromosome 6p21.1, and G) chromosome 1q41. In each panel, purple diamonds indicate the top
SNPs, which have the strongest evidence of association. Each circle shows a SNP with a color scale relating the \( r^2 \) value for that SNP and the top SNP from HapMap CEU. Blue lines indicate estimated recombination rates from HapMap. The bottom panels illustrate the relative position of genes near each locus. Candidate genes are indicated by red ovals.

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log odds ratio of T2D), increased TG (\( \beta = 0.25, p = 2.6 \times 10^{-14} \))

Discussion

In this comprehensive multi-ethnic analysis of the genetic influences on adiponectin levels and their impact on metabolic traits and T2D, we have identified 10 novel loci and confirmed the associations of variants in the ADIPOQ and CDH13 loci with adiponectin levels. The adiponectin risk alleles were associated with T2D and related metabolic traits such as BMI, WHR, TG, HDL-C, 2-hour glucose, HOMA-IR and fasting insulin. These findings demonstrate that adiponectin, T2D and metabolic syndrome have a shared allelic architecture.

Biological Relevance of the GWAS Loci

In the first step toward understanding the biological relevance of the identified regions, we examined the genes harbored by the associated loci using human disease and animal databases. Although some of the genes in these loci do not have a known function, several signify diverse biological functions.

On chromosome 1, the lead SNP was located 300 kb from the LIPMALS gene, a protein that regulates phospholipids on cellular membranes. Independent efforts have also identified this locus in other metabolic/obesity related traits GWAS: first with BMI (rs2605100; \( p = 0.49 \) [21]), and rs4846567; \( p = 0.55 \) [27] respectively with the lead adiponectin SNP, rs3001032), and more recently with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication). In the same report by MAGIC, variants near IRS1 (insulin receptor substrate 1) and PEPI (a protein that hydrolyzes dipeptides and tripeptides) have also been associated with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication). In the same report by MAGIC, variants near IRS1 (insulin receptor substrate 1) and PEPI (a protein that hydrolyzes dipeptides and tripeptides) have also been associated with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication). In the same report by MAGIC, variants near IRS1 (insulin receptor substrate 1) and PEPI (a protein that hydrolyzes dipeptides and tripeptides) have also been associated with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication). In the same report by MAGIC, variants near IRS1 (insulin receptor substrate 1) and PEPI (a protein that hydrolyzes dipeptides and tripeptides) have also been associated with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication). In the same report by MAGIC, variants near IRS1 (insulin receptor substrate 1) and PEPI (a protein that hydrolyzes dipeptides and tripeptides) have also been associated with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication). In the same report by MAGIC, variants near IRS1 (insulin receptor substrate 1) and PEPI (a protein that hydrolyzes dipeptides and tripeptides) have also been associated with fasting insulin by a joint meta-analysis including the interaction between SNP and BMI (MF Hivert for the MAGIC investigators, personal communication).

The lead SNP at 3p21.1 falls within a region that is located in a genomic region containing many genes which could have potential functions in metabolism. Our data provide evidence that adiponectin levels were correlated with human adipocyte mRNAS levels of many genes in this region (GLYCTK, SEM1G, STAB1, PBRM1, SMBT1; see Table 4). However, this association does not imply a direct influence of these genes on adiponectin level. Among those genes, STAB1 encodes for stabilin 1, described as an endocytic receptor for advanced glycation end products and may have a function in angiogenesis, lymphocyte homing, cell adhesion, or receptor scavenging for acetylated low-density lipoprotein [30].

Interestingly, several of the genes near lead genome-wide significant SNPs have been implicated in angiogenesis, which might be important for adipose tissue expansion, highlighting the recurring theme of “adipose tissue expandability’’ in the genetic origins of obesity-related complications [31]. For example, VEGFA is the vascular endothelial growth factor A gene, known in a variety of vascular endothelial cell functions, such as angiogenesis and maintenance of the glomerular endothelium in nephrons [32]. Variants in this gene are also associated with diabetic retinopathy and WHR [27,33]. Moreover, the product of VEGFA interacts with resveratrol, which has been shown to have a beneficial influence in some metabolic traits, including diabetes [34]. Rodent studies show that resveratrol decreases blood glucose, blood insulin, and glycated hemoglobin, as well as increases insulin sensitivity in animals with hyperglycemia (reviewed in [35]). Resveratrol also inhibits TNF-a-induced reductions in adiponectin levels in 3T3-L1 adipocytes [36]. Furthermore, it has been shown that resveratrol modulates adiponectin expression and improves insulin sensitivity, likely through the inhibition of inflammatory-like response in adipocytes [37]. At this locus, VEGFA mRNA levels in adipocytes were the strongest association with adiponectin levels (Table 4). Also likely involved in vascular biology, TRIB1 encodes for a protein-coupled receptor-induced protein interacting with MAP kinases that regulates proliferation and chemotaxis of vascular smooth muscle cells [38]. TRIB1 expression was shown to be elevated in human atherosclerotic arteries [39]. Several variants (rs2954029, rs2954021, rs17321515; all in moderate LD with our lead SNP) in the TRIB1 gene have been associated with HDL-C, LDL-C and CHD risk in European and Asian populations [22,40,41,42,43]. These two loci (TRIB1 and VEGFA) argue for the importance of vascular biology in adiponectin regulation as underlined previously by findings of adiponectin levels associated with variants near CDH13 (a receptor for adiponectin expressed by endothelial smooth muscle) [44].

All three homologous genes GPR109A/B/81 are located on chromosome 12 are predominantly expressed in adipocytes and mediate anti-inflammatory effects [45]. Our eQTL results (Table 3) and the correlation between mRNA and adiponectin levels (Table 4) argue strongly for a role of GPR109A at this locus. GPR109A (also known as NiAcR1) is a receptor with a high-affinity, concentration-dependent response to nicotinic acid (niacin) [45]. Treatment by niacin increases serum adiponectin levels by up to 94% in obese men with metabolic syndrome in a time- and dose-dependent manner [46]. Functional studies in GPR109A receptor knockout mice demonstrate that niacin increases serum total and HMW adiponectin concentrations and decreases lipolysis following GPR109A receptor activation [47]. Moreover, a recent meta-analysis on cohorts containing extremes of HDL-C provided evidence suggestive of association in GPR109A/B/81 [48].

Finally, variants in ZNF664 have been associated with CHD, HDL-C and TG levels in a large meta-analysis of over 100,000 individuals of European ancestry [22]. The sex heterogeneity observed in this study is comparable to our finding that the more loci associated with adiponectin at genome wide significance level have been shown in female stratified analysis.

Taken together, the loci identified in this large-scale GWAS for adiponectin levels highlight many genes with demonstrated relationships with metabolic disease.

Shared Allelic Architecture of Adiponectin Levels and Metabolic Traits

Using a multi-SNP genotypic risk score we attempted to understand if the allelic architecture of adiponectin levels was shared with T2D and metabolic traits. This risk score was...
### Table 2. Genome-Wide Significant SNPs from the Sex-Combined Multi-Ethnic Meta-Analysis.

<table>
<thead>
<tr>
<th>Nearby* Gene</th>
<th>Lead SNP;</th>
<th>Gene region chr/position†</th>
<th>EA/NEA*</th>
<th>EAF†† (CEU/EA/AA)</th>
<th>Multi-Ethnic Fixed Effects Meta-analysis Beta (SE)</th>
<th>pvalue</th>
<th>Q-Value</th>
<th>I2</th>
<th>Multi-Ethnic Random Effects Meta-analysis Beta (SE)</th>
<th>pvalue</th>
<th>BF ‡</th>
<th>phet‡</th>
<th>MANTRA</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYPLAL1</td>
<td>rs2791553</td>
<td>1q41 1/217742665</td>
<td>G/A</td>
<td>0.6/0.46/0.54</td>
<td>-0.02(0.004)</td>
<td>4.91E-07</td>
<td>25.18</td>
<td>0</td>
<td>-0.02(0.004)</td>
<td>4.91E-07</td>
<td>6.3</td>
<td>0.06</td>
<td>37,665</td>
<td></td>
</tr>
<tr>
<td>IRS1</td>
<td>rs925735</td>
<td>2q36.3 2/226887874</td>
<td>G/C</td>
<td>0.64/0.89/0.74</td>
<td>-0.02(0.004)</td>
<td>1.88E-08</td>
<td>22.15</td>
<td>0.01</td>
<td>-0.02(0.004)</td>
<td>2.12E-08</td>
<td>8.1</td>
<td>0.06</td>
<td>37,638</td>
<td></td>
</tr>
<tr>
<td>GNL3</td>
<td>rs2590838</td>
<td>3p21.1 3/52597126</td>
<td>G/A</td>
<td>0.5 1/0.34/0.54</td>
<td>-0.03(0.004)</td>
<td>4.08E-15</td>
<td>28.85</td>
<td>0.06</td>
<td>-0.03(0.004)</td>
<td>1.88E-13</td>
<td>14.1</td>
<td>0.05</td>
<td>37,680</td>
<td></td>
</tr>
<tr>
<td>ADIPOQ</td>
<td>rs6810075</td>
<td>3q27.3 3/188031259</td>
<td>T/C</td>
<td>0.93/1/0.86</td>
<td>0.06(0.004)</td>
<td>1.10E-43</td>
<td>27.44</td>
<td>0.02</td>
<td>0.06(0.004)</td>
<td>2.41E-42</td>
<td>43.6</td>
<td>0.16</td>
<td>31,533</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>rs592423</td>
<td>6q24.1 6/139882386</td>
<td>C/A</td>
<td>0.54/0.36/0.41</td>
<td>0.02(0.004)</td>
<td>3.59E-07</td>
<td>15.46</td>
<td>0</td>
<td>0.02(0.004)</td>
<td>3.59E-07</td>
<td>6.5</td>
<td>0.03</td>
<td>37,430</td>
<td></td>
</tr>
<tr>
<td>TRB1</td>
<td>rs2980179</td>
<td>8q24.13 8/126550657</td>
<td>T/A</td>
<td>0.69/0.77/0.67</td>
<td>0.03(0.004)</td>
<td>9.91E-10</td>
<td>21.08</td>
<td>0</td>
<td>0.03(0.004)</td>
<td>9.91E-10</td>
<td>8.2</td>
<td>0.04</td>
<td>32,426</td>
<td></td>
</tr>
<tr>
<td>GPR109A</td>
<td>rs611339</td>
<td>12q24.31 12/121740696</td>
<td>G/A</td>
<td>0.19/0.39/0.31</td>
<td>0.03(0.005)</td>
<td>3.77E-09</td>
<td>36.11</td>
<td>0.25</td>
<td>0.03(0.006)</td>
<td>4.31E-06</td>
<td>8.3</td>
<td>0.09</td>
<td>37,666</td>
<td></td>
</tr>
<tr>
<td>CMP</td>
<td>rs2952979</td>
<td>16q23.2 16/80092291</td>
<td>T/C</td>
<td>0.3 0.43/0.31</td>
<td>-0.04(0.004)</td>
<td>3.12E-21</td>
<td>23.12</td>
<td>0</td>
<td>-0.04(0.004)</td>
<td>3.12E-21</td>
<td>19.8</td>
<td>0.31</td>
<td>37,687</td>
<td></td>
</tr>
<tr>
<td>CDH13</td>
<td>rs12051272</td>
<td>16q23.3 16/81220789</td>
<td>T/G</td>
<td>0.03/0.33/0.03</td>
<td>-0.26(0.017)</td>
<td>4.74E-51</td>
<td>39.17</td>
<td>0.62</td>
<td>-0.26(0.032)</td>
<td>1.10E-14</td>
<td>66.0</td>
<td>1.00</td>
<td>24,216</td>
<td></td>
</tr>
<tr>
<td>PEPD</td>
<td>rs4805885</td>
<td>19q13.11 19/38597963</td>
<td>T/C</td>
<td>0.39/0.64/0.41</td>
<td>-0.03(0.004)</td>
<td>1.65E-11</td>
<td>34.94</td>
<td>0.23</td>
<td>-0.03(0.005)</td>
<td>2.05E-08</td>
<td>9.9</td>
<td>0.05</td>
<td>37,479</td>
<td></td>
</tr>
</tbody>
</table>

The novel loci identified using Multi-Ethnic Meta-analysis (that were not identified in the European only analysis) are listed in **bold**.

*When possible, plausible biological candidate genes have been listed; otherwise, the closest gene is designated.

†Lead SNP is the SNP with the lowest \( p \)-value for each locus.

‡Positions are relative to Human Genome NCBI Build 36.

§\( \log_{10} \) Bayes factor (BF) from the MANTRA analysis. A \( \log_{10} \) BF of 6 and higher was considered as a conservative threshold for genome-wide significance.

††The posterior probability of heterogeneity between studies.

*EA: effect allele, NEA: non-effect allele.

**EAF: Frequency of effect allele in CEU, East Asian, and AA, populations respectively.

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associated with increased risk of T2D and traits associated with insulin resistance and the metabolic syndrome. However, unexpectedly, adiponectin decreasing alleles were associated with a decrease in BMI. In our adiponectin GWAS, BMI was included as a covariate in order to avoid direct identification of obesity SNPs since BMI is strongly related to adiponectin levels [49,50]. Furthermore, this unexpected direction of effect was entirely explained by SNPs at the ZNF664 and PEPD loci; when these loci were removed from the analysis, the association of the genotypic risk score with BMI disappeared (results not shown). Therefore, adiponectin risk alleles at ZNF664 and PEPD are of considerable interest since they impart deleterious changes on aspects of the metabolic syndrome (increased TC, TG, LDL-C and WHR and decreased HDL-C), but also act to decrease BMI and percent fat.

Table 3. The Association of Lead Genome-Wide Significant SNPs for Adiponectin with mRNA Levels of Their Nearest Gene.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lead SNP-Cis-eQTL Chr</th>
<th>Transcript Start Site</th>
<th>Transcript End Site</th>
<th>EA†</th>
<th>EAF‡</th>
<th>Beta (SE)</th>
<th>P-Exp*</th>
<th>P-GWAS**</th>
<th>lead SNP-GWAS††</th>
<th>r²‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT5DC2</td>
<td>rs130810283 3</td>
<td>52533424</td>
<td>52544133</td>
<td>G</td>
<td>0.444</td>
<td>0.14(0.02)</td>
<td>1.32E-19</td>
<td>1.05E-09</td>
<td>rs1108842</td>
<td>0.84</td>
</tr>
<tr>
<td>GPR109A</td>
<td>rs2454722* 12</td>
<td>121771085</td>
<td>121781028</td>
<td>G</td>
<td>0.166</td>
<td>–0.15(0.03)</td>
<td>1.71E-09</td>
<td>3.87E-11</td>
<td>rs601339</td>
<td>1</td>
</tr>
<tr>
<td>CCD92</td>
<td>rs10773049 12</td>
<td>122996907</td>
<td>123023116</td>
<td>T</td>
<td>0.611</td>
<td>0.15(0.02)</td>
<td>8.09E-22</td>
<td>2.67E-08</td>
<td>rs7133378</td>
<td>0.02</td>
</tr>
<tr>
<td>ZNF664</td>
<td>rs825453 12</td>
<td>123074711</td>
<td>123065922</td>
<td>T</td>
<td>0.615</td>
<td>–0.04(0.01)</td>
<td>4.51E-05</td>
<td>4.03E-08</td>
<td>rs7978610</td>
<td>0.03</td>
</tr>
<tr>
<td>PEPD</td>
<td>rs8182584 19</td>
<td>38506964</td>
<td>38704639</td>
<td>T</td>
<td>0.364</td>
<td>–0.13(0.02)</td>
<td>9.96E-10</td>
<td>6.64E-11</td>
<td>rs731839</td>
<td>1</td>
</tr>
</tbody>
</table>

†Lead SNP is the SNP with the lowest p-value for each gene in gene expression data.
‡EAF: Frequency of effect allele.
*P value for lead SNP is the SNP in gene expression data.
**P value for lead SNP from meta-analysis from discovery phase.
‖LD between lead SNP from expression and lead SNP from meta-analysis.

Table 4. The Association of mRNA Levels from Genes in Candidate Loci in Human Adipocytes with Circulating Adiponectin Levels.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene region</th>
<th>GeneStart</th>
<th>GeneEnd</th>
<th>Beta‡</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLYCTK</td>
<td>3p21.1</td>
<td>52296875</td>
<td>52304311</td>
<td>0.060</td>
<td>1.77E-20</td>
</tr>
<tr>
<td>SEMA3G</td>
<td>3p21.1</td>
<td>52442307</td>
<td>52454083</td>
<td>–0.018</td>
<td>9.28E-06</td>
</tr>
<tr>
<td>STAB1</td>
<td>3p21.1</td>
<td>52504395</td>
<td>52533551</td>
<td>–0.039</td>
<td>2.26E-14</td>
</tr>
<tr>
<td>PBK</td>
<td>3p21.1</td>
<td>52554407</td>
<td>52688779</td>
<td>0.007</td>
<td>2.49E-04</td>
</tr>
<tr>
<td>SFMBT1</td>
<td>3p21.1</td>
<td>52913666</td>
<td>53055110</td>
<td>0.010</td>
<td>2.53E-08</td>
</tr>
<tr>
<td>DNAB1</td>
<td>3q27.3</td>
<td>18777160</td>
<td>18778283</td>
<td>–0.014</td>
<td>3.31E-07</td>
</tr>
<tr>
<td>EIF4A2</td>
<td>3q27.3</td>
<td>187984054</td>
<td>18799379</td>
<td>0.021</td>
<td>1.53E-08</td>
</tr>
<tr>
<td>ADIPQO</td>
<td>3q27.3</td>
<td>188043156</td>
<td>188058944</td>
<td>0.054</td>
<td>1.03E-13</td>
</tr>
<tr>
<td>MAD2L1BP</td>
<td>6q21.1</td>
<td>43711554</td>
<td>43761666</td>
<td>0.009</td>
<td>4.09E-09</td>
</tr>
<tr>
<td>VEGFA</td>
<td>6q21.1</td>
<td>43845923</td>
<td>43862199</td>
<td>0.012</td>
<td>2.15E-09</td>
</tr>
<tr>
<td>ZCCH8</td>
<td>12q24.31*</td>
<td>121523387</td>
<td>121551471</td>
<td>0.011</td>
<td>2.60E-04</td>
</tr>
<tr>
<td>GPR109A</td>
<td>12q24.31</td>
<td>121765255</td>
<td>121767392</td>
<td>0.010</td>
<td>3.74E-06</td>
</tr>
<tr>
<td>GPR109A</td>
<td>12q24.31</td>
<td>121778105</td>
<td>121781082</td>
<td>0.026</td>
<td>1.80E-11</td>
</tr>
<tr>
<td>PITPNM2</td>
<td>12q24.31*</td>
<td>122033972</td>
<td>122068287</td>
<td>0.011</td>
<td>2.68E-04</td>
</tr>
<tr>
<td>U1SRRNBPB</td>
<td>12q24.31</td>
<td>122508604</td>
<td>122516289</td>
<td>0.001</td>
<td>5.90E-06</td>
</tr>
<tr>
<td>ATP6V0A2</td>
<td>12q24.31</td>
<td>122762817</td>
<td>122812252</td>
<td>0.008</td>
<td>5.90E-06</td>
</tr>
<tr>
<td>ZNF664</td>
<td>12q24.31</td>
<td>123023622</td>
<td>123065922</td>
<td>0.010</td>
<td>8.28E-06</td>
</tr>
<tr>
<td>SLCA1A</td>
<td>19q13.11</td>
<td>38391409</td>
<td>38408596</td>
<td>0.072</td>
<td>1.66E-14</td>
</tr>
</tbody>
</table>

*These two loci are independent loci.

Adiponectin: Multiethnic Genome-Wide Meta-Analysis

In conclusion, the data presented in this study provide strong evidence of association for 10 novel loci for adiponectin levels.
Our study consisted of three stages. First, in the discovery stage we performed a meta-analysis of the GWAS summary statistics of 16 studies involving 29,347 participants of white European origin to detect SNPs that are associated with adiponectin levels. All signals with \( p < 5 \times 10^{-6} \) were followed up in seven additional cohorts \( (n = 6,623) \) with GWAS data (in-silico phase) that later joined the consortium and then a subset of SNPs \( (n = 10) \) by de-novo genotyping in 3,913 additional participants from three cohorts \( (n = 39,883) \) for the combined analysis in Europeans. We also performed a multi-ethnic meta-analysis by combining summary statistics from the 16 studies of individuals of white European origin to obtain a total 35,355 individuals for the multi-ethnic meta-analysis involving different ethnicities. After identifying variation near two genes of pharmaceutical importance (GPR109A and GPR109B), which encode the putative niacin receptors, we typed additional rare coding and tagging variants in a subset of cohorts. Second, we examined whether the identified SNPs of the first stage also associate with mRNA levels of nearest gene(s) expressed using adipose tissue of 776 European women. We also tested for association between adiponectin levels and mRNA levels of the genes in our candidate loci in adipose tissue of a subgroup of 436 individuals [25]. Third, we calculated a multi-SNP genotypic risk score using genome-wide significant adiponectin-lowering alleles and tested the association of this risk score with T2D and related metabolic traits. Figure 3 shows a flow chart detailing the study design.

**Materials and Methods**

**Ethical Consideration**

All participants provided informed written consent. The research protocol of all studies were reviewed and approved by institutional ethics review committees at the involved institutions.

**Study Design**

Our study consisted of three stages. First, in the discovery stage we performed a meta-analysis of the GWAS summary statistics of 16 studies involving 29,347 participants of white European origin to detect SNPs that are associated with adiponectin levels. All signals with \( p < 5 \times 10^{-6} \) were followed up in seven additional cohorts \( (n = 6,623) \) with GWAS data (in-silico phase) that later joined the consortium and then a subset of SNPs \( (n = 10) \) by de-novo genotyping in 3,913 additional participants from three cohorts \( (n = 39,883) \) for the combined analysis in Europeans. We also performed a multi-ethnic meta-analysis by combining summary statistics from the 16 studies of individuals of white European discovery cohorts \( (n = 29,347) \) with those of five cohort studies that included African Americans subjects \( (n = 4,232) \) and one East Asian cohort \( (n = 1,776) \) to obtain a total 35,355 individuals for the GWAS meta-analysis involving different ethnicities. After identifying variation near two genes of pharmaceutical importance (GPR109A and GPR109B), which encode the putative niacin receptors, we typed additional rare coding and tagging variants in a subset of cohorts. Second, we examined whether the identified SNPs of the first stage also associate with mRNA levels of nearest gene(s) expressed using adipose tissue of 776 European women. We also tested for association between adiponectin levels and mRNA levels of the genes in our candidate loci in adipose tissue of a subgroup of 436 individuals [25]. Third, we calculated a multi-SNP genotypic risk score using genome-wide significant adiponectin-lowering alleles and tested the association of this risk score with T2D and related metabolic traits. Figure 3 shows a flow chart detailing the study design.

**Study Populations**

In total, 45,891 individuals from 26 European and 7 non-European cohorts participated in the different phases of this meta-analysis. Participating cohorts were either population-based \( (n = 23) \), family-based \( (n = 4) \), or case-control \( (n = 4) \) studies. The age of participants ranged from 10 to 95 years. Adiponectin levels were measured using ELISA or RIA methods. More details on the study cohorts and adiponectin measurement are presented in the Text S1 and Table S1. In addition, genotyping of four coding and tagging SNPs in the candidate genes, GPR109A and GPR109B, was undertaken in samples from the Lausanne, Lolipop, MRC Ely, and Fenland cohorts.

**Genotyping and Imputation**

All cohorts were genotyped using commercially available Affymetrix or Illumina genome-wide genotyping arrays. Quality control was performed for each study independently and genotype imputation was carried out using IMPUTE, MACH, BimBam or Beagle with reference to either the Phase II CEU, CEU+YRI, or CHB+JPT+CEU HapMap according to the origin of population. Imputation of East Asian genotypes was undertaken by first masking genotypes of 200 SNPs and then imputing them based on the CEU+CHB+JPT panel from HapMap. This resulted in an allelic concordance rate of \( 96.7\% \). For the African Americans, a combined CEU+YRI reference panel was created. This panel included SNPs segregating in both CEU and YRI, as well as SNPs

---

**Table 5. Results of Association of Multi-SNP Genotypic Risk Score with Diabetes and Related Traits.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>Effect (95% CI)</th>
<th>P</th>
<th>Consortium</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2D**</td>
<td>22,044</td>
<td>0.301 (0.09, 0.51)</td>
<td>4.3E-03</td>
<td>DIAGRAM+</td>
</tr>
<tr>
<td>BMI (SD units)</td>
<td>121,335</td>
<td>-0.162 (-0.25, -0.08)</td>
<td>1.4E-04</td>
<td>GIANT</td>
</tr>
<tr>
<td>WHR*</td>
<td>77,167</td>
<td>0.177 (0.1, 0.26)</td>
<td>1.8E-05</td>
<td>GIANT</td>
</tr>
<tr>
<td>Percent Fat</td>
<td>34,853</td>
<td>-0.052 (-0.15, 0.05)</td>
<td>0.31</td>
<td>Body Fat Percent</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>46,186</td>
<td>0.011 (-0.03, 0.05)</td>
<td>0.58</td>
<td>MAGIC</td>
</tr>
<tr>
<td>Fasting Insulin**(pmol/L)</td>
<td>38,238</td>
<td>0.05 (0.01, 0.09)</td>
<td>1.5E-02</td>
<td>MAGIC</td>
</tr>
<tr>
<td>HomaB</td>
<td>36,466</td>
<td>0.033 (0, 0.07)</td>
<td>5.1E-02</td>
<td>MAGIC</td>
</tr>
<tr>
<td>Homa IR</td>
<td>37,037</td>
<td>0.042 (0, 0.08)</td>
<td>4.7E-02</td>
<td>MAGIC</td>
</tr>
<tr>
<td>2hr Glucose**(mmol/L)</td>
<td>15,234</td>
<td>0.245 (0.06, 0.44)</td>
<td>1.1E-02</td>
<td>MAGIC</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>35,908</td>
<td>-0.002 (-0.04, 0.03)</td>
<td>0.91</td>
<td>MAGIC</td>
</tr>
<tr>
<td>TG**(SD units)</td>
<td>93,440</td>
<td>0.248 (0.18, 0.31)</td>
<td>2.6E-14</td>
<td>GLGC</td>
</tr>
<tr>
<td>HDL-C**(SD units)</td>
<td>96,748</td>
<td>-0.243 (-0.31, -0.18)</td>
<td>4.5E-13</td>
<td>GLGC</td>
</tr>
<tr>
<td>LDL-C (SD units)</td>
<td>92,348</td>
<td>0.023 (-0.05, 0.09)</td>
<td>0.52</td>
<td>GLGC</td>
</tr>
<tr>
<td>TC (SD units)</td>
<td>97,021</td>
<td>0.0003 (-0.07, 0.07)</td>
<td>0.99</td>
<td>GLGC</td>
</tr>
</tbody>
</table>


1 Effect is mean change in trait or disease per adiponectin-decreasing allele.

2 Waist to hip ratio adjusted for BMI.

3 Significantly associated trait is coded in bold.

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Further analyses confirmed that the level of expression of some of these candidate genes in human adipocytes correlated directly with adiponectin levels. A multi-SNP genotypic risk score, and several of the identified variants, directly influence parameters of the metabolic syndrome and, in particular, markers of insulin resistance. These findings identify novel genetic determinants of adiponectin levels, which, taken together, influence risk of T2D and markers of insulin resistance.
segregating in one panel and monomorphic and non-missing in the other (2.74 million SNPs). Due to the overlap of African American individuals on the Affymetrix 6.0 and IBC arrays [51], it was possible to analyze imputation performance at SNPs not genotyped on Affymetrix 6.0. For imputation based on Affymetrix data, the use of the CEU+YRI panel resulted in an allelic concordance rate of ~95.6% (calculated as 1−0.5 * [imputed−dosage−chip dosage]). This rate is comparable to rates calculated for individuals of African descent imputed with the HapMap 2 YRI individuals. Table S1 summarizes the genotyping methods used for each cohort, genotype-calling algorithms, imputation algorithms and exclusion thresholds. SNP-level quality control metrics were applied prior to meta-analysis for each cohort. These were: call rate ≥95%, minor allele frequency (MAF) ≥1%, Hardy-Weinberg equilibrium (HWE) p > 10−5, and quality measures for imputed SNPs (r2 ≥ 0.3, or proper info ≥ 0.4, for cohorts imputing their data with MACH and IMPUTE, respectively).

Eleven coding and tagging variants in two candidate genes of pharmaceutical importance (GPR109A encoding the niacin receptor and GPR109B) were genotyped in a parallel study in Lausanne, MRC Ely, and Fenland white subjects. Genotyping was performed using a KASPar-On-Demand SNP Genotyping Assay (KBioscience Ltd., Hoddesdon, UK). In Lausanne and Fenllopap samples the genotyping assay was carried out on 3.75 ng of genomic DNA in 1 µl 1536-well plate reactions, dispensed with a Meridian, microfluidic dispenser (KBioscience Ltd., Hoddesdon, UK), thermocycled using a Hydrocycler (KBioscience Ltd., Hoddesdon, UK). A Pharmacia (MG GmbH, Germany) was used for end-point detection and Kraken-LIMS (KBioscience Ltd., Hoddesdon, UK) was used for automated allele calling. In MRC Ely and Fenland samples, the genotyping assay was carried out on 10 ng of genomic DNA in 5 µl 384-well plate reactions using a G-Storm GS4 Thermal Cycler (GRI, Rayne, UK). The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK) was used for end-point detection and allele calling.

Statistical Analysis

Genome-wide association studies. All cohorts independently tested for the additive genetic association of common (MAF ≥ 1%) genotyped and imputed SNPs with natural log transformed adiponectin levels, while adjusting for age, sex, body mass index (BMI), principal components of population stratification and study site (where appropriate), and for family structure in cohorts with family members [49,50,52]. The analyses were performed for men and women combined, as well as for men and women separately. The Cardiovascular Health Study cohort (CHS) also provided GWA results for high molecular weight (HMW) adiponectin using the same methods as described above.

Meta-analysis of GWAS. The meta-analysis was performed by two analysts independently each using different methods; inverse variance-weighted methods using both fixed and random effect models available through either the METAL (http://www.sph.umich.edu/csg/abecasis/metal/) or GWAMA version 2.0.5 (http://www.well.ox.ac.uk/gwama/) software packages [53]. Summary statistics were crosschecked to ensure consistency of results. Prior to the meta-analysis, study-specific summary statistics were corrected using genomic control (lambda range = 0.99–1.25) and the overall meta-analytic results were additionally corrected for genomic control (lambda = 1.06). To examine whether associations with adiponectin were sex-specific, we performed meta-analyses for men and women separately. A p-value threshold of 5 × 10−8 was considered to be genome-wide significant. Ethnicity-specific meta-analyses were performed for white European and non-European populations separately, using the same methods as described above.

Presence of heterogeneity in the meta-analysis was assessed by the I² statistic and Q-test [54]. Since cohorts measured adiponectin concentrations using either RIA or ELISA methods, we also performed a GWA meta-analysis stratified by the method of measurement to test whether this contributed to heterogeneity.

Follow-up phase. The follow-up phase comprised two stages; in-silico follow-up and de-novo follow-up.

—In silico follow-up: 468 SNPs with p < 5 × 10−8 from the discovery phase (which includes both genome-wide significant [n = 196, p < 5 × 10−8] and “suggestive” [n = 272, 5 × 10−8 < p < 5 × 10−5] SNPs, Table S3) were tested for their association in 6,523 individuals from seven additional cohorts with GWAS data that joined the consortium after the discovery stage had been finalized.

—De novo follow-up: We next selected the lead SNP arising from selected loci from the joint analysis of the discovery and in-silico follow-up phase with p-values greater than 5 × 10−8 but less than 5 × 10−6 and genotyped 10 SNPs in 3,164 samples from the SAPPHIR cohort and an additional subgroup of the KORA cohort. Finally, these same SNPs, or their proxy SNPs (n = 2), were tested for association in the THISEAS cohort (n = 738), which had been genotyped using the Metabochip [55]. Study-level summary statistics from the follow-up phases were meta-analyzed with the data from the discovery phase.

Multi-ethnic meta-analysis. In order to perform a meta-analysis of GWAS data from cohorts of different ethnic backgrounds, we utilized the novel MANTRA (Meta-Analysis of Trans-ethnic Association studies) software [24]. This method combines GWAS from different ethnic groups by taking advantage of the expected similarity in allelic effects between the most closely related populations. Fixed-effects meta-analysis assumes the allelic effect to be the same in all populations, and cannot account for heterogeneity between ethnic groups. Conversely, random effects meta-analysis assumes that each population has a different underlying allelic effect, however, populations from the same ethnic group would be more homogeneous than those that are more distantly related. To address this challenge we accounted for the expected similarity in allelic effects between the most closely related populations by means of a Bayesian partition model. For each variant, allelic effects and corresponding standard errors are estimated within each population under the assumption of an additive model. Populations are then clustered according to their similarity in terms of relatedness as measured by the mean allele frequency difference at 10,000 independent SNPs, and to their allelic effects at the variant. If all populations are assigned to the same cluster, this is equivalent to a fixed allelic effect across all populations (i.e. no trans-ethnic heterogeneity). The posterior distribution of the allelic effect in each population under the Bayesian partition model is approximated by means of a Monte-Carlo Markov chain algorithm. Evidence in favor of association of the trait with the variant was assessed by means of a Bayes’ factor (BF). A log10 BF of 6 or higher is considered a relatively conservative threshold for genome-wide significance. We also performed meta-analysis by using both random and fixed effects models including all ethnicities. Those loci that achieved both a BF > 6 in MANTRA and a P-value less than 5 × 10−7 in multiethnic analysis are presented in Table 2.

Association of Genome-Wide Significant SNPs with Gene Expression (Stage 2)

In order to identify cis-expression quantitative trait loci (cis-eQTLs) and test whether mRNA levels of candidate genes arising from our GWASs were associated with adiponectin levels, we used...
expression profiles in human adipocytes from the Multiple Tissue Human Expression Resource (MuTHER) Consortium, (856 female twins from the UK) [25]. mRNA expression profiles from subcutaneous fat and genome-wide genotypes were available for 776 individuals and circulating adiponectin levels for 436 of these women. We note that while adiponectin levels were measured at an earlier time point than the fat biopsies, the BMI at time of adipose expression measurement and time of adiponectin measurement was highly correlated (r^2 = 0.9).

cis-eQTLs were defined as associations between SNPs and a transcript within 1 Mb of the identified SNP. To correct for multiple testing, we used QVALUE software [56], and estimated that a genome-wide false discovery rate of 1% corresponds to a p-value threshold of 5.06 x 10^-8 (this conservative threshold accounts for all multiple arising from the use of the array, rather than multiple testing arising from assessing only transcripts in the genome-wide significant regions). To test whether mRNA levels of candidate genes identified in the GWAS meta-analysis are associated with circulating adiponectin levels, we applied a Bonferroni corrected threshold of p < 5 x 10^-8 and accounted for all multiple testing arising from assessing only transcripts in the genome-wide significant regions).

Association of Genome-Wide Significant SNPs with T2D and Metabolic Traits (Stage 3)
The DIAGRAM+ (effective n = 22,044) [19], MAGIC (n = up to 46,186) [20], GLGC (n = up to 97,021) [22], GIANT (n = up to 121,335) [21], and Body Fat GWAS (n = up to 36,625) consortia provided summary statistics for the association of each SNP that was genome-wide significant in the discovery phase. Since 196 SNPs (which were estimated to be equivalent to 96 independent statistical tests due to linkage disequilibrium [LD]) [26] were tested for their association, we employed a Bonferroni-corrected threshold of p = 0.0005 (where 0.0005 = 0.05/96) to define the threshold of association for any individual SNP association with T2D and related traits.

While any individual SNP may demonstrate a relationship with T2D or related traits, it can be more informative to test whether a multi-SNP genotypic risk score is associated with the outcome of interest. In the absence of pleiotropic effects arising from loci other than ADIPOQ, such a multi-SNP genotypic risk score would enable testing of whether adiponectin levels are causally related to risk of T2D or metabolic traits through a Mendelian randomization framework. Since most of the SNPs that we identified to be genome-wide significant for adiponectin levels were not in the ADIPOQ locus, the presence of such pleiotropy precluded a formal Mendelian randomization study. To create a multi-SNP genotypic risk score we implemented a novel method that approximates the average effect of adiponectin decreasing alleles on T2D or related traits. Further, this method allows the use of consortium-level meta-analytic results for a set of SNPs, rather than requiring the re-analysis of individual-level data in each cohort, thereby providing more accurate effects of each allele (due to the larger sample size in the consortium-level meta-analysis). The weighted sum of the individual SNP coefficients leads not only to an estimate of the average combined allelic effect, but also to an approximate estimate of the explained variance (when scaled by the inverse of the total meta-analysis sample size) from a multivariate regression model containing these SNPs.
Specifically, suppose $m$ SNPs have shown association in the discovery phase, and effects are denoted $a_j$. However, suppose that the goal of interest is to estimate the joint effect of these SNPs on an outcome of interest, $y$. Let $j$ index the individuals in the outcome of interest dataset and let

$$s_j = \sum_{i=1}^m w_i x_{ij}$$

be a risk score based on the discovery data SNPs, and their associated parameter estimates $w_i$. Therefore, the desired goal is to estimate the parameter in the following equation:

$$y_j = y_0 + a_j + e_j$$

in the outcome of interest dataset. The proportion of variance in $y_j$ explained by the previous equation, (i.e. the $R^2$) attributable to the risk score can be estimated. Standard linear model model theory shows that the change in log likelihood is proportional to the $R^2$,

$$2[\ln(L(M_1)) - \ln(L(M_0))] \cong nR^2$$

If the SNPs are uncorrelated, and if the total percentage of variance explained is small, then the change in log likelihood can be approximated by

$$C = \sum_{i=1}^m \left( \frac{\hat{\beta}_i - \tilde{\beta}_i}{2\sigma_i^2} \right)^2$$

where $\beta_i$ now refers to the effect of SNP $i$ in the outcome data, $\hat{\beta}_i$ is the outcome data estimate, and $\sigma_i$ is the associated standard error estimate. Assuming that this log likelihood difference approximation is maximized with an appropriate value of $C_i$, then it can be shown that $a$ can be estimated by:

$$\hat{a} \cong \sum_{i=1}^m w_i \hat{\beta}_i s_i^{-2}$$

with a standard error estimate of

$$se(\hat{a}) \cong \sqrt{\frac{1}{\sum_{i=1}^m w_i^2 s_i^{-2}}}$$

Therefore, under the assumption of uncorrelated SNPs, their joint effect can be estimated in external data by a weighted mean of the individual SNP effects, weighted by the estimates from the discovery data. All these quantities can be obtained from meta-analysis or summary data, so that individual-level data are not required to obtain these results.

To implement this method, we first selected LD-independent adiponectin associated alleles by LD pruning the set of genome-wide significant adiponectin SNPs from the discovery phase with an LD threshold of $r^2 \leq 0.05$ in the HapMap CEU population, yielding 20 independent LD blocks from the 196 SNPs in Table S2. (We also applied the method using an LD threshold of $r^2 \leq 0.01$ and found no relevant change in results). Since many SNPs from the same independent blocks were associated with adiponectin, we selected the SNP from the LD block that explained the most variance in adiponectin levels. Next, we approximated the effect of the multi-SNP genetic risk score using $\beta$ and its standard error as derived from the consortium-level meta-analysis in DIAGRAM+, MAGIC, GLGC, GIANT and Body Fat GWAS consortium.

**Supporting Information**

**Figure S1** The comparison between two independent meta-analyses performed in different centers for quality control purposes. The $-\log_{10}(p)$-value of all SNPS with MAF$\geq 0.01$ in the first analysis are plotted against the $-\log_{10}(p)$-value from the second analysis.

**(TIF)**

**Figure S2** The Manhattan plots of sex-stratified meta-analyses in the discovery phase in the European population. The meta-analysis shown in panel a) is stratified for women and that in panel b) is stratified for men. Manhattan plots demonstrate $-\log_{10}(p)$-value measures for association between single nucleotide polymorphisms (SNPs) and chromosomal position. The SNPs that achieved genome-wide significance are highlighted in green in the plots. The red ovals identify loci found only in women.

**(TIF)**

**Figure S3** Association Results Near Peaks for Sex-specific Analysis of Adiponectin. SNPs in regions near peak associations are shown for a) chromosome 8 female, b) chromosome 8 males, c) chromosome 12 females and d) chromosome 12 males. Purple diamonds indicate the top SNPs, which have the strongest evidence of association in women. Each circle shows a SNP with a color scale proportional to the $r^2$ value for that SNP and the top SNP from HapMap CEU. Blue lines show the estimated recombination rates from HapMap. The bottom panels illustrate the relative position of each gene in the locus.

**(TIF)**

**Table S1** Cohort characteristics.

**(XLSX)**

**Table S2** Comparing the Genome-Wide Significant SNPS from fixed effect model with random effect model. *SNP with I^2 less than 0.5 are listed in bold, EA: Effect Allele, NEA: Non-Effect Allele.

**(PDF)**

**Table S3** Association Results of SNPs achieving $p \leq 5 \times 10^{-6}$ in the Discovery phase in European Populations (Sex-Combined Analysis). *Denotes SNPs typed in the de-novo follow-up phase.

**(PDF)**

**Table S4** Genome-Wide Significant SNPs ($p < 5 \times 10^{-8}$) Associated with Adiponectin Levels in Non-Europeans Populations. EA: Effect Allele, NEA: Non-Effect Allele. EA-Freq: Frequency of Effect Allele.

**(PDF)**

**Table S5** SNPs associated with adiponectin at genome-wide significant levels ($p < 5 \times 10^{-8}$) using the fixed-effect model in women only in European populations (including Discovery and Follow-Up phases).

**(PDF)**

**Table S6** SNPs associated with adiponectin at genome-wide significant levels ($p < 5 \times 10^{-8}$) using fixed-effect models in men only in European populations.

**(PDF)**

**Table S7** Association results of nominally significant SNPs with Type 2 Diabetes in the DIAGRAM+ Consortium. EA: Effect Allele, NEA: Non-Effect Allele. B) Association results of nominally significant SNPs with diabetes-related traits in the MAGIC Consortium. Fasting glucose and 2 h glucose in mmol/L; Insulin in pmol/L, EA: Effect Allele, NEA: Non-Effect Allele. C) Association results of nominally significant SNPs with diabetes-related traits in the GIANT Consortium.
related traits in the GIANT and Body fat GWAS consortia. The beta expressed in inverse normally transformed BMI units (i.e., interpretable as SD or Z-score), shows the change in BMI per additional effect allele. *Results that are statistically significant, accounting for the number of independent SNPs, are highlighted in bold. EA: Effect Allele, NEA: Non-Effec Allele, EA-Freq: Frequency of Effect Allele. D) Association results of nominally significant SNPs with lipid traits in the GLGC Consortium. For these traits the effect size is in SD units, based on standard error-weighted meta-analysis. *Results that are statistically significant, accounting for the number of independent SNPs are highlighted in bold. EA: Effect Allele, NEA: Non-Effec Allele, EA-Freq: Frequency of Effect Allele.

Text S1 Supplemental data include description of study cohorts and funding.

(DOCX)

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