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Genome-wide association study for adiponectin levels in Filipino women identifies *CDH13* and a novel uncommon haplotype at *KNG1–ADIPOQ*

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Adiponectin is an adipocyte-secreted protein involved in a variety of metabolic processes, including glucose regulation and fatty acid catabolism. We conducted a genome-wide association study to investigate the genetic loci associated with plasma adiponectin in 1776 unrelated Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). Our strongest signal for adiponectin mapped to the gene *CDH13* (rs3865188, $P \leq 7.2 \times 10^{-16}$), which encodes a receptor for high-molecular-weight forms of adiponectin. Strong association was also detected near the *ADIPOQ* gene (rs864265, $P = 3.8 \times 10^{-9}$) and at a novel signal 100 kb upstream near *KNG1* (rs11924390, $P = 7.6 \times 10^{-7}$). All three signals were also observed in 1774 young adult CLHNS offspring and in combined analysis including all 3550 mothers and offspring samples (all $P \leq 1.6 \times 10^{-9}$). An uncommon haplotype of rs11924390 and rs864265 (haplotype frequency = 0.050) was strongly associated with lower adiponectin compared with the most common C–G haplotype in both CLHNS mothers ($P = 1.8 \times 10^{-25}$) and offspring ($P = 8.7 \times 10^{-32}$). Comprehensive imputation of 2653 SNPs in a 2 Mb region using as reference combined CHB, JPT and CEU haplotypes from the 1000 Genomes Project revealed no variants that perfectly tagged this haplotype. Our findings provide the first genome-wide significant evidence of association with plasma adiponectin at the *CDH13* locus and identify a novel uncommon *KNG1–ADIPOQ* haplotype strongly associated with adiponectin levels in Filipinos.

INTRODUCTION

Adiponectin, an adipokine secreted by adipocytes, is believed to play important roles in various metabolic processes, including glucose regulation and fatty acid catabolism (1). Plasma adiponectin level is negatively correlated with body mass index (BMI), glucose, insulin and triglyceride levels and is positively associated with high-density lipoprotein cholesterol (HDL-C) concentration and insulin-stimulated glucose disposal (1). Animal studies have reported that administration of adiponectin attenuated insulin resistance and improved endothelial dysfunction (2,3). The protective effect of adiponectin is further supported by epidemiological studies in which individuals with obesity, type 2 diabetes and coronary artery disease had decreased circulating adiponectin concentrations (4,5). Therefore, hypoadiponectinemia has been suggested as an independent risk factor for metabolic syndrome and may lead to obesity, type 2 diabetes and atherosclerosis (6,7).

Circulating adiponectin level is under substantial genetic influence. An estimated 30–70% of the variability in plasma

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adiponectin is explained by genetic variation (8–10). Genome-wide linkage scans for adiponectin level produced linkage signals, but replications were inconsistent across different ethnic populations (8–10). Encoding the adiponectin protein, the gene *ADIPOQ* has been widely investigated for variants associated with circulating adiponectin. Several SNPs including rs17300539 (−11391G>A), rs2241766 (+45T>G) and rs1501299 (+276G>T) have been reproducibly reported to be associated with circulating adiponectin (11–16). Two reports suggested the presence of distinct association signals for adiponectin levels represented by 5′-promoter SNP rs17300539 and either rs6773957 (17) or rs182052 (18), and a gene-wide tagSNP investigation revealed that two haplotype blocks mapping to the *ADIPOQ* promoter and the relevant exons showed association with adiponectin (19). Only limited evidence has been provided for the functional relevance of these SNPs (20,21), and the common genetic variants identified thus far explain only a fraction of the estimated heritability for adiponectin (22).

Genome-wide association studies (GWAS) provide a more complete characterization of common genetic determinants across the genome. The first GWAS for adiponectin, conducted in a population of northern and western European origin, confirmed the strong association with variants in *ADIPOQ* and reported initial evidence of association with *CDH13* (rs7195409, $P = 2.0 \times 10^{-5}$), although the latter finding did not reach a level of genome-wide significance (23). A recent meta-analysis of three GWAS revealed a new locus, *ARL15* (rs4311394, $P = 2.9 \times 10^{-8}$), and provided further evidence that variants at *ARL15* may be associated with risk of type 2 diabetes in European populations (24). The evidence for loci associated with circulating adiponectin remains unclear in populations of non-European ancestry. Therefore, the primary goal of this study was to perform a GWAS to investigate SNP associations with plasma adiponectin level in 1776 Filipino mothers from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). We also genotyped selected SNPs and tested their association with adiponectin concentration in 1774 young adult offspring of the CLHNS mothers to test evidence in both sexes and in a younger Asian population.

RESULTS

GWAS results in CLHNS mothers

A GWAS for plasma adiponectin in 1776 CLHNS women (Table 1) showed 38 SNPs associated at $P < 10^{-6}$ that clustered in three chromosome regions. The observed genomic control inflation factor (λ_{GC}) was 1.03, suggesting no substantial population stratification among the CLHNS mothers sample (Fig. 1). The strongest signal mapped to *CDH13* locus on chromosome 16 (rs3865188, $P = 7.2 \times 10^{-16}$) and two signals were located on chromosome 3, near *ADIPOQ* (rs864265, $P = 3.8 \times 10^{-9}$) and ∼100 kb upstream of *ADIPOQ* near *KNG1* (rs11924390, $P = 7.6 \times 10^{-7}$). Conditioning on rs3865188, the remaining 22 nearby *CDH13* SNPs showed no evidence for a secondary signal ($P > 0.13$, Supplementary Material, Fig. S1). Conditional analysis for SNPs within the *KNG1–ADIPOQ* gene region on chromosome 3 showed that the two nearby loci were not completely independent (Supplementary Material, Fig. S2). In models accounting for the *ADIPOQ* variant rs864265, the strength of association slightly increased for the *KNG1* SNP rs11924390 (conditioned $P = 8.7 \times 10^{-8}$), but was greatly attenuated for other *ADIPOQ* variants ($P > 0.13$). Similarly, when the *KNG1* variant rs11924390 was included in the statistical model, the strength of association for the *ADIPOQ* SNP rs864265 became more significant (conditioned $P = 4.4 \times 10^{-10}$), whereas the association strength with other *KNG1* SNPs was attenuated ($P > 0.53$).

We next tested whether these associations with adiponectin were affected by adjustment for waist circumference. The association became stronger for *CDH13* ($P = 4.1 \times 10^{-21}$) and *ADIPOQ* ($P = 9.2 \times 10^{-11}$), but did not change substantially for *KNG1* ($P = 2.4 \times 10^{-6}$). These findings may indicate that adjusting for waist circumference removes an independent source of variation in adiponectin levels.

Follow-up genotyping results in CLHNS mothers and offspring samples

To confirm the evidence of the adiponectin association based on imputed SNPs, we directly genotyped *CDH13* rs3865188 and *KNG1* rs11924390 in 1776 CLHNS mothers. Compared with the original association based on imputed SNPs, the evidence remained comparably significant when using experimentally determined genotypes (rs3865188 $P = 3.8 \times 10^{-16}$; rs11924390 $P = 2.2 \times 10^{-6}$, Table 2); high quality imputed genotypes were analyzed for rs864265 (MACH $r^2 > 0.99$, see also Materials and Methods).

We also genotyped the three index SNPs in the CLHNS young adult offspring (Table 1). We first examined the association in a subgroup of the offspring sample consisting of 336 individuals whose mothers were not present in CLHNS mothers GWAS sample. Significant association (Bonferroni corrected $P < 0.017$, 0.05/3 tests) was observed for rs3865188 ($P = 3.7 \times 10^{-6}$), rs864265 ($P = 5.1 \times 10^{-4}$) and rs11924390 ($P = 0.0059$) (Table 2). When all 1774 offspring were included in the analysis, we observed significant evidence of association for rs3865188 ($P = 2.3 \times 10^{-18}$), rs864265 ($P = 1.3 \times 10^{-14}$) and rs11924390 ($P = 2.2 \times 10^{-4}$) (Table 2). When the mother and offspring samples were combined using a linear mixed effect model that accounted for their relationships, the associations reached genome-wide significance (rs3865188, $P = 4.1 \times 10^{-30}$; rs864265, $P = 1.4 \times 10^{-19}$ and rs11924390, $P = 1.6 \times 10^{-9}$, Table 2).

Two-SNP haplotype effect of rs11924390 (*KNG1*) and rs864265 (*ADIPOQ*) on plasma adiponectin

Given the close proximity of the *KNG1* and *ADIPOQ* loci, we performed haplotype analyses to investigate the association between adiponectin and the specific two-SNP haplotypes consisting of the lead SNPs at *KNG1* (rs11924390) and *ADIPOQ* (rs864265) in both the CLHNS mothers and the offspring. The effect on adiponectin of each additional copy of the specific haplotype compared with the homozygote reference haplotype was assessed. The uncommon C–T haplotype with a frequency of 0.050 was significantly associated with a

Table 1. General characteristics of CLHNS mothers and young adult offspring

	Mothers	Independent offspring	All offspring
N	1776	336	1774
Female (%)	100	44.4	47.3
Adiponectin (μg/ml)	2.48 (1.90, 3.32)	2.37 (1.88, 3.12)	2.47 (1.94, 3.10)
Age in 2005 (years)	48.4 ± 6.1	21.5 ± 0.3	21.5 ± 0.3
Household income in 2005 (pesos/week)	396.3 (243.7, 623.0)	326.7 (196.1, 563.8)	358.1 (213.6, 585.2)
Household assets in 2005 (0 to 11)	5.2 ± 2.0	5.1 ± 2.1	5.2 ± 2.0
Number of previous pregnancies	6.5 ± 3.0	–	–
Post-menopausal (%)	38.2	–	–
Waist circumference (cm)	81.1 ± 10.9	71.2 ± 8.3	70.3 ± 7.8
BMI (kg/m^2)	24.1 (21.4, 27.0)	20.5 (18.8, 22.7)	20.2 (18.7, 22.2)

Data are mean ± SD, median (25th percentile, 75th percentile) or %.
Independent offspring ($n = 336$) are a subgroup of individuals within the 1774 offspring sample whose mother was not included in the 1776 CLHNS mothers cohort for GWA analysis.

decreased level of adiponectin compared with the most common C−G haplotype in CLHNS mothers ($P = 1.8 \times 10^{-25}$, Table 3). Similar findings of haplotype association were observed in the 336 independent offspring ($P = 9.5 \times 10^{-12}$) and in the entire set of 1774 offspring ($P = 8.7 \times 10^{-32}$, Table 3). Further analyses that computed score statistics provided significant evidence for an overall association between haplotypes and adiponectin level (global $P = 1.1 \times 10^{-27}$, 5.0×10^{-4} and 5.9×10^{-35} in CLHNS mothers, independent offspring subgroup and all offspring, respectively). In both mothers and offspring, the uncommon C−T haplotype showed the strongest evidence of association, suggesting that this haplotype is largely responsible for the observed associations between adiponectin and the individual SNPs. Comprehensive imputation of 2653 SNPs in a 2 Mb region using as reference combined CHB, JPT and CEU haplotypes from the 1000 Genomes Project revealed no variants that perfectly tagged this haplotype (Supplementary Material, Fig. S3).

Adiponectin association with previously reported SNPs

We next examined whether associations in the CLHNS mother sample replicated evidence for loci previously reported in either candidate gene studies or GWAS (Table 4). Among *ADIPOQ* variants identified by a candidate gene approach, the widely reported promoter SNP rs17300539 (−11391 G>A) with potential biological function (16) was monomorphic in the CLHNS mothers. Four other candidate gene variants rs266729 (−11377 C>G), rs2241766 (+45 T>G), rs1501299 (+276 G>T) and rs1063537 (11,13,16) were marginally associated with adiponectin ($P = 0.018$, 0.0077, 0.059 and 0.017, respectively). The GWAS SNP rs3774261 (23) displayed significant association in the same direction in CLHNS ($P = 7.0 \times 10^{-5}$). Although rs3774261 was in weak LD with the *ADIPOQ* index SNP rs864265 ($r^2 = 0.02$ based on HapMap CHB and JPT combined sample), reciprocal conditional analyses suggested that adiponectin associations with these variants are not independent (P for rs3774261 conditional on rs864265 = 0.066, Table 4; P for rs864265 conditional on rs3774261 $= 2.4 \times 10^{-6}$). We did not replicate associations with other GWAS SNPs (23,24) in *ARL15* (rs4311394, $P = 0.53$) and *LYZL1* (rs1774950, $P = 0.73$). Notably, the most strongly associated *CDH13* SNP reported previously (rs7195409) (23) was not significantly associated with adiponectin in the CLHNS mothers ($P = 0.16$). The strongest *CDH13* SNP in the CLHNS mothers (rs3865188, $P = 3.8 \times 10^{-16}$) exhibited only limited LD with rs7195409 ($r^2 = 0$ and $D' = 0.06$ in HapMap CHB and JPT combined samples, Table 4).

Association of *CDH13*, *ADIPOQ* and *KNG1* variants with other metabolic-related traits

Consistent with findings in previous studies (25,26), plasma adiponectin was highly correlated with other metabolic-related traits including BMI, waist circumference, lipid profiles, insulin and homeostasis model assessment (HOMA) index in both the mothers and offspring (Supplementary Material, Table S1). We next assessed the strongest adiponectin SNPs at *CDH13*, *ADIPOQ* and *KNG1* for their associations with these related quantitative traits (Table 5). In the CLHNS mothers, nominal associations were detected for *CDH13* SNP rs3865188 with waist circumference ($P = 0.042$) and for *ADIPOQ* SNP rs864265 with triglycerides ($P = 0.047$). In the offspring, the G allele of *ADIPOQ* SNP rs864265 that was associated with higher adiponectin level displayed nominally significant association with lower BMI ($P = 0.0086$), lower waist circumference ($P = 0.0066$), lower insulin level ($P = 0.0011$), decreased HOMA-IR ($P = 0.0027$) and decreased HOMA-β ($P = 6.3 \times 10^{-4}$). The association between rs864265 and HOMA-β remained significant after Bonferroni correction for multiple testing ($P < 0.0008$, 0.05/60 tests). After including adiponectin level as a covariate in the model, the *P*-values for the associations with insulin, HOMA-IR and HOMA-β remained nominally significant at 0.014, 0.026 and 0.0072, respectively, indicating that the genetic effects of rs864265 on these traits may be partially independent of adiponectin level.

DISCUSSION

The present study provides strong evidence of association with adiponectin at *CDH13*, *ADIPOQ* and *KNG1* that together explain approximately 7.5 and 8.9% of the variability in natural log-transformed adiponectin levels in 1776 Filipino mothers and 1774 offspring, respectively. These data provide the first genome-wide significant association at *CDH13* and compelling evidence for a novel uncommon haplotype near *ADIPOQ*. Only nominally significant associations were detected between *ADIPOQ* SNP rs864265 and multiple metabolic-related traits in the CLHNS offspring, and the effects of the associations with insulin, HOMA-IR and HOMA-β were partially independent of adiponectin level.

Our strongest main effect signal mapped to the gene *CDH13* on chromosome 16 and explained 3.7% of the variability in the

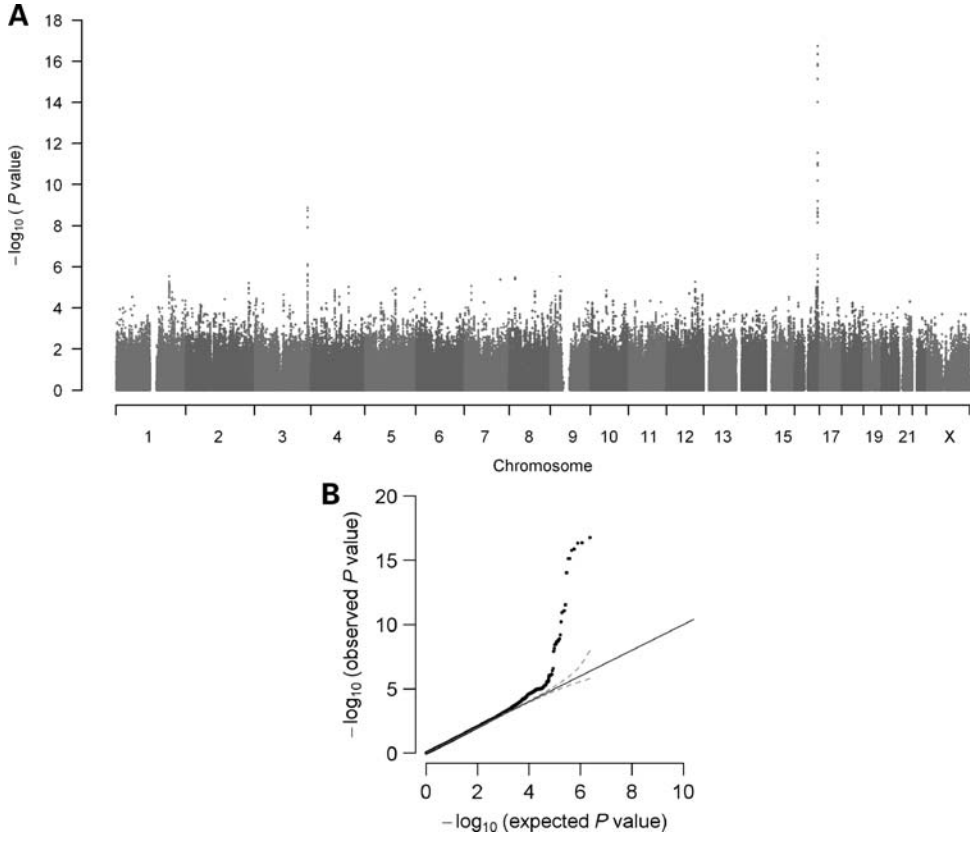

Figure 1. Genome-wide association with plasma adiponectin in CLHNS mothers cohort. (**A**) Manhattan plot, (**B**) quantile–quantile plot of observed and expected *P*-values for association with the adiponectin level. The observed genomic control inflation factor (λ_{GC}) was 1.03.

mothers and 4.3% in the offspring. The *CDH13* gene encodes the protein T-cadherin, which is a receptor for hexameric and high-molecular-weight forms of adiponectin (27). T-cadherin is highly expressed in endothelial and smooth muscle cells, where it interacts with adiponectin and may protect vascular endothelial cells from apoptosis (28). The association of adiponectin with a SNP near *CDH13* was recently reported in a GWAS consisting of 2280 northern and western Europeans (rs7195409, $P = 1.99 \times 10^{-5}$) (23), but we did not strongly support association with that SNP in the CLHNS mothers ($P = 0.16$). Nonetheless, our data provided solid evidence that the *CDH13* locus is associated within adiponectin levels. We observed a stronger and separate signal for adiponectin in the *CDH13* locus at rs3865188 ($P = 6.7 \times 10^{-16}$), which resides in the gene's promoter region, about 887 kb upstream of the reported variant rs7195409. Very weak linkage disequilibrium exists between rs7195409 and rs3865188 ($r^2 = 0$, $D' = 0.12$ in CEU and $r^2 = 0$, $D' = 0.06$ in CHB and JPT combined samples), and conditional analyses further supported their independence (P for rs3865188 conditional on rs7195409 $= 5.0 \times 10^{-16}$; P for rs7195409 conditional on rs3865188 $= 0.11$). In addition, for the *CDH13* variant rs11646213, which was previously reported in a study of 4659 European individuals ($P = 0.10$), we observed stronger association in the 1776 CLHNS mothers ($P = 1.27 \times 10^{-6}$) (29). Together, these results suggest that a difference in genetic architecture between Europeans and Asians within the *CDH13* locus may explain our lack of replication of the previous SNP as well as our strong association with other SNPs near the same gene.

The CLHNS samples also showed strong evidence for an association between adiponectin and rs11924390 near the gene *KNG1*, which has not been reported previously. *KNG1* encodes two different proteins of high-molecular-weight kininogen (HMWK) and low-molecular-weight kininogen via alternative splicing. HMWK is essential for blood coagulation and bradykinin release. A common variant (rs710446) was recently reported to associate with activated partial thromboplastin time (aPTT), which is related to the risk of thrombosis and coagulation disorders (30). The SNP rs710446 is located approximately 27 kb away from rs11924390 and we found no association of this SNP with adiponectin in the CLHNS mothers ($P = 0.45$). The two SNPs are in weak pair-wise linkage disequilibrium ($r^2 = 0.07$, $D' = 0.59$ based on HapMap CHB and JPT combined samples), suggesting that the aPTT signal in *KNG1* is distinct from the adiponectin signal we detected.

The gene region of *ADIPOQ* had strong prior evidence of association with adiponectin level as a candidate gene and was validated by recent GWA studies (11,16–18, 20,21,23,29). The Framingham Offspring Study, which systematically examined the associations with 22 SNPs that captured all common variation at $r^2 > 0.8$ across *ADIPOQ* and its flanking regions, observed that adiponectin level was

Table 2. Strongest loci associated with adiponectin in CLHNS mother and young adult offspring cohorts

SNP	Gene	Allele 1	Allele 2	Mothers ($n = 1776$)			Independent offspring ($n = 336$)		All offspring ($n = 1774$)		Combined ($n = 3550$)	
				MAF	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
rs3865188	*CDH13*	T	A	T: 0.467	−0.111 (0.014)	3.8E−16	−0.131 (0.028)	3.7E-06	−0.106 (0.012)	2.3E−18	−0.109 (0.009)	4.1E−30
rs864265	*ADIPOQ*	G	T	T: 0.124	0.123 (0.021)	3.8E−09	0.144 (0.041)	0.0005	0.141 (0.018)	1.3E−14	0.130 (0.014)	1.4E−19
rs11924390	*KNG1*	C	T	T: 0.499	−0.067 (0.014)	2.2E−06	−0.086 (0.031)	0.0059	−0.046 (0.012)	2.2E−04	−0.059 (0.010)	1.6E−09

Directly genotyped SNPs were used in analysis except rs864265, which was imputed in CLHNS mothers (MACH $r^2 > 0.99$). CLHNS mothers analysis was adjusted for age, age^2, household assets, natural log-transformed household income and menopausal status; CLHNS offspring analyses were adjusted for sex, household assets and natural log-transformed household income. The mixed model analysis of combined samples was adjusted for age, age^2, sex, household assets, natural log-transformed household income and generation (mothers/offspring). MAF, minor allele frequency; β, change in natural log-transformed adiponectin (μg/ml) with each additional copy of Allele 1. All SNPs are aligned to the HapMap forward DNA strand.

significantly associated with SNPs in two different regulatory regions, the 5′-promoter (rs17300539) and the 3′-untranslated regions (rs6773957) (17). A recent study dissecting the genetic architecture of plasma adiponectin suggested that two *ADIPOQ* variants (rs17300539 and rs182052) exhibited independent association (18), and a recent GWAS of 4659 European individuals suggested association with at least nine SNP groups near *ADIPOQ* (29). In the CLHNS samples, conditional analyses suggest that common SNPs in or near the *ADIPOQ* gene (chr3:188 032–188 070 kb, spanning 11 kb up- and downstream of exons) likely represent a single signal for plasma adiponectin (Supplementary Material, Fig. S2), although none of the SNPs imputed with high quality perfectly tags the strongly associated rs11924390–rs864265 haplotype.

Great efforts have been made by previous studies to pinpoint the functional variant(s) that influence circulating adiponectin levels. Two *ADIPOQ* promoter SNPs, rs17300539 (−11391G>A) and rs266729 (−11377C>G), have been investigated frequently in functional experiments (9,11,12,31) due to their strong reproducible correlations with circulating adiponectin and to the potential role of the promoter region in regulating gene transcriptional activity. *In vitro* data revealed a functional effect of rs17300539 on adiponectin, suggesting that the A allele was likely to increase *ADIPOQ* promoter activity and enhance transcription (31). A subsequent study demonstrated that SNPs rs17300539, rs266729 and a third *ADIPOQ* promoter variant rs16861194 (−11426A>G) modulate gene transcription by altering the DNA binding activity in mouse 3T3-L1 adipocytes; the rs16861194 G allele substantially reduced basal promoter and DNA binding activity, although the exact binding factors remained largely unclear (21). Bioinformatic prediction suggests that SNP rs266729 (−11377C>G) alters the sequence of one of four transcriptional stimulatory protein (SP1) binding sites in the *ADIPOQ* promoter region (32). Our results, however, did not support the importance of these promoter SNPs in CLHNS mothers who reside in Metro Cebu, Philippines. SNP rs17300539 is monomorphic in mothers, while rs266729 showed only modest association with plasma adiponectin ($P = 0.018$), and no evidence for association was found for rs16861194 ($P = 0.26$). While rs266729 shows similar minor allele frequencies between Europeans and Asians (CEU: G 0.30 and CLHNS: G 0.27), frequencies differ for rs17300539 (CEU: A 0.08 and CLHNS: A 0.00) and rs16861194 (CEU: G 0.07 and CLHNS: G 0.18), suggesting different genetic architecture in the *ADIPOQ* promoter region between populations.

Given the differences in local genetic structure, the possible functional relevance of other nearby genetic variants should be considered. The SNP rs864265 (chr3: 188 036 986), located 5 kb upstream of the widely discussed *ADIPOQ* promoter region and 16 kb upstream of the translation start site, showed the strongest association with plasma adiponectin in the CLHNS sample ($P = 3.8 \times 10^{-9}$). Perhaps more relevant, our findings of association with the uncommon C–T haplotype of the rs11924390 and rs864265 SNPs suggest that a functional variant might be tagged by the uncommon haplotype. Compared with the previously reported haplotypes that cover the *ADIPOQ* gene (19), this novel haplotype spans the

Table 3. Adiponectin association with haplotypes consisting of SNPs rs11924390 (*KNG1*) and rs864265 (*ADIPOQ*)

rs11924390	rs864265	Estimated haplotype frequency	β	SE	*P*-value
CLHNS mothers ($n = 1776$)					
C	G	0.450	Reference		
T	G	0.426	0.021	0.015	0.16
T	T	0.074	0.064	0.028	0.020
C	T	0.050	−0.385	0.036	1.8E−25
Independent CLHNS offspring ($n = 336$)					
C	G	0.442	Reference		
T	G	0.405	0.027	0.030	0.38
T	T	0.096	0.098	0.052	0.063
C	T	0.056	−0.504	0.071	9.5E−12
All CLHNS offspring ($n = 1774$)					
C	G	0.444	Reference		
T	G	0.427	0.004	0.013	0.78
T	T	0.076	0.027	0.025	0.27
C	T	0.052	−0.386	0.032	8.7E−32

The 'haplo.glm' function implemented in the 'haplo.stats' R package was used to calculate the coefficient β and *P*-value for each haplotype compared with the reference haplotype, which was set as the most common haplotype C–G. The same covariates used for genotype analysis were applied in haplotype analysis. Using the 'haplo.score' function implemented in the 'haplo.stats' R package, the global *P*-values for association between haplotypes and adiponectin level were 1.1×10^{-27}, 5.0×10^{-4} and 5.9×10^{-35} in CLHNS mothers, independent offspring subgroup and all offspring sample, respectively.

KNG1–ADIPOQ locus and suggests a more extensive gene region where the potential causal SNP(s) may reside. We investigated additional variation within 2 Mb (187–189 Mb) flanking *KNG1* and *ADIPOQ* by testing the association of SNPs imputed based on 1000 Genomes Project Pilot data, but no additional SNPs showed stronger association than SNPs imputed based on HapMap (Supplementary Material, Fig. S3). The failure to identify a stronger signal suggests either that the 1000 Genomes Project Pilot did not include the single functional variant, that such a variant was poorly imputed and thus its association with adiponectin was inaccurate or that more than one functional variant exists. Non-synonymous amino acid substitutions may contribute to the variation of circulating adiponectin by affecting the spatial organization of the protein and interfering with post-transcriptional modification, multimerization or interaction of the protein with its receptors (16). Variants including G84R, G90S, H111Y and R112C have been reported to be associated with adiponectin level and suggested to be functionally relevant (16,33,34). However, these variants were rare in initial studies (MAF < 0.02) and are not included as HapMap or 1000 Genomes Project Pilot imputed SNPs. Thus, their association with adiponectin level in the CLHNS sample remains unknown.

In conclusion, this study expands our understanding of genetic association with adiponectin in a population of mostly non-European ancestry and underscores a difference in the genetic architecture of plasma adiponectin levels between Europeans and Asians. Our findings provide compelling evidence of a strong association for plasma adiponectin with the *CDH13* locus. We also observed a haplotype association with plasma adiponectin of an uncommon C–T haplotype consisting of two variants at the *KNG1–ADIPOQ* locus, which may motivate further studies to investigate potential functional variants and to elucidate the underlying biological mechanisms.

MATERIALS AND METHODS

The original study population, study design and recruitment protocols of the CLHNS have been described in detail previously (35). The CLHNS is a community-based birth cohort study which originally enrolled 3327 pregnant women in 1983–1984, and the women and their offspring have since been followed. In the 2005 survey, the available study samples consisted of 1895 Filipino mothers and 1779 offspring. Among the mothers, we excluded 81 estimated first-degree relatives, 14 participants without genotyping data and 24 individuals with missing adiponectin outcome or covariates, and among the offspring we excluded one of each twin pair ($n = 5$). The final sample set included 1776 CLHNS women and 1774 offspring, of which 336 independent offspring did not have a mother in the CLHNS mothers GWA cohort and 1438 are members of offspring–mother pairs.

GWA SNP genotyping, quality control and genotype imputation based on HapMap CEU + CHB + JPT using MACH have also been previously described (36). Briefly, SNP genotyping was performed using the Affymetrix Genomewide Human SNP Array 5.0, according to the manufacturer's standard protocol. Genotyping calling was performed using Birdseed (Version 2). Genotyping was conducted on 1895 unique CLHNS mothers samples, 40 CLHNS duplicates and 5 HapMap CEPH trios. Fourteen CLHNS samples were excluded due to genotyping failure ($n = 10$) or a <97% call rate ($n = 4$). The final sample call rate was 99.6% in the remaining 1881 CLHNS mother samples. In the marker quality control for the initial 424 670 genotyping SNPs, those with poor mapping, call rate <90% and/or deviation from Hardy–Weinberg equilibrium ($P < 10^{-6}$) were removed from the follow-up imputation ($n = 13\,287$). An additional 3277 SNPs were discarded due to three or more discrepancies between genotypes in 40 duplicate pairs, Mendelian inheritance errors in five CEPH trios and/or three or more genotype discrepancies with HapMap. We applied a hidden Markov model algorithm implemented in MACH software version 1.0 (37) to impute genotypes in CLHNS mothers samples for 352 264 directly genotyped SNPs that were polymorphic in both the 60 CEU founders and the 89 combined CHB + JPT HapMap samples. After exclusion of SNPs with poor imputation quality (MACH $r^2 < 0.3$) or with low minor allele frequency (MAF ≤ 0.01), a total of 2 073 674 HapMap imputed SNPs were tested for association with plasma adiponectin level in 1776 CLHNS mothers. Additional imputation within 2 Mb gene region (187–189 Mb) of *KNG1–ADIPOQ* locus was performed based on haplotypes created from the 1000 Genomes Project pilot release (August 2009) of CEU+CHB+JPT samples. Regional plots were created using LocusZoom (38).

We constructed principal components (PCs) using the software EIGENSOFT to capture population substructure among CLHNS subjects (39). We assessed the association between

Table 4. Evidence of association in CLHNS mothers of SNPs previously reported to be associated with adiponectin

SNP (alias)	Closest gene	Chr	Position	Allele 1 > 2	MAF	Imputation quality[a]	Baseline model β (SE)	*P*-value	[b]LD r^2	Conditioned *P*[c]	Reference
rs16861194 (−11426 A>G)	*ADIPOQ*	3	188,042,119	A>G	G: 0.18	0.95	−0.021 (0.019)	0.26	0.013	0.95	(21)
rs17300539 (−11391 G>A)	*ADIPOQ*	3	188,042,154	G>A	A: 0.00	–	–	–	ND	ND	(16)
rs266729 (−11377 C>G)	*ADIPOQ*	3	188,042,168	G>C	G: 0.27	0.96	−0.038 (0.016)	0.018	0.029	1.7E−04	(16)
rs182052	*ADIPOQ*	3	188,043,476	A>G	A: 0.45	1.00	−0.017 (0.014)	0.22	0.057	9.1E−04	(19)
rs17366568	*ADIPOQ*	3	188,053,147	G>A	A: 0.03	0.98	0.034 (0.043)	0.44	0.001	0.54	(29)
rs2241766 (+45 T>G)	*ADIPOQ*	3	188,053,594	G>T	G: 0.26	0.79	0.047 (0.017)	0.0077	ND	0.10	(13)
rs1501299 (+276 G>T)	*ADIPOQ*	3	188,053,825	G>T	T:0.39	0.90	−0.028 (0.015)	0.059	0.000	0.75	(13)
rs3774261	*ADIPOQ*	3	188,054,253	G>A	G:0.37	1.00	−0.056 (0.014)	7.0E−05	0.019	0.066	(23)
rs6773957	*ADIPOQ*	3	188,056,407	G>A	G:0.37	1.00	−0.056 (0.014)	7.0E−05	0.019	0.066	(23)
rs1063537	*ADIPOQ*	3	188,056,769	C>T	T:0.22	0.96	−0.041 (0.017)	0.017	0.020	0.12	(11)
rs4311394	*ARL15*	5	53,336,419	A>G	G:0.44	0.99	0.009 (0.014)	0.53	ND	ND	(24)
rs1774950	*LYZL1*	10	82,085,093	T>C	T:0.44	0.99	0.005 (0.014)	0.73	ND	ND	(23)
rs7195409	*CDH13*	16	82,085,093	A>G	G:0.10	0.99	0.032 (0.023)	0.16	0	0.10	(23)

Analyses were adjusted for age, age^2, household assets, natural log-transformed household income and menopausal status ($n = 1776$). β (SE) indicates the effect size and standard error of each additional copy of Allele 1 on natural log-transformed adiponectin level. The SNP rs17300539 (−11391G>A) was monomorphic in direct TaqMan genotyping.

[a]MACH r^2 was applied to indicate the imputation quality of the imputed SNPs.

[b]Linkage disequilibrium (LD) measure r^2 with the index SNP of each locus (*ADIPOQ*: rs864265 and *CDH13*: rs3865188), based on the HapMap CHB + JPT combined sample.

[c]*P*-values for SNP association when conditional analysis was performed using the index SNP of each locus; ND, no data. Position for all SNPs is relative to NCBI Build 36 assembly.

each of the first 10 PCs and the adiponectin level to identify any potential ancestry explanatory PC; none were significantly associated and thus no PCs were included as covariates in the linear regression models for GWAS (All $P > 0.06$, Supplementary Material, Table S2). The genomic control value (λ_{GC}) was calculated to evaluate the type I error inflation due to population stratification.

Plasma samples were analyzed for adiponectin with a commercially available enzyme-linked immunosorbent assay (R&D Systems #DY1065). All samples were assayed in duplicate, and control samples were included with each assay to monitor between-assay variation. The percent coefficient of variation (SD/mean) for low, middle and high controls was 9.5, 9.6 and 7.8, respectively. Informed consent was obtained from all CLHNS participants, and the study protocol was approved by the University of North Carolina Institutional Review Board for the Protection of Human Subjects.

Follow-up genotyping was performed for four SNPs (rs3865188, rs11924390, rs864265 and rs17300539). The first three SNPs were genotyped to validate their significant associations with adiponectin observed in the mothers and also to test for supporting evidence in the offspring. The fourth SNP (rs17300539) was genotyped because it was a reproducibly reported associated SNP for adiponectin, but imputed genotypes were unavailable in the CLHNS mothers. Three SNPs (rs17300539, rs3865188 and rs11924390) were genotyped using TaqMan allelic discrimination (Applied Biosystems, Foster City, CA, USA) in both the CLHNS mothers and offspring. The genotyping success rate was >97%, and no discrepancies among 80 duplicate pairs were observed. The *ADIPOQ* SNP rs864265 was successfully genotyped in all 1774 CLHNS offspring using the Cardio-MetaboChip (Illumina, San Diego, CA, USA). Direct genotype data for rs864265 from the Cardio-MetaboChip were also available for 85 (4.8%) mothers. Due to both the high imputation quality of rs864265 ($r^2 > 0.99$) and the high allele concordance rate (>99.4%) between the Cardio-MetaboChip genotypes and the posterior expected genotypes based on HapMap imputed genotypes in the 85 mothers, rs864265 was not directly genotyped in the remaining 1691 mothers. The imputed genotypes were then used in statistical analyses.

Adiponectin level was natural log-transformed (log-adiponectin) to satisfy the model assumption of normally distributed residuals. GWA analyses were performed in Array Studio software version 3.6 (Omicsoft Corporation, Research Triangle Park, NC, USA). A multiple linear regression model assuming an additive effect and adjusting for age, age^2, household assets, natural log-transformed household income and menopausal status during the 2005 survey was applied to test for the association between each SNP and log-adiponectin in CLHNS mothers (Supplementary Material, Table S2). A one degree-of-freedom likelihood ratio test was used to examine the statistical significance.

In follow-up analyses, multiple linear regression models were performed to test the association of three genotyped SNPs (rs3865188, rs864265 and rs11924390) with log-adiponectin in both the mother and offspring samples. In the offspring, the analyses were adjusted for sex, household

Table 5. Association of adiponectin-associated SNPs with other metabolic traits

SNP		Log_BMI	Waist circumference	Log_TG	Log_HDL-C	Log_LDL-C	Log_glucose	Log_insulin	Log_HOMA-IR	Log_HOMA-β
CLHNS mothers ($n = 1776$)										
rs3865188 *CDH13*	β (SE)	−0.007 (0.006)	−0.72 (0.35)	−0.009 (0.016)	−0.000 (0.009)	0.004 (0.010)	−0.005 (0.007)	−0.022 (0.021)	−0.027 (0.024)	−0.008 (0.021)
	P-value	0.25	0.042	0.58	0.97	0.66	0.49	0.29	0.25	0.72
rs864265 *ADIPOQ*	β (SE)	0.004 (0.009)	0.30 (0.54)	0.049 (0.025)	0.002 (0.013)	0.016 (0.015)	0.005 (0.011)	0.041 (0.031)	0.046 (0.036)	0.026 (0.032)
	P-value	0.64	0.58	0.047	0.89	0.26	0.68	0.19	0.20	0.42
rs11924390 *KNG1*	β (SE)	0.006 (0.006)	0.30 (0.36)	0.005 (0.017)	0.000 (0.009)	0.011 (0.010)	−0.004 (0.007)	0.032 (0.021)	0.028 (0.024)	0.040 (0.022)
	P-value	0.34	0.41	0.77	0.99	0.25	0.56	0.13	0.25	0.063
CLHNS offspring ($n = 1774$)										
rs3865188 *CDH13*	β (SE)	−0.001 (0.005)	0.08 (0.26)	−0.017 (0.017)	−0.007 (0.008)	−0.000 (0.010)	−0.005 (0.003)	−0.021 (0.019)	−0.026 (0.020)	−0.008 (0.019)
	P-value	0.77	0.75	0.32	0.38	0.99	0.091	0.27	0.18	0.67
rs864265 *ADIPOQ*	β (SE)	−0.019 (0.007)	−1.07 (0.39)	−0.008 (0.025)	0.021 (0.013)	−0.007 (0.015)	0.002 (0.005)	−0.091 (0.028)	−0.089 (0.030)	−0.097 (0.028)
	P-value	0.0086	0.0066	0.76	0.091	0.63	0.61	0.0011	0.0027	6.3E−04
rs11924390 *KNG1*	β (SE)	−0.003 (0.005)	−0.06 (0.26)	−0.009 (0.017)	0.000 (0.008)	0.002 (0.010)	0.003 (0.003)	0.007 (0.019)	0.010 (0.020)	−0.004 (0.019)
	P-value	0.51	0.82	0.58	0.99	0.88	0.34	0.73	0.63	0.85

CLHNS mothers analysis was adjusted for age, age^2, household assets, natural log-transformed household income and menopausal status; CLHNS offspring analysis was adjusted for sex, household assets and natural log-transformed household income. The reference alleles are the same as those indicated in Table 2.

assets and natural log-transformed household income. Age, age^2 and menopausal status that were used as covariates for the analysis of CLHNS mothers were not included in offspring analyses because the ages of all offspring were within 2 years of each other (mean ± SD = 21.5 ± 0.3, Table 1) and none had reached menopause. In light of the relatedness between mother–child pairs, follow-up analysis in offspring was first conducted in a subgroup sample consisting of 336 CLHNS offspring whose mothers were not included in the CLHNS mother GWA samples. Partial correlations were determined for each SNP to estimate the proportion of variation in log-adiponectin explained by these genetic loci. To assess the association on the combined sample of mothers and offspring, a general linear mixed model was used to account for the correlation of adiponectin levels between mother–child pairs due to shared genetic and environmental exposures.

Partial correlation analyses were used to evaluate the relationship between adiponectin level and other metabolic-related traits including BMI, waist circumference, triglycerides, HDL-C, low-density lipoprotein cholesterol (LDL-C), fasting glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β). HOMA-IR was calculated as fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5 while HOMA-β was computed as 20 × fasting insulin (μU/ml)/[fasting glucose (mmol/l) − 3.5]. All traits except for waist circumference were natural log-transformed before analyses. Multiple linear regression models, accounting for the same covariates as for the log-adiponectin analyses, were performed to test for genotype effects on the metabolic-related traits. All analyses for follow-up association with log-adiponectin and other metabolic-related traits were performed with SAS version 9.2 (SAS Institute, Cary, NC, USA).

Haplotype analyses were performed using the 'haplo.stat' R package. Haplotypes and haplotype frequencies were estimated using the R function 'haplo.em'. The association between haplotypes and adiponectin was assessed using the R function 'haplo.glm'. An additive model was assumed, in which the regression coefficient β represents the expected change in natural log-transformed adiponectin level with each additional copy of the specific haplotype compared with the reference haplotype. The most common C–G haplotype of *KNG1* (rs11924390)–*ADIPOQ* (rs864265) was set as the reference haplotype. The R function 'haplo.score' was used to compute the global score statistics to test the overall association between haplotypes and adiponectin. The same covariates used for genotype analysis were also applied in the haplotype analysis models.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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