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# A meta-analysis of genome-wide association studies for adiponectin levels in East Asians identifies a novel locus near *WDR11-FGFR2*

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**Blood levels of adiponectin, an adipocyte-secreted protein correlated with metabolic and cardiovascular risks, are highly heritable. Genome-wide association (GWA) studies for adiponectin levels have identified 14 loci harboring variants associated with blood levels of adiponectin. To identify novel adiponectin-associated loci, particularly those of importance in East Asians, we conducted a meta-analysis of GWA studies for adiponectin in 7827 individuals, followed by two stages of replications in 4298 and 5954 additional individuals. We identified a novel adiponectin-associated locus on chromosome 10 near *WDR11-FGFR2* ($P = 3.0 \times 10^{-14}$) and provided suggestive evidence for a locus on chromosome 12 near *OR8S1-LALBA* ($P = 1.2 \times 10^{-7}$). Of the adiponectin-associated loci previously described, we confirmed the association at *CDH13* ($P = 6.8 \times 10^{-165}$), *ADIPOQ* ($P = 1.8 \times 10^{-22}$), *PEPD* ($P = 3.6 \times 10^{-12}$), *CMIP* ($P = 2.1 \times 10^{-10}$), *ZNF664* ($P = 2.3 \times 10^{-7}$) and *GPR109A* ($P = 7.4 \times 10^{-6}$). Conditional analysis at *ADIPOQ* revealed a second signal with suggestive evidence of association only after conditioning on the lead SNP ($P_{initial} = 0.020$; $P_{conditional} = 7.0 \times 10^{-7}$). We further confirmed the independence of two pairs of closely located loci (<2 Mb) on chromosome 16 at *CMIP* and *CDH13*, and on chromosome 12 at *GPR109A* and *ZNF664*. In addition, the newly identified signal near *WDR11-FGFR2* exhibited evidence of association with triglycerides ($P = 3.3 \times 10^{-4}$), high density lipoprotein cholesterol (HDL-C, $P = 4.9 \times 10^{-4}$) and body mass index (BMI)-adjusted waist–hip ratio ($P = 9.8 \times 10^{-3}$). These findings improve our knowledge of the genetic basis of adiponectin variation, demonstrate the shared allelic architecture for adiponectin with lipids and central obesity and motivate further studies of underlying mechanisms.**

## INTRODUCTION

Adiponectin is an adipocyte-secreted protein and blood adiponectin levels are positively associated with high density lipoprotein cholesterol (HDL-C) concentration and negatively correlated with the risk of type 2 diabetes (T2D), glucose, insulin, insulin resistance, triglycerides and anthropometric measures of obesity (1–3). Twins and family studies demonstrated an estimated 30–70% heritability for circulating adiponectin levels (4–6). A recent multi-ethnic meta-analysis of genome wide association (GWA) studies, including ∼40 000 Europeans, ∼4200 African Americans and ∼1800 East Asians, identified 10 novel loci associated with adiponectin levels (7), in addition to the previously reported *ADIPOQ*, *CDH13*, *ARL15* and *FER* (8–14). A multi-SNP genotype risk score that accounted for 5% of the variance of adiponectin levels exhibited significant association with T2D and markers of insulin resistance, suggesting a shared allelic architecture of adiponectin and other metabolic traits (7).

To date, only variants at *CDH13* and *ADIPOQ* exhibited significant association at $P < 5 \times 10^{-8}$ in studies of Asians (11,12,14,15). Large-scale meta-analysis of these and other GWA studies should increase the statistical power to detect and confirm additional loci. The *CDH13* signal that was initially identified in Asians and had a consistently stronger genetic effect in this population than in Europeans suggested that the genetic contributions may differ across populations (7,11,12,15). Meta-analyses of GWA studies in East Asians for T2D, body mass index (BMI), blood pressure and other metabolic traits have identified novel loci that show Asian-specific associations either due to differences in allele frequencies or due to genuine heterogeneity of genetic effects across continental populations (16–20).

Allelic heterogeneity is frequently observed in large genetic association studies (21–23). A deep resequencing of *ADIPOQ* in Europeans revealed seven variants exerting independent effects on the adiponectin level (24). A previous GWA study for adiponectin in Koreans suggested the existence of two signals at *CDH13*, but did not evaluate their independence (12). Two pairs of adiponectin loci are located <2 Mb apart, including *CDH13* and *CMIP* at 16q23.2–23.3 and *GPR109A* and *ZNF664* at 12q24.31; however, it is unclear whether these two nearby loci are independent of each other. Although a physical distance is frequently used to define independent signals, genomic regions have been reported with LD that extended >1 Mb (25,26). These findings motivated our analysis of closely co-localized adiponectin loci to evaluate independence.

We carried out the first meta-analysis of GWA studies for adiponectin in East Asians of the Asian Genetic Epidemiology

Network (AGEN). We aimed to identify novel adiponectin-associated variants/loci, evaluate whether previously identified loci are shared across ancestries and investigate the allelic heterogeneity at these loci, as well as the independence of the associations for SNPs at nearby loci. We further characterized novel loci by evaluating evidence of association with obesity and lipid traits in East Asians and Europeans.

## RESULTS

The meta-analysis included three stages, including GWA discovery and two stages of follow-up of selected SNPs (Supplementary Material, Fig. S1). Descriptions of collection, phenotyping and genotyping for study samples in each participating cohort are shown in the Supplementary Material, text and Table S1. The results of meta-analyses using the inverse-variance weighted and sample size-weighted meta-analysis methods were similar. No substantial difference was observed in results analyzed from Models 1 and 2, with or without the adjustment for BMI. We showed results based on Model 1 that accounted for BMI and meta-analyzed using an inverse-variance weighted method.

### Stage 1 GWA discovery

The meta-analysis of seven GWA studies including 7827 East Asians in discovery stage revealed three loci significantly associated with the adiponectin level at $P < 5.0 \times 10^{-8}$ (Table 1, Supplementary Material, Fig. S2). These loci included the previously described *CDH13* (rs4783244, $P = 2.0 \times 10^{-104}$) and *ADIPOQ* (rs10937273, $P = 1.1 \times 10^{-22}$), and a novel signal on chromosome 10, ~300 kb from *WDR11* and ~300 kb from *FGFR2* (rs3943077, $P = 1.2 \times 10^{-9}$) (Table 1). Our data also showed suggestive evidence of association ($P < 10^{-4}$) for four novel signals at *KCNH8* (rs12714975, $P = 1.2 \times 10^{-6}$), *OR8S1-LALBA* (rs11168618, $P = 1.7 \times 10^{-5}$), *HIVEP2* (rs12211360, $P = 1.0 \times 10^{-5}$) and *GAL3ST1* (rs6518702, $P = 4.5 \times 10^{-5}$). In addition, the signals previously reported in Europeans at *CMIP*, *PEPD*, *ZNF664*, *GPR109A* and *IRS1* also exhibited suggestive association with adiponectin at $P < 10^{-4}$ in East Asians (Table 1). The AGEN evidence of adiponectin association at other previously reported loci are described in Supplementary Material, Table S2. Furthermore, we did not observe evidence of sex-specific signals at $P < 5 \times 10^{-8}$, and all *P*-values for heterogeneity between sexes were $> 10^{-6}$ (uncorrected for multiple testing). All loci associated with the adiponectin level in the sex-combined analysis and all loci previously reported in other populations exhibited *P* for heterogeneity >0.02 in East Asians (Supplementary Material, Table S3).

### Stage 2 *in silico* follow-up

A total of 115 SNPs exhibiting genome-wide significant or suggestive association ($P < 10^{-4}$) in Stage 1 were tested for association with adiponectin level in three additional cohorts including up to 4298 individuals (Table 1). The meta-analysis of 10 cohorts consisting of 12 125 East Asians in combined Stages 1 and 2 confirmed the novel adiponectin locus near *WDR11-FGFR2* ($P = 1.8 \times 10^{-13}$). Four loci *KCNH8*, *OR8S1-LALBA*, *HIVEP2* and *GAL3ST1* that exhibited association at $P < 10^{-4}$ in Stage 1 also provided suggestive evidence of association in Stages 1 and 2 combined analysis with *P*-values between $2.8 \times 10^{-7}$ and $7.6 \times 10^{-6}$. In addition to *CDH13* and *ADIPOQ*, associations for SNPs at the previously reported *PEPD* (rs889140, $P = 3.6 \times 10^{-12}$) and *CMIP* (rs2925979, $P = 2.1 \times 10^{-10}$) loci reached genome-wide significance in Stages 1 and 2 combined meta-analysis. We also observed associations for SNPs at *ZNF664* (rs1187415, $P = 2.3 \times 10^{-7}$) and *GPR109A* (rs10847980, $P = 7.4 \times 10^{-6}$), whereas little evidence of association was observed at *IRS1* ($P = 1.4 \times 10^{-3}$).

### Stage 3 further follow-up

To further examine the possible novel signals that exhibited genome-wide significant or suggestive evidence of association in Stages 1 and 2 combined meta-analysis ($P < 10^{-5}$), five SNPs were investigated in four additional cohorts including up to 5954 individuals (Table 1). The meta-analysis combining all 14 cohorts including 18 079 individuals in the discovery and two follow-up stages provided additional evidence for the signal near *WDR11-FGFR2* which had already achieved genome-wide significance in Stages 1 and 2 ($P = 3.0 \times 10^{-14}$, Fig. 1A). The data also provided supporting yet still suggestive evidence of another locus near *OR8S1-LALBA*, which did not reach but approximated to the genome-wide significance ($P = 1.2 \times 10^{-7}$) (Fig. 1B). However, the Stages 1, 2 and 3 combined meta-analysis did not strongly support the association at *HIVEP2*, *GAL3ST1* and *KCNH8*, which showed less evidence of association despite an increased statistical power when additional subjects were included in the analysis (Table 1).

### Conditional analysis

To explore the presence of additional signals at adiponectin-associated loci, we performed conditional analyses at *WDR11-FGFR2*, *ADIPOQ*, *GPR109A*, *ZNF664*, *CDH13*, *CMIP* and *PEPD* loci by conditioning on the lead SNP at each of the seven loci and testing the residual association with all remaining SNPs within ± 500 kb flanking regions of the lead SNPs. We also carried out conditional analyses to evaluate independence of the association for signals at two pairs of closely located (<2 Mb) loci, *GPR109A* and *ZNF664* on 12q24.31, and at *CMIP* and *CDH13* on 16q23.2-23.3. Meta-analysis of the seven cohorts in Stage 1 revealed a second signal near *ADIPOQ* exhibiting suggestive evidence of association only after conditioning on the lead SNP rs10937273 (*EIF4A2*-rs266719: $P_{\text{initial}} = 0.020$, $P_{\text{conditional}} = 7.0 \times 10^{-7}$; Table 2, Supplementary Material, Fig. S3). The other six loci each had only one signal ($P_{\text{conditional}} > 10^{-4}$) within the ± 500 kb flanking region of the index SNPs. We next performed conditional analysis on the 2 Mb genomic region (chr12: 121.4–123.4 Mb) that included *GPR109A* and *ZNF664*. When we conditioned on *ZNF664* rs1187415, the second best signal in this region was rs10847980 near *GPR109A*, with no reduction of association in both magnitude and significance (Table 2, Supplementary Material, Fig. S4A and B). In reciprocal conditional analysis accounting for *GPR109A* rs10847980, the effect size and *P*-value of association for rs1187415 did not change (Table 2, Supplementary Material, Fig. S4A and C). When both rs1187415 and rs10847980 were included in

**Table 1.** Loci associated with adiponectin

| Locus/ nearby gene | Index SNP | Chr | Position (hg18) | Effect/ non-effect alleles | Stage 1 ($n = 7827$) | | | Stages 1 + 2 ($n = 12\,125$) | | Stages 1 + 2 + 3 ($n = 18\,079$) | | Direction[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | EAF[a] | $\beta$ (SE) | $P$ | $\beta$ (SE) | $P$ | Beta (SE) | $P$ | |
| Novel locus exhibiting GWA with adiponectin | | | | | | | | | | | | |
| *WDR11-FGFR2* | rs3943077 | 10 | 122 935 076 | A/G | 0.567 | 0.09 (0.02) | 1.2E−09 | 0.09 (0.01) | 1.8E−13 | 0.07 (0.01) | 3.0E-14 | +++++++++++++++− |
| Loci exhibiting suggestive association with adiponectin | | | | | | | | | | | | |
| *OR8S1-LALBA* | rs11168618 | 12 | 47 219 500 | T/C | 0.137 | −0.10 (0.02) | 1.7E−05 | -0.08 (0.02) | 1.1E−06 | −0.06 (0.01) | 1.2E−07 | −−+−−−−−−−−−−−+ |
| *HIVEP2* | rs12211360 | 6 | 143 161 525 | A/G | 0.966 | −0.21 (0.05) | 1.0E−05 | −0.21 (0.04) | 2.8E−07 | −0.16 (0.03) | 5.5E−06 | −−−?−−−?−−+−−+ |
| *KCNH8* | rs12714975 | 3 | 19 060 378 | C/G | 0.047 | 0.21 (0.04) | 1.2E−06 | 0.16 (0.04) | 7.6E−06 | 0.12 (0.03) | 8.9E−05 | ++++−++++−−−++ |
| *GAL3ST1* | rs6518702 | 22 | 29 278 752 | T/C | 0.249 | −0.08 (0.02) | 4.5E−05 | −0.06 (0.01) | 5.2E−06 | −0.04 (0.01) | 5.3E−04 | −−−−−−−−−+−++−− |
| Known loci with previous evidence of association with adiponectin ($P < 10^{-4}$ in stage 1) | | | | | | | | | | | | |
| *CDH13* | rs4783244 | 16 | 81 219 769 | T/G | 0.360 | −0.34 (0.02) | 2.0E−104 | −0.33 (0.01) | 6.8E−165 | n.a. | n.a. | −−−−−−−−−−− |
| *ADIPOQ* | rs10937273 | 3 | 188 032 389 | A/G | 0.404 | 0.15 (0.02) | 1.1E−22 | 0.12 (0.01) | 1.8E−22 | n.a. | n.a. | ++++++++++ |
| *PEPD* | rs889140 | 19 | 38 580 840 | A/G | 0.450 | 0.07 (0.02) | 8.4E−06 | 0.08 (0.01) | 3.6E−12 | n.a. | n.a. | ++−+++++++ |
| *CMIP* | rs2925979 | 16 | 80 092 291 | T/C | 0.411 | −0.07 (0.02) | 5.3E−06 | −0.08 (0.01) | 2.1E−10 | n.a. | n.a. | −−+−−−+−−− |
| *ZNF664* | rs1187415 | 12 | 123 057 482 | C/G | 0.920 | −0.14 (0.03) | 1.2E−06 | −0.11 (0.02) | 2.3E−07 | n.a. | n.a. | −−−−−−−−−− |
| *GPR109A* | rs10847980 | 12 | 121 953 875 | T/G | 0.771 | −0.08 (0.02) | 7.2E−06 | −0.06 (0.01) | 7.4E−06 | n.a. | n.a. | −−−−−−−−−+ |
| *IRS1* | rs7558386 | 2 | 227 270 383 | A/G | 0.341 | −0.06 (0.02) | 7.8E−05 | −0.04 (0.01) | 1.4E−03 | n.a. | n.a. | −−−−−−−−−− |

[a]EAF, effect allele frequency based on the data in Stage 1.
[b]Effect direction of each individual studies in the order of SP2_1M, SP2_610 K, SP2_550 K, KCPS-II, CLHNS, NHAPC Beijing, and NHAPC Shanghai in Stage 1, Ansan, KING_GWAS, SAPPHIRe in Stage 2 and followed by KING_noGWAS, ACC, Nomura and SMHS in Stage 3 if the cohorts were included in the analysis.

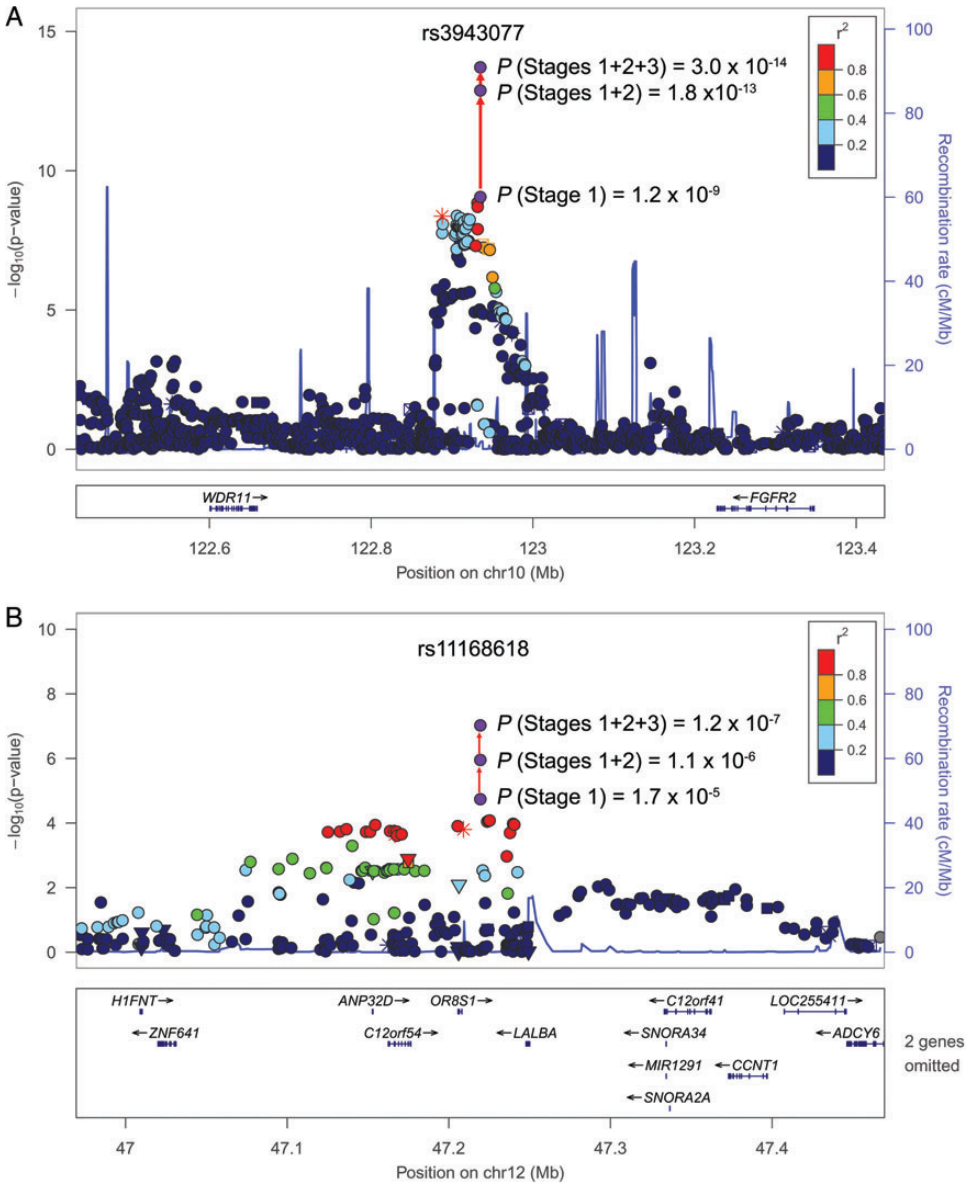


**Figure 1.** Regional plots of the novel and suggestive adiponectin-associated loci identified in individuals of East Asian ancestry. (**A**) The novel locus near *WDR11-FGFR2* on chromosome 10. The purple circle represents the index SNP rs3943077 (chr10:122 935 076; Build 36, hg18), which exhibits the strongest evidence of association at this locus based on HapMap-imputed data. SNPs are colored based on HapMap Phase II CHB + JPT linkage disequilibrium with rs3943077. Nearby gene *WDR11* is located 122.601–122.659 Mb and *FGFR2* is located at 123.228–123.348 Mb. (**B**) The suggestive locus at *OR8S1-LALBA* on chromosome 12. The index SNP rs11168618 (chr12:47 219 500) has the strongest evidence of association near *OR8S1* (47.206–47.208 Mb) and *LALBA* (47.248–47.250 Mb). The LD $r^2$ is also based on HapMap Phase II CHB + JPT data.

conditional analysis, the association for all other SNPs in the 2 Mb region were not significant (all $P_{\text{conditional}} > 10^{-4}$ in stage 1 meta-analysis), providing no evidence for a third signal at this region. Similarly, when rs2925979 at *CMIP* was conditioned on rs4783244 at the strong signal *CDH13*, and vice versa, little change of association was observed, indicating two independent loci at 16q23.2–23.3 (Table 2, Supplementary Material, Fig. S5).

## Characterization of novel loci

We looked up the lead SNPs near *WDR11-FGFR2* and *OR8S1-LALBA* loci for evidence of adiponectin association in the publicly released data of ADIPOGen European discovery meta-analysis (http://www.mcgill.ca/genepi/adipogen-consortium). The SNP rs3943077 near *WDR11-FGFR2* showed consistent direction of allelic effect, but did not exhibit strong evidence of association ($P = 0.093$) in $>$ 29 000 Europeans (Table 3). Despite a lower allele frequency of rs3943007 in ADIPOGen (A allele = 0.24) compared with that in AGEN (A allele = 0.57), the European study has a $>$ 96% power to detect the effect size ($\beta_Z = 0.07$) observed in AGEN at a threshold of $P < 5 \times 10^{-8}$. The differences in allele frequency and significant level of association suggested that variants at *WDR11-FGFR2* might have a larger genetic effect on levels

**Table 2.** Regions with multiple signals or independent loci associated with adiponectin ($P_{conditional} < 10^{-4}$)

| Index SNP | Chr | Position | Effect/ non-effect alleles | EAF | Main effect analysis[a] | | Conditional analysis[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $\beta$ (SE) | $P$ | $\beta$ (SE) | $P$ |
| *ADIPOQ* | | | | | | | | |
| rs10937273 | 3 | 188 032 389 | A/G | 0.404 | 0.15 (0.02) | 5.7E−23 | 0.16 (0.02) | 6.9E−26 |
| rs266719 | 3 | 187 984 342 | T/C | 0.096 | 0.06 (0.03) | 0.020 | 0.13 (0.03) | 7.0E−07 |
| *GPR109A-ZNF664* | | | | | | | | |
| rs1187415 | 12 | 123 057 482 | C/G | 0.920 | −0.14 (0.03) | 1.0E−06 | −0.14 (0.03) | 1.2E−06 |
| rs10847980 | 12 | 121 953 876 | T/G | 0.771 | −0.08 (0.02) | 6.8E−06 | −0.08 (0.02) | 9.6E−06 |
| *CMIP-CDH13* | | | | | | | | |
| rs4783244 | 16 | 81 219 769 | T/G | 0.450 | −0.34 (0.02) | 9.5E−106 | −0.34 (0.02) | 1.8E−106 |
| rs2925979 | 16 | 80 092 291 | T/C | 0.411 | −0.07 (0.02) | 5.1E−06 | −0.07 (0.02) | 4.8E−06 |

[a]The standard errors (SEs) and *P*-values from Stage 1 main effect analysis were not corrected for genomic control, thus the statistics can be compared with those from the regional conditional analyses.
[b]Reciprocal conditional analyses were performed; The effect sizes and *P*-values in conditional analysis for one SNP were conditioned on the other, and vice versa.
EAF, effect allele frequency.

**Table 3.** Association of the novel and suggestive loci with adiponectin and obesity-related traits in other consortium

| Trait | Consortium | Ethnicity | *WDR11-FGFR2*-rs3943077 | | | *OR8S1-LALBA*-rs11168618 | | |
|---|---|---|---|---|---|---|---|---|
| | | | Direction[a] | $P$ | N | Direction[a] | $P$ | N |
| Adiponectin | ADIPOGen | European | + | 0.093 | 29 202 | − | 0.47 | 29 328 |
| TG | AGEN | East Asian | − | 3.3E-04 | 8311 | − | 0.16 | 18 393 |
| HDL-C | AGEN | East Asian | + | 4.9E-04 | 15 035 | + | 0.040 | 25 112 |
| LDL-C | AGEN | East Asian | + | 0.82 | 12 651 | + | 0.81 | 22 470 |
| TC | AGEN | East Asian | + | 0.89 | 12 672 | + | 0.31 | 22 756 |
| Obesity (BMI $\geq$ 27.5 kg/m$^2$) | AGEN | East Asian | + | 0.17 | 32 380 | − | 0.25 | 46 355 |
| BMI | AGEN | East Asian | + | 0.43 | 32 380 | − | 0.49 | 46 355 |
| WC | AGEN | East Asian | + | 0.41 | 22 174 | − | 0.51 | 33 202 |
| WCadjBMI | AGEN | East Asian | − | 0.91 | 22 174 | − | 0.68 | 33 202 |
| WHR | AGEN | East Asian | − | 0.094 | 17 560 | + | 0.77 | 26 397 |
| WHRadjBMI | AGEN | East Asian | − | 9.8E-03 | 17 560 | + | 0.61 | 26 397 |
| BMI | GIANT | European | + | 0.72 | 123 862 | − | 0.48 | 123 855 |
| WHRadjBMI | GIANT | European | − | 0.013 | 77 165 | − | 0.82 | 77 163 |

[a]The directions of effect are based on the alleles (rs3943077-A; rs11168618-C) associated with increased adiponectin levels in this study. The A allele frequency of rs3943077 is 0.57 in AGEN and 0.24 in AdipoGEN; the C allele frequency of rs11168618 is 0.86 and 0.46 in AGEN and AdipoGEN, respectively. TG: triglycerides; TC: total cholesterol; WC: waist circumference; WCadjBMI: BMI-adjusted waist circumference; WHR: waist–hip ratio; WHRadjBMI: BMI-adjusted waist–hip ratio.

of adiponectin in East Asians than Europeans, or the pairwise LD between the index SNP and the untyped causal variant vary across different populations. No evidence of association was detected for rs11168618 at *OR8S1-LALBA* in ADIPOGen Europeans ($P = 0.47$).

HDL-C level is usually the trait most strongly correlated with adiponectin in both Europeans and East Asians, while measures of insulin resistance or obesity are the next closest correlates (2,3,27–29). We confirmed these correlations in our study populations (Supplementary Material, Table S4), and next investigated the SNP association with other phenotypes, including lipid profiles and obesity-related anthropometric traits, which were available in AGEN or other consortia (Table 3). We found that the adiponectin-increasing allele of rs3943077 at *WDR11-FGFR2* was significantly associated with decreased triglycerides ($P = 3.3 \times 10^{-4}$) and increased HDL-C ($P = 4.9 \times 10^{-4}$) levels in East Asians from the AGEN consortium. The SNP rs11618618 at *OR8S1-LALBA* exhibited a borderline association with HDL-C in East Asians ($P = 0.040$). In addition, the A allele of rs3943077 associated with increased adiponectin level was associated with decreased WHRadjBMI in East Asians ($P = 9.8 \times 10^{-3}$). GIANT data including up to 77 000 Europeans also showed a borderline association between rs3943077 and WHRadjBMI ($P = 0.013$) with consistent direction of effect.

The novel signal near *WDR11-FGFR2* explained 0.6% of the total variation in adiponectin. To assess whether this signal could be refined, we investigated additional variants within $\pm$500 kb of rs3943077 by testing the association of SNPs imputed from the 1000 Genomes Project in a subset of 3778 individuals from the Singapore prospective study program (SP2)_1M, SP2_610 and the Cebu Longitudinal Health and Nutrition Survey (CLHNS) that had imputed data available. The most strongly associated SNP (rs72631105, EAF = 0.632, $\beta = 0.13$, $P = 5.4 \times 10^{-7}$) was located 30 kb away and in a moderate LD ($r^2/D' = 0.63/0.89$ in Genomes Project Phase 1 ASN) with rs3943077 (EAF = 0.541, $\beta = 0.10$, $P = 1.4 \times 10^{-6}$) (Supplementary Material, Fig. S6). All seven variants that exhibited stronger evidence of association were located 0.16–35 kb from rs3943077 and were not present in the HapMap reference panel. Six of these variants were in moderate to high LD ($r^2$ 0.63–1.00) with rs3943077, except rs10886862 (EAF =

0.339, $r^2/D' = 0.23/0.84$). Considering that imputation inaccuracy (e.g. rs72631105: IMPUTE proper info ~0.75; MAHC Rsq ~0.65) may introduce uncertainty into the association results, the lead SNP from the 1000 Genomes imputation in a subset of samples may not be a better candidate causal variant.

The index SNP rs3943077 was located at an uncharacterized large intergenic non-coding RNA (lincRNA) ENST00000 429809, with two predicted exons and a long intergenic region. Twenty-two variants spanning 65 kb are in moderate to high LD with the index SNP rs3943077 ($r^2 > 0.6$ based on the 1000 Genomes Project Phase 1 ASN) and seven of them overlap the lincRNA. We successfully amplified and sequence-verified this transcript from RNA of testes where the transcript was initially identified, but not other tissues including adipose and liver (data not shown). At least four LD proxies of rs3943077 are located at or near enhancer marks in adipose nuclei and predicted to possibly alter the transcriptional activity of nearby genes (30,31). *FGFR2* implicated in adipocyte hyperplasia and hypertrophy (32,33) is a good candidate gene; however, luciferase reporter assays in differentiated adipocytes showed no allelic difference in transcriptional activity for the five SNPs tested (data not shown).

## DISCUSSION

This study is the largest GWAS meta-analysis conducted for adiponectin association in populations of East Asian ancestry to date. The three-stage meta-analyses provided convincing evidence of a novel adiponectin-associated locus near *WDR11-FGFR2*. Our data also suggested a potential new locus near *OR8S1-LALBA* that did not reach traditional threshold of GWA significance. In addition to confirming the previously described loci of *CDH13*, *ADIPOQ*, *PEPD*, *CMIP*, *GPR109A* and *ZNF664*, we identified a second signal at *EIF4A2* near *ADIPOQ* that exhibited suggestive evidence of association only after conditioning the lead SNP. Our findings demonstrated the independence of two pairs of closely located loci on chromosome 16 at *CMIP* and *CDH13*, and on chromosome 12 at *GPR109A* and *ZNF664*. The adiponectin-increasing allele of the index SNP near *WDR11-FGFR2* was also associated with increased HDL-C, decreased triglycerides and decreased BMI-adjusted WHR.

We hypothesize that the novel locus would likely act by regulating the expression or function of a transcript that could affect adiponectin production or secretion. A nearby transcript *FGFR2*, located ~ 300 kb away and encoding fibroblast growth factor receptor type 2, is a strong candidate gene. Abundantly expressed in human and mouse adipocytes, FGFR2 includes two alternatively spliced isoforms, FGFR2b and FGFR2c, which have different specificities for ligands and patterns of expression (34,35). FGFR2b is a receptor for FGF10 and regulates the proliferation of preadipocytes and the subsequent differentiation into mature adipocytes (32). Adiponectin is not expressed in preadipocytes; differentiation into mature adipocytes is necessary for adiponectin expression and secretion (36). In mouse white adipose tissue, Fgfr2c is a receptor for Fgf9 and affects hypertrophy of mature adipocytes (33). An increase in the size of mature adipocytes dysregulates the expression of adipokines, including adiponectin (37). Hence, the involvement of FGFR2b and FGFR2c in the processes of adipocyte hyperplasia and hypertrophy suggests possible mechanisms that link FGFR2 to adiponectin regulation.

While consistent evidence supports the adiponectin association with variants in or near *ADIPOQ* in diverse populations, the most strongly associated SNPs are not shared across studies. The lead SNP rs6810075 reported in Europeans by ADIPOGen, though also significantly associated with adiponectin in East Asians ($\beta = 0.12$, $P = 4.7 \times 10^{-16}$, effect allele frequency = 0.55), exhibited weaker evidence of association compared with that for our index SNP rs10937273 ($\beta = 0.15$, $P = 1.1 \times 10^{-22}$, effect allele frequency = 0.41). The magnitude and significance level for rs6810075 were substantially attenuated ($\beta = 0.01$, $P_{\text{conditional}} = 0.36$) when conditioning on our index SNP rs10937273. The two variants were moderately correlated, with LD estimates of $r^2/D' = 0.58/1.00$ and 0.44/1.00 in the 1000 Genomes Project Phase 1 ASN and EUR, respectively. Therefore, rs10937273 and rs6810075 likely represent the same signal at *ADIPOQ*.

However, there is a suggestion of a secondary signal rs266719 located ~60 kb upstream of *ADIPOQ* at *EIF4A2*, the gene encoding eukaryotic initiation factor 4A (EIF4A), isoform 2. EIF4A is an ATP-dependent RNA helicase and forms the translational initiation complex EIF4F (38), which has been shown to regulate the expression of C/EBPs that affect adipocyte differentiation, adipogenesis and insulin sensitivity (39). Genetic variants may affect adiponectin levels by influencing *EIF4A2* expression or by acting more distantly on *ADIPOQ* expression. The identification of this second signal that showed association with adiponectin only after conditioning on the lead signal suggests allelic heterogeneity at this locus but a complex pattern of association (40). The trait-lowering allele of rs266719 (C allele frequency = 0.904) is coupled with the trait-increasing allele of rs10937273 (A allele frequency = 0.404) on the same haplotype (LD $r^2/D' = 0.03/0.77$; frequencies of rs266719–rs10937273 haplotypes: CG = 0.559, CA = 0.360, TG = 0.077 and TA = 0.007, 1000 Genomes Project Phase 1 ASN), thus the significance of residual association increased when accounting for the other signal. An SNP–SNP interaction might underlie the association. Prior evidence exists for multiple signals at *ADIPOQ* (24,41); however, these SNPs may still be partially tagged by untyped variants (40). Therefore, more detailed characterization of allelic heterogeneity requires deeper sequencing and functional assessment.

Our data from conditional analysis demonstrate that the locus *CDH13* is independent of *CMIP* located 1 Mb away (7), but did not support the previous evidence of two signals at *CDH13* (rs3865188, rs3865186, $r^2/D' = 0.34/0.97$) (12) (Supplementary Material, Fig. S7). Our index SNP rs4783244 is highly correlated with the previously reported first signal rs3865188 (LD $r^2/D' = 0.90/0.97$), and conditioning on this signal substantially attenuated the association with the previously described second signal (rs3865186, $P_{\text{initial}} = 2.3 \times 10^{-49}$, $P_{\text{conditional}} = 0.058$). Although the pairwise LD is modest, conditional analysis suggested that the second signal could be explained by the initial signal. The signal at *CDH13* consistently has been reported to exhibit stronger evidence of association compared with that at *ADIPOQ* in all published GWA studies for adiponectin in Asian populations (11,12,14,15), while the *ADIPOQ* has shown the strongest adiponectin association in populations of

European ancestry (7–10,13). At *CDH13*, the index SNPs from our East Asian samples (rs4783244) and the ADIPOGen Europeans (rs12922394) are weakly correlated (LD $r^2/D' = 0.36/0.71$ and 0.04/0.75 in the 1000 Genomes Project Phase 1 ASN and EUR, respectively) and have varied allele frequencies (rs4783244: 0.36 in East Asians and 0.46 in Europeans; rs12922394: 0.24 in East Asians and 0.07 in Europeans). Similarly, the lead SNP rs12051272 from the ADIPOGen multiethnic meta-analysis was common in Asians (minor allele frequency, MAF = 0.33), but rare in Europeans and African Americans (MAF = 0.03 for both) (7), and the pairwise LD between rs12051272 and rs4783244 differs across populations ($r^2/D' = 0.95/0.99$, 0.03/1.00 and 0.10/1.00 in the 1000 Genomes Project Phase 1 ASN, EUR and AFR, respectively). These varied allele frequencies and LD structures may explain the differences in strength of genetic association across continental populations. The differences may also be influenced by differing environmental exposures that modulate the effect of a gene (42). Consistent with previous findings (11,43), we found little evidence of the association between *CDH13* and other metabolic and cardiovascular-related traits in East Asians (all $P > 0.05$).

In this study, we also generalized the adiponectin association with *GPR109A* and *ZNF664* at 12q24.31 to populations of East Asian ancestry, and confirmed that the two loci located ~1 Mb apart were independently associated with adiponectin. The *ZNF664* index SNPs identified in Europeans (rs7133378) and East Asians (rs1187415) were in moderate to high LD ($r^2/D' = 0.64/1.00$ and 0.90/1.00 in the 1000 Genomes Project Phase 1 ASN and EUR, respectively), suggesting that both the groups shared the same signal. At *GPR109A*, the lead SNP rs10847980 identified in this study was ~200 kb away from the European index rs601339 and these two SNPs were weakly correlated ($r^2/D' = 0.02/0.31$ and 0.03/0.30 in the 1000 Genomes Project Phase 1 ASN and EUR). The SNP rs601339 only exhibited borderline association with adiponectin in East Asians ($P_{\text{initial}} = 0.014$), and this association can be explained by rs10847980 ($P_{\text{conditional}} = 0.20$ for rs601339). The differences in lead SNPs from the different populations might reflect different frequencies, different causal variants or that index SNPs may be only correlated with one or more underlying causal variants not analyzed. Further study of biological mechanisms is warranted to determine whether the signals at *GRP109A* and *ZNF664* act independently on distinct genes or on the same gene. Among nearby candidates, GPR109A has been shown to be required for niacin-stimulated adiponectin secretion (44).

The adiponectin-increasing allele of the *WDR11-FGFR2* index SNP was associated with an increased HDL-C, decreased triglycerides and decreased BMI-adjusted WHR in East Asians. This direction of the genetic effects on these traits agrees with the consistently observational positive correlation of adiponectin with HDL-C and the inverse correlation with triglycerides and indices of abdominal obesity (Supplementary Material, Table S4) (45–47). In addition, the more pronounced evidence of SNP association with BMI-adjusted WHR ($P = 9.8 \times 10^{-3}$) compared with BMI ($P = 0.43$) suggests that *WDR11-FGFR2* variants directly or indirectly influence abdominal obesity, a better predictor of metabolic and cardiovascular risk (48,49) than the overall obesity. Several other known adiponectin loci also exhibited evidence of association with other metabolic and cardiovascular risks. A SNP rs3786897 at *PEPD* was previously reported to be associated with the risk of T2D in East Asians (16); this SNP is in complete LD with the adiponectin index rs889140 ($r^2/D' = 0.99/1.00$ in the 1000 Genomes Project Phase 1 ASN), demonstrating a shared signal for adiponectin and T2D in this population. SNPs near *ZNF664*, associated with HDL-C and triglycerides in Europeans (23), are highly correlated with the adiponectin signal in both Europeans and Asians (rs4765127 and rs1187415, $r^2/D' = 0.97/0.99$ in the 1000 Genomes Project Phase 1 EUR and 0.92/0.96 in 1000Genomes ASN). In addition, the same index SNP rs2925979 at *CMIP* exhibited association with HDL-C in Europeans (23) and with adiponectin in our data. *CMIP* also displayed suggestive evidence of association with T2D in East Asians; however, the signals for T2D and adiponectin were weakly correlated ($r^2/D' = 0.14/0.51$ in the 1000 Genomes Project Phase 1 ASN). The South Asian-specific T2D locus *ST6GAL1* (50) was ~100 kb away from *ADIPOQ*; but the T2D index SNP rs16861329 is not in LD with either of the two adiponectin-associated signals at *ADIPOQ* (LD $r^2 = 0$). Given our current data, we were unable to determine whether the genetic effect of adiponectin loci on related metabolic traits is due to a pleiotropic effect or through SNP influence on adiponectin. Nevertheless, these findings support the prior suggestions of a shared allelic architecture of adiponectin levels and related metabolic traits (7) and motivate further studies to investigate potential cause–effect relationships between traits (51,52).

In conclusion, this GWAS meta-analysis for adiponectin in East Asians provides the first evidence for a novel locus near *WDR11-FGFR2* and expands the understanding of the genetic basis of adiponectin levels at several known loci. The findings that the novel adiponectin locus near *WDR11-FGFR2* also displayed association with HDL-C, triglycerides and BMI-adjusted WHR demonstrate the shared allelic architecture for adiponectin with lipid traits and central obesity, and motivate further studies of underlying biological mechanisms.

## MATERIALS AND METHODS

### Study population and phenotype

The Asian Genetic Epidemiology Network (AGEN) is a consortium of genetic epidemiology studies of metabolic and cardiovascular diseases and related traits conducted in individuals of East Asian ancestry (http://www.agenconsortium.org/). This AGEN adiponectin study consisted of a total of 18 079 individuals from 14 cohorts that participated in three stages of meta-analysis. The participating cohorts are either population-based ($n = 13$) or family-based ($n = 1$). Stage 1 of GWA discovery consisted of 7827 Chinese, Korean and Filipino individuals from SP2, the Korean Cancer Prevention Study II (KCPS-II), CLHNS and the Nutrition and Health of Aging Population in China (NHAPC). SP2 consisted of three independent cohorts of SP2_1M, SP2_610 K and SP2_550 K genotyped with different platforms. NHAPC included two independent cohorts of NHAPC Beijing and NHAPC Shanghai based on the sites where individuals were recruited. Stage 2 of *in silico* replication included 4298 individuals from the Ansan cohort (Ansan), Kita-Nagoya Genomic Epidemiology Study (KING) and the Stanford Asian Pacific Program in Hypertension and Insulin

Resistance (SAPPHIRe). Stage 3 contains 5954 individuals from three Japanese cohorts of KING, the anti-aging center cohort study (AAC) and Nomura cohort study (Nomura) and one Chinese cohort of Shanghai Men's Health Study (SMHS). Plasma or serum adiponectin levels were measured via an enzyme-linked immunosorbent assay method, a latex enhanced imunoturbidimetric assay or Luminex xMAPTM Technology. Total adiponectin was measured in all studies, except Nomura, in which high-molecular weight adiponectin was assessed. Further description of the sample characteristics is given in detail in the Supplementary Material, text and Table S1. The correlation structures between adiponectin and these traits are shown in the Supplementary Material, Table S4. The sex-stratified measures of adiponectin and other metabolic/cardiovascular-related traits are described in Supplementary Material, Table S5. All study protocols were approved by Institutional Review Boards at their respective sites, and written informed consent was obtained from all participants.

### Genotyping, imputation and quality control

Individuals in Stages 1 and 2 were genotyped using commercially available Illumina or Affymetrix genome-wide genotyping arrays. Supplementary Material, Table S1, summarizes the genotyping platforms, quality control criteria across studies, including SNP call rate, sample success rate, Hardy–Weinberg equilibrium and MAF. Imputation of HapMap haplotypes (CHB + JPT for all samples except CLHNS which used CHB + JPT + CEU) of ~2 million SNPs was carried out for each study using IMPUTE or MACH. Additional imputation within ± 500 kb flanking region of rs3943077 at *WDR11-FGFR2* was performed based on the haplotypes from the 1000 Genomes Project Phase 1 release (November 2010) of all Asian samples (ASN) in a subset of 3778 individuals from three Stage 1 cohorts, including SP2_1M, SP2_610K and CLHNS. SNPs with poor imputation quality (proper info <0.5 for IMPUTE or Rsq < 0.3 for MACH) were excluded from association analysis. In Stage 3, genotyping for individuals from the KING_noGWAS, ACC and Nomura cohorts ($n = 5724$) was carried out using TaqMan, and all five SNPs had call rates >98.8%. SNP genotyping and imputation in SMHS ($n = 230$) were carried out using Affymetrix 6.0 and MACH, respectively. All five SNPs analyzed in SMHS were imputed from phased haplotypes of HapMap (R22 CHB + JPT), with imputation quality (MACH_Rsq) >0.76.

### Statistical analysis and SNP prioritization

*Association analyses within each cohort*

In each individual cohort, adiponectin was natural log transformed to approximate normal distribution. Outliers defined as values greater than mean ± 4 SD were truncated. As the ranges of adiponectin levels substantially varied across studies (Supplementary Material, Table S1), natural log-transformed adiponectin was standardized to *z*-scores. In population-based studies, multiple linear regression models assuming an additive mode of inheritance were applied to test for association with genotyped or imputed SNPs by accounting for age, sex and BMI in Model 1, and without the adjustment for BMI in Model 2. The family-based study used regression models by the generalized estimating equation approach to adjust for the same covariates while also accounting for correlations among related individuals. Software applied for association analysis in each study is described in Supplementary Material, Table S1.

*Meta-analysis of GWAS in Stage 1*

The meta-analysis for adiponectin association with ~2.5 million SNPs was performed by two analysts independently each using two different methods of sample size weighted and inverse-variance weighted models implemented in METAL. Prior to meta-analysis, cohort-specific summary statistics were corrected using genomic control ($\lambda_{GC}$ ranges 0.997–1.033), and the overall meta-analytic results were additionally corrected for genomic control ($\lambda_{GC} = 1.009$). The presence of heterogeneity was assessed by $I^2$ statistic and Cochran's *Q*-test. After meta-analysis, ~226 000 (9%) SNPs were removed due to an effective sample size of <50% of the total sample size in Stage 1 and/or evidence of heterogeneity across cohorts (*P* for Cochran's *Q*-test $< 10^{-6}$). We applied the genome-wide association meta-analysis software to perform the sex-specific meta-analysis and test for heterogeneity between sex using the whole genome association data (53,54).

In silico *follow-up in Stage 2*

A total of 612 SNPs had a meta-analyzed *P*-value of $<10^{-4}$ in either Model 1 or 2. To prioritize SNPs for Stage 2 follow-up, we applied the '—clump' command implemented in PLINK (55) (http://pngu.mgh.harvard.edu/~purcell/plink/), by setting the LD threshold of $r^2 < 0.1$ in HapMap reference panel of CHB + JPT_r23a and disregarding the physical distance between SNPs. A total of 115 SNPs, including 110 clumped SNPs and 5 extra variants at/near each locus of *WDR11-FGFR2*, *CDH13*, *ADIPOQ*, *PEPD* and *ZNF664*, were tested for association with adiponectin in 4298 individuals from three cohorts with GWAS data. The cohort-level summary statistics from the *in silico* follow-up were meta-analyzed with the data from the seven individual cohorts in Stage 1.

*Further follow-up in Stage 3*

We selected lead SNPs representing the five novel genome-wide significant or suggestive loci ($P < 10^{-5}$; *WDR11-FGFR2*, *KCNH8*, *OR8S1-LALBA*, *HIVEP2* and *GAL3ST1*) from the Stages 1 and 2 combined meta-analysis, and followed up these loci in 5954 individuals from the four cohorts in Stage 3. Joint meta-analysis was carried out by combining the cohort-level summary statistics from all the 14 individual cohorts in Stages 1, 2 and 3.

*Conditional analysis*

Conditional analysis was conducted in the seven cohorts in Stage 1 by adding the most strongly associated SNP at a locus into the regression model as a covariate and testing the residual association with all remaining SNPs within ± 500 kb flanking regions of the lead SNP. Sequential conditional analyses were performed until the strongest SNP displayed a conditional *P*–value of $>10^{-4}$ in meta-analysis of the seven cohorts. Reciprocal conditional analyses were also carried out at two pairs of closely located (<2 Mb) loci, *GPR109A* and *ZNF664* on 12q24.31, and at *CMIP* and *CDH13* on 16q23.2–23.3, to

evaluate the independence of the association for these nearby loci. The regions for conditional analyses and the SNPs used as conditioning variables are shown in Supplementary Material, Table S6.

The explained phenotypic variance was calculated as: $2 \times \text{MAF} \times (1 - \text{MAF}) \times \beta_z^2$ (56). Regional association plots were created using LocusZoom (57).

*SNP association with lipid and obesity-related anthropometric traits in Asians*

We investigated the evidence of association for the two variants of rs3943077 at *WDR11-FGFR2* and rs11618618 at *OR8S1-LALBA* with lipid and obesity-related anthropometric traits that were available in other AGEN studies (Supplementary Material, text). The on-going AGEN lipids study provided the summary statistics for the SNP associations with triglycerides, HDL-C, LDL-C and total cholesterol in up to 25 413 Asians from 13 cohorts in the discovery stage. Association results for obesity and obesity-related anthropometric traits, including BMI, waist circumference and waist–hip ratio, were provided by the AGEN BMI study, the discovery stage of which consisted of 86 757 Asians from 21 individual studies.

## SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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