Adiponectin Concentrations: A Genome-wide Association Study

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Adiponectin is associated with obesity and insulin resistance. To date, there has been no genome-wide association study (GWAS) of adiponectin levels in Asians. Here we present a GWAS of a cohort of Korean volunteers. A total of 4,001 subjects were genotyped by using a genome-wide marker panel in a two-stage design (979 subjects initially and 3,022 in a second stage). Another 2,304 subjects were used for follow-up replication studies with selected markers. In the discovery phase, the top SNP associated with mean log adiponectin was rs3865188 in *CDH13* on chromosome 16 ($p = 1.69 \times 10^{-15}$ in the initial sample, $p = 6.58 \times 10^{-39}$ in the second genome-wide sample, and $p = 2.12 \times 10^{-32}$ in the replication sample). The meta-analysis p value for rs3865188 in all 6,305 individuals was 2.82×10^{-83}. The association of rs3865188 with high-molecular-weight adiponectin ($p = 7.36 \times 10^{-58}$) was even stronger in the third sample. A reporter assay that evaluated the effects of a *CDH13* promoter SNP in complete linkage disequilibrium with rs3865188 revealed that the major allele increased expression 2.2-fold. This study clearly shows that genetic variants in *CDH13* influence adiponectin levels in Korean adults.

Adiponectin in serum decreases insulin resistance and body weight by increasing lipid oxidation in muscle and other organs, such as the pancreas and liver.[1] Adiponectin is reduced among obese individuals, as well as those with diabetes mellitus or coronary heart disease.[2,3] Adiponectin circulates in several forms, principally as a low-molecular-weight hexamer (~180 kDa) and a high-molecular-weight multimer (~360 kDa).[4] Recent evidence has suggested that the high-molecular-weight adiponectin may be more strongly related to several characteristics of the metabolic syndrome complex.[5]

A recent family-based study reported a shared heritability of adiponectin and the metabolic syndrome.[6] Identification of genes controlling adiponectin levels may aid our understanding of how genes influence metabolic syndrome and possibly obesity.[7,8] Recently, several genome-wide association studies (GWAS) for adiponectin have identified *ADIPOQ* (MIM 605441) and *ARL15* as possibly causal.[9–11] Because these genome-wide studies were conducted primarily in samples from European-derived populations, it remains uncertain whether these findings can be applied to other populations, especially Asian populations. Continental Asian populations have a higher percentage of body fat for a given unit of body mass index (BMI) than do Europeans.[12] However, there has been no published GWAS for adiponectin yet in an Asian population.

We conducted a GWAS of adiponectin levels with the Human SNP Array 5.0 (Affymetrix) on a discovery sample of volunteers from the Korean Metabolic Syndrome Research Initiative study in Seoul. For replication purposes, we selected samples from two other areas in South Korea: Ansan and Bundang-gu, both in Gyeonggi Province; where a genome-wide marker panel was available from the former. We also tested for association with high-molecular-weight adiponectin by using SNPs identified from the Seoul discovery sample in a third replication sample.

Subjects for the GWAS were recruited from the Korean Metabolic Syndrome Research Initiative study in Seoul, initiated in December 2005. A total of 9,128 individuals were recruited in 2006, and an additional 17,569 individuals were recruited in 2007.[13,14] Therefore, the total Seoul cohort included 26,697 volunteers. Volunteers from the first round had routine health examinations at the Health Promotion Center in University Hospitals between January 2006 and December 2007. From this total, 6,563 individuals were randomly selected for measurement of adiponectin levels. Of the 6,563 individuals with adiponectin, 1,004 individuals were genotyped. A total of 305 individuals were selected for having very low (33rd percentile) or very high (66th percentile) adiponectin levels and waist circumference. Another 699 individuals were randomly selected for genome-wide genotyping. A total of 1,004

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individuals were selected from our Seoul discovery set (see Figure S1 available online).

Subjects in a second genome-wide cohort were drawn from the Ansan cohort, initiated in 2001 as part of the Korean Genome Epidemiology Study (KoGES). Initial Ansan samples included 5,020 participants aged 40–69.[15] The sampling base for this cohort is Gyeonggi Province, about 30 km west of Seoul. Members of this cohort have been examined every 2 years since their baseline visit, with the third scheduled follow-up study (including family members) completed in 2008. A total of 5,020 samples were genotyped. From these 5,020 samples, 3,022 subjects were randomly selected for measurement of adiponectin levels.

Subjects for a third cohort were selected from the Korean Metabolic Syndrome Research Initiative study in the Bundang-gu area. Bundang-gu is also in Gyeonggi Province, about 30 km south of Seoul. A total of 2,304 individuals from Bundang-gu were recruited in 2008 and had both total and high-molecular-weight adiponectin levels measured.

The Institutional Review Board of Human Research of Yonsei University approved the study protocol, and written informed consent was obtained from all subjects.

For all three cohorts, each participant was interviewed via a structured questionnaire to collect personal history of cigarette smoking (never smoked, ex-smoker, or current smoker), alcohol consumption (nondrinker or drinker of any amount of alcohol), demographic characteristics (age, gender, etc.), and family history of diabetes. Waist circumference was measured midway between the lower rib and iliac crest. For measurement of weight and height, light clothing was worn. Body mass index was calculated as weight (kg) divided by height squared (m^2).

For clinical chemistry assays, serum was separated from peripheral venous blood samples obtained from each participant after a 12 hr fast and stored at −70°C. From this stored serum, adiponectin levels were measured via ELISA (Mesdia Co., Ltd.). Intra- and interassay variances for adiponectin ranged from 6.3% to 7.4% and 4.5% to 8.6%, respectively.[16] Quality control (QC) of data was in accordance with procedures of the Korean Association of Laboratory Quality Control.

Seoul samples (cohort 1) were genotyped on the Affymetrix Genome-Wide Human SNP Array 5.0 at DNALink. For the data obtained from this chip, internal QC measures were used: the QC call rate (dynamic model algorithm) always exceeded 86%, and heterozygosity of X chromosome markers identified gender for each individual. Genotype calling was performed with the Birdseed (v2) algorithm. A total of 1,004 individuals were genotyped via this platform in the first discovery phase. However, 10 of 1,004 individuals were removed because of low genotyping call rates (<95%). PLINK (v1.06) was used to estimate identity by state (IBS) over all SNPs, and four individuals were shown to be biological relatives, so one member of each pair was excluded. Eleven individuals were also excluded as a result of gender mismatches. Therefore, 979 individuals were available for this genome-wide analysis. A default set of 400,794 SNPs were used for further analysis, as recommended by Affymetrix. In quality assurance screening, we flagged SNPs with genotype call rates < 95%, minor allele frequencies (MAF) < 0.01, and SNPs showing deviation from Hardy-Weinberg equilibrium (HWE) at $p < 0.0001$. The final set of acceptable markers included 317,859 autosomal SNPs.

The majority of genomic DNAs from Ansan cohort participants (cohort 2) were genotyped on this same panel. Where DNA samples for genotyping were inadequate (mostly owing to degradation; $n = 129$), DNA extracted from Epstein-Barr virus-immortalized lymphoblastoid cell lines was substituted. DNA samples with low concentration ($n = 55$) were amplified prior to genotyping according to the manufacturer's protocol (QIAGEN). A total of 5,020 samples were genotyped with the Affymetrix Genome-Wide Human SNP Array 5.0 using 500 ng of genomic DNA. Markers with low call rate (<95%), low MAF (<0.01), and/or significant deviation from HWE ($p < 0.0001$) were excluded, leaving a total of 354,357 markers from the Ansan cohort.

Genotyping of a total of 2,304 subjects (cohort 3) as a replication study was conducted for the two strongest signals selected from the Seoul discovery sample; in addition, six other SNPs were tagged from the HapMap Japanese sample panel in International HapMap data as $r^2 > 0.3$. These eight SNPs were genotyped by using the TaqMan reaction.[17] Duplicate genotyping for about 1%–2.5% of all samples was performed as a QC check. Only those SNPs showing a concordance rate in duplicates of over 99% and a genotype success rate of over 98% were included in subsequent association analyses.

The distribution of observed p values for the given SNPs were plotted against the theoretical distribution of expected p values to construct quantile-quantile (Q-Q) plots for $\log_{10}$(total adiponectin).[18] Concentration bands (the shaded region in all Q-Q plots) represent the 95% confidence interval and were drawn by calculating the 2.5th and 97.5th percentiles of p values under the null hypothesis, assuming random sampling (Figure S2).

Genotype calls for the Affymetrix Genome-Wide Human SNP Array 5.0 were determined in batches of approximately 200–300 samples under the BRLMM algorithm. In creating a cluster plot for any given SNP, total signal information was processed to generate an integrated summary file. The summary file was then translated into a cluster plot format by using an algorithm similar to SST1.0 (SNP signal tool, Affymetrix) (Figure S3).

For expression experiments, we generated four different constructs for analysis of *CDH13* promoter activity. We generated the constructs composed of 0.6 or 2.7 kb promoter sequences containing −629 to +3 and −2782 to +3 of *CDH13*, respectively. These DNA fragments were amplified by PCR from genomic DNAs of the female donors whose genotypes were different from each other,

one being TT and the other GG, for rs12444338 SNP. The forward primers were 5′-GCAAGCTCGAATTGATCTGTC AT-3′ and 5′-AAGGTTTACTGGAGCCACTCT-3′ for the 0.6 and 2.7 kb constructs, respectively. The same reverse primer, 5′-CATTTTGACCGACTAGAAGC-3′, was used for both constructs. The PCR product was cloned into pGEM-T Easy (Promega), and an EcoRI restriction fragment of this construct was inserted into the EcoRI-digested pGLuc basic vector (NEB). This yielded the construct with Gaussian luciferase reporter gene under control of the 0.6 or 2.7 kb *CDH13* promoter sequences. The authenticity of the constructs was determined by DNA sequencing of the plasmids. There was no variation in DNA sequences between T and G variant promoters except rs12444338SNP itself.

HEK293 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified 5% CO_2 incubator. Cells were plated 1 day ahead of transfection at a density of 4.0×10^5 cells/ml in six-well culture plates. For each plasmid or empty pGLuc vector (control without promoter), 500 ng was transiently cotransfected with 100 ng of β-galactosidase expression vector using polyethylenimine. Twenty-four hours posttransfection, luciferase activity was determined by using a BioLux Gaussia Luciferase Assay kit (NEB) following the manufacturer's manual and measured with a luminometer (Turner Designs). Luciferase activity was normalized against β-galactosidase activity for transfection efficiency. Three independent experiments were performed in duplicate. The values were normalized against background activity of empty vector control, and the fold difference was calculated against the T type promoter. Data were compared by two-tailed Student's t test.

All biomarkers except adiponectin appeared to be normally distributed. Therefore, only adiponectin levels were log transformed ($\log_{10}$). Each SNP was tested for possible effects on $\log_{10}$(total adiponectin) under an additive model in PLINK. Multivariate linear regression models used in the study incorporated covariates (age, sex, smoking status, and BMI). The Seoul discovery data set and two other data sets were combined via an inverse-variance meta-analysis method assuming fixed effects with Cochran's Q test used to assess between-study heterogeneity.[19] All meta-analysis calculations were performed with the R program (v2.7.1). We also analyzed the combined Seoul and Ansan cohorts (n = 4,001).

The majority of individuals were middle-aged (Table 1). This sample of Korean volunteers had a low BMI on average, with only 24.1% and 0.8% of men and 26.9% and 2.5% of women having BMI values ≥ 25 kg/m^2 and ≥ 30 kg/m^2 (conventional cutoff points defining overweight and obese), respectively.

Table 2 lists SNPs yielding the top 20 $-\log_{10}$(p values) from a linear regression model for $\log_{10}$(total adiponectin) in the 979 discovery set samples when the regression model included age, sex, smoking status, and BMI as covariates. Table S1 lists the next top 30 SNPs. The top SNP found to be associated with $\log_{10}$(total adiponectin) was rs3865188 in *CDH13* (MIM 601364) on chromosome 16 ($p = 1.69 \times 10^{-15}$ in the Seoul sample; $p = 6.58 \times 10^{-39}$ in the Ansan sample; $p = 2.12 \times 10^{-32}$ in the Bundang-gu sample) (Figure 1). Five other SNPs in *CDH13* were among the top six SNPs associated with mean $\log_{10}$(total adiponectin). These six SNPs in *CDH13* in the original discovery sample were replicated in the Ansan cohort, showing very similar estimated regression coefficients. The major allele served as the reference allele in these regression models. In three top SNPs in *CDH13*, the minor allele was associated with lower $\log_{10}$(total adiponectin). However, the minor allele was associated with higher $\log_{10}$(total adiponectin) for three other SNPs in *CDH13*. Two top SNPs (rs3865188 and rs12596316) among these top 20 were further genotyped using the Bungdang Gu sample and gave very similar estimated regression coefficients (specifically β = −0.079 for rs3865188 and

Table 1. General Characteristics of Study Population

	Cohort 1	Cohort 2	Cohort 3
Location	Seoul	Ansan	Bundang-gu
n	979	3,022	2,304
Males, %	56.5	52.4	55.1
Age, years	41.5 ± 8.5	54.6 ± 7.4	42.9 ± 7.8
Waist circumference, cm	81.1 ± 9.7	80.1 ± 8.6	80.1 ± 9.5
Height, cm	166.0 ± 8.5	161.8 ± 8.2	166.0 ± 8.3
Weight, kg	65.6 ± 12.1	64.6 ± 10.0	64.8 ± 11.7
Body mass index, kg/m^2	23.7 ± 3.1	24.6 ± 2.9	23.4 ± 3.0
Total adiponectin, μg/ml	6.7 ± 6.4[a]	5.4 ± 5.0[a]	4.4 ± 3.3[a]
Log total adiponectin, μg/ml	0.82 ± 0.29	0.71 ± 0.31	0.64 ± 0.24
HMW adiponectin, μg/ml	–	–	2.7 ± 2.0
Log HMW adiponectin, μg/ml	–	–	0.32 ± 0.31
Fasting blood sugar, mg/dl	93.8 ± 16.4	99.7 ± 31.1	93.7 ± 16.7
Systolic blood pressure, mm Hg	120.8 ± 13.9	111.7 ± 14.1	117.8 ± 14.1
Diastolic blood pressure, mm Hg	73.8 ± 10.4	74.9 ± 9.8	76.7 ± 11.8
HDL cholesterol, mg/dl	54.1 ± 12.8	44.9 ± 10.7	52.2 ± 12.7
LDL cholesterol, mg/dl	108.7 ± 29.2	127.7 ± 31.6	117.9 ± 30.7
Triglyceride, mg/dl	118.0 ± 93.6	141.3 ± 89.7	124.9 ± 81.9
Smoking status, %			
Ex	16.8	24.1	21.9
Current	28.1	16.6	23.2

The following abbreviations are used: HMW, high molecular weight; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data shown with ± are given as mean ± standard deviation unless indicated otherwise.
[a] Data given as median ± interquartile range.

Table 2. Twenty Most Strongly Associated SNPs from the Seoul Project for Log_{10}(Total Adiponectin) Based on Linear Regression Model

				Cohort 1 (n = 979)			Cohort 2 (n = 3,022)			Combined Set (n = 4,001)	
Chromosome	SNP	Position	Nearest Gene	MAF	Effect (μg/ml)	p Value	MAF	Effect (μg/ml)	p Value	Effect (μg/ml)	p Value
16	rs3865188	81208218	*CDH13*	0.309	−0.095	1.685×10^{-15}	0.296	−0.096	6.582×10^{-39}	−0.096	7.793×10^{-51}
16	rs12596316	81203653	*CDH13*	0.311	−0.088	4.763×10^{-13}	0.298	−0.096	1.731×10^{-37}	−0.094	8.287×10^{-47}
16	rs7193788	81213661	*CDH13*	0.470	−0.057	3.643×10^{-07}	0.449	−0.071	6.304×10^{-24}	−0.068	2.484×10^{-28}
16	rs3865186	81204473	*CDH13*	0.448	0.057	5.844×10^{-07}	0.458	0.063	9.984×10^{-19}	0.062	1.634×10^{-23}
16	rs3852724	81203595	*CDH13*	0.448	0.057	6.225×10^{-07}	0.458	0.063	6.727×10^{-19}	0.062	1.046×10^{-23}
16	rs3865185	81203963	*CDH13*	0.448	0.057	6.225×10^{-07}	0.458	0.064	4.621×10^{-19}	0.062	7.568×10^{-24}
3	rs1438545	106535094	*ALCAM*	0.105	0.089	6.286×10^{-07}	0.086	−0.014	0.2577	0.029	0.006585
1	rs12072620	188794697	*FAM5C*	0.177	−0.074	0.0000009	0.166	0.008	0.4387	−0.011	0.1741
1	rs1501501	188795068	*FAM5C*	0.177	−0.074	0.0000009	0.166	0.007	0.4664	−0.012	0.1629
13	rs4943398	36083064	*LOC400120*	0.217	−0.064	0.0000029	0.227	−0.020	0.02218	−0.032	0.00002418
11	rs4936310	110405970	*C11orf53*	0.229	0.066	0.0000043	0.182	0.018	0.04669	0.035	0.00001446
8	rs436753	88599977	*CNBD1*	0.318	0.063	0.0000063	0.268	−0.014	0.08527	0.006	0.3774
14	rs7148411	19629122	*OR4K17*	0.230	0.063	0.0000099	0.245	−0.008	0.3319	0.008	0.2529
12	rs17251474	91089449	*BTG1*	0.198	−0.064	0.0000114	0.195	0.008	0.3818	−0.010	0.1821
5	rs17156226	103533627	*NUDT12*	0.204	0.068	0.0000119	0.164	0.003	0.7811	0.026	0.002127
15	rs7171526	60497216	*FLJ38723*	0.118	−0.078	0.0000119	0.114	0.008	0.4576	−0.010	0.3096
8	rs10957333	66104003	*CYP7B1*	0.106	−0.080	0.0000163	0.106	−0.001	0.9349	−0.019	0.05887
1	rs2889921	220406261	*KIAA1822L*	0.167	−0.087	0.0000194	0.183	−0.018	0.05355	−0.036	0.00002391
4	rs11722604	157084201	*CTSO*	0.192	0.077	0.0000231	0.071	0.004	0.7631	0.052	0.000006402
10	rs2817677	98811818	*SLIT1*	0.229	−0.058	0.0000234	0.214	0.000	0.9617	−0.012	0.1117

Table lists the minor allele frequency (MAF), estimated effect size (β) for the 20 most significant SNPs identified from the Seoul cohort (cohort 1; n = 979), and their p values in a multiple linear regression model considering age, sex, smoking status, and body mass index under an additive model. MAF, estimated effect size (β), and p value for the Ansan cohort (cohort 2) are also shown.

β = −0.076 for rs12596316, both of which were highly significant) (Table 3).

BMI was omitted as a covariate in the regression model, and these results are presented in Table S2. SNP rs3865188 in *CDH13* was still among the top SNPs, although strength of association became a bit weaker ($p = 2.3 \times 10^{-12}$). The other five SNPs in *CDH13* were still among the top 20 SNPs associated with $\log_{10}$(total adiponectin). The p value for rs3865188 combined across all three data sets was 3.33×10^{-76} when BMI was omitted (see Table S3).

We also tested these two SNPs for association with high-molecular-weight adiponectin as a separate phenotype using the 2,304 replication samples from a third cohort (Table 4). Six additional SNPs flanking rs3865188 and rs12596316 were also included in this replication analysis (Figure S4). The association between high-molecular-weight adiponectin and rs3865188 was even stronger ($p = 7.36 \times 10^{-58}$) than seen with $\log_{10}$(total adiponectin). Another SNP, rs4783244, was also very significantly associated with high-molecular-weight adiponectin ($p = 5.72 \times 10^{-61}$) (Table 4).

As a first step in understanding the underlying mechanism for genotype AA of rs3865188 with higher adiponectin level at a molecular level, we hypothesized that this SNP may be in linkage disequilibrium (LD) with another variant that controls the promoter activity of *CDH13*, and DNA sequences containing the A allele may have higher transcriptional activity than those with the T allele based on its position in the genomic sequences. We tested this hypothesis by comparing promoter activities with both alleles controlling reporter gene expression in transient transfection experiments. We selected the rs12444338 SNP, which is in strong LD with rs3865188 and is located 543 bp upstream of the transcription start site for *CDH13*. The promoter activity of the G variant occurring with the A allele at rs3865188 was 1.6 ± 0.2, and this is 2.2-fold higher than that of the T variant for the 0.6 and 2.7 kb promoters, respectively (Figure S5). This result suggests that the G variant at rs12444338 has a strongly increased promoter activity in vitro.

Table S4 lists SNPs yielding the top 20 $-\log_{10}$(p values) from this linear regression model for mean $\log_{10}$(total

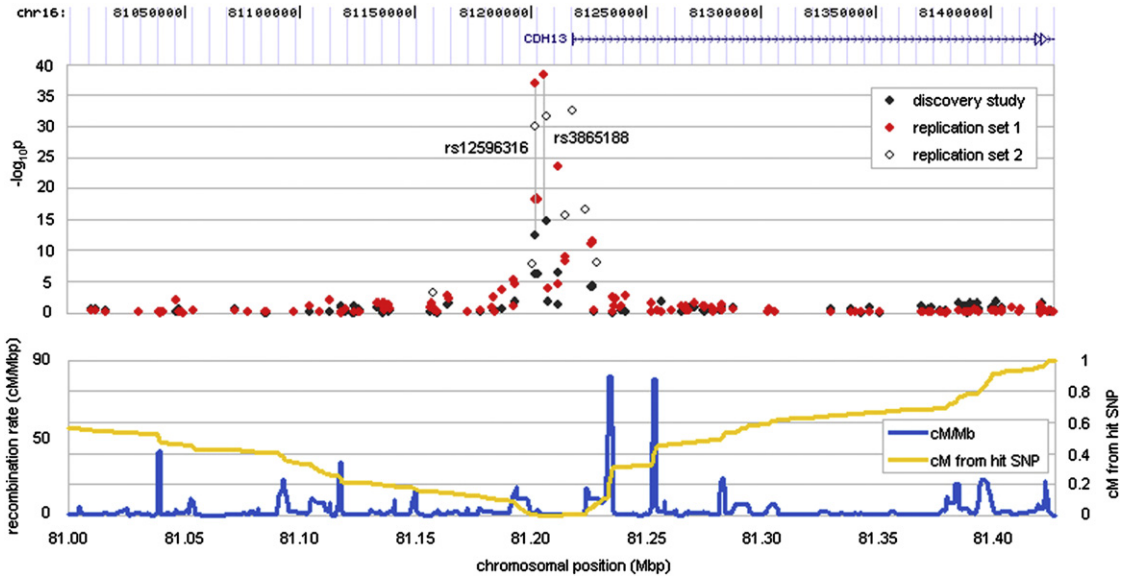

Figure 1. Significance of SNPs in *CDH13* for All Three Cohorts
Discovery set, n = 979; replication set 1, n = 3,022; replication set 2, n = 2,034. Here, a linear regression model including age, gender, smoking status, and body mass index was used for each individual SNP. Top panel: plot showing the $-\log_{10}$(p value). Black, red, and white symbols represent data from the discovery study, replication set 1, and replication set 2, respectively. Bottom panel: plot showing the recombination rate (blue) and cumulative recombination rate measured away from the most highly associated SNP of rs3865188 (yellow). Positions of highly significant SNPs were upstream of *CDH13*.

adiponectin) in the 3,022 Ansan subjects when the regression model included age, sex, smoking status, and BMI as covariates (Figure S6). Three SNPs in *ADIPOQ* were among the top 20 SNPs associated with mean $\log_{10}$(total adiponectin). Among these three SNPs, two SNPs (rs2241767 and rs266733) were replicated in the 979 Seoul samples. The most significant SNP in *ADIPOQ* influencing mean $\log_{10}$(total adiponectin) was rs2241767 ($p = 3.29 \times 10^{-8}$ in the 3,022 Ansan sample; $p = 0.0012$ in the 979 Seoul sample). We also examined SNPs in *ADIPOQ* from our genome-wide marker panel in the combined sample (Seoul and Ansan cohorts combined had n = 4,001). Two of these three SNPs gave p values at or near genome-wide significance in the combined sample (rs2241767, $p = 6.72 \times 10^{-10}$; rs864265, $p = 1.37 \times 10^{-7}$), and four other SNPs gave nominally significant evidence of an effect on $\log_{10}$(total adiponectin) (Figure 2). There were a total of eight SNPs in the genome-wide marker panel, and we imputed ~16 additional SNPs by using PLINK with the HapMap Chinese/Japanese sample as the reference population to provide better coverage of this gene. Figure 2 summarizes results of this analysis of SNPs in *ADPIOQ*.

In a cohort of 979 subjects from Seoul genotyped with the Affymetrix Human SNP Array 5.0 marker panel, linear regression models incorporating age, gender, smoking status, and BMI as covariates identified six SNPs in *CDH13* as significantly associated with $\log_{10}$(total adiponectin) levels. A second cohort of 3,022 adults genotyped on this same platform also showed significant effects from these same six SNPs (achieving genome-wide significance with identical direction of effect). A third replication cohort of 2,304 Koreans also showed similar regression coefficients for $\log_{10}$(total adiponectin) and even stronger effects when levels of high-molecular weight adiponectin were analyzed.

The cadherin 13 preprotein (*CDH13*; also known as T-cadherin) gene spans 1.2 Mb and contains 14 exons. *CDH13* has been reported as an adiponectin receptor.[20,21]

Table 3. Two Most Strongly Associated SNPs from the Seoul Project for Log_{10}(Total Adiponectin) Based on Linear Regression Model and Meta-analysis Results in 6,305 Samples

	Seoul	Ansan	Bundang-gu			
SNP	Effect Size (SE) (μg/ml)	Effect Size (SE) (μg/ml)	Effect Size (SE) (μg/ml)	Meta-analysis Effect Size (μg/ml)	Meta-analysis p Value	Meta-analysis Heterogeneity Q (p)
rs3865188	−0.095 (0.0118)	−0.096 (0.0073)	−0.079 (0.0066)	−0.09	2.82×10^{-83}	3.58 (0.167)
rs12596316	−0.088 (0.012)	−0.096 (0.0074)	−0.076 (0.0065)	−0.09	3.09×10^{-77}	4.13 (0.1268)

p values were calculated under a linear regression under an additive model incorporating age, sex, smoking status, and body mass index as covariates. Effect sizes are given with standard error (SE).

Table 4. Association of SNPs in *CDH13* with Log_{10}(Total Adiponectin) and Log_{10}(High-Molecular-Weight Adiponectin) Based on Linear Regression Model in 2,304 Korean Adults in the Bundang-gu Sample

			Total Adiponectin		High-Molecular-Weight Adiponectin	
SNP	**Position**	**MAF**	**Effect**	**p Value**	**Effect**	**p Value**
rs17244777	81159584	0.232	−0.025	0.00069	−0.05	8.17×10^{-8}
rs7200895	81202107	0.459	0.035	2.05×10^{-8}	0.069	5.31×10^{-18}
rs12596316	81203653	0.305	−0.076	1.08×10^{-30}	−0.13	8.30×10^{-55}
rs3865188	81208218	0.298	−0.079	2.12×10^{-32}	−0.315	7.36×10^{-58}
rs7204454	81216695	0.352	−0.053	1.78×10^{-16}	−0.084	5.05×10^{-25}
rs4783244	81219769	0.299	−0.079	2.74×10^{-33}	−0.138	5.72×10^{-61}
rs8047711	81225172	0.194	−0.067	3.15×10^{-17}	−0.112	3.47×10^{-29}
rs12922394	81229828	0.212	−0.043	1.19×10^{-8}	−0.073	2.66×10^{-14}

p values were calculated under a linear regression under an additive model, incorporating age, sex, smoking status, and body mass index as covariates.

The most significant SNP, rs3865188, is located 17.9 kb upstream of *CDH13* itself. T-cadherin was identified as a receptor for the hexameric and high-molecular-weight species of adiponectin, but not for the trimeric or globular species.[20] T-cadherin is expressed in endothelial and smooth muscle cells, where it may interact with adiponectin. Although transcriptional regulation of *CDH13* is not well understood, evidence that a nucleotide variant in the promoter region is associated with increased promoter activity of *CDH13* suggests that this variant plays an important role in expression. Furthermore, we showed that the variants in LD with the promoter are positively associated with adiponectin levels. Thus, our findings suggest that level of the receptor, *CDH13*, may regulate adiponectin level.

Several GWAS in Western countries have reported that *ADIPOQ* exerts a major effect on plasma adiponectin levels.[6,9,10] Ling et al. recently reported a genome-wide study showing that SNPs within *ADIPOQ* gave the strongest evidence of association with adiponectin levels ($p < 10^{-7}$).[9] Another recent GWAS also reported that *ADIPOQ* showed the strongest association with adiponectin levels

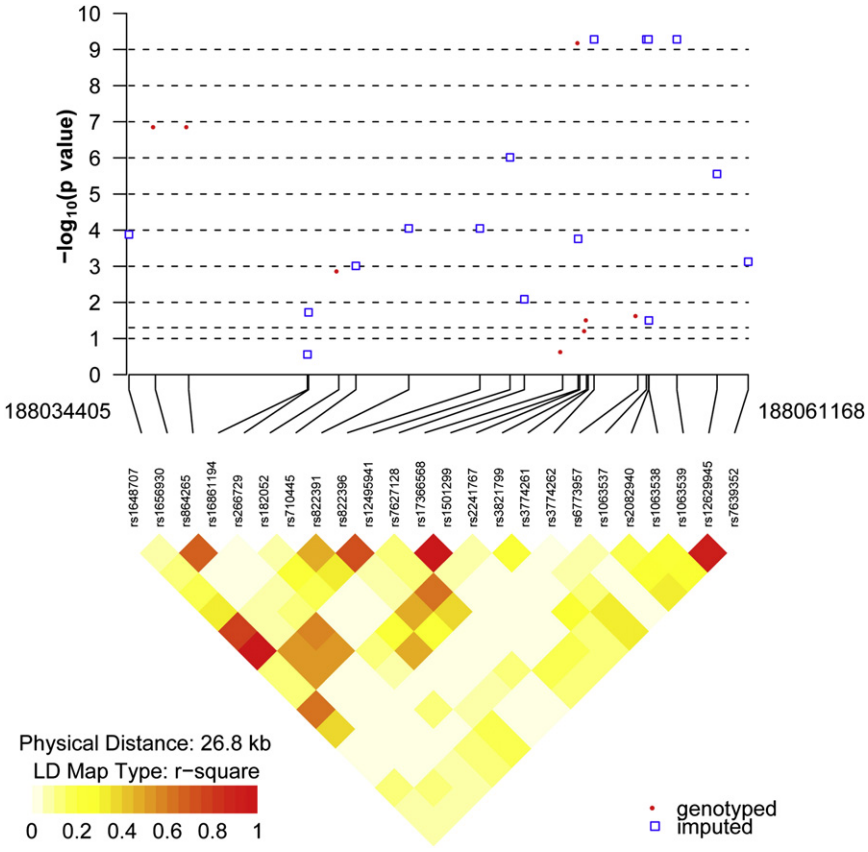

Figure 2. $-log_{10}$(p Value) for both Imputed and Genotyped SNPs in *ADIPOQ* in 4,001 Korean Adults
Red dots are genotyped SNPs; open blue squares are imputed SNPs.

($p = 4.3 \times 10^{-24}$).[10] In the present study, five SNPs in *ADIPOQ* were associated with serum total adiponectin at a more modest p value ($p = 1.45 \times 10^{-8}$) in the Ansan sample; however, some of the SNPs were not replicated in the smaller Seoul sample (Table S4).

The present study showed a weaker association for SNPs in *ADIPOQ* with adiponectin levels but much stronger association for SNPs in *CDH13* with adiponectin levels than in previous studies in Western populations. A recent GWAS reported a SNP in *CDH13* gave the fourth strongest test of association ($p < 2 \times 10^{-5}$), which was a much weaker association than our results.[9] One possible reason is the differences in allele frequency of these SNPs in *CDH13*. In the case of rs3865188, allele frequency information obtained from HapMap samples showed that European populations were more polymorphic than Asian populations at this marker.

In the present study, the association of SNPs in *CDH13* became even stronger when high-molecular-weight adiponectin levels were used, as compared with total adiponectin levels. Recent studies have demonstrated that the high-molecular-weight multimer form of adiponectin is the active form of this protein.[22] This high-molecular-weight form is most active in suppression of hepatic glucose production.[22] Kobayashi et al. reported that only high-molecular-weight adiponectin selectively suppressed endothelial cell apoptosis, whereas neither the middle- nor the low-molecular-weight form of adiponectin had such an effect.[23]

The strength of our study lies in our assessment of a unique physiologic measure of adiposity (serum adiponectin) in an Asian population. We also measured high-molecular-weight adiponectin levels in one sample. We adjusted for potential confounders, including age, sex, smoking status, and BMI. When BMI was included as a covariate, rs3865188 in *CDH13* was still among the top SNPs, with even stronger evidence. The question of whether or not findings from these studies can be generalized to all populations remains uncertain. Western populations have both different genetic backgrounds and different dietary patterns. Genetic studies of adiposity in Asian populations may not necessarily identify the same set of susceptibility genes as those in European-derived populations. However, these three Korean cohorts show strong evidence that *CDH13* on chromosome 16 is associated with serum adiponectin levels.

Supplemental Data

Supplemental Data include six figures and five tables and can be found with this article online at http://www.cell.com/AJHG.

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Web Resources

The URLs for data presented herein are as follows:

HapMap, http://hapmap.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
PLINK, http://pngu.mgh.harvard.edu/purcell/plink/

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