SUPPLEMENTARY DATA

Study populations

The Singapore Prospective Study Programme (SP2) was a follow-up of participants from four previous cross-sectional studies that all included a random sample of Singapore residents with over-sampling of ethnic minority groups [1-3]. From 2003 to 2007, all 10,747 participants from these studies were invited to participate in the current study. Clinical data from health examination was available for 5,139 participants, among whom only individuals of Chinese ethnicity (n = 2,483) were genotyped. Written informed consent was obtained from all participants and ethics approval was obtained from the Singapore General Hospital and the National University Hospital Institutional Review Boards.

We sought for replication of the results from the SP2 study in Japanese and Korean study populations. The Japanese sample was a combination of two population-based cohorts. The Nomura cohort was based on a community (Nomura town) of 11,000 inhabitants in Ehime Prefecture, a largely rural area located in western Japan [4]. Participants were recruited through a community-based annual medical check-up in 2002 and the study population consisted of 2,895 middle-aged and elderly residents. The other Japanese cohort was composed of participants in the medical check-up program at the Ehime University Hospital Anti-aging Center (AAC) [5]. These cross-sectional investigations were carried out as part of the longitudinal Shimanami Health Promoting Program (J-SHIPP) study [6]. The Japanese studies were approved by the ethics committee of Ehime University Graduate School of Medicine. All study participants provided informed consent.

The Korean replication sample consisted of the community-based Yangpyeong Cohort Study [7]. Five out of 12 districts of the Yangpyeong county were selected and 1,841 residents over 40 years old were recruited from 2005 to 2006. Written informed consent was obtained from all participants. The Institutional Review Board of Hanyang University approved the study.

Laboratory analyses

In the SP2 study, fasting blood samples were analyzed for glucose using enzymatic methods (ADVIA 2400, Siemens, Germany), for insulin using micro-particle enzyme immunoassay (Abbot AXSYM, Abbott Laboratories, Chicago, IL), and for total and HMW adiponectin using an enzyme linked immune-sorbent assay (Sekisui Medical Co Ltd, Tokyo, Japan). The intra- and inter-batch coefficient of variations percent were as follows: glucose (2.5, 6.6), insulin (4.0, 4.5), total adiponectin (18.1, 15.9), HMW adiponectin (6.8, 18.3).

In the replication samples, the plasma concentration of total adiponectin was determined using a latex enhanced imunoturbidimetric assay (Mitsubishi Chemical Medience, Tokyo, Japan) and HMW adiponectin using an ELISA system (Fujirebio Inc., Tokyo, Japan) in the Japanese studies [6]. The intra- and inter-assay coefficients of variation of the HMW adiponectin assay were 4.4% and 9.7%, respectively. In the Yangpyeong Study, total adiponectin was determined by Radioimmunoassay (Gamma Counter, PacKard, USA) using Human adiponectin RIA Kit (LINCO Research, Inc., USA). The intra- and inter-assay coefficients of variation at adiponectin concentration in the range of 1.5 to 7.5 ug/mL were 6.9 to 9.3% and 1.8 to 6.2%, respectively.

Genotyping and quality control

2,865 blood-derived DNA samples from the SP2 Chinese participants were genotyped using Illumina HumanHap 550, 610 Quad, and 1Mduov3 BeadChips. (http://www.illumina.com). The data went through quality control (QC) procedures which were described in detail elsewhere [8]. SNPs with call

rate < 0.95, minor allele frequency < 0.01 or Hardy-Weinberg equilibrium *P*-value < 1×10^{-6} were filtered out. 431 individuals did not pass the QC due to high rates of missingness, excessive heterozygosity, cryptic relatedness, discordant ethnicity and gender discrepancy. Imputation was done with IMPUTE (http://mathgen.stats.ox.ac.uk/impute/ impute.html) on 22 autosomes using NCBI build 36 HapMapII CHB and JPT data (release 22) as the reference panel. Imputation results of SNPs that were actually genotyped were replaced with experimentally determined genotypes before the association tests were conducted.

Statistical Analysis

A genome-wide association study for total and HMW adiponectin was carried out on 2,434 post-QC samples of the SP2 study. Adiponectin levels were natural log-transformed and standardized to z-scores. Additive genetic models adjusting for age and sex were used to test the associations and BMI was further adjusted for in a second model. The software used for the association study was SNPTEST v2.2.0 (https://mathgen.stats.ox.ac.uk/genetics_software/ snptest/snptest.html). Samples genotyped on different chips were treated as separate studies and the results were meta-analyzed under fixed-effect model weighted by inverse variance using METAL (http://www.sph.umich.edu/csg/abecasis/metal/). Sample size weighted meta-analysis was also performed and results were similar. Genomic control was applied to each study as well as the first-round meta-analysis results (λ=1.003 and 1.007 for total and HMW adiponectin) to correct for inflation and no obvious population stratification was detected.
Genotypes for the top SNP from the meta-analysis, rs4783244 in the *CDH13* gene, were successfully called for 2,429 individuals in the SP2 sample. After exclusion of participants with missing values for adiponectin (n=39), HOMA-IR (n=4), age (n=1), and BMI (n=2) and those taking anti-diabetic medications (n=101), 2,282 individuals remained for the analysis.

A total of 3,290 Japanese from the Nomura and AAC studies (1,226 with both total and HMW adiponectin levels) and 1,610 Koreans in the Yangpyeong Study (total adiponectin levels only) had clinical and genotyping data for rs4783244 available and were included in the replication analysis. For the Nomura and AAC studies, potential cohort effect was accounted for by adjustment for cohort as a covariate in the statistical models.

SUPPLEMENTARY DATA

Supplementary Table 1. Associations between adiponectin levels and metabolic traits in Singaporean Chinese, Japanese and Korean study populations.

	Singaporean Chinese				**Japanese**				**Korean**	
	Total adiponectin (N=2,282)		HMW adiponectin (N=2,282)		Total adiponectin (N=1,266)		HMW adiponectin (N=3,290)		Total adiponectin (N=1,610)	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value	β (95% CI)	*P*-value
HOMA-IR										
Model 1	-0.48 (-0.53, -0.43)	4.5E-74	-0.27 (-0.30, -0.24)	1.6E-53	-0.27 (-0.30, -0.24)	6.6E-55	-0.25 (-0.27, -0.23)	1.6E-84	-0.63 (-0.72, -0.54)	8.0E-39
Model 2	-0.26 (-0.30, -0.21)	3.9E-26	-0.14 (-0.17, -0.10)	7.2E-18	-0.19 (-0.22, -0.16)	2.9E-31	-0.15 (-0.18, -0.13)	1.1E-39	-0.53 (-0.63, -0.43)	7.6E-24
HOMA-B										
Model 1	-0.24 (-0.30, -0.19)	2.7E-19	-0.14 (-0.17, -0.11)	4.0E-15	-0.17 (-0.20, -0.14)	3.2E-27	-0.11 (-0.13, -0.08)	2.8E-20	-0.14 (-0.23, -0.05)	3.7E-03
Model 2	-0.11 (-0.17, -0.06)	5.4E-05	-0.06 (-0.10, -0.03)	4.9E-04	-0.10 (-0.13, -0.07)	2.6E-11	-0.04 (-0.06, -0.01)	1.7E-03	-0.05 (-0.15, 0.04)	0.256
HOMA-B(adj)*										
Model 1	0.04 (-0.00,0.08)	0.065	0.02 (-0.01, 0.05)	0.176	0.01 (-0.01, 0.03)	0.411	0.05 (0.04, 0.07)	9.1E-10	0.10 (0.00, 0.19)	0.044
Model 2	0.04 (-0.01,0.08)	0.090	0.02 (-0.01, 0.05)	0.238	0.02 (0.00, 0.04)	0.108	0.06 (0.04, 0.08)	1.5E-11	0.11 (0.01, 0.20)	0.026
Fasting glucose										
Model 1	-0.05 (-0.06, -0.04)	1.6E-24	-0.03 (-0.03, -0.02)	4.0E-17	-0.03 (-0.04, -0.02)	3.3E-15	-0.04 (-0.04, -0.03)	1.2E-44	-1.24 (-1.54, -0.95)	3.3E-16
Model 2	-0.03 (-0.04, -0.02)	3.4E-09	-0.01 (-0.02, -0.01)	5.7E-06	-0.03 (-0.03, -0.02)	1.5E-11	-0.03 (-0.04, -0.03)	3.5E-30	-1.02 (-1.31, -0.73)	1.3E-11
Fasting insulin										
Model 1	-0.43 (-0.48, -0.38)	5.4E-68	-0.24 (-0.27, -0.21)	2.6E-49	-0.24 (-0.27, -0.21)	4.0E-52	-0.21 (-0.24, -0.19)	9.3E-73	-0.65 (-0.76, -0.55)	5.0E-32

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Model 2	-0.22 (-0.27, -0.18)	7.7E-23	-0.12 (-0.15, -0.09)	1.1E-15	-0.17 (-0.20, -0.14)	2.0E-28	-0.12 (-0.14, -0.10)	1.8E-30	-0.52 (-0.64, -0.41)	3.0E-18
LDL cholesterol										
Model 1	-0.12 (-0.19, -0.06)	3.2E-04	-0.06 (-0.10, -0.01)	0.011	-2.73 (-4.67, -0.80)	5.7E-03	-5.25 (-6.47, -4.04)	3.8E-17	0.05 (-0.07, 0.18)	0.408
Model 2	-0.06 (-0.13, 0.01)	0.115	-0.02 (-0.06, 0.03)	0.466	-1.32 (-3.36, 0.71)	0.203	-3.98 (-5.24, -2.72)	6.1E-10	0.11 (-0.02, 0.23)	0.090
HDL cholesterol										
Model 1	0.19 (0.17, 0.20)	2.0E-97	0.11 (0.10, 0.12)	3.2E-75	8.37 (7.43, 9.32)	5.1E-61	5.68 (5.08, 6.29)	4.5E-72	1.18 (0.99, 1.37)	1.2E-31
Model 2	0.15 (0.13, 0.17)	7.4E-62	0.09 (0.07, 0.10)	3.6E-47	7.16 (6.18, 8.14)	1.9E-43	4.50 (3.88, 5.11)	1.9E-45	1.03 (0.84, 1.23)	8.9E-25
Total cholesterol										
Model 1	-0.02 (-0.03, -0.00)	0.014	-0.01 (-0.02, 0.00)	0.080	1.43 (-0.67, 3.53)	0.181	-3.77 (-5.10, -2.44)	3.1E-08	-0.38 (-0.62, -0.13)	2.4E-03
Model 2	-0.01 (-0.02, 0.01)	0.496	-0.00 (-0.01, 0.01)	0.861	2.41 (0.20, 4.63)	0.033	-2.98 (-4.36, -1.59)	2.6E-05	-0.22 (-0.46, 0.01)	0.066
Triglycerides										
Model 1	-0.41 (-0.44, -0.37)	5.8E-84	-0.24 (-0.26, -0.21)	2.8E-66	-0.18 (-0.21, -0.16)	2.0E-39	-0.18 (-0.20, -0.16)	1.1E-79	-0.58 (-0.66, -0.50)	7.9E-41
Model 2	-0.32 (-0.36, -0.28)	4.7E-50	-0.18 (-0.21, -0.15)	2.2E-39	-0.15 (-0.17, -0.12)	1.0E-24	-0.15 (-0.16, -0.13)	2.6E-53	-0.51 (-0.60, -0.43)	2.3E-31
CRP										
Model 1	-0.72 (-0.81, -0.63)	6.0E-53	-0.42 (-0.48, -0.36)	2.8E-42	-0.24 (-0.30, -0.18)	3.5E-15	-0.27 (-0.31, -0.23)	1.7E-37	-0.12 (-0.16, -0.07)	1.9E-07
Model 2	-0.41 (-0.50, -0.32)	2.5E-19	-0.24 (-0.29, -0.18)	5.3E-16	-0.16 (-0.22, -0.10)	5.7E-07	-0.19 (-0.23, -0.15)	6.2E-19	-0.07 (-0.12, -0.03)	1.8E-03

SUPPLEMENTARY DATA

HMW adiponectin, High molecular weight adiponectin; HOMA-IR, Homeostasis model assessment-insulin resistance; HOMA-B, Homeostasis model assessment-β cell function; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; CRP, C-reactive protein.

* HOMA-IR adjusted HOMA-B

Model 1 adjusted for age and sex; Model 2 adjusted for age, sex and BMI. Total and HMW adiponectin were natural log transformed and standardized in all the studies. All the metabolic traits were natural log transformed for normality except for LDL cholesterol in Singaporean Chinese, LDL, HDL and total cholesterol in Japanese. β represents SD change in the outcome variable per SD change in the explanatory variable, on the natural log scale if applicable.

SUPPLEMENTARY DATA

Supplementary Table 2 . Association between SNPs in reported loci and plasma adiponectin from a genome-wide association study in Singaporean Chinese.

							Total adiponectin			**HMW adiponectin**		
SNP	Chromosome	Position	Nearest Gene	Allele*	EAF	N	β	SE	*P-value*	β	SE	*P-value*
rs4783244†	16	81219769	*CDH13*	T/G	0.342	2,387	-0.328	0.027	2.1×10^{-33}	-0.446	0.028	6.9×10^{-58}
rs8062260	16	81215591	*CDH13*	A/G	0.273	2,391	-0.386	0.032	5.6×10^{-33}	-0.537	0.033	4.2×10^{-59}
rs3865188	16	81208218	*CDH13*	A/T	0.675	2,392	0.326	0.028	4.3×10^{-32}	0.447	0.028	1.7×10^{-56}
rs12922394	16	81229828	*CDH13*	T/C	0.223	2,391	-0.177	0.031	1.0×10^{-8}	-0.253	0.032	1.0×10^{-15}
rs10937273†	3	188032389	*ADIPOQ*	A/G	0.424	2,392	0.150	0.026	6.4×10^{-9}	0.157	0.026	2.2×10^{-9}
rs1648707†	3	188034405	*ADIPOQ*	A/C	0.553	2,391	0.122	0.026	2.4×10^{-6}	0.137	0.026	2.1×10^{-7}
rs6810075	3	188031259	*ADIPOQ*	T/C	0.575	2,391	0.125	0.027	2.5×10^{-6}	0.127	0.027	2.3×10^{-6}
rs3001032	1	217794402	*LYPLAL1*	T/C	0.775	2,390	-0.027	0.030	0.379	-0.052	0.031	0.089
rs925735	2	226887874	*IRS1*	C/G	0.076	2,392	0.045	0.048	0.350	0.015	0.049	0.765
rs1108842†	3	52695120	*GNL3*	A/C	0.577	2,391	0.013	0.025	0.618	-0.002	0.026	0.938
rs4311394†	5	53336419	*ARL15*	G/A	0.554	2,390	0.029	0.025	0.258	0.047	0.026	0.070
rs10447248	5	107943635	*FER*	A/G	0.685	2,390	0.049	0.028	0.082	0.023	0.028	0.417
rs998584	6	43865874	*VEGFA*	A/C	0.581	2,387	-0.001	0.027	0.962	0.025	0.028	0.370
rs592423	6	139882386	---	A/C	0.720	2,391	-0.011	0.029	0.695	-0.020	0.029	0.496
rs2980879	8	126550657	*TRIB1*	A/T	0.309	2,392	0.003	0.028	0.922	-0.018	0.028	0.530

rs7955516	12	20389303	*PDE3A*	A/C	0.895	2,392	-0.089	0.043	0.037	-0.080	0.043	0.063
rs601339	12	121740696	*GPR109A*	A/G	0.519	2,392	-0.097	0.025	1.3×10^{-4}	-0.081	0.026	1.6×10^{-3}
rs7133378	12	122975455	*DNAH10*	A/G	0.113	2,391	0.077	0.046	0.089	0.066	0.046	0.154
rs2925979†	16	80092291	*CMIP*	T/C	0.426	2,390	-0.084	0.026	1.0×10^{-3}	-0.075	0.026	4.1×10^{-3}
rs731839	19	38590905	*PEPD*	A/G	0.445	2,392	0.064	0.026	0.012	0.058	0.026	0.026

EAF, effect allele frequency; HMW adiponectin, high molecular weight adiponectin.
SNPs presented here include the top hits for *ADIPOQ* and *CDH13* in our study as well as previously implicated loci associated with adiponectin level. Results are based on the additive genetic model adjusting for age, gender and BMI.
* Effect allele, followed by other allele. †The SNP is genotyped on all the three arrays used for this study.

Supplementary Table 3. Correlations between the first four principal components (PCs) and adiponectin levels in Singaporean Chinese.

	Total adiponectin	**HMW adiponectin**
PC1	0.004 (0.87)	0.005 (0.80)
PC2	-0.025 (0.24)	-0.032 (0.13)
PC3	0.025 (0.23)	0.045 (0.03)
PC4	-0.004 (0.84)	-0.013 (0.54)

The numbers are correlation coefficient followed by *P*-value of significance in the bracket.

SUPPLEMENTARY DATA

Supplementary Table 4. Associations between rs4783244 genotypes and different forms of adiponectin under general genetic model.

Population	Genotype	Total adiponectin			HMW adiponectin			HMW-to-total adiponectin ratio		
		N	β (95% CI)	*P*-value	N	β (95% CI)	*P*-value	N	β (95% CI)	*P*-value
Singaporean Chinese	GT	2,282	-0.37 (-0.45, -0.29)	5.3×10^{-20}	2,282	-0.5 (-0.57, -0.42)	5.1×10^{-36}	2,282	-0.55 (-0.63, -0.47)	1.4×10^{-42}
	TT		-0.57 (-0.70, -0.45)	9.9×10^{-19}		-0.79 (-0.91, -0.67)	3.3×10^{-36}		-0.89 (-1.02, -0.77)	7.1×10^{-44}
Japanese	GT	1,266	-0.34 (-0.44, -0.23)	3.1×10^{-10}	3,290	-0.41 (-0.48, -0.35)	1.2×10^{-38}	1,266	-0.44 (-0.55, -0.33)	4.7×10^{-15}
	TT		-0.69 (-0.86, -0.53)	1.9×10^{-15}		-0.73 (-0.82, -0.63)	1.5×10^{-48}		-0.64 (-0.82, -0.47)	1.7×10^{-12}
Koreans	GT	1,610	-0.37 (-0.46, -0.28)	4.1×10^{-14}	---	---	---	---	---	---
	TT		-0.74 (-0.89, -0.58)	5.0×10^{-20}	---	---	---	---	---	---
Fixed effect meta-analysis	GT	5,158	-0.36 (-0.41, -0.31)	7.7×10^{-43}	5,572	-0.45 (-0.49, -0.40)	2.4×10^{-75}	3,548	-0.52 (-0.58, -0.45)	2.8×10^{-58}
	TT		-0.65 (-0.74, -0.57)	9.0×10^{-52}		-0.75 (-0.83, -0.68)	6.2×10^{-85}		-0.81 (-0.91, -0.71)	1.8×10^{-55}
Random effect meta-analysis	GT	5,158	-0.36 (-0.41, -0.31)	8.4×10^{-43}	5,572	-0.45 (-0.49, -0.40)	2.6×10^{-74}	3,548	-0.51 (-0.58, -0.45)	1.8×10^{-58}
	TT		-0.65 (-0.74, -0.57)	1.1×10^{-51}		-0.75 (-0.83, -0.68)	1.6×10^{-85}		-0.81 (-0.91, -0.71)	1.8×10^{-55}

Results are based on linear regression with rs4783244 as independent variable, adjusted for age and sex. Total and HMW adiponectin were natural log transformed and all the adiponectin forms were standardized to the z-scores. rs4783244 genotypes refer to GG, GT and TT with GG as the reference. A general genetic model is assumed and each β represents the effect of the alternative genotype on the specific form of adiponectin as compared with the reference genotype.

SUPPLEMENTARY DATA

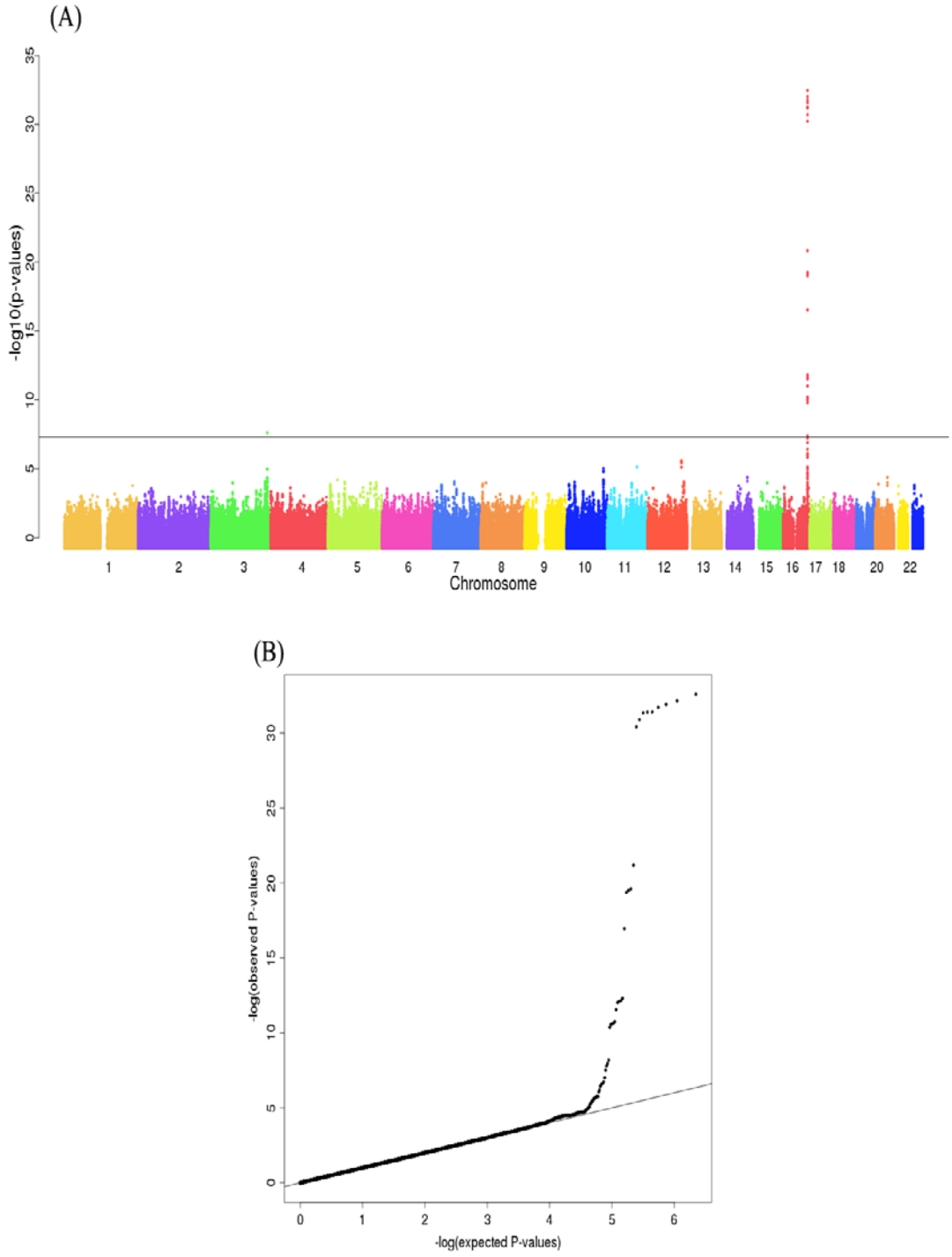
Supplementary Table 5. Association between rs4783244 in *CDH13* and glucometabolic traits after adjusting for the top four principal components in Singaporean Chinese.

Metabolic trait		**Model 1**	**Model 2**	**Model 3**
BMI	β (95% CI)	-0.04 (-0.10, 0.02)	-0.21 (-0.27, -0.14)	---
	P-value	0.178	3.8×10^{-11}	---
HOMA-IR	β (95% CI)	-0.06 (-0.12, 0.00)	-0.23 (-0.29, -0.16)	-0.12 (-0.18, -0.07)
	P-value	0.065	5.9×10^{-13}	6.6×10^{-6}
HDL-C	β (95% CI)	0.01 (-0.05, 0.06)	0.18 (0.13, 0.24)	0.14 (0.08, 0.19)
	P-value	0.847	1.2×10^{-10}	7.0×10^{-7}
Triglycerides	β (95% CI)	-0.02 (-0.08, 0.04)	-0.19 (-0.25, -0.14)	-0.15 (-0.20, -0.09)
	P-value	0.449	3.1×10^{-11}	3.3×10^{-7}

Model 1: adjusted for age and sex; Model 2: adjusted for the covariates in Model 1 plus HMW adiponectin; Model 3: adjusted for the covariates in model 2 plus BMI.

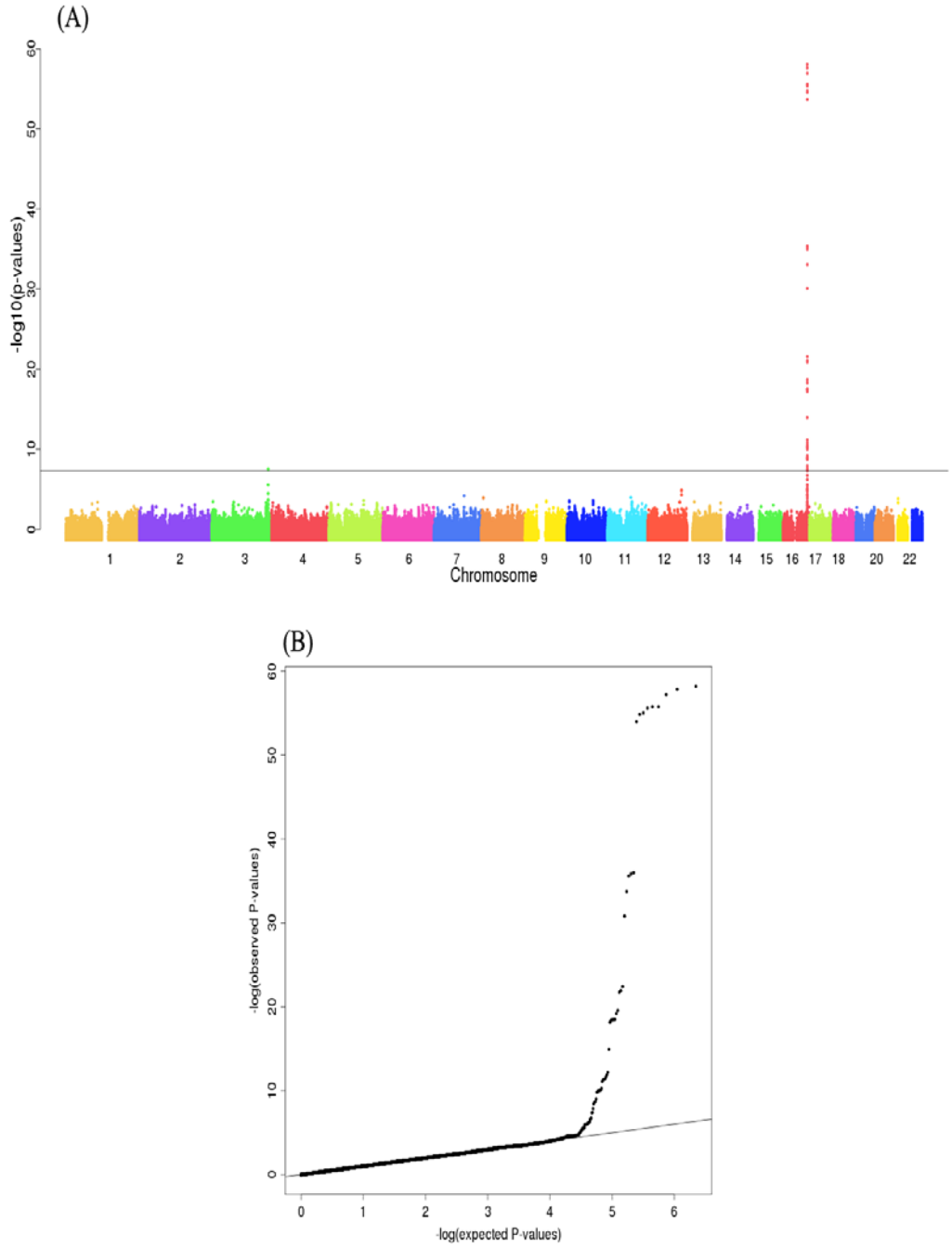
SUPPLEMENTARY DATA

Supplementary Figure 1. Whole genome association with total plasma adiponectin in Singapore Prospective Study Programme (SP2). (A) Manhattan plot with significance level set at 5×10^{-8} as indicated by the gray horizontal line. (B) Quantile-quantile plot of the observed and expected *P*-values for the association with total plasma adiponectin. Covariates adjusted for include age, gender and BMI.



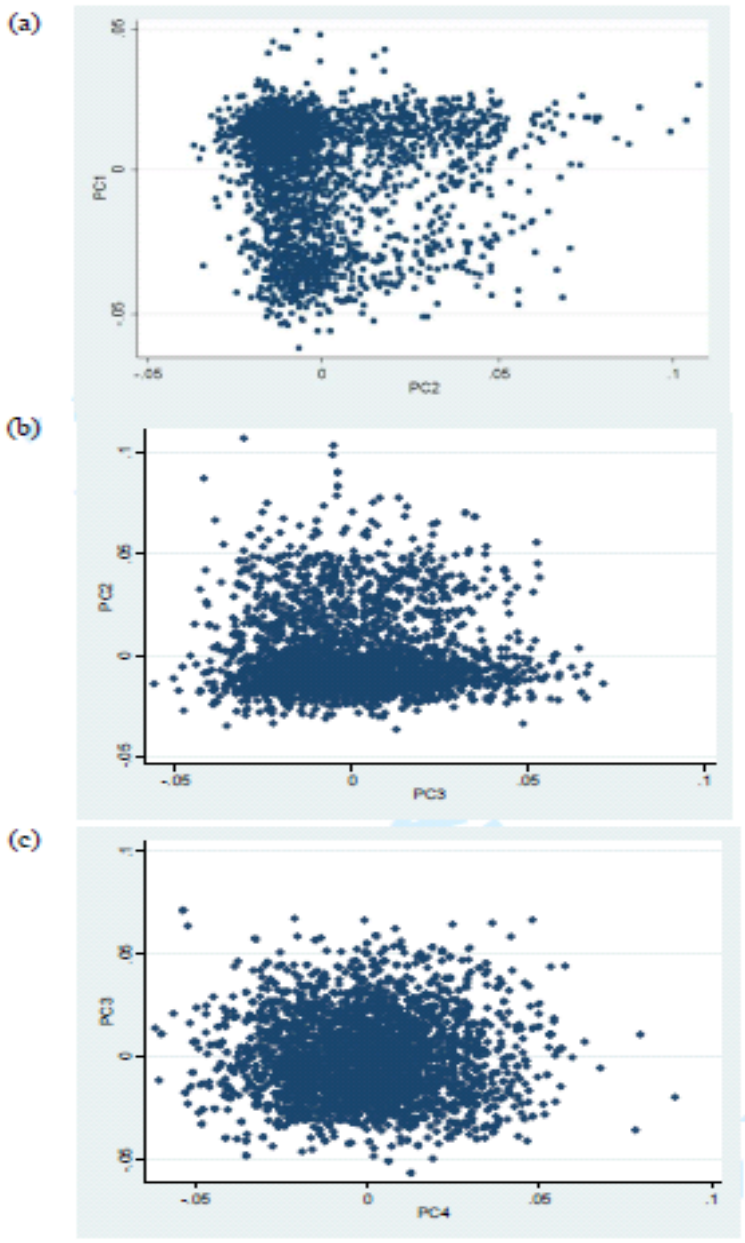
SUPPLEMENTARY DATA

Supplementary Figure 2. Whole genome association with HMW adiponectin in Singapore Prospective Study Programme (SP2). (A) Manhattan plot with significance level set at 5×10^{-8} as indicated by the gray horizontal line. (B) Quantile-quantile plot of the observed and expected *P*-values for the association with HMW adiponectin. Covariates adjusted for include age, gender and BMI.



SUPPLEMENTARY DATA

Supplementary Figure 3. Plot of principal components (PCs) to identify population structure in the Singaporean Chinese population. (a), the first and the second PCs; (b), the second and the third PCs; (c), the third and the fourth PCs.



References

1. Hughes, K., et al., *Central obesity, insulin resistance, syndrome X, lipoprotein(a), and cardiovascular risk in Indians, Malays, and Chinese in Singapore.* J Epidemiol Community Health, 1997. **51**(4): p. 394-9.
2. Tan, C.E., et al., *Prevalence of diabetes and ethnic differences in cardiovascular risk factors. The 1992 Singapore National Health Survey.* Diabetes Care, 1999. **22**(2): p. 241-7.
3. Cutter, J., B.Y. Tan, and S.K. Chew, *Levels of cardiovascular disease risk factors in Singapore following a national intervention programme.* Bull World Health Organ, 2001. **79**(10): p. 908-15.
4. Tabara, Y., et al., *Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project.* Hypertension, 2010. **56**(5): p. 973-80.
5. Tabara, Y., et al., *Composition of lower extremity in relation to a high ankle-brachial index.* Journal of hypertension, 2009. **27**(1): p. 167-73.
6. Tabara, Y., et al., *Reduced high-molecular-weight adiponectin and elevated high-sensitivity C-reactive protein are synergistic risk factors for metabolic syndrome in a large-scale middle-aged to elderly population: the Shimanami Health Promoting Program Study.* J Clin Endocrinol Metab, 2008. **93**(3): p. 715-22.
7. Yang, Y.J., et al., *Dietary zinc intake is inversely related to subclinical atherosclerosis measured by carotid intima-media thickness.* Br J Nutr, 2010. **104**(8): p. 1202-11.
8. Sim, X., et al., *Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia.* PLoS Genet, 2011. **7**(4): p. e1001363.