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Meta-analysis of genome-wide association studies in East Asian-ancestry populations identifies four new loci for body mass index

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Recent genetic association studies have identified 55 genetic loci associated with obesity or body mass index (BMI). The vast majority, 51 loci, however, were identified in European-ancestry populations. We conducted a meta-analysis of associations between BMI and ~2.5 million genotyped or imputed single nucleotide polymorphisms among 86 757 individuals of Asian ancestry, followed by *in silico* and *de novo* replication among 7488–47 352 additional Asian-ancestry individuals. We identified four novel BMI-associated loci near the *KCNQ1* (rs2237892, $P = 9.29 \times 10^{-13}$), *ALDH2/MYL2* (rs671, $P = 3.40 \times 10^{-11}$; rs12229654, $P = 4.56 \times 10^{-9}$), *ITIH4* (rs2535633, $P = 1.77 \times 10^{-10}$) and *NT5C2* (rs11191580, $P = 3.83 \times 10^{-8}$) genes. The association of BMI with rs2237892, rs671 and rs12229654 was significantly stronger among men than among women. Of the 51 BMI-associated loci initially identified in European-ancestry populations, we confirmed eight loci at the genome-wide significance level ($P < 5.0 \times 10^{-8}$) and an additional 14 at $P < 1.0 \times 10^{-3}$ with the same direction of effect as reported previously. Findings from this analysis expand our knowledge of the genetic basis of obesity.

INTRODUCTION

To date, genome-wide association studies (GWAS) have identified 55 genetic loci associated with obesity or body mass index (BMI) (1–14). Fifty-one of these loci were reported by studies conducted in populations of European ancestry. The remaining four loci were identified by our meta-analyses conducted among East Asians (9,10). However, these loci together explain only a small portion of observed variation in BMI [1.45% in Europeans (8), 1.18% in East Asians (9)], suggesting that additional BMI-related loci remain to be discovered. Since the publication of our previous meta-analysis in East Asians (9,10), nine additional GWAS with 18 352 additional participants have joined the Asian Genetic Epidemiology Network (AGEN) BMI-Consortium. We carried out a new round of meta-analyses that included data from 86 757 Asians recruited from 21 studies conducted in mainland China, Japan, Singapore, South Korea, Taiwan, the Philippines and the USA to identify new BMI loci and re-confirm associations with BMI that have been previously reported.

RESULTS

Our initial meta-analysis used BMI as the outcome and analyzed the association of BMI with ~2.5 million genotyped or imputed single nucleotide polymorphisms (SNPs) generated from these 21 studies, comprising 86 757 individuals of East Asian or Southeast Asian ancestry (Stage I). This was followed by a replication analysis (Stage II) of eight selected SNPs from four study sites, comprising 7488–47 352 Asian-ancestry individuals based on the availability of *de novo* and/or *in silico* data for each SNP. Details of the study design are presented in Supplementary Material, Figure S1. Participating studies are described in the Supplementary Information and Supplementary Material, Tables S1–S3.

The Stage I meta-analysis found eight SNPs at seven loci near the *KCNQ1* (rs2237892, $P = 7.32 \times 10^{-10}$), *ALDH2/MYL2* (rs671, $P = 5.96 \times 10^{-10}$, rs12229654, $P = 1.26 \times 10^{-8}$), *ITIH4* (rs2535633, $P = 1.33 \times 10^{-8}$), *NT5C2* (rs11191580, $P = 7.59 \times 10^{-6}$), *LINC00461* (rs6893807, $P = 1.81 \times 10^{-7}$) and *SEMA6D* (rs1912631, $P = 6.06 \times 10^{-8}$) genes and the intergenic region at 2p25.3 (rs4854307, $P = 9.21 \times 10^{-7}$) that

were associated with BMI at or near the genome-wide significance level (Table 1, Supplementary Material, Table S4). These eight SNPs were taken forward to the Stage II replication analyses (Supplementary Material, Table S3), which included *de novo* genotyping data from three study sites with a total of 40 422 participants and *in silico* replication data from the Tai Chi study ($N = 7369$) genotyped with Illumina's iSelect 200 k Cardio-MetaboChip (Supplementary Material, Table S4). In the Stage II analysis, five of these eight SNPs had the same direction of association as in Stage I and were nominally significant ($P < 0.05$). Combined analysis of data from Stages I and II showed that the association for all five of these SNPs at four genetic loci reached the genome-wide significance level: *KCNQ1* (rs2237892, $P = 9.29 \times 10^{-13}$), *ALDH2/MYL2* (rs671, $P = 3.40 \times 10^{-11}$, rs12229654, $P = 4.56 \times 10^{-9}$), *ITIH4* (rs2535633, $P = 1.77 \times 10^{-10}$) and *NT5C2* (rs11191580, $P = 3.83 \times 10^{-8}$) (Table 1, Supplementary Material, Table S4). Data obtained from the GIANT consortium (8,15) (Supplementary Material, Table S5) revealed significant associations for two of the SNPs ($P = 9.18 \times 10^{-3}$ for rs2535633 and $P = 1.06 \times 10^{-8}$ for rs11191580) with the same direction of association as the current study. The SNPs in the *ALDH2* (rs671) and *MYL2* (rs12229654) genes had a minor allele frequency (MAF) of 0.24 and 0.20, respectively, in the current study, but are monomorphic in HapMap European-ancestry data; no GIANT consortium data were available for these two SNPs. The variation explained by each newly identified SNP ranged from 0.03% to 0.05% (Table 1, Supplementary Material, Table S4). The variation explained for all four of these newly identified BMI loci combined was 0.16% based on Stage II data.

The two newly identified SNPs, rs671 in the *ALDH2* gene (12q24.12) and rs12229654 in the *MYL2* gene (12q24.11), are located 827 kb apart and are in LD ($r^2 = 0.58$) in Asians (Fig. 1). To examine their independent effects, we conducted a conditional analysis that included these two SNPs in the same regression model using available data. The conditional analysis showed that only rs671 had a significant independent effect on BMI (Supplementary Material, Table S6).

To evaluate the possible modifying effect of alcohol consumption on the association between *ALDH2* and BMI, we analyzed the association of BMI with rs671 by gender and alcohol consumption status (drinkers *versus*. non-drinkers) using data from the two studies (SGWAS for Chinese and KCPS-II for Koreans) for which we had direct access to individual data. We found that, among both men and women, the association either was significantly stronger (KCPS-II, P for interaction test = 0.0178) or was only significant (SGWAS) among non-drinkers (Supplementary Material, Table S7).

The *ALDH2/SH2B3* locus at 12q24 has been reported to be a target of recent selection in European- and East Asian-ancestry populations (16), with reduction of haplotype diversity. Using the same six representative SNPs (rs4646777, rs671, rs3742000, rs12422941, rs10850014 and rs2301757) reported by Kato *et al.* (16), we derived the same four common haplotypes (H1, H4, H5, H6) in the two Chinese (SGWAS) and Korean (KCPS-II) data sets mentioned above. The haplotype class specific to East Asians (H5) had the strongest association with BMI in our populations (data not shown).

As shown in Table 2, of the 51 BMI-associated loci that were identified among European-ancestry individuals, the index SNPs at eight loci (rs2890652, rs13078807, rs7638110, rs13107325, rs11847697, rs12444979, rs17024258 and rs10508503) were monomorphic in Asians (Supplementary Material, Table S8). Of the remaining 43 loci, Stage I data revealed that all but one (rs5996074 at *SREBF2*) had the same direction of association as reported previously ($P = 1.0 \times 10^{-11}$ by the binomial test), eight known loci (near the *FTO, BDNF, SEC16B, MC4R, TMEM18, GIPR/QPCTL, ADCY3/RBJ* and *GNPDA2* genes) were associated with BMI at the genome-wide significance level ($P < 5 \times 10^{-8}$), and another 14 known loci (near the *ADCY9, MAP2K5, TFAP2B, TMEM160, OLFM4, FLJ35779, FAIM2, MTCH2, RPL27A, SFRS10/ETV5, NUDT3, HOXB5, ZNF608* and *FANCL* genes) were associated with BMI at a Bonferroni-corrected significance level ($P < 0.05/51$ known loci $= 1.0 \times 10^{-3}$). The variation explained by each SNP in these known BMI loci ranged from 0.02–0.15%. The variation explained by all 22 of these re-confirmed BMI-associated loci combined was 1.14%. We compared BMI–SNP associations in East Asian- and European-ancestry populations using data from this study and the GIANT consortium (Supplementary Material, Table S5, S8) and found correlations of effect sizes of $r = 0.80$ ($P = 6.49 \times 10^{-6}$) for all genome-wide significant loci and $r = 0.62$ ($P = 8.07 \times 10^{-7}$) for all newly and previously identified loci combined between the two populations.

To compare the genetic architecture of regions associated with BMI between Asians and Europeans, we investigated the

Table 1. Newly identified loci associated with BMI variation in Asian-ancestry populations

Nearby gene	Cytoband	SNP	Alleles[a]	EAF[b]	Stage I P	Stage I and II Stage II P	Number of samples	β (SE)[c]	P[d]	EV (%)[e]
KCNQ1	11p15.4	rs2237892	T/C	0.36	7.32E−10	1.73E−04	133 312	0.0298 (0.0042)	9.29E−13	0.04
ALDH2	12q24.12	rs671	G/A	0.76	5.96E−10	6.64E−03	97 990	0.0378 (0.0057)	3.40E−11	0.05
MYL2	12q24.11	rs12229654	T/G	0.80	1.26E−08	1.89E−02	110 211	0.0341 (0.0058)	4.56E−09	0.04
ITIH4	3p21.1	rs2535633	G/C	0.42	1.33E−08	2.56E−03	111 673	0.0288 (0.0045)	1.77E−10	0.04
NT5C2	10q24.33	rs11191580	C/T	0.27	7.59E−06	6.78E−04	98 883	0.0295 (0.0054)	3.83E−08	0.03

[a]Shown as: effect allele/other allele.
[b]Effect allele frequency in Asian-ancestry populations, estimated from Stage I and II studies.
[c]Per allele effects of SNPs on BMI are presented in standard deviations, which were derived from the meta-analysis.
[d]Derived from the meta-analysis. The P-values for combined data were adjusted for both study-specific inflation factors and the estimated inflation factor for the Stage I meta-analysis statistic.
[e]Explained variance, estimated from combined Stages I and II data.

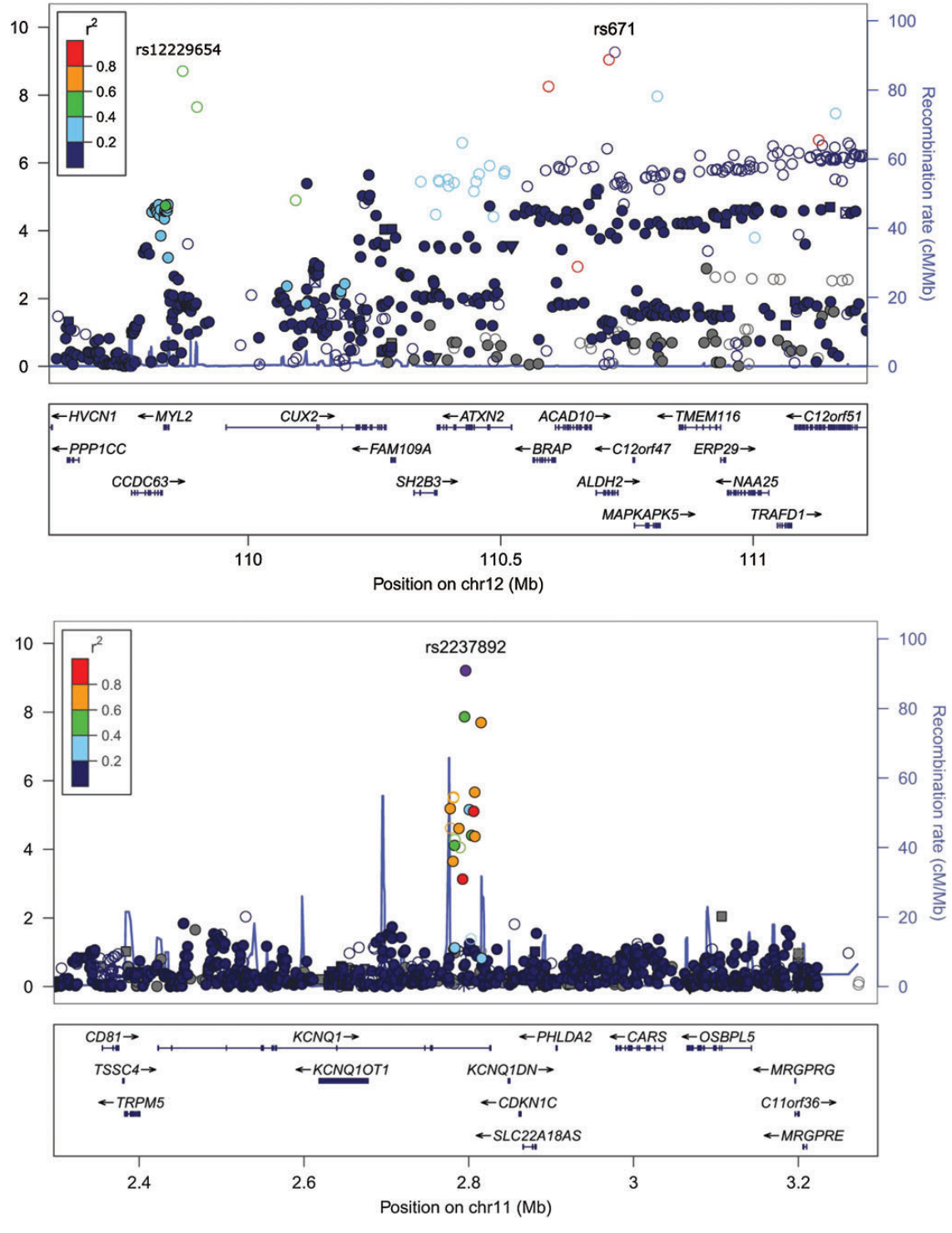

Figure 1. Regional plots for the four novel loci identified in this study. SNPs are plotted by their position on the chromosome against their association ($-\log 10$ *P*-value) with BMI using Stage I (GWAS meta-analysis) data. The name and *P*-value for the top SNP shown on the plots is based on all combined data with full genomic control adjustment (Table 1). Estimated recombination rates (from HapMap) are plotted in cyan to reflect the local LD structure. The SNPs surrounding the top SNP (rs671 was used for the *ALDH2/MYL2* locus) are color-coded (see inset) to reflect their LD with the top SNP (using pair-wise r^2 values from HapMap CHB + JPT data). Genes and positions of exons, as well as directions of transcription, are shown below the plots (using data from the UCSC Genome Browser, genome.ucsc.edu). Plots were generated using LocusZoom.

linkage disequilibrium (LD; by r^2) of SNPs in the 200 kb flanking all previously (Supplementary Material, Table S8) and newly (Table 1) identified BMI loci in both populations. We calculated the pairwise distance and LD (r^2) for each locus in each population based on HapMap3 SNP data through the public SNP Annotation and Proxy Search (SNAP) tool.

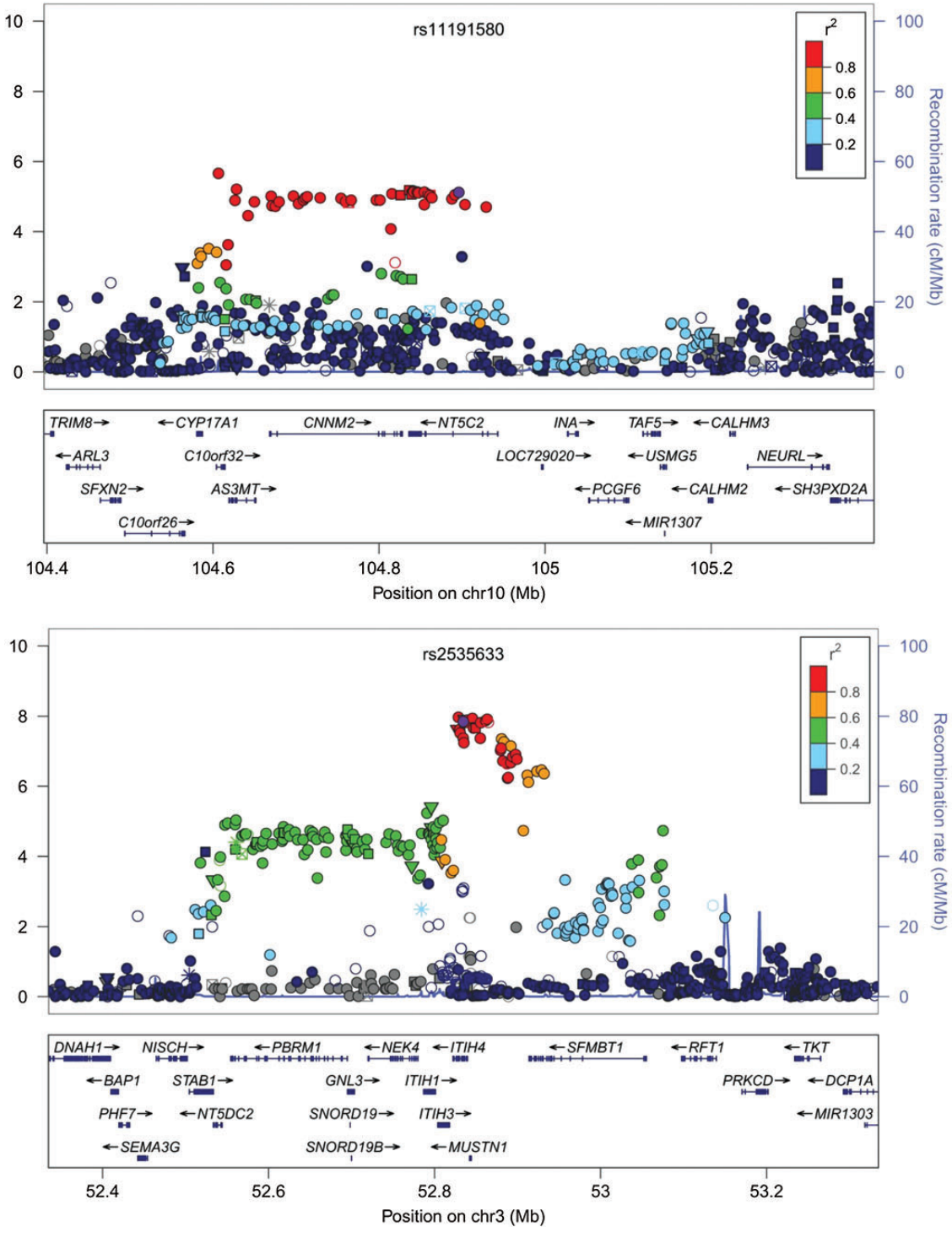

Fig. 1. *Continued*

The average LD decay over distance for the two populations showed similar patterns, suggesting that the genetic structure of those regions is similar (Supplementary Material, Fig. S2).

The reported effect sizes for all BMI-related SNPs in studies of European-ancestry populations are usually >3% of the standard deviation of BMI (4). Given the size of our study ($N = 86\,757$ for Stage I), we had adequate statistical power (>80% at a significance level of $P < 1.0 \times 10^{-3}$) to detect a SNP with such an effect size and a MAF of >0.12. Previously reported loci that were not replicated in our study at $P < 1.0 \times 10^{-3}$ had either a very small effect size or a low MAF (Supplementary Material, Table S8).

Of the four BMI-associated loci we identified in our previous studies conducted among East Asians (9,10), Stage I data

showed that 3 loci (in the *PCSK1, CDKAL1* and *KLF9* genes) remained genome-wide significant ($P < 5.0 \times 10^{-8}$), while the *GP2* locus did not reach the genome-wide significance level ($P = 6.13 \times 10^{-7}$) (Table 2, Supplementary Material, Table S8). The variation explained by all four of these loci combined was 0.22%. Altogether, the overall variation explained by the 30 re-confirmed or newly identified BMI-associated loci (22 loci originally identified in Europeans, 4 loci originally identified in East Asians and 4 newly identified loci) was 1.52%, which is an improvement over the previously reported value of 1.18% in East Asians (9). Assuming that the 21 BMI loci identified in European-ancestry populations that we did not confirm in this study could be confirmed with a larger sample size, the variation explained by all known BMI loci would be 1.65%. We anticipate that the variation explained by genetics will increase when rare variants are considered.

Additional analyses examined effect sizes for differences across sex, population, individual studies and obesity status. Analyses stratified by sex (Table 3) showed that associations with BMI among men were significantly stronger than

Table 2. Associations of SNPs in previously identified loci with BMI in East Asian-ancestry populations

Nearby gene	Chr	SNP	Alleles[a]	EAF[b]	Number of samples	β (SE)[c]	P[d]	Explained variance	References
Eight BMI loci identified in populations of European ancestry were significant at $P < 5.0 \times 10^{-8}$ in East Asian populations									
FTO	16	rs1558902	A/T	0.15	86 668	0.0756 (0.0070)	6.63E−27	0.15%	1–5,7,8
BDNF	11	rs11030104	A/G	0.55	86 637	0.0478 (0.0052)	2.36E−20	0.11%	4,8
SEC16B	1	rs574367	T/G	0.21	86 493	0.0580 (0.0064)	1.93E−19	0.11%	4,8
MC4R	18	rs591166	A/T	0.24	80 605	0.0464 (0.0062)	7.24E−14	0.08%	3–5,7,8
TMEM18	2	rs12463617	C/A	0.91	84 166	0.0634 (0.0090)	2.08E−12	0.07%	4,5,7,8
GIPR/QPCTL	19	rs11671664	G/A	0.49	70 606	0.0406 (0.0058)	3.47E−12	0.08%	8,9
ADCY3/RBJ	2	rs6545814	G/A	0.45	86 669	0.0331 (0.0052)	1.30E−10	0.05%	8,9
GNPDA2	4	rs16858082	T/C	0.35	84 150	0.0324 (0.0055)	3.79E−09	0.05%	5,8
Fourteen BMI loci identified in populations of European ancestry were significant at $P < 1.0 \times 10^{-3}$ in East Asian populations									
ADCY9	16	rs2531995	T/C	0.33	75 987	0.0315 (0.0058)	7.29E−08	0.04%	14
MAP2K5	15	rs4776970	A/T	0.22	84 217	0.0317 (0.0062)	3.49E−07	0.03%	8,9
TFAP2B	6	rs9473924	T/G	0.29	76 551	0.0308 (0.0061)	3.77E−07	0.04%	8
TMEM160	19	rs3810291	A/G	0.24	79 328	0.0333 (0.0068)	8.98E−07	0.04%	8
OLFM4	13	rs9568867	A/G	0.23	75 149	0.0310 (0.0067)	3.98E−06	0.03%	11
FLJ35779	5	rs888789	A/G	0.46	83 977	0.0240 (0.0052)	4.42E−06	0.03%	8
FAIM2	12	rs897057	C/T	0.79	75 542	0.0287 (0.0068)	2.24E−05	0.03%	8
MTCH2	11	rs11604680	G/A	0.30	86 354	0.0235 (0.0056)	2.95E−05	0.02%	5,8
RPL27A	11	rs10160804	A/C	0.47	86 569	0.0212 (0.0051)	3.50E−05	0.02%	8
SFRS10/ETV5	3	rs10513801	T/G	0.97	84 121	0.0616 (0.0153)	5.43E−05	0.02%	4,8
NUDT3	6	rs4713766	A/C	0.12	61 708	0.0420 (0.0104)	5.49E−05	0.04%	8
HOXB5	17	rs9299	T/C	0.56	72 384	0.0227 (0.0057)	7.27E−05	0.03%	11
ZNF608	5	rs7701094	C/G	0.48	55 908	0.0292 (0.0080)	3.78E−04	0.04%	8
FANCL	2	rs1861411	A/G	0.41	86 623	0.0183 (0.0053)	5.14E−04	0.02%	12
Four BMI loci identified in populations of Asian ancestry in the current Stage I meta-analysis									
CDKAL1	6	rs9356744	T/C	0.57	86 052	0.0374 (0.0052)	5.40E−13	0.07%	9
PCSK1	5	rs261967	C/A	0.41	86 488	0.0376 (0.0052)	7.96E−13	0.07%	9
KLF9	9	rs11142387	C/A	0.41	70 553	0.0324 (0.0058)	2.79E−08	0.05%	10
GP2	16	rs12597579	C/T	0.78	86 314	0.0316 (0.0063)	6.13E−07	0.03%	9

[a]Shown as effect allele/other allele.
[b]Effect allele frequency, estimated from Stages I and II studies for Asians.
[c]Per allele effects of SNPs on BMI are presented in standard deviations, which were derived from the meta-analysis.
[d]Derived from the meta-analysis and adjusted for both study-specific inflation factors (for Stages I and II) and for the estimated inflation factor for the Stage I meta-analysis statistic.

Table 3. Newly identified loci associated with BMI variation in East Asian-ancestry populations, by gender

Nearby gene	Chr	SNP	Alleles[a]	Among men			Among women			Test for homogeneity
				Number	β (SE)[b]	P[c]	Number	β (SE)[b]	P[c]	P
KCNQ1	11	rs2237892	T/C	59 365	0.0411 (0.0059)	4.54E−12	72 300	0.0204 (0.0055)	2.18E−04	1.07E−02
ALDH2	12	rs671	G/A	42 896	0.0560 (0.0080)	1.97E−12	53 421	0.0234 (0.0077)	2.32E−03	3.11E−03
MYL2	12	rs12229654	T/G	48 395	0.0543 (0.0083)	5.45E−11	60 141	0.0190 (0.0077)	1.38E−02	1.76E−03
ITIH4	3	rs2535633	G/C	48 927	0.0289 (0.0065)	8.29E−06	61 184	0.0266 (0.0059)	6.13E−06	7.96E−01
NT5C2	10	rs11191580	C/T	42 636	0.0252 (0.0079)	1.47E−03	53 382	0.0332 (0.0070)	2.03E−06	4.49E−01

[a]Shown as effect allele/other allele.
[b]Per-allele effects of SNPs on BMI are presented in standard deviations, which were derived from the meta-analysis.
[c]Derived from the meta-analysis and adjusted for both study-specific inflation factors (for Stages I and II) and the estimated inflation factor for the Stage I meta-analysis statistic.

associations among women for rs2237892 in *KCNQ1* (effect size: 0.0411 versus 0.0204, P for homogeneity $= 1.07 \times 10^{-2}$), rs671 in *ALDH2* (effect size: 0.0560 versus 0.0234, P for homogeneity $= 3.11 \times 10^{-3}$) and rs12229654 in *MYL2* (effect size: 0.0543 versus 0.0190, P for homogeneity $= 1.76 \times 10^{-3}$). In addition, we also observed a stronger association among men than among women in two of our previously reported loci at *CDKAL1* (P for homogeneity $= 5.74 \times 10^{-3}$) and *PCSK1* (P for homogeneity $= 5.95 \times 10^{-3}$) (Supplementary Material, Table S8). Analyses stratified by population (Supplementary Material, Table S9) showed that associations with BMI for all four new loci were similar (P for homogeneity ≥ 0.15) across Chinese, Japanese and Korean populations, although none were statistically significant among Malay/Filipino populations. No significant heterogeneity across individual studies was found for these four new loci (data not shown). Meta-analyses of obesity as a dichotomous outcome (BMI ≥ 27.5 kg/m^2) (17) also showed similar associations with odds ratios per allele ranging from 1.03 to 1.09, although the statistical power for this analysis was lower (Supplementary Material, Table S10).

In an effort to search for potential functional variants, we systemically examined expression quantitative trait loci (eQTL) in the 1 Mb regions flanking the four newly identified loci. A total of 178 eQTLs (Supplementary Material, Table S11) were identified in public databases and the previous literature. We next investigated whether these eQTL SNPs were located in certain functional elements using the online tool HaploReg (18). We found that of the 178 eQTL SNPs, 69.7% were located in enhancer regions. This percentage is significantly higher ($P = 2.2 \times 10^{-16}$) than the percentage of enhancer regions in the human genome (19.8%). In particular, the four newly identified loci are all located in motif binding sites and are associated with enhancer regions (Supplementary Material, Table S12).

To further explore over-represented biological pathways among the genes located near the newly and previously identified BMI loci listed in Table 1 and Supplementary Material, Table S8, we examined their functional enrichment in biological pathway analyses using the ingenuity pathway analysis (IPA) tool in Ingenuity (version 17199142). We found that two relevant BMI pathways, CDK5 signaling ($P = 1.94 \times 10^{-4}$) and corticotropin-releasing hormone signaling ($P = 3.74 \times 10^{-4}$), were significantly enriched.

DISCUSSION

Of the four newly identified BMI-associated loci in this study, SNP rs2237892 is located in an intron of the *KCNQ1* gene, which encodes a voltage-gated potassium channel. This locus is involved in long QT syndrome in Europeans and African Americans (19,20) and is associated with type 2 diabetes (T2D) in both Asian and European populations (21–23). The T2D risk-associated *C* allele of rs2237892 has been related to lower fasting insulin levels (24) and a reduction in insulin secretion (25). The current study found that this risk allele is also associated with lower BMI. Adjusting for BMI in logistic regression models has been shown to strengthen rather than attenuate the association of rs2237892 with T2D (26). Given the strong link between T2D and obesity, we carried out additional analyses after excluding participants with T2D and found that the association of rs2237892 with BMI remained ($P = 3.72 \times 10^{-8}$). While the relationships of T2D with insulin secretion and insulin resistance are clear, the cause-and-effect relationships between hyperinsulinemia, insulin resistance, obesity and T2D remain unresolved. One study has suggested that suppression of insulin secretion was associated with loss of body weight and fat mass (27).

The locus represented by rs671 contains the *ALDH2* gene, which is involved in dehydrogenation of acetaldehyde and is associated with alcohol consumption behavior and alcohol-flushing responses in Asians (22,28,29). GWAS have reported that the BMI-increasing allele of this SNP is associated with diverse traits, including alcohol consumption behavior (22), increased intracranial aneurysm (30), triglycerides (31), gamma glutamyl transferase levels (32), elevated blood pressure (16), lower risk of coronary heart disease (33), decreased alcohol-flushing responses and esophageal cancer (34). rs671 results in a glutamine to lysine missense change at position 504 in the ALDH2 protein (accession ID NP_000681.2), known as the *ALDH*2* allele, and is predicted by both PolyPhen-2 (35) and SIFT (36) to be functionally important. A recent Mendelian randomization study suggested that *ALDH2* may influence the risk of hypertension by affecting alcohol consumption behavior, with *ALDH*1* allele carriers having higher blood pressure due to higher alcohol consumption (37). However, our study (Supplementary Material, Table S7) suggested an antagonistic effect of alcohol consumption on the *ALDH2*–BMI association. The *ALDH*1* BMI-increasing effect was mainly observed among non-drinkers.

While rs671 appears to be the most likely candidate in the 12q24 region, it is also in strong LD with the *A* allele of rs3782886 ($r^2 = 0.95$), which reached the genome-wide significance level in our Stage I data ($P = 1.24 \times 10^{-8}$) and is associated with decreased levels of alanine aminotransferase (32). Although its association with BMI was no longer significant after adjustment for rs671 in our study, another SNP in the 12q24 region, rs12229654 near the *MYL2* gene, has been associated with HDL cholesterol (38), levels of gamma glutamyl transpeptidase (38) and alcohol consumption (39) in Asian-ancestry populations. SNP rs12229654 is in LD ($r^2 = 0.67$) with 3 SNPs (rs11065756, rs3782888 and rs12231049) that are predicted to be among the strongest eQTLs in the region in HapMap lymphoblastoid cell lines for the *MYL2* gene (40) ($P < 0.05$, Supplementary Material, Table S11). *MYL2* encodes the myosin light chain and is involved in heart morphogenesis, and downregulation of this gene has been posited to play a role in coronary artery disease (41). In a Korean population, new loci in *MYL2* were recently shown to be associated with plasma glucose levels (42) and HDL levels (38). A SNP in the 12q24 region that is in LD ($r^2 = 0.58$) with rs671, rs2074356, has been previously associated with waist-to-hip ratio (43).

The third new locus, rs2535633, is in an intron of the *ITIH4* gene, which has been reported to be involved in the stabilization of the extracellular matrix and shows wide expression in the blood and liver (44). Obesity in rats has been positively correlated with rat blood levels of the ITIH4 protein, which has led to the suggestion that this protein may act as a biomarker for obesity (45). Fujita *et al.* (46) reported an association of the *ITIH4* gene with total cholesterol levels in individuals of

Japanese ancestry. SNP rs2535633 is in LD with two non-synonymous SNPs in the *ITIH4* gene, rs13072536 and rs4687657 ($r^2 = 0.83$ and 0.71, respectively), that reached the genome-wide significance level in Stage I ($P = 2.05 \times 10^{-8}$ for rs13072536 and $P = 2.63 \times 10^{-8}$ for rs4687657). Whereas rs13072536 is predicted by PolyPhen-2 (35) to be 'probably damaging', rs4687657 is predicted to be 'damaging' by SIFT (36). SNP rs2535633 is also an eQTL in HapMap lymphoblastoid cell lines for the *ITIH4* ($P = 5.5 \times 10^{-7}$), *FLJ12442* ($P = 1.7 \times 10^{-6}$) and *TMEM110* ($P = 2.2 \times 10^{-19}$) (47,48) genes and is in strong LD with other SNPs also predicted to act as eQTLs in lymphoblastoid cell lines and monocytes for *ITIH4*, *ITIH3*, *NT5DC2*, *WRD51A* and *FLJ12442* (40,47–49). This, in combination with biomarker studies in rats suggest that ITIH4 levels (45), which may be higher in those with the risk allele, may help identify individuals at risk for obesity. In addition, rs11918800 ($r^2 = 1.0$ with rs2535633) is located in a predicted transcription factor binding site, and rs6445538 ($r^2 = 0.73$ with rs2535633) is in a predicted hsa-miR-1301 miRNA binding site (50). The precise mechanisms by which one or more of these SNPs act on gene function and BMI remain to be determined.

Finally, the index SNP for the fourth new locus, rs11191580, resides in an intron of the *NT5C2* gene and has been associated with a number of psychiatric disorders, including autism and schizophrenia (51–53). Another SNP, rs11191548, which is in complete LD with rs11191580 ($r^2 = 1$), has been associated with measures of blood pressure in both European- and Asian-ancestry populations in four previous GWAS (16,54–56). Genetic variations in this gene were recently found to be associated with reduced subcutaneous and visceral fat mass in Japanese women (57). Further, rs11191580 is in strong LD with a number of SNPs that are predicted eQTLs for the *USMG5* gene according to two different datasets [$P = 4.5 \times 10^{-7}$ by Veyrieras *et al.* (40), $P = 9.7 \times 10^{-55}$ by Zeller *et al.* (47)]. The *USMG5* gene has been identified as coding a diabetes-associated protein in insulin-sensitive tissue (58). A recent study (59) reported a locus (rs12413409) that was associated with coronary artery disease. This SNP is in strong LD with rs11191580 ($r^2 = 1$ in Europeans, $r^2 = 0.895$ in Asians) and was associated with BMI ($P = 6.67 \times 10^{-7}$) in our Stage I data.

We observed similarities in the genetic architecture of BMI loci between Asian- and European-ancestry populations, despite notable differences in allele frequencies for some BMI loci, such as loci that were monomorphic. However, BMI distribution in Asians is very different from that in Europeans, supporting the notion that non-genetic factors, such as diet and physical activity, play a more important role in obesity than genetic factors. In fact, only a small percentage of BMI variation can be explained by genetic loci (1.52% in Asians). Clearly, further research is needed to investigate the interaction between genetic and lifestyle factors on the worldwide obesity epidemic.

The eQTL analysis suggested evidence of a potential functional role for the newly identified loci. Pathway analysis found two BMI-related pathways. One is cyclin-dependent kinase (CDK5) signaling, which can result in phosphorylation of the nuclear receptor PPARγ, which is encoded by the *PPARG* gene, a 'master' gene for fat cell biology and differentiation (60,61). Another top pathway was corticotropin-releasing hormone signaling ($P = 3.74 \times 10^{-4}$), which has been associated with depression and type 2 diabetes (62). A more thorough investigation and experimental verification are warranted to definitively establish the causal connections.

It is worth noting that four of the newly identified BMI-associated loci, *KCNQ1*, *ALDH2*, *ITIH4* and *NT5C2*, showed substantial pleiotropic effects, as mentioned above, on multiple obesity-related chronic-disease traits, such as T2D, blood pressure, coronary heart disease and schizophrenia. Of note, the BMI-decreasing alleles are associated with increased risk of T2D (*KCNQ1*), elevated blood pressure (*NT5C2*) and schizophrenia (*ITIH4* and *NT5C2*). However, the BMI-decreasing allele of rs671 in *ALDH2* is associated with decreased blood pressure and increased risk of coronary heart disease. Further studies are warranted to elaborate on the causal relationship between these genes, chronic-disease traits and obesity.

In conclusion, our study confirmed 22 previously reported BMI-associated loci in studies of European-ancestry populations and identified four novel loci near the *KCNQ1*, *ALDH2*/*MYL2, ITIH4* and *NT5C2* genes that are associated with BMI at the genome-wide significance level. The SNPs in the *KCNQ1* and *ALDH2*/*MYL2* genes showed stronger effects among men compared with women. SNPs rs671 and rs12229654 in *ALDH2*/*MYL2* are monomorphic in European-ancestry populations. Our study demonstrates the value of conducting genetic studies in different ethnic populations and expands our knowledge of the genetic basis for obesity.

MATERIALS AND METHODS

Study design

This study had two stages. Stage I was a meta-analysis of study-specific results on the association between SNPs and BMI from the 21 GWAS that participated in the consortium and included a total of 86 757 individuals of Asian ancestry. Promising SNPs selected from the Stage I meta-analysis were further examined by *de novo* or *in silico* replication analyses (Stage II). Supplementary Material, Tables S1–3, Figure S1 and the Supplementary Information summarize the basic information for all participating studies.

Stage I samples and genotyping

The sample sizes of the 21 GWAS in Stage I varied from 821 to 33 530, with a total of 86 757 individuals. Nine studies used Affymetrix arrays, and 12 studies used the Illumina platform (detailed information is provided in the Supplementary Information). To allow for combination of the data derived from different genotyping platforms and to improve coverage of the genome, genotype imputation was performed by each participating study using either MACH or IMPUTE with HapMap CHD + JPT data (release #22, build 36) as the imputation reference panel (Supplementary Material, Table S2).

Stage I statistical analysis

A uniform statistical analysis protocol was followed by each participating study. BMI was calculated by dividing weight in kilograms by the square of height in meters. To improve the

normality of the BMI distribution and alleviate the impact of outliers, rank-based inverse normal transformation (INT) was applied to BMI data separately for each gender by each study. INT involves ranking all BMI values, transforming these ranks into quantiles and, finally, converting the resulting quantiles into normal deviates. Associations between SNPs and the inverse normal-transformed BMI were analyzed with a linear regression model; associations between SNPs and obesity were analyzed as a dichotomous outcome, in which obesity was defined as BMI $\geq$ 27.5 (17), by using a logistic regression model, assuming an underlying additive genetic model and adjusting for age (continuous), age-squared and gender (if applicable). Stratified analyses by gender and disease status (with or without cancer and T2D) were also performed by each study.

Next, we carried out meta-analyses using a weighted average method with inverse-variance weights. The meta-analyses were carried out on all data combined and also stratified by gender and disease status using the freely available METAL software. The presence of heterogeneity across studies and between genders was tested with Cochran's Q statistics (63).

To correct each study for residual population stratification or cryptic relatedness, the meta-analyses were performed with genomic control correction (64) by adjusting for the study-specific inflation factor (λ), which ranged from 1.000 to 1.123 in Stage I (Supplementary Material, Table S2). After study-specific genomic control adjustment, the estimated inflation factor for the Stage I meta-analysis statistic was 1.128, which was further adjusted for when calculating the Stage I results.

Stage II replication analysis

Eight SNPs that were not near any previously reported BMI-associated loci and that had $P < 7.59 \times 10^{-6}$ in the Stage I data were taken forward into the Stage II replication analysis. The Stage II studies included a total of 47 791 individuals and consisted of *de novo* genotyping data from three study sites and *in silico* replication data from the Tai Chi study, which had been previously genotyped with Illumina's iSelect 200k Cardio-MetaboChip (Supplementary Material, Table S3). Due to the differing availability of replication data, for each SNP the sample size for the Stage II analysis varied from 7488 for rs4854307 to 47 352 for rs2237892.

Each study individually conducted a similar analysis of the association between BMI and the selected SNPs, using the same protocol used in Stage I. The Stage II data were combined using the same meta-analysis methods as in Stage I. Finally, we used meta-analysis to combine all data from both Stages I and II.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

URLS

METAL program, http://www.sph.umich.edu/csg/abecasis/Metal/; Cardio-MetaboChip, http://www.sph.umich.edu/csg/kang/MetaboChip/; HaploReg, http://www.broadinstitute.org/mammals/haploreg/haploreg.php/; Ingenuity, http://www.ingenuity.com/. SNAP, http://www.broadinstitute.org/mpg/snap/ldsearchpw.php

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