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Genome-Wide Association Meta-analysis Identifies Novel Variants Associated With Fasting Plasma Glucose in East Asians

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Fasting plasma glucose (FPG) has been recognized as an important indicator for the overall glycemic state preceding the onset of metabolic diseases. So far, most indentified genome-wide association loci for FPG were derived from populations with European ancestry, with a few exceptions. To extend a thorough catalog for FPG loci, we conducted meta-analyses of 13 genome-wide association studies in up to 24,740 nondiabetic subjects with East Asian ancestry. Follow-up replication analyses in up to an additional 21,345 participants identified three new FPG loci reaching genome-wide significance in or near *PDK1-RAPGEF4*, *KANK1*, and *IGF1R*. Our results could provide additional insight into the genetic variation implicated in fasting glucose regulation.

Fasting plasma glucose (FPG) levels are tightly regulated as a part of metabolic homeostasis (1). Failure in blood glucose regulation can lead to elevated FPG levels, representing an independent risk factor for type 2 diabetes (T2D) and a predictor of cardiovascular disease (2,3). The fasting glucose level is a moderately heritable trait with the heritability around 30% (4–6). A considerable number of genetic determinants influencing fasting glucose levels has been identified from numerous genetic studies in the past few years. The total heritability of fasting glucose levels, however, is yet to be fully explained.

To date, 39 genetic loci harboring variants associated with FPG have been identified from genome-wide association (GWA) studies and GWA meta-analyses that were conducted in populations of European ancestry (7,8). The genetic basis of glycemic regulation has not been fully explored in non-European populations, with only one study in East Asians that identified a single locus associated with FPG (rs895636 at the *SIX2-SIX3* loci) (9). Considering differences in the allele frequencies and linkage disequilibrium (LD) structures among ethnic groups, large-scale genetic studies in populations of non-European ancestries may increase the chance to detect additional novel genetic loci for FPG.

GWA meta-analyses have an advantage to identify genetic variants with small effect size and low allele frequency that were hardly detected in a single GWA study (10). Therefore, in this study, we aimed to identify novel loci influencing fasting glucose variation by conducting GWA meta-analysis in East Asian populations. We conducted a two-stage association study, comprising a discovery set (stage 1) of 24,740 individuals from the Asian Genetic Epidemiology Network (AGEN) and follow-up de novo genotyping replication set (stage 2) of 21,345 individuals from independent East Asian populations (Table 1 and Supplementary Fig. 1).

RESEARCH DESIGN AND METHODS

Study Subjects

Stage 1 subjects were drawn from 13 GWA studies participating in the AGEN consortium, which was organized

in 2010 to enable GWA studies of metabolic traits such as diabetes, hypertension, and obesity. These 13 studies consist of 24,740 subjects from the Korea Association REsource (KARE) project, Health Examinee shared control study (HEXA), Cardiovascular Disease Association Study (CAVAS), three Singapore Prospective Study Programs (SP2), Shanghai Breast Cancer Study (SBCS), Shanghai Men's Health Study (SMHS), Genetic Epidemiology Network of Salt Sensitivity (GenSalt), Cardiometabolic Genome Epidemiology (CAGE) Network, Cebu Longitudinal Health and Nutrition Survey (CLHNS), Cardiometabolic Risk in Chinese (CRC) study, and the Korean Cancer Prevention Study-II (KCPS-II). Stage 2 included 21,345 subjects from five independent studies for de novo replication analysis. Each study obtained approval from the appropriate institutional review board, and all participants provided written informed consent across the studies. Information including the study design and descriptive characteristics of each participating study is outlined in Supplementary Table 1 and the Supplementary Data.

Phenotype Measurement

Fasting glucose levels were measured from whole blood, plasma, or serum for each cohort. Fasting whole-blood glucose levels were multiplied by 1.13 to convert to FPG

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Table 1—Study design and samples

	Representative	Study	Ethnic group	Genotyping method	Sample size
Stage 1 (discovery)	KNIH	KARE	Korean	Affymetrix 5.0	7,696
		HEXA	Korean	Affymetrix 6.0	3,385
		CAVAS	Korean	Illumina 1M	3,205
	NUS	SP2(1)	Chinese	Illumina 1M	933
		SP2(2)	Chinese	Illumina 610K	1,044
		SP2(3)	Chinese	Illumina 550K	305
	VU/SCI	SBCS	Chinese	Affymetrix 6.0	2,017
		SMHS	Chinese	Affymetrix 6.0/Illumina 660K	291
	Tulane University	GenSalt	Han Chinese	Affymetrix 6.0	1,832
	NCGM	CAGE	Japanese	Illumina 550K	756
	UNC	CLHNS	Filipino	Affymetrix 5.0	1,624
	Harvard University	CRC	Chinese	Illumina 610K	733
	Yonsei University	KCPS-II	Korean	Affymetrix 5.0	919
	Stage 1 total				24,740
Stage 2 (de novo replication)	KNIH	Health2	Korean	TaqMan	5,277
	RIKEN/UT	BBJ	Japanese	Multiplex PCR invader assay	1,883
	SJTU	SJTUDS	Han Chinese	MassARRAY	3,412
	Ehime University	JMGP	Japanese	TaqMan	10,299
	CUHK	CUHKS	Han Chinese	Sequenom MassARRAY	474
	Stage 2 total				21,345
Overall	AGEN	AGEN-FPG	East Asian		46,085

Stage 1 includes 13 studies that provided full GWA analysis results for FPG. Stage 2 includes five studies that provided de novo replication results of SNPs selected from stage 1. BBJ, BioBank Japan; CUHK, Chinese University of Hong Kong; CUHKS, Chinese University of Hong Kong Diabetes Study; Health2, Health2 Study; JMGP, The Japanese Millennium Genome Project; KNIH, Korea National Institute of Health; NUS, National University of Singapore; SCI, Shanghai Cancer Institute; SJTU, Shanghai Jiao Tong University; SJTUDS, Shanghai Jiao Tong University Diabetes Study; UNC, University of North Carolina; UT, The University of Tokyo; VU, Vanderbilt University.

levels. Anthropometric measurements, such as BMI, were obtained by standardized procedures.

Genotyping, Imputation, and Quality Control

Genotyping and quality control methods for individual studies are outlined in Supplementary Table 2. A variety of genotyping platforms from Affymetrix or Illumina were applied to each individual GWA analysis to obtain the entire genome scan data. Imputation of genotypes to the HapMap Phase 2 (CHB + JPT except for CLHNS, which used HapMap CHB + JPT + CEU) as the reference panel was carried out using the programs MACH, IMPUTE, or BEAGLE. Imputed single nucleotide polymorphisms (SNPs) with high imputation quality (proper-info >0.5 for IMPUTE and Rsq >0.3 for MACH and BEAGLE) were used for subsequent association analysis. Genotyping for de novo replication in stage 2 was carried out by TaqMan, Multiplex PCR invader assay, or Sequenom MassArray method.

Statistical Analyses

Only nondiabetic individuals were tested for FPG by excluding diabetic patients, individuals using antidiabetes medicine, and individuals with fasting glucose ≥ 7 mmol/L. The rank-based inverse normal transformed FPG was tested for the association analyses to improve the normality of the FPG distribution and alleviate the impact of outliers. Association analyses were adjusted for sex and BMI (plus recruitment area in KARE and National Center for Global Health and Medicine [NCGM] studies) to compensate for multivariate linear regression analyses in the additive genetic mode. For the family design of the GenSalt study, family relationship was adjusted using a linear mixed model in which family identification was used as a random effect. Association analyses were performed using the programs SNPTEST, Mach2qtl, or PLINK (Supplementary Table 2). The meta-analysis was conducted using an inverse-variance method assuming fixed effects with a Cochran Q test to assess heterogeneity between the 13 studies. All meta-analyses were performed using the METAL software, and study-specific genomic control adjustment was applied. Genomic control inflation factor (λ) was estimated from the median of the χ^2 statistic divided by 0.456. The λ for the meta-analysis was 1.06 (and was less than 1.029 for individual studies), indicating that the results seen in stage 1 were probably not the result of population stratification. The Manhattan plot showing the negative log P value distribution for stage 1 meta-analysis results was generated by WGAViewer software. The quantile-quantile plot of trend test P values showed deviations from the null distribution due to the strong associations observed for FPG. Regional association plots from genome-wide meta-analysis results were generated using the LocusZoom software.

Gene Relationships Across Implicated Loci Analysis

To understand gene relationships across implicated loci, a Gene Relationships Across Implicated Loci (GRAIL)

analysis was conducted as described previously (11,12). A total of 43 FPG genes comprising 40 previously known genes (Supplementary Table 3) and 3 genes newly implicated in this study (Table 2) were included for the analysis. PubMed abstracts published after December 2006 were not included for the analysis to reduce confounding by results from FPG GWA studies.

RESULTS

Our stage 1 meta-analyses from 24,740 AGEN subjects revealed signals showing strong evidence for FPG associations. Most of them were in known FPG loci (Fig. 1). Twenty-three of 40 FPG loci that were detected mostly in the European populations were replicated in our stage 1 meta-analyses (with $P < 0.05$ and a consistent direction of effect) (Supplementary Table 3). Of these, 11 (*GCKR, SIX2-SIX3, G6PC2-ABCC11, CDKAL1, TMEM195, GCK, SLC30A8, GLIS3, CDKN2A/B, MTNR1B*, and *FOXA2*) reached genome-wide significance and showed similar direction of association as in the original reports (Table 2).

After removing signals within previously identified FPG loci, SNPs showing the deviation between the distributions of the observed and expected *P* values were still observed on the quantile-quantile plot (Supplementary Fig. 2). Those signals likely represent new FPG loci that require validation in additional investigations. For follow-up replication, we selected three independent signals (i.e., with pairwise LD statistics $r^2 < 0.2$ and minor allele frequency ≥ 0.05 within a 500-kb window of the genomic region) from the stage 1 meta-analysis based on our arbitrary inclusion threshold ($P < 5 \times 10^{-7}$), heterogeneity *P* value >0.01, and at least 10 studies having been included in the meta-analysis. To consolidate genetic associations for the promising three new variants, we conducted de novo genotyping. The stage 2 replication analysis (five studies, up to 21,345 subjects) showed a statistically significant association of these three variants with FPG and the same direction of association as in the stage 1 analysis results (Table 2).

An overall meta-analysis of the total samples (18 studies, up to 46,085 subjects) identified three novel loci for FPG reaching genome-wide significance ($P < 5 \times 10^{-8}$). These FPG-associated loci were located close to *PDK1-RAPGEF4* (rs733331, $P_{overall} = 6.98 \times 10^{-11}$), *KANK1* (rs10815355, $P_{overall} = 1.26 \times 10^{-9}$), and *IGF1R* (rs2018860, $P_{overall} = 2.99 \times 10^{-8}$) (Table 2 and Fig. 2). The newly identified loci in our study showed less significant association in European ancestry subjects studied by the MAGIC investigators ($P < 5 \times 10^{-3}$) (2). Notably, two of the new loci (near *PDK1-RAPGEF4* and *KANK1*) have very low minor allele frequencies (<0.01) in Europeans (Supplementary Table 4).

DISCUSSION

SNP rs733331 is located on chromosome 2q31 between *PDK1* (pyruvate dehydrogenase kinase (PDK) isozyme 1) and *RAPGEF4* (Rap guanine nucleotide exchange factor 4).

Table 2—Identified genetic loci associated with FPG at genome-wide significance in East Asian populations

	Chr	Candidate gene	Effect allele	Other allele	Stage 1 (discovery)		Stage 2 (de novo replication)		Combined (stage 1+2)	
					β ± SEM	*P*	β ± SEM	*P*	β ± SEM	*P*
New loci identified in this study										
rs733331	2	*PDK1-RAPGEF4*	A	G	0.048 ± 0.009	7.18E-08	0.029 ± 0.007	3.85E-05	0.036 ± 0.006	6.98E-11
rs10815355	9	*KANK1*	T	G	0.074 ± 0.012	3.73E-10	0.027 ± 0.009	3.59E-03	0.045 ± 0.007	1.26E-09
rs2018860	15	*IGF1R*	A	T	0.050 ± 0.009	1.72E-08	0.019 ± 0.007	7.21E-03	0.031 ± 0.006	2.99E-08
Previously reported loci										
rs780094	2	*GCKR*	C	T	0.052 ± 0.009	3.58E-09	—	—	0.052 ± 0.009	3.58E-09
rs895636	2	*SIX2-SIX3*	T	C	0.069 ± 0.010	2.53E-13	—	—	0.069 ± 0.010	2.53E-13
rs13387347	2	*G6PC2-ABCC11*	C	T	0.114 ± 0.009	2.35E-36	—	—	0.114 ± 0.009	2.35E-36
rs9356744	6	*CDKAL1*	C	T	0.057 ± 0.009	9.24E-10	—	—	0.057 ± 0.009	9.24E-10
rs1974620	7	*TMEM195*	T	C	0.063 ± 0.009	2.79E-11	—	—	0.063 ± 0.009	2.79E-11
rs730497	7	*GCK*	A	G	0.121 ± 0.011	7.72E-27	—	—	0.121 ± 0.011	7.72E-27
rs3802177	8	*SLC30A8*	G	A	0.063 ± 0.009	5.23E-12	—	—	0.063 ± 0.009	5.23E-12
rs4237150	9	*GLIS3*	C	G	0.053 ± 0.009	4.31E-09	—	—	0.053 ± 0.009	4.31E-09
rs10811661	9	*CDKN2A/B*	T	C	0.062 ± 0.009	8.66E-12	—	—	0.062 ± 0.009	8.66E-12
rs3847554	11	*MTNR1B*	T	C	0.059 ± 0.009	2.20E-11	—	—	0.059 ± 0.009	2.20E-11
rs6048216	20	*FOXA2*	T	C	0.095 ± 0.013	1.91E-12	—	—	0.095 ± 0.013	1.91E-12

Chr, chromosome.

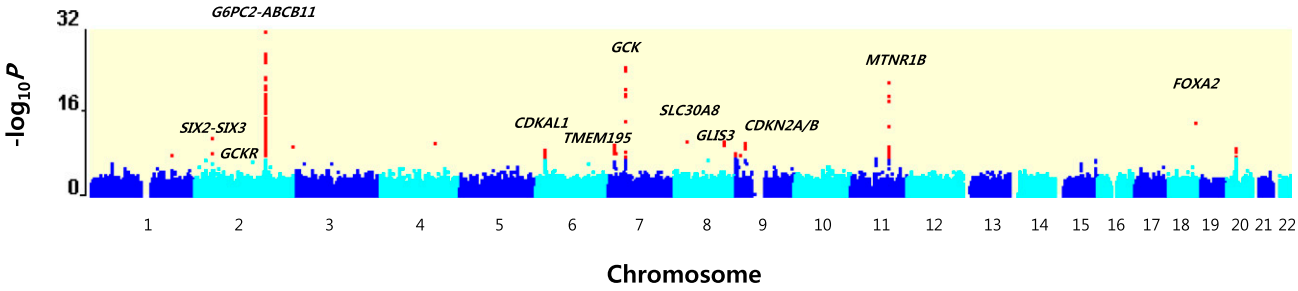

Figure 1—Genome-wide Manhattan plot of the meta-analysis for FPG in East Asian populations. Shown are the $-\log_{10} P$ values using the trend test for SNPs distributed across the entire autosomal genome (NCBI build 37). The red dots at each locus indicate the signals with $P < 10^{-6}$ detected in the GWA meta-analysis. Approximately 2.4 mol/L SNPs that were present in at least 13 stage 1 studies were used to generate the plot.

Pyruvate dehydrogenase, a mitochondrial multienzyme complex, is one of the important key enzymes responsible for glucose homeostatic regulation. The enzyme activity by cyclic de-phosphorylation cascades is regulated by a specific PDK. A previous functional study reported that liver-specific *Pdk1* deficiency in mice was associated with postprandial hyperglycemia (13). In addition, a potential regulator of PDK1, the pancreas-specific miR-375, was directly implicated in the regulation of glucose-induced biological responses (14). *RAPGEF4* has a role in initiating insulin secretion and mediating cAMP-dependent pulsatile insulin release (15).

The rs10815355 signal on chromosome 9p24 is located in an intron of *KANK1* (KN motif and ankyrin repeat domains 1), which has a role in the formation of the cytoskeleton by regulating actin polymerization. KANK1 negatively regulates the formation of actin stress fibers and cell migration through the inhibition of ρ-associated kinase activity (16).

Recently, the population-based Metabolic Syndrome in Men (METSIM) study conducted exome array analysis in 8,229 nondiabetic Finnish males (17). This study demonstrated that genetic variant rs3824420, encoding Arg667His in KANK1 and located 90 kb from rs10815355, was associated with circulating proinsulin levels and related insulin processing and secretion traits (17). In the METSIM study, rs10815355 also exhibited association with proinsulin levels at GWA significance (Supplementary Table 5). The substantial attenuation in association for both SNPs in conditional analysis suggests that rs10815355 likely represents the same signal as rs3824420 for the proinsulin levels. This result is supported by the strong LD (r^2 = 0.787, D' = 1.000 [K.L.M. and METSIM scientists, unpublished data]) calculated by the genotypes in METSIM (Supplementary Table 5).

Both rs10815355 and rs3824420 were not significantly associated with fasting glucose levels in METSIM (Supplementary Table 5). On the other hand, in addition to the strong association of rs10815355 for FPG, our AGEN stage 1 meta-analysis (seven studies, up to 11,822 subjects) demonstrated that rs3824420 was also marginally associated with fasting glucose ($P_{stage\ 1}$ = 0.035). Considering the substantial differences in SNP minor allele frequency and LD between the two ethnic groups, these discrepancies between AGEN and METSIM are not surprising (Supplementary Table 6). It is known that genetic variants with low allele frequency are hardly detected in the GWA studies (18).

Unlike the case of FPG, both rs10815355 and rs3824420 were not associated with the insulin-related traits such as fasting insulin and HOMA of β-cell function in one of the AGEN stage 1 studies (KARE, up to 7,183 subjects) (Supplementary Table 7). To detect the evidence of association for these traits in the East Asian populations, meta-analysis combining all AGEN stage 1 data (thus improving power by increasing the sample size) should be carried out (Supplementary Table 7).

Although two SNPs, rs10815355 and rs3824420, are weakly linked in East Asians (r^2 = 0.087, D' = 0.362 in HapMap CHB/JPT), the association strength of one SNP was moderately diminished after adjustment for the other SNP in our conditional analyses using data from three AGEN stage 1 studies, KARE, HEXA, and CAVAS (up to 12,178 subjects) (Supplementary Table 8). These results plausibly indicate the functional relevance of rs10815355 to rs3824420 in the *KANK1* region for FPG association in the East Asian populations. Further study will be needed to determine whether these signals share an underlying unrevealed causal variant for FPG levels. Other conditional analyses demonstrated that association signals of rs10815355 and rs3824420 for FPG remained after adjustment for the insulin-related traits (Supplementary Table 9). These results suggested that the FPG association of the *KANK1* region was not simply secondary to the association for insulin-related traits in our study.

SNP rs2018860 in 15q26 is located in an intron of *IGF1R* (insulin-like growth factor receptor), which is involved in cell growth, differentiation, migration, and metabolism and is a major aspect of glucose homeostasis (19). IGF1R, the protein encoded by *IGF1R*, has tyrosine kinase activity that stimulates growth in many different cell types and blocks apoptosis in multiple signaling pathways (20,21). Recent GWA analyses detected the association of *IGF1R* with higher serum uric acid concentrations in European populations (22). Serum

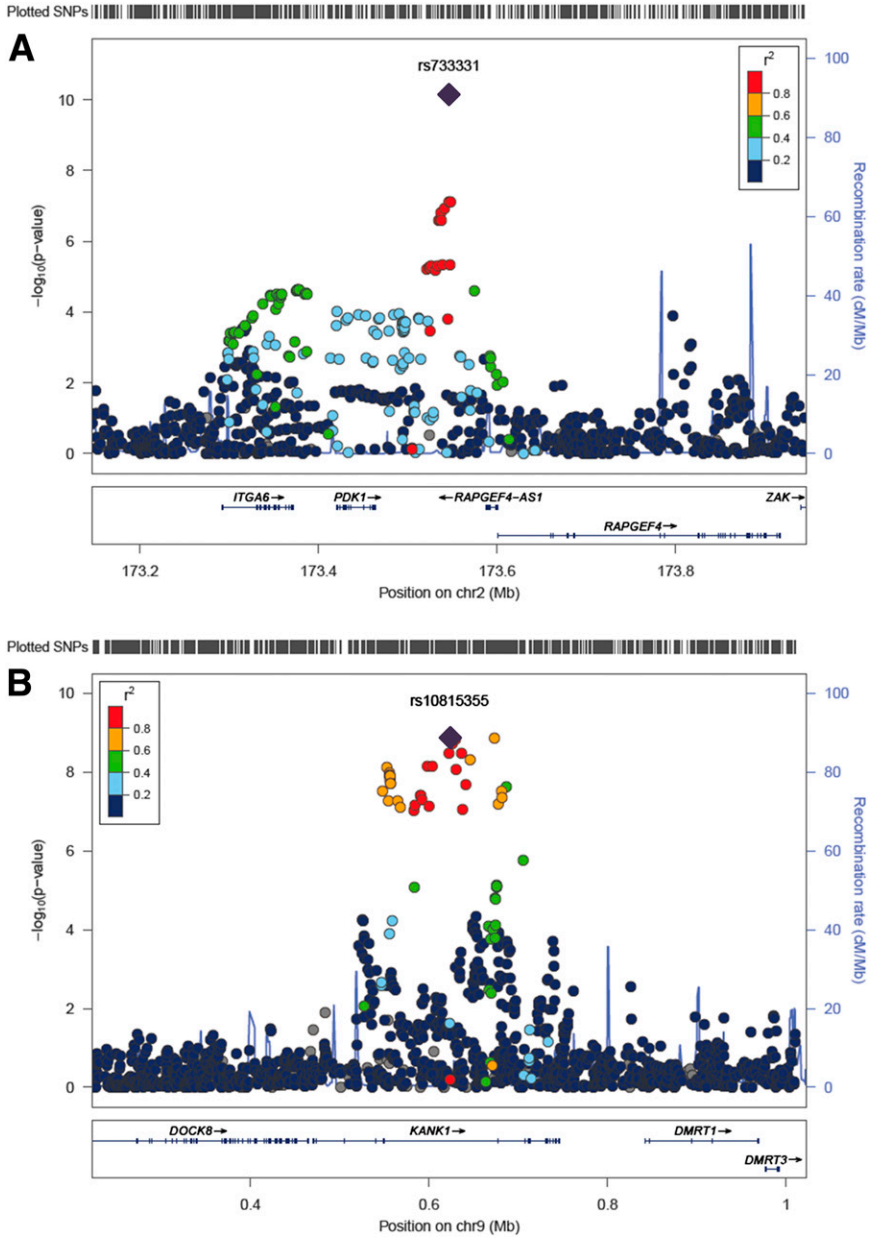

Figure 2—Regional association plots of three newly discovered FPG loci. *A*–*C*: The SNP positions are shown at the top and the regional association results from the GWA meta-analysis are shown in the middle. The trend test $-\log_{10} P$ values are shown for SNPs distributed in a 0.8-Mb genomic region centered on the most strongly associated signal, which is depicted as a purple diamond for the combined stage 1 and 2 results. The locations of known genes in the region are shown at the bottom. The genetic information is from the Human Genome hg19, and the LD structure is based on the 1000 Genomes East Asian Ancestry data (March 2012). chr, Chromosome.

uric acid levels as a potential biomarker have been reported in association with impaired glucose metabolism (23). In addition, a two-stage study reported a putative role of *IGF1R* variants on insulin resistance and arterial hypertension (24). Expression of a dominant-negative IGF1R in muscle leads to severely impaired insulin-mediated glucose uptake (25). β-Cell–specific knockout of IGF1R results in hyperinsulinemia and impaired glucose tolerance (26). IGF1R is involved in mediating GLP-1 increase in glucose competence and proliferation on the β-cell (27).

Given the knowledge that the substantial elevation of FPG is one of the typical signs of T2D, we investigated the relevance of the three new FPG signals to T2D risk from AGEN-T2D meta-analysis data (11). None showed evidence for association with T2D (Supplementary Table 10). These results indicate that the three new variants influencing FPG likely have limited impact on T2D risk, as exemplified by *MADD* and *SLC2A2* loci in the previous report (2).

We performed the GRAIL literature-based annotation analysis (12) to investigate functional connectivity among the three new FPG genes from this study and the 40 known

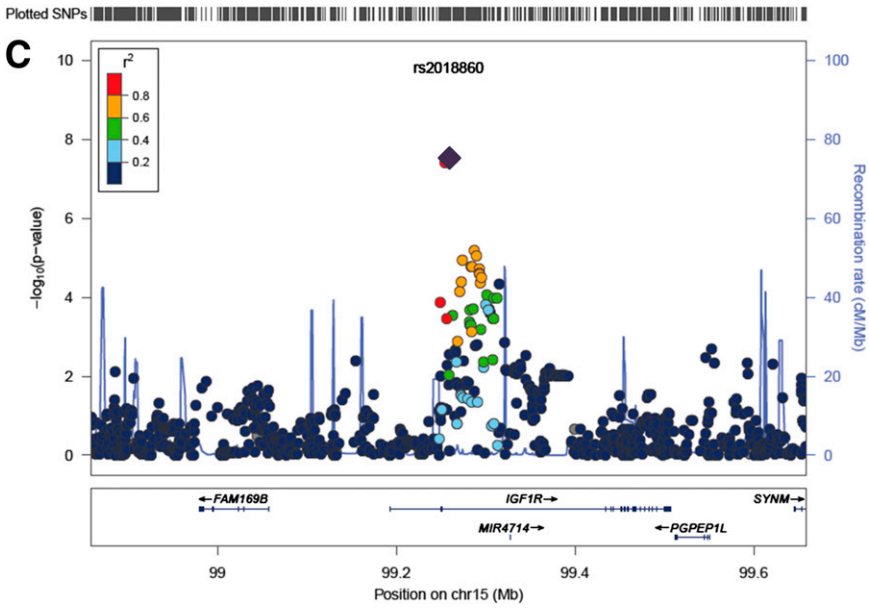

Figure 2—*Continued*.

genes from previous studies (Supplementary Table 11). The strongest connections were observed in biological pathways, such as insulin secretion, circadian rhythm, and carbohydrate digestion, along with the most frequently connecting terms, including insulin, glucose, circadian, and growth. The results highlighted biological functions of newly identified loci in the regulation of glucose metabolism (Supplementary Table 12 and Supplementary Fig. 3).

This study is the largest GWA study meta-analysis, to our knowledge, conducted for FPG in East Asians. In conclusion, our meta-analysis identified three novel loci in or near the *PDK1*, *KANK1*, and *IGF1R* genes at genome-wide significance levels. This study was also able to replicate many of the FPG risk loci that were previously established in Europeans. The identification of these loci provides the possibility to further the functional connection and the causal evidence in fasting glucose regulation and related diseases.

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Y.S.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

The programs discussed in the Research Design and Methods section are available from the following Web sites: PLINK, http://pngu.mgh.harvard.edu/~purcell/plink; WGAViewer, http://compute1.lsrc.duke.edu/softwares/WGAViewer; METAL, http://www.sph.umich.edu/csg/abecasis/Metal/index.html; SNAP, http://www.broadinstitute.org/mpg/snap; HapMap, http://hapmap.ncbi.nlm.nih.gov; LocusZoom, http://csg.sph.umich.edu/locuszoom; GRAIL, http://www.broadinstitute.org/mpg/grail.

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