A genome-wide search for gene-by-obesity interaction loci of dyslipidemia in Koreans shows diverse genetic risk alleles[S]

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Abstract **Dyslipidemia is a well-established risk factor for CVD. Studies suggest that similar fat accumulation in a given population might result in different levels of dyslipidemia risk among individuals; for example, despite similar or leaner body composition compared with Caucasians, Asians of Korean descent experience a higher prevalence of dyslipidemia. These variations imply a possible role of gene-obesity interactions on lipid profiles. Genome-wide association studies have identified more than 500 loci regulating plasma lipids, but the interaction structure between genes and obesity traits remains unclear. We hypothesized that some loci modify the effects of obesity on dyslipidemia risk and analyzed extensive gene-environment interactions (G×Es) at genome-wide levels to search for replicated gene-obesity interactive SNPs. In four Korean cohorts (n = 18,025), we identified and replicated 20 gene-obesity interactions, including novel variants (*SCN1A* and *SLC12A8*) and known lipid-associated variants (*APOA5*, *BUD13*, *ZNF259*, and *HMGCR*). When we estimated the additional heritability of dyslipidemia by considering G×Es, the gain was substantial for triglycerides (TGs) but mild for LDL cholesterol (LDL-C) and total cholesterol (Total-C); the interaction explained up to 18.7% of TG, 2.4% of LDL-C, and 1.9% of Total-C heritability associated with waist-hip ratio. Our findings suggest that some individuals are prone to develop abnormal lipid profiles, particularly with regard to TGs, even with slight increases in obesity indices; ethnic diversities in the risk alleles might partly explain the differential dyslipidemia risk between populations. Research about these interacting variables may facilitate knowledge-based approaches to personalize health guidelines according to individual genetic profiles.**—Kang, M., and J. Sung. **A genome-wide search for gene-by-obesity interaction loci of dyslipidemia in Koreans shows diverse genetic risk alleles. *J. Lipid Res.* 2019.** 60: **2090–2101.**

Supplementary key words dyslipidemias • lipids • high density lipoprotein • low density lipoprotein • triglycerides • obesity • gene-environment interaction • genome-wide interaction scan • missing heritability • meta-analysis

Dyslipidemia is a well-established risk factor for CVD. Genome-wide association studies (GWASs) have identified more than 500 loci influencing plasma lipid levels in European ancestry cohorts (1–11). The total variance explained by these loci in the Framingham Heart Study was 15.0% for total cholesterol (Total-C) levels, 13.7% for HDL-C levels, 14.6% for LDL-C levels, and 11.7% for triglyceride (TG) levels, corresponding to 25–30% of the heritability of each lipid trait (1–3).

Previous GWASs on lipids have been successful in terms of both the richness of robustly replicated loci and the genetic variances explained by these loci, but the current list in the GWAS Catalog is based on marginal association models assuming a lack of gene-gene or gene-environment interactions (G×Es). Ethnic differences in lipid abnormalities reactive to obesity, however, suggest that the interplay between genetic background and obesity traits may play a role in regulating lipid concentrations (12). Compared with non-Hispanic whites, for example, non-Hispanic blacks have a lower dyslipidemia subtype prevalence despite a higher obesity risk (12, 13). Conversely, despite similar or even leaner body compositions than Caucasians of the same

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1A2B2002136). The authors declare that they have no conflicts of interest with the contents of this article.

Manuscript received 11 July 2019 and in revised form 21 October 2019.

Published, JLR Papers in Press, October 29, 2019
DOI https://doi.org/10.1194/jlr.P119000226

Abbreviations: CC, case-control test; CO, case-only test; CT1, cocktail I test; CT2, cocktail II test; DG, disease-gene association test; DG1, disease-gene association test to screen and empirical Bayesian test to test; DG2, disease-gene association test to screen and case-control test to test; EB, empirical Bayesian test; EDG×E, environment-gene correlation test + disease-gene association test to screen and case-control test to test; EG, environment-gene correlation test; EG2, environment-gene correlation test to screen and case-control test to test; GWAS, genome-wide association study; GWIS, genome-wide interaction scan; G×E, gene-environment interaction; H2, hybrid test; HC, hip circumference; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; OR_D, marginal odds ratio; OR_G, genetic odds ratio; $OR_{G\times E}$, gene-environment interactive odds ratio; Remnant-C, remnant cholesterol; TG, triglyceride; Total-C, total cholesterol; WC, waist circumference; WHR, waist-hip ratio.

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[S] The online version of this article (available at http://www.jlr.org) contains a supplement.

age and sex, the prevalence of dyslipidemia is increased in Asians (12, 14, 15), particularly those of Korean descent (12, 16, 17).

A growing body of evidence from candidate-gene studies also supports the presence of gene-obesity interactions in lipid profiles, as the effects of lipid-associated loci are modified by obesity traits such as BMI, waist circumference (WC), and waist-hip ratio (WHR) (18–22). However, few studies have been performed at a genome-wide scale to identify SNPs influencing lipid levels based on obesity status, and even these studies were carried out using two-step analyses and genetic risk scores rather than exhaustive methods (23–26). Moreover, current findings of gene-obesity interactions are predominantly based on European populations (18–20, 22–26), and the G×E effects could have been underestimated if non-Caucasians are more susceptible to obesity-reactive dyslipidemia genes.

We hypothesized that some genetic susceptibility loci modify the effects of obesity on the risk of dyslipidemia and assumed that these G×E loci might include both novel and known genes with different reactive effect sizes. We also hypothesized that the novel gene-obesity interactions underlying lipid parameters explain more of the total and genetic variances of each lipid trait than do the genetic loci with only marginal effects. G×E analyses are generally believed to suffer from weak study power rather than type 1 errors or false-positives (27). Partly due to this, replicating the findings has not been widely accepted as a prerequisite for reporting G×Es, but we believe replications in G×E studies are as important as in GWASs. To search for and replicate gene-obesity interactive SNPs influencing the risk of dyslipidemia, we carried out a genome-wide interaction scan (GWIS) of four independent genome cohort studies in Korea. We believe that the identification of genes interacting with potentially modifiable risk factors will facilitate knowledge-based approaches to personalize health guidelines according to an individual's genetic profile.

MATERIALS AND METHODS

Participants

A total of 18,025 individuals from four independent Korean cohorts with a genome-wide set of variants were included in this study: 4,637 individuals from the Ansan cohort, 4,205 individuals from the Ansung cohort, 3,700 individuals from the urban cohort, and 5,483 individuals from the rural cohort. The baseline characteristics of each cohort are described in **Table 1**. Participants in the Ansan and Ansung cohorts were aged 40–69 years and recruited from industrialized suburban and rural areas in Korea. The urban and rural cohorts were population-based cohorts aged over 40 years and were respectively recruited from urban medical institutions and rural regions in Korea: Gangwha, Goryeong, Namwon, Pyeongchang, Wonju, and Yangpyeong. These cohorts are part of the Korean Genome and Epidemiology Study (KoGES), an ongoing population-based cohort study initiated in 2001 to understand chronic diseases in Korea. The research was performed according to the Declaration of Helsinki principles. The research protocol and data in this study were approved by the Institutional Review Board of Seoul National University (Institutional Review Board number: E1805-003-001).

Measurements

Data on health status, health-related behaviors, and medical and medication histories were collected through a standardized questionnaire. Trained experts at clinical centers performed anthropometric measurements, specimen collection, and laboratory tests. All participants provided informed consent for the baseline data and specimens; the detailed protocols are described in previous reports (28, 29). Total-C, HDL-C, and TG were measured using traditional enzymatic methods in blood samples drawn after an 8 h fast. For individuals with TG under 4.52 mmol/l, LDL-C was calculated using the Friedewald's formula (30); remnant cholesterol (Remnant-C) was determined as the level of Total-C minus HDL-C minus LDL-C (31, 32). BMI and WHR were calculated using directly measured height, weight, WC, and hip circumference (HC).

Phenotypes

We defined dyslipidemia based on thresholds of high-risk CVD groups reported by the National Cholesterol Education Program (33): Total-C over 6.21 mmol/l or LDL-C over 4.14 mmol/l or TG over 2.26 mmol/l or the lowest quintile of HDL-C or the highest quintile of Remnant-C. Individuals with a medical history of hyperlipidemia or the use of any lipid-lowering drugs like statins were considered as cases of dyslipidemia in the statistical analyses. Obesity traits were defined using clinical guidelines of the National Institutes of Health and the Korean Society for the Study of Obesity (KSSO) (34, 35): overweight class 1 (BMI $\geq$23.0 kg/m^2), overweight class 2 (BMI $\geq$25.0 kg/m^2), obesity (BMI $\geq$30.0 kg/m^2), abdominal obesity class 1 (WC >90 cm for males, 80 cm for females), abdominal obesity class 2 (WC >102 cm for males, 88 cm for females), and abdominal obesity due to WHR (WHR >0.90 for males, 0.85 for females). Individuals with inaccurate lipid levels or any history of cancer or diabetes were excluded from this study.

Genotype information

The following genome-wide dense SNP arrays were used to generate genetic data: the Affymetrix Genome-Wide Human SNP Array 5.0 for the Ansan and Ansung cohorts, the Affymetrix Genome-Wide Human SNP Array 6.0 for the urban cohort and part of the rural cohort (n = 1,816), and the Illumina HumanOmni1-Quad BeadChip for the rest of the rural cohort (n = 3,667). All variants violating Hardy-Weinberg equilibrium (P-value $<1 \times 10^{-6}$), with genotype call rates below 95%, and with minor allele frequency (MAF) values below 0.01 were excluded. After quality control, the remaining markers were imputed using the 1000 Genomes Project's haplotypes phase I in NCBI build 37 (GRCh37/hg19) of the Asian reference panels. SHAPEIT2 and IMPUTE2 were used for phasing and imputation, respectively (36, 37). Only SNPs with imputation quality scores higher than 0.6 were retained, yielding 4,780,608 SNPs for the Ansan and Ansung cohorts and 5,729,661 SNPs for the urban and rural cohorts. After comparing each cohort, 3,914,038 overlapping SNPs were selected as the final genetic markers.

Statistical analyses for the GWIS

The risk of dyslipidemia was adjusted for age, age^2, sex, and each obesity trait one by one; the logarithm of the odds ratio (OR) of dyslipidemia was corrected using a logit model. Before the GWIS, we conducted marginal disease-gene association (DG) and environment-gene correlation (EG) tests for the 3.9 million SNPs. Genetic markers associated with both lipid and obesity traits (P-value $<1 \times 10^{-3}$) were excluded to reduce potential pleiotropy. Exhaustive scans, including case-control (CC), case-only (CO) (38), and empirical Bayesian (EB) tests (39), are direct one-step methods; CC tests the null hypothesis $\beta_{G\times E} = 0$ using a standard G×E model, CO checks the EG in affected individuals, and

TABLE 1. Basic characteristics of the participants in each Korean cohort

	Reference Set		Replication Set	
	Ansan Cohort	Ansung Cohort	Urban Cohort	Rural Cohort
Participants	4,236	3,606	3,436	4,736
Age (years)	50.1 ± 7.7	56.9 ± 8.8	52.7 ± 8.2	60.2 ± 9.3
Sex, male (%)	2,136 (50.4)	1,531 (42.5)	1,494 (43.5)	1,914 (40.4)
BMI (kg/m^2)	24.5 ± 2.8	24.4 ± 3.2	23.9 ± 2.9	23.9 ± 3.2
WC (cm)	80.4 ± 7.9	85.6 ± 8.5	82.2 ± 8.8	83.5 ± 8.8
HC (cm)	94.1 ± 4.7	91.0 ± 5.5	95.3 ± 5.8	93.2 ± 6.6
WHR	0.85 ± 0.06	0.94 ± 0.06	0.86 ± 0.06	0.90 ± 0.06
Total-C (mmol/l)	5.11 ± 0.82	4.87 ± 0.82	5.13 ± 0.89	5.13 ± 0.96
HDL-C (mmol/l)	1.18 ± 0.24	1.17 ± 0.24	1.42 ± 0.34	1.18 ± 0.29
LDL-C (mmol/l)	3.18 ± 0.75	2.96 ± 0.75	3.08 ± 0.83	3.20 ± 0.88
TG (mmol/l)	1.64 ± 0.98	1.63 ± 1.00	1.37 ± 1.00	1.62 ± 1.09
Remnant-C (mmol/l)	0.75 ± 0.45	0.75 ± 0.46	0.63 ± 0.46	0.74 ± 0.50

LDL-C was calculated using the Friedewald's formula for individuals with TG under 4.52 mmol/l; Remnant-C was determined as the level of Total-C minus HDL-C minus LDL-C. Detailed features stratified by obesity status into subgroups based on BMI, WC, and WHR are presented in supplemental Table S2.

EB integrates the results from CC with those of CO. Two-step methods, on the other hand, comprise screening and hypothesis testing. We carried out emerging two-step approaches in parallel: DG|EB (DG1), DG|CC (DG2) (40), EG|CC (EG2) (41), hybrid (H2) (27), Cocktail I (CT1), Cocktail II (CT2) (42), and EDG×E (43). H2, Cocktail, and EDG×E adopt both DG and EG information to screen genetic markers in step-1; H2 uses these tests in parallel, Cocktail applies the information flexibly depending on the *P*-values of each DG and EG test, and EDG×E combines DG and EG statistics to generate new screening statistics. For testing in step-2, H2 and EDG×E adopt the results from CC; Cocktail applies EB when step-1 is based on DG and adopts CC if it uses EG to screen genetic markers.

After finding novel G×E SNPs, we applied the standard genome-wide significance level (*P*-value $<5 \times 10^{-8}$) for exhaustive scans. We assumed a screening threshold of 1×10^{-4} for step-1 of the DG1, DG2, EG2, and H2 methods; for step-2, the subset of SNPs passing step-1 were tested at a more liberal cut-off (0.05 divided by the number of screened SNPs) (27). We applied weighted hypothesis testing in step-2 of the CT1, CT2, and EDG×E methods rather than testing only SNPs passing step-1; stepwise penalties according to the marginal *P*-value were applied for each SNP in step-2 (44). We removed one of a pair of the identified SNPs if the linkage disequilibrium (LD) was greater than 0.5 (variance inflation factor greater than 2) for informed LD pruning (LD clumping). We identified novel G×E loci using a reference set consisting of the Ansan and Ansung cohorts; all detected G×E loci were reconfirmed using a replication set composed of the urban and rural cohorts. We considered the effect size, magnitude of standard error, *P*-value, and the direction of effect to calculate the final results of meta-analyses. We used PLINK (45), METAL (46), and R in the analyses.

We carried out additional continuous and categorical analyses for TG to clarify the scale and categorization issues; we tested whether the different scales of phenotypes (continuous vs. dichotomous) and the different categorization methods (quantiles vs. clinical guidelines) had affected the results. We conducted GWISs using TG levels as continuous traits; TG, a nonnormalized trait, was transformed into a logarithmic scale and adjusted for age, age^2, sex, and each obesity trait one by one. For categorical analyses, we used quantiles as cut-offs to determine outcomes and environmental factors instead of using clinical guidelines. We conducted GWISs *1*) using the highest quintile of TG as a categorical phenotype and *2*) using the highest quintile of BMI or WC or WHR as obesity traits. We also adjusted the risk of hypertriglyceridemia for age, age^2, sex, and each obesity trait one by one. All the results of additional analyses were identified using the reference set and replicated using the replication set.

Methods of evaluating impacts

We estimated the genetic variances due to the genetic susceptibility SNPs using the simplified equation $2p(1-p)[\log(OR)]^2$, where p is the MAF of a variant and OR is the estimated odds ratio from a logistic regression model for marginal associations (47). The contribution of each G×E marker was estimated using $2p(1-p)[\log(OR_G)]^2/V_P + 2ep[(1-p) + 2p(e-1)^2][\log(OR_{G\times E})]^2/V_P$. In this equation, e is the prevalence of an environmental factor, V_P is the phenotypic variance, and OR_G and $OR_{G\times E}$ are estimated additive and gene-obesity interactive ORs from a logistic regression model for G×Es. We used GenABEL, the R package for genome-wide association analyses (48), to estimate the total heritability of dyslipidemia from the Healthy Twin Study, a family-based cohort study in Korea (supplemental Table S2.e) (49, 50). GCTA, an analysis tool for genome-wide complex traits (51), was also used to estimate the SNP-based heritability attributable to all GWAS variants genotyped on a microarray. To transform the estimate of variance explained on the observed scale to that on the underlying scale, we assessed the prevalence of dyslipidemia using data from the Korean National Health and Nutrition Examination Survey (KNHANES) (52).

RESULTS

Table 1 shows the baseline characteristics of the participants in each Korean genome cohort. We observed the age, sex, obesity-related traits, and age- and sex-standardized plasma lipid levels of the cohorts; all features were stratified by obesity status into subgroups based on BMI, WC, and WHR (supplemental Tables S1, S2). We focused on the adjusted lipid concentrations to assess the trends of lipids in each obesity and abdominal obesity subgroup. As expected, age- and sex-adjusted lipid levels significantly worsened as the degree of obesity status increased in the combined Korean cohort (supplemental Fig. S1).

We identified 55 SNPs showing genome-wide significant G×E effects on the risk of abnormal lipid profiles with at least one of the six obesity traits (supplemental Table S3). By conducting LD clumping based on the genetic contribution to the risk of dyslipidemia, we detected 20 gene-obesity interactions due to novel SNPs near *SCN1A* and *SLC12A8* and to lipid-associated SNPs near *APOA5*, *BUD13*, *ZNF259*, and *HMGCR* that were reported in previous GWASs. **Table 2** shows the marginal and gene-obesity

TABLE 2. Novel gene-obesity interactive loci modifying the risk of dyslipidemia identified from the meta-analysis of the Korean cohorts

Trait	Environment	Gene	Marker	CHR	Position	MAF	A1/A2	GWAS	G×E Interaction		Test
								OR_D (*P*)	OR_G (*P*)	$OR_{G\times E}$ (*P*)	
Total-C	BMI	*COL4A3BP*	rs7733436	5	74666492	0.48	C/T	0.81 (3.88E-09)	0.79 (4.26E-10)	1.47 (7.32E-03)	CT1/CT2/EDG×E
	WC	*HMGCR*	rs2878417	5	74617262	0.48	G/A	0.81 (4.04E-09)	0.76 (3.07E-08)	1.18 (1.62E-02)	CT1/CT2/EDG×E
		COL4A3BP	rs7733436	5	74666492	0.48	C/T	0.81 (5.38E-09)	0.78 (1.97E-10)	1.27 (1.13E-02)	CT1/CT2/EDG×E
	WHR	*HMGCR*	rs7702895	5	74612893	0.48	G/A	0.81 (7.29E-09)	0.72 (3.15E-08)	1.22 (5.40E-03)	CT1/CT2/EDG×E
HDL-C	BMI	*LOC101929680/SCN1A*	rs11890028	2	166943277	0.09	G/T	0.96 (4.19E-01)	0.92 (8.78E-02)	2.30 (2.79E-08)	CO
LDL-C	BMI	*LOC101928271*	rs11693076	2	21140033	0.20	C/T	0.75 (1.25E-09)	0.96 (6.54E-01)	0.70 (6.79E-04)	CT1/CT2/EDG×E
	WC	*ANKDD1B*	rs7703282	5	74906963	0.46	A/C	0.77 (7.72E-10)	0.74 (6.12E-11)	1.30 (2.45E-02)	CT1/CT2/EDG×E
	WHR	*ANKDD1B*	rs7703282	5	74906963	0.46	A/C	0.77 (9.08E-10)	0.70 (4.41E-08)	1.19 (4.57E-02)	EDG×E
TG	BMI	*LOC105374079/SLC12A8*	rs77008808	3	124868173	0.06	T/C	0.97 (6.37E-01)	0.89 (9.29E-02)	2.70 (4.33E-08)	CO
		BUD13	rs1558860	11	116607368	0.22	A/C	1.55 (5.10E-35)	1.76 (1.01E-14)	0.80 (3.27E-03)	EDG×E
	WC	*APOA5*	rs651821	11	116662579	0.29	C/T	1.87 (1.56E-73)	2.01 (5.00E-47)	0.81 (4.06E-04)	CT1/CT2/EDG×E
		BUD13	rs918144	11	116633825	0.47	T/C	0.71 (4.22E-28)	0.66 (1.47E-19)	1.17 (7.75E-03)	EDG×E
	WHR	*BUD13*	rs180378	11	116588909	0.32	A/G	1.37 (8.00E-22	1.65 6.04E-18	0.76 1.26E-05	DG1/CT1/CT2/EDG×E
		APOA5/ZPR1(ZNF259)	rs2075291	11	116661392	0.08	A/C	1.98 (8.80E-42)	2.23 (4.26E-21)	0.82 (2.43E-02)	CT1/CT2
		APOA5	rs651821	11	116662579	0.29	C/T	1.86 (6.82E-73)	2.26 (8.52E-41)	0.74 (3.92E-06)	DG1/CT1/CT2/EDG×E
Remnant-C	BMI	*BUD13*	rs7926828	11	116586423	0.31	C/T	1.35 (1.76E-18)	1.33 (5.07E-16)	1.37 (4.88E-02)	EDG×E
	WC	*BUD13*	rs2075295	11	116628401	0.47	C/T	0.75 1.53E-27	0.73 2.21E-27	1.16 1.16E-02	EDG×E
	WHR	*BUD13*	rs180378	11	116588909	0.32	A/G	1.35 (5.63E-26)	1.47 (1.92E-15)	0.84 (1.07E-03)	EDG×E
		APOA5	rs651821	11	116662579	0.29	C/T	1.82 (1.52E-87)	1.99 (4.07E-41)	0.82 (1.76E-04)	CT1/CT2/EDG×E

The results for each gene-obesity interaction were summarized according to the discriminators of obesity traits: BMI, WC, and WHR. Detailed results are presented in supplemental Table S3.

interactive effects of the newly identified variants on the risk of dyslipidemia; we summarized the novel G×Es according to the discriminators of obesity traits, such as BMI, WC, and WHR. **Figure 1** (supplemental Table S4) describes the risk of abnormal lipid profiles for each genetic and environmental factor; we estimated the OR as the ratio of the probability of dyslipidemia occurring in each exposed group ($G \neq 0$ or $E \neq 0$) to the probability in a nonexposed group ($G = 0$ and $E = 0$).

We identified three novel SNPs interacting with obesity traits to modify the risk of abnormal elevation of Total-C: rs2878417, rs7702895, and rs7733436. In particular, *COL4A3BP* exhibited synergistic effects with BMI and WC on the risk of abnormalities in Total-C. For the interplay between *HMGCR* and WHR, the marginal odds ratio (OR_D) was 0.81 (95% CI, 0.78–0.84); OR_G and $OR_{G\times E}$ were 0.72 (95% CI, 0.68–0.77) and 1.22 (95% CI, 1.13–1.30), respectively. As shown in **Fig. 1A** (supplemental Table S4.a), the multiplicative effect of abdominal obesity was 1.12 (95% CI, 0.96–1.31) for individuals with two wild-type alleles at rs7702895. The magnitude of the effect of abdominal obesity, however, increased with the number of minor alleles, with values of 1.46 (95% CI, 1.28–1.67) for heterozygous and 1.57 (95% CI, 1.26–1.95) for homozygous minor alleles.

SCN1A marked by rs11890028 was detected as a novel locus interacting with obesity to change the risk of abnormal reduction of HDL-C. Although the marginal effect of this variant was negligible ($P = 4.19 \times 10^{-1}$), a noticeable G×E effect ($P = 2.79 \times 10^{-8}$) was observed in an exhaustive CO analysis. The estimated OR_D, OR_G, and $OR_{G\times E}$ for the interplay between *SCN1A* and BMI were 0.96 (95% CI, 0.91–1.01), 0.92 (95% CI, 0.87–0.96), and 2.30 (95% CI, 1.98–2.67). Figure 1E (supplemental Table S4.e) describes the gene-obesity interactive effect on the risk of abnormal HDL-C reduction; the multiplicative effect for common homozygous or heterozygous and rare homozygous genotypes was 1.42 (95% CI, 1.22–1.65), 1.99 (95% CI, 1.57–2.52), and 6.24 (95% CI, 4.03–9.64), respectively.

LOC101928271 showed antagonistic effects on the risk of abnormal elevation of LDL-C due to BMI. For the gene-obesity interaction, the estimated OR_D, OR_G, and $OR_{G\times E}$ were 0.75 (95% CI, 0.71–0.78), 0.96 (95% CI, 0.88–1.05), and 0.70 (95% CI, 0.63–0.78), respectively. As shown in Fig. 1B (supplemental Table S4.b), the multiplicative effect of overweight class 1 was 1.00 (95% CI, 0.54–1.82) for rare homozygous genotypes. For common homozygous or heterozygous genotypes, on the other hand, obesity acted as a risk factor for abnormalities in LDL-C; the multiplicative effect was 1.82 (95% CI, 1.61–2.06) and 1.34 (95% CI, 1.12–1.61), respectively.

We identified six novel SNPs with modifiable effects on the risk of abnormalities in TG due to obesity indices: rs1558860, rs180378, rs2075291, rs651821, rs918144 on chromosome 11, and rs77008808 on chromosome 3. *BUD13* and *APOA5* have previously been reported as lipid-associated loci, and the marginal effects of these loci on the risk of abnormal TG elevation were also markedly significant in this study. *BUD13* marked by rs918144 showed a risky G×E effect attributable to WC; OR_D, OR_G, and $OR_{G\times E}$

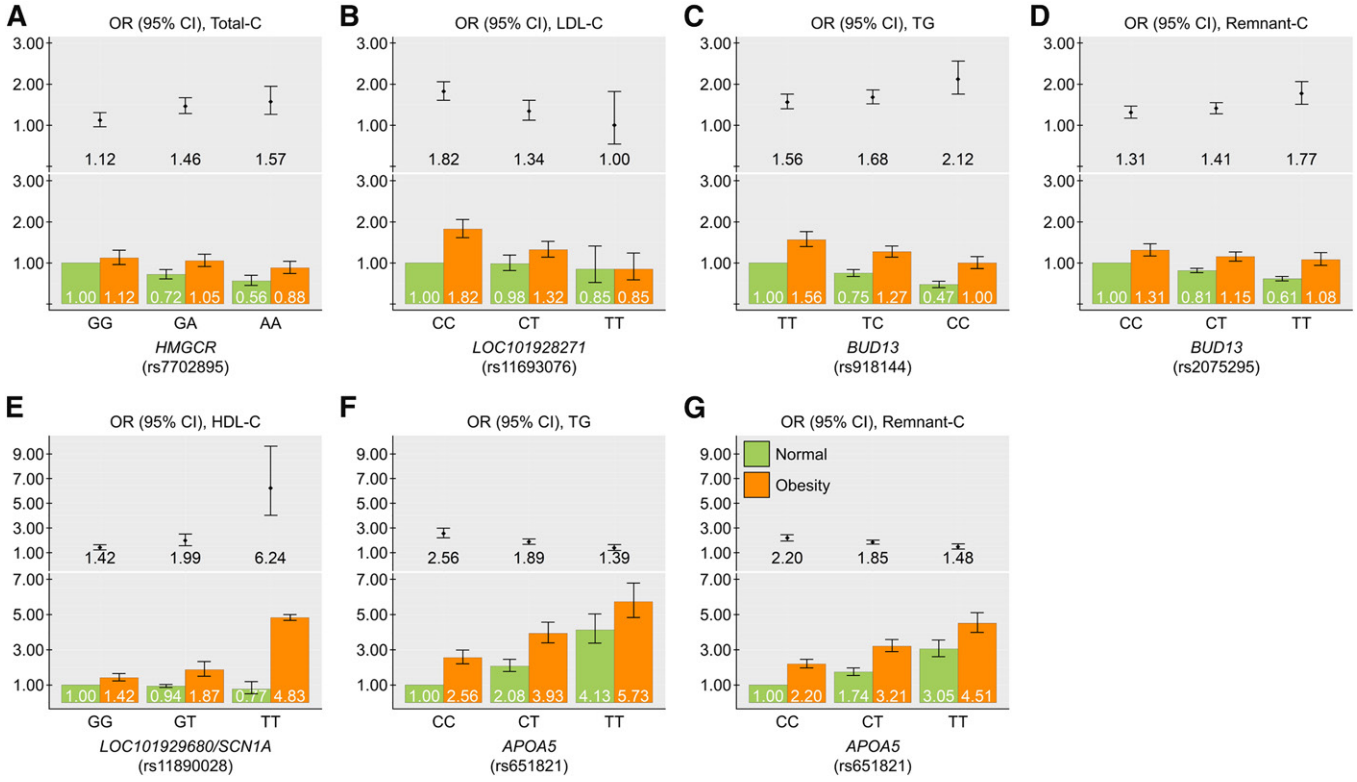

Fig. 1. Gene-obesity interactive effects on the risk of dyslipidemia. The bar plots on the lower side of each graph describe the OR as the ratio of the probability of dyslipidemia occurring in each exposed group ($G \neq 0$ or $E \neq 0$) to the probability in a nonexposed group ($G = 0$ and $E = 0$). The upper plots, on the other hand, show multiplicative effects of obesity traits for each genetic group. The figure above describes the estimated OR of each lipid abnormality due to the interplay between *HMGCR* and abdominal obesity based on WHR (A), *LOC101928271* and overweight class 1 (B), *BUD13* and abdominal obesity class 1 (C), *BUD13* and abdominal obesity class 2 (D), *LOC101929680/SCN1A* and obesity (E), *APOA5* and abdominal obesity based on WHR (F), and *APOA5* and abdominal obesity based on WHR (G). Further details are provided in supplemental Table S4.

were 0.71 (95% CI, 0.69–0.73), 0.66 (95% CI, 0.64–0.70), and 1.17 (95% CI, 1.10–1.24), respectively. As shown in Fig. 1C (supplemental Table S4.c), the multiplicative effect of abdominal obesity for common homozygous or heterozygous and rare homozygous genotypes was 1.56 (95% CI, 1.40–1.76), 1.68 (95% CI, 1.52–1.86), and 2.12 (95% CI, 1.76–2.56), respectively. Conversely, *APOA5* marked by rs651821 showed a protective G×E effect due to WHR; OR_D, OR_G, and $OR_{G\times E}$ were 1.86 (95% CI, 1.80–1.93), 2.26 (95% CI, 2.13–2.41), and 0.74 (95% CI, 0.69–0.79), respectively. The multiplicative effect of abdominal obesity for each genotype is illustrated in Fig. 1F (supplemental Table S4.f), with values of 2.56 (95% CI, 2.20–2.99) for common homozygous, 1.89 (95% CI, 1.68–2.12) for heterozygous, and 1.39 (95% CI, 1.16–1.66) for rare homozygous genotypes.

BUD13 and *APOA5* also showed significant marginal associations with the risk of abnormal elevation of Remnant-C. *BUD13* interacted with WC to modify the risk of dyslipidemia; OR_D, OR_G, and $OR_{G\times E}$ were 0.75 (95% CI, 0.73–0.77), 0.73 (95% CI, 0.71–0.75), and 1.16 (95% CI, 1.10–1.23), respectively. The multiplicative effect of abdominal obesity class 2 is illustrated in Fig. 1D (supplemental Table S4.d), with values of 1.31 (95% CI, 1.17–1.47), 1.41 (95% CI, 1.28–1.55), and 1.77 (95% CI, 1.51–2.07) for individuals with common homozygous or heterozygous and rare homozygous genotypes, respectively. *APOA5*, on the other hand, had an antagonistic effect due to WHR; OR_D, OR_G, and $OR_{G\times E}$ were 1.82 (95% CI, 1.77–1.88), 1.99 (95% CI, 1.89–2.09), and 0.82 (95% CI, 0.78–0.86), respectively. As shown in Fig. 1G (supplemental Table S4.g), the multiplicative effect of abdominal obesity was 2.20 (95% CI, 1.96–2.46) for individuals with two wild-type alleles at rs651821. The effects decreased as the number of minor alleles increased, with values of 1.85 (95% CI, 1.69–2.03) for heterozygous and 1.48 (95% CI, 1.28–1.71) for rare homozygous genotypes.

We ascertained the identified G×Es from different points of view; **Fig. 2** (supplemental Table S5) shows the trends of lipid levels due to changes in BMI for each subgroup stratified by the number of risk alleles of G×E SNPs. In normal weight ($18.5\ kg/m^2 \leqslant BMI < 25.0\ kg/m^2$) individuals with no risk alleles (low-risk group), HDL-C levels decreased by 0.032 mmol/l (95% CI, 0.030–0.034 mmol/l) for each unit ($1\ kg/m^2$) increase in BMI; the HDL-C decrement was 0.039 mmol/L (95% CI, 0.035-0.043 mmol/L) for individuals with at least one risk allele (high-risk group) and 0.038 mmol/l (95% CI, 0.033–0.043 mmol/l) for the upper 50% of the high-risk group (higher-risk group). Differences in the HDL-C decrement for each genetic subgroup were far clearer in obese individuals; for individuals with BMI over $25\ kg/m^2$, for example, the HDL-C decrements for the low-, high-, and higher-risk groups were 0.004 mmol/l (95%

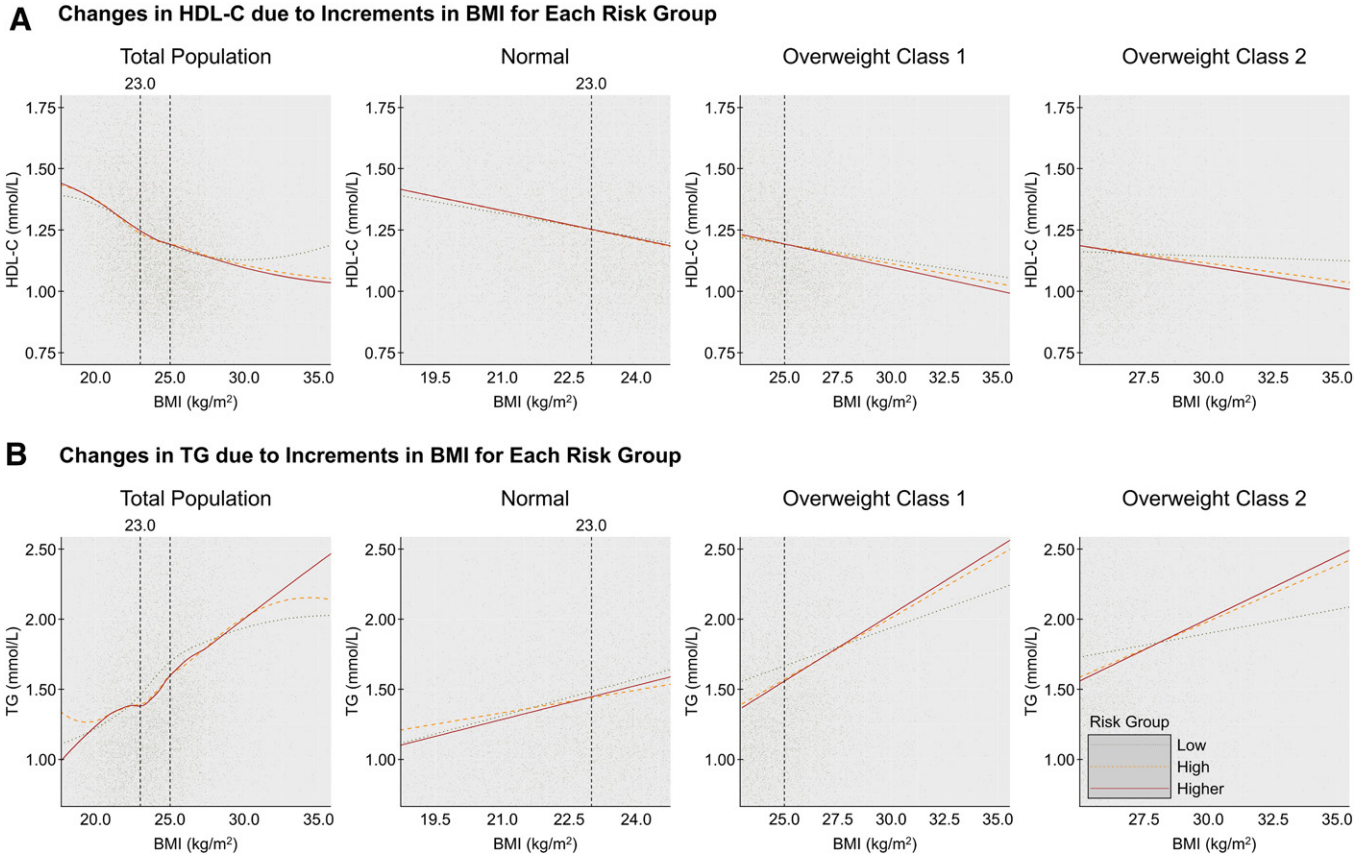

Fig. 2. Changes in lipid levels due to increments in BMI for each risk group. The participants in each study population were classified into three groups by the number of risk alleles on G×E markers: the low-risk group (individuals with no risk alleles), the high-risk group (individuals with at least one risk allele), and the higher-risk group (the upper 50% of the high-risk group). The figure above describes the trends of lipid levels due to an increment of 1 kg/m^2 in BMI for each subgroup. A: The differences in the decrement of HDL-C for each genetic subgroup were far clearer in the obese group than in the group with normal BMI. B: The differences in the increment of TG for each risk group were far clearer in the obese group than in the group with normal BMI; further details are presented in supplemental Table S5.

CI, 0.002–0.006 mmol/l), 0.014 mmol/l (95% CI, 0.010–0.018 mmol/l), and 0.017 mmol/l (95% CI, 0.012–0.022 mmol/l). Similarly, we ascertained that the TG increment was different for each genetic subgroup, and the trends of change were clearer in individuals with obesity.

We conducted GWISs using TG levels as continuous traits in supplemental Table S6 and newly found five genetic markers near *APOA5*, *BUD13*, and *ZNF259* interacting with BMI. All the identified variants had strong marginal effects ($P \leq 9.70 \times 10^{-25}$) on the traits of interest and were in LD with our previous finding, *BUD13* marked by rs1558860, except for rs2041967 ($r^2 = 0.20$). We could not detect any interactions of G×E markers with WC or WHR. In supplemental Table S7, we compared the identified gene-obesity interactive variants of hypertriglyceridemia defined by two different methods: by clinical guidelines and by the quantile distribution in our data. All the interactions of loci (*APOA5* and *BUD13*) with WC or WHR were replicated with our previous findings. On the other hand, we could not detect any interactions of G×E markers with BMI; only the variants, previously detected with multiple analytical methods for testing G×Es (Table 2), were consistently identified. Similarly, we identified only one G×E SNP of dyslipidemia, located on *APOA5*, by using the alternative definition of obesity, the highest quintile of BMI or WC or WHR (supplemental Table S8); we could not find any loci interacting with BMI or WC.

Table 3 shows the contributions of marginal associations and gene-obesity interactions to abnormal lipid profiles; we present the proportion of total heritability for each lipid explained by GWAS-identified SNPs, novel G×E loci, and the combined set of both lipid-associated and gene-obesity interactive variants (total genetic impact). The total and SNP-based heritabilities of the risk of abnormalities in Total-C were approximately 35.5% and 17.7–24.9%, respectively, after adjusting the risk of dyslipidemia by age, age^2, and sex. The genetic contributions increased when we considered both marginal associations and gene-obesity interactions, with differences between the GWAS-identified and total genetic impact of 1.1–1.9%. The total and SNP-based heritabilities of the risk of LDL-C abnormalities, on the other hand, were approximately 31.7% and 17.2–25.6%, respectively. For each obesity trait, the total genetic contributions including gene-obesity interactions to the risk of dyslipidemia were 0.9–2.4% higher than the marginal impact due only to direct associations.

The contributions of the combined set of both GWAS-identified and GxE variants were markedly higher when several independent gene-obesity interactive loci were present for each pair of lipid traits and environmental

TABLE 3. Contributions of gene-obesity interactive loci to abnormal lipid profiles

Trait	Heritability (%) (SNP-Based Heritability)	Environment	Gene	Marker	A1/A2	Contribution (%) of Genetic Variants							Total Genetic Contribution in KOR Population
						Contribution of GWAS-Identified Loci for KOR (Number of SNPs)	Additional Contribution of Gene-Obesity Interaction (Allele Frequency in Each Ethnic Group[a])						
							KOR	EAS	SAS	EUR	AMR	AFR	
Total-C	35.5 (17.7–24.9)	BMI	*COL4A3BP*	rs7733436	C/T	9.5 (20)	1.2 (0.48)	1.2 (0.49)	1.1 (0.41)	1.2 (0.61)	1.2 (0.54)	0.3 (0.06)	10.7
		WC	*HMGCR*	rs2878417	G/A	8.4 (17)	1.3 (0.48)	1.3 (0.50)	1.2 (0.40)	1.3 (0.58)	1.3 (0.53)	1.3 (0.57)	10.8
			COL4A3BP	rs7733436	C/T		1.1 (0.48)	1.2 (0.49)	1.1 (0.41)	1.2 (0.61)	1.2 (0.54)	0.2 (0.06)	
		WHR	*HMGCR*	rs7702895	G/A	7.2 (15)	1.9 (0.48)	1.9 (0.49)	1.8 (0.40)	1.9 (0.56)	1.9 (0.46)	0.2 (0.03)	9.1
HDL-C	34.8 (18.4–26.2)	BMI	*LOC101929680/SCN1A*	rs11890028	G/T	19.5 (31)	0.3 (0.09)	0.4 (0.13)	0.8 (0.28)	0.8 (0.29)	0.6 (0.21)	0.4 (0.16)	19.8
LDL-C	31.7 (17.2–25.6)	BMI	*LOC101928271*	rs11693076	C/T	5.5 (11)	0.9 (0.20)	0.9 (0.20)	1.0 (0.22)	1.6 (0.46)	1.6 (0.48)	1.7 (0.85)	6.4
		WC	*ANKDD1B*	rs7703282	A/C	6.3 (14)	1.8 (0.46)	1.8 (0.48)	1.7 (0.43)	1.8 (0.62)	1.8 (0.54)	0.4 (0.06)	8.1
		WHR	*ANKDD1B*	rs7703282	A/C	5.6 (12)	2.4 (0.46)	2.4 (0.48)	2.4 (0.43)	2.3 (0.62)	2.4 (0.54)	0.5 (0.06)	8.0
TG	38.3 (18.4–26.4)	BMI	*LOC105374079/SLC12A8*	rs77008808	T/C	48.2 (53)	0.3 (0.06)	0.3 (0.07)	0.2 (0.04)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	52.7
			BUD13	rs1558860	A/C		4.2 (0.22)	4.5 (0.24)	3.8 (0.19)	2.0 (0.09)	2.8 (0.13)	0.2 (0.01)	
		WC	*APOA5*	rs651821	C/T	36.6 (40)	7.4 (0.29)	7.3 (0.29)	5.5 (0.19)	2.6 (0.08)	4.5 (0.15)	4.8 (0.16)	47.1
			BUD13	rs918144	T/C		3.2 (0.47)	3.0 (0.38)	3.2 (0.56)	3.2 (0.48)	3.1 (0.60)	2.9 (0.68)	
		WHR	*BUD13*	rs180378	A/G	39.3 (48)	4.6 (0.32)	4.7 (0.32)	5.4 (0.60)	5.3 (0.43)	5.2 (0.40)	5.4 (0.46)	58.0
			APOA5/ZPR1(ZNF259)	rs2075291	A/C		3.4 (0.08)	1.8 (0.04)	0.5 (0.01)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	
			APOA5	rs651821	C/T		10.7 (0.29)	10.7 (0.29)	7.9 (0.19)	3.8 (0.08)	6.6 (0.15)	6.9 (0.16)	
Remnant-C	48.6 (11.3–14.2)	BMI	*BUD13*	rs7926828	C/T	35.8 (55)	1.3 (0.31)	1.3 (0.32)	1.5 (0.52)	1.0 (0.22)	1.1 (0.25)	1.3 (0.31)	37.0
		WC	*BUD13*	rs2075295	C/T	36.7 (56)	1.8 (0.47)	1.7 (0.38)	1.8 (0.46)	1.4 (0.27)	1.8 (0.44)	1.6 (0.34)	38.5
		WHR	*BUD13*	rs180378	A/G	38.5 (59)	2.4 (0.32)	2.4 (0.32)	2.7 (0.60)	2.7 (0.43)	2.7 (0.40)	2.8 (0.46)	47.8
			APOA5	rs651821	C/T		6.9 (0.29)	6.8 (0.29)	5.1 (0.19)	2.4 (0.08)	4.2 (0.15)	4.5 (0.16)	

KOR, Korean; EAS, East Asian; SAS, South Asian; EUR, European; AMR, American; AFR, African.
[a]Allele frequencies of each genetic marker in various ethnic groups were referred to the 1000 Genomes Project database (GRCh37/hg19).

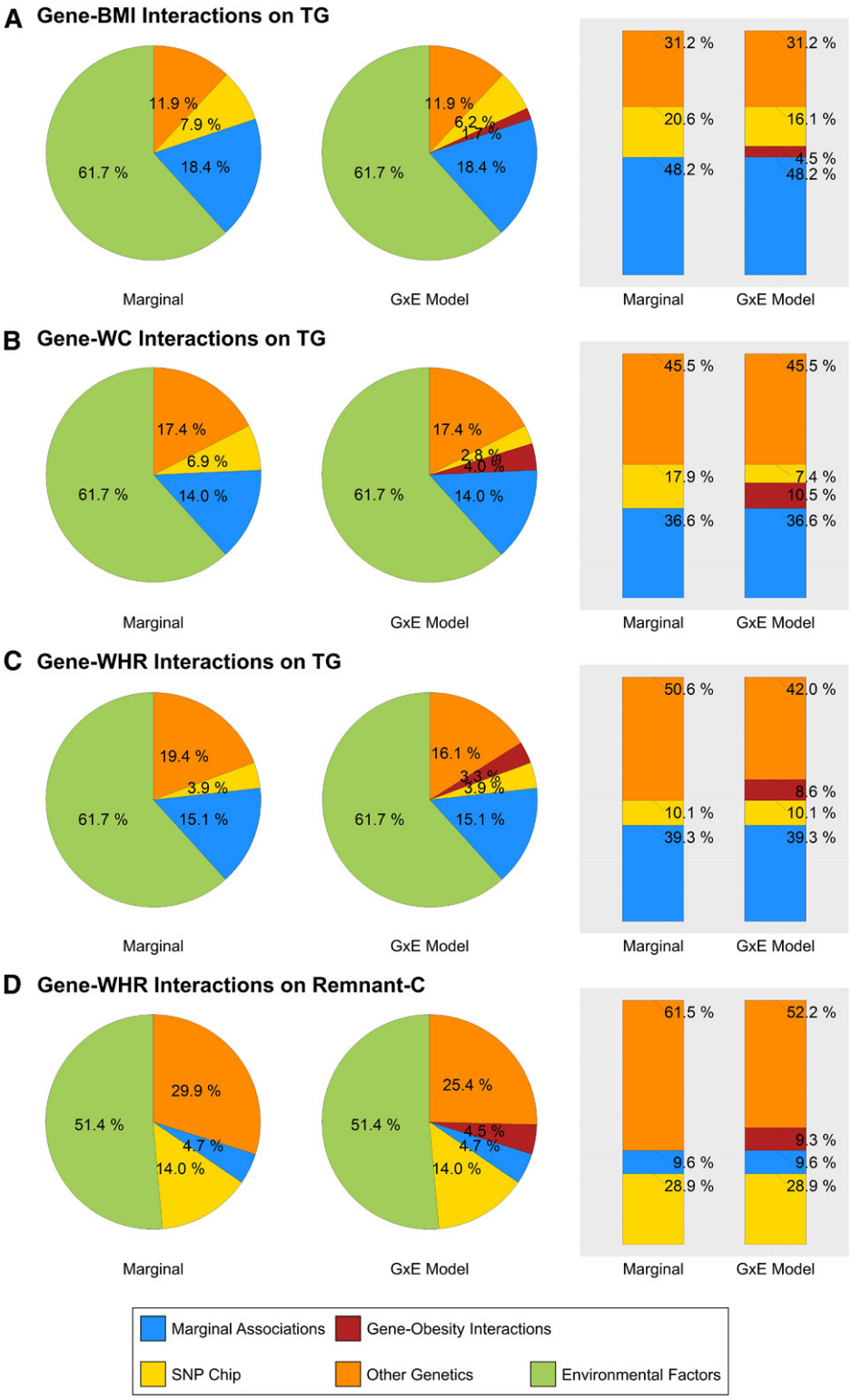

Fig. 3. Contributions of marginal associations and gene-obesity interactions to the risk of dyslipidemia. The pie plots describe the proportion of phenotypic variation attributable to the overall genetic variation (total heritability), genetic markers assayed by SNP arrays (SNP-based heritability), and the combined set of both GWAS-identified and novel G×E variants. The bar plots, on the other hand, show the proportion of genetic variation explained by marginal and gene-obesity interactive effects. Parts (A) to (C) describe the genetic contributions to abnormal TG due to the interplay between genes and obesity indices classified by BMI (A), WC (B), and WHR (C). D: The genetic contributions to abnormal Remnant-C due to the interplay between genes and obesity indices classified by WHR; further details are presented in Table 3.

factors. **Figure 3A**, B, and C (Table 3) present the risk of abnormal elevation of TG. Genetic factors accounted for approximately 38.3% of the total variance of the risk of abnormalities in TG after adjusting the risk by age, age^2, and sex. Genetic markers located on the genome-wide dense SNP microarray accounted for 18.4–26.4% of the overall variance for the risk of hypertriglyceridemia. Approximately 36.6% of the total heritability was due to 40 independent GWAS-identified SNPs only; the genetic contribution increased to 47.1% when we considered the interactions of *APOA5* or *BUD13* with WC. Similarly, the total genetic impact increased from 39.3% to 58.0% when we considered both marginal associations and newly found genetic interactions attributable to WHR. For Caucasians, the additional TG heritability due to the interactions of G×E variants with WC or WHR was 5.8% and 9.1%; the gain was 10.6% and 18.7% for Koreans, respectively.

The genetic contributions to the risk of abnormal elevation of Remnant-C are described in Fig. 3D (Table 3). Genetic factors explained approximately 48.6% of the total variance after adjusting for age, age^2, and sex. Genotyped loci on the SNP microarray accounted for 11.3–14.2% of the overall variance for Remnant-C. Approximately 38.5% of the total heritability was explained by 59 independent GWAS-identified SNPs only. When both marginal associations and interactions of *APOA5* or *BUD13* with WHR that modify the risk of abnormal Remnant-C were considered, the genetic contribution increased to 47.8%; the difference between marginal and total genetic impact was 9.3%. For Caucasians, on the other hand, the additional heritability for Remnant-C was just 5.1%.

DISCUSSION

One of the main purposes of human genome studies is to personalize treatment and health guidelines according to an individual's genetic constitution. GWISs are approaches intended for achieving this end, particularly when genetic loci interacting with modifiable risk factors are examined at a genome-wide level. Such studies permit the identification of higher- or lower-risk individuals depending on changes in known risk factors. In this study, we identified novel and known genes interacting with obesity indices to modify the risk of dyslipidemia. We also replicated our findings using independent genome cohorts and assessed how much phenotypic variance or heritability was additionally explained by considering the gene-obesity interactions.

Our study focused on increasing power to detect gene-obesity interactions by applying a variety of strategies for testing G×Es. We carried out emerging exhaustive scans and two-step methods in parallel because each analytical model provided differential power to detect G×Es, mainly according to marginal genetic and G×E effects. We tested interactions of SNPs at a genome-wide scale with several obesity traits, including Korean-specific parameters defined by additional ranges of BMI and WC. Besides, we adopted liberal cut-offs and stepwise penalties due to marginal *P*-values as well as the standard genome-wide significance level to find gene-obesity interactive loci influencing the risk of dyslipidemia. Type 1 errors are generally considered to be less problematic than possible underpowered findings (27); it is recommended to use multiple models for G×Es. Further verification can be done by replication and stratified analyses for candidate G×E regions.

Our findings reveal a genome-wide set of variants with a wide range of marginal effects on the risk of dyslipidemia. We identified novel G×E markers near *SCN1A* and *SLC12A8* with little or no direct association with lipid parameters as well as gene-obesity interactions related to lipid-associated loci reported in previous GWASs on lipids: *APOA5*, *BUD13*, *ZNF259*, and *HMGCR*. We identified *SCN1A* and *SLC12A8* through exhaustive CO analyses, while all other G×Es due to lipid-associated loci were detected using two-step methods due to the marginal effects of each locus in the first step. These trends are consistent with the results of an earlier simulation study of statistical power for G×E detection, which showed that exhaustive CO analysis is more powerful than other two-step methods when the marginal effects of genetic variants are small (43). To our knowledge, *SCN1A* and *SLC12A8* have not been previously associated with any lipid parameters.

We replicated the novel findings in four Korean genome cohorts; one strength of using cohorts formulated on identical protocols is the ability to examine gene-obesity interactions with high-quality health outcomes, genetic and environmental factors. In addition, conducting meta-analyses with the independent Korean cohorts permitted the estimation of more precise effects of susceptibility loci interacting with obesity traits. We also classified individuals in this study into three groups according to the number of risk alleles at G×E loci and compared the changes in lipid levels when BMI, WC, and WHR increased by one unit between the three groups. This comparison reconfirmed the identified gene-obesity interactions from different points of view, as the changes in lipids due to the elevation of obesity indices worsened as the number of risk alleles increased.

Although generating interesting findings, our approaches for testing gene-obesity interactive effects on lipid profiles are not free of limitations. Our study did not include G×Es due to loci marked by rare variants (MAF <0.01) and other essential obesity indices, such as body fat percentage and visceral fat level. We focused only on the G×E effects due to a set of common variants because our study populations did not include enough information for rare genetic variants. In addition, current analytical methods do not provide adequate power for detecting G×E effects on rare variants; the latest approaches using gene-set analyses and sum of powered score tests are also limited to testing G×Es at a variant-by-variant level (53, 54). Some rare variants in *NPC1L1*, however, are known to be associated with cholesterol absorption and LDL-C levels (55), and the interplay between rare variants and obesity traits might play a role in regulating lipid levels.

Furthermore, our new findings primarily concern G×Es based on indirect obesity indices, as BMI, WC, and WHR

are surrogate measures of overall and abdominal adiposity (56). Other vital indices measuring adipose tissue distribution, such as body fat percentage and visceral fat level, have been reported to be related to higher risks of CVD and metabolic syndrome in large-scale epidemiological studies (57–59) but were not addressed in this study. Considering the evidence that cardio-metabolic abnormalities are more closely linked with body shape and fat distribution than with conventional obesity indices (60, 61), interactions of genetic susceptibility loci of dyslipidemia with indicators that directly reflect adiposity warrant greater concern.

We used dyslipidemia as a dichotomous outcome variable instead of using quantitative lipid levels because the statistical power would be improved by the following reasons. *1*) Some analytical approaches for testing G×Es use dichotomous traits as a prerequisite, such as CO and EB, and these methods are optimized to detect genetic markers with weak marginal effects. *2*) Some methods, such as CC, DG2, EG2, H2, and EDG×E, could be extended to quantitative outcomes and these analyses have more power for G×E markers with strong marginal genetic effects (43). 3) In the analysis of statistical interactions, quantitative scales are sensitive to distribution; it is well-known that some nonnormal distribution would generate false-positive results of interactions. Despite concerns about the scale issues, our approaches could cover a broader range of gene-obesity interactive variants, having strong marginal effects as well as weak marginal effects, than the G×E analyses using TG levels quantitatively (supplemental Table S6). For GWISs, it is more important to use multiple analytical models as possible to generate a consistent and powerful result.

For categorical analyses, the cut-offs were decided by clinical guidelines for managing hyperlipidemia and obesity. For HDL-C, on the other hand, we used quantiles because the clinical cut-off points (HDL-C <1.03 mmol/l for males, 1.29 mmol/l for females) resulted in too many dyslipidemia cases (46.8%) in our study population; the arbitrary cut-offs could affect the results for interactions. To clarify the issue, we conducted GWISs using two different methods of categorization: by clinical guidelines and by the quantile distribution in our datasets. Compared with using quantiles to determine dyslipidemia and obesity traits, the categorical G×E analyses using clinical thresholds could find out a more extensive range of gene-obesity interactions (supplemental Tables S7, S8). The clinical cut-off points, commonly accepted and ascertained by several epidemiological studies, were more appropriate to detect interactions at a genome-wide scale than quantiles dividing the study population into equal-sized bins for phenotypes.

Our ability to extend these novel findings from Korean populations to other ethnic groups is limited by differences in the MAFs of genetic markers, distributions of obesity traits, and prevalences of each lipid abnormality. Our results were estimated and reconfirmed in four independent Korean cohorts sharing phenotyping and genotyping protocols, and the identified gene-obesity interactions in the risk of dyslipidemia might not be supported when racial differences in lipid profiles and the distribution of genetic and environmental factors are considered. *ZNF259* marked by rs2075291, for example, could be a useful therapeutic target for managing TG in the Korean population; the *ZNF259*-WHR interactive impact on the risk of hypertriglyceridemia was 3.4%. This genetic variant, however, is not a suitable target for the other ethnic groups; the minor allele of rs2075291 is infrequent for South Asians and too rare for Europeans, Americans, and Africans (Table 3). Conversely, some minor findings in this study could be useful for managing lipids and obesity traits in other ethnic groups.

Many human traits and complex diseases are known to be a consequence of both genetic and environmental factors, and thus G×E analyses may hold the key to further insights on disease biology and the development of better prediction models. Our exploration of G×Es at a genome-wide level in Koreans revealed novel genetic susceptibility loci of dyslipidemia interacting with modifiable obesity traits. Our results were replicated in independent genome cohorts and confirmed by comparing changes in lipid levels due to an increment of obesity for each genetic subgroup. Compared with lipid-associated loci with only marginal effects, the inclusion of gene-obesity interactive loci clearly explained more of the total and genetic variances of each lipid. Based on the different allele frequencies between Caucasians and Asians, besides, Asians have a higher risk of dyslipidemia, particularly for TG, even with a small increase in obesity indices. These newly identified gene-obesity interactions can be used to classify individuals into higher- or lower-risk groups and to develop personalized guidelines for managing lipid and obesity traits according to the genetic constitution.

This study was provided with bioresources from National Biobank of Korea, Centers for Disease Control and Prevention, Ministry for Health and Welfare, Republic of Korea. Data in this study were from the Korean Genome and Epidemiology Study (4851-302), National Research Institute of Health, Centers for Disease Control and Prevention, Ministry for Health and Welfare, Republic of Korea.

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