Association and interaction of APOA5, BUD13, CETP, LIPA and health-related behavior with metabolic syndrome in a Taiwanese population

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Increased risk of developing metabolic syndrome (MetS) has been associated with the APOA5, APOC1, BRAP, BUD13, CETP, LIPA, LPL, PLCG1, and ZPR1 genes. In this replication study, we reassessed whether these genes are associated with MetS and its individual components independently and/or through complex interactions in a Taiwanese population. We also analyzed the interactions between environmental factors and these genes in influencing MetS and its individual components. A total of 3,000 Taiwanese subjects were assessed in this study. Metabolic traits such as waist circumference, triglyceride, high-density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure, and fasting glucose were measured. Our data showed a nominal association of MetS with the APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444 single nucleotide polymorphisms (SNPs). Moreover, APOA5 rs662799, BUD13 rs11216129, and BUD13 rs623908 were significantly associated with high triglyceride, low HDL, triglyceride, and HDL levels. Additionally, we found the interactions of APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444, alcohol consumption, smoking status, or physical activity on MetS and its individual components. Our study indicates that the APOA5, BUD13, CETP, and LIPA genes may contribute to the risk of MetS independently as well as through gene-gene and gene-environment interactions.

The metabolic syndrome (MetS), a chronic and complex disease, is characterized by having large waist circumference plus two or more of the following factors: raised triglyceride levels, low high-density lipoprotein (HDL) cholesterol levels, raised blood pressure, and raised glucose levels1. Due to escalating prevalence rates and its risk for the development of several chronic complications such as cardiovascular diseases, MetS has become a major public health challenge in Taiwan and at the global scale2. MetS is primarily caused by a combination of genetics and environmental factors such as health-related behaviors2,3. While more and more MetS risk loci have been identified, it has long been noted that genetic variants conferring susceptibility may vary across ethnicities4. Among the genes involved in the development of MetS and/or cardiovascular diseases are the apolipoprotein A5 (APOA5), apolipoprotein C1 (APOC1), BRCA1 associated protein (BRAP), BUD13 homolog (BUD13), cholesteryl ester transfer protein (CETP), lipase A lysosomal acid type (LIPA), lipoprotein lipase (LPL), phospholipase C gamma 1 (PLCG1), and ZPR1 zinc finger (ZPR1) gene.

The APOA5 gene is located on chromosome 11q23 and encodes an apolipoprotein protein that has been implicated in regulating the plasma triglyceride levels, a major risk factor for coronary artery disease (CAD).
common single nucleotide polymorphism (SNP), rs662799 (−1131T > C), located in the promoter region of the APOA5 gene is one of the most extensively studied variants. The relationship between the MetS and the APOA5 rs662799 SNP has been ambiguous. The APOA5 rs662799 SNP has been reported to increase the risk of acquiring MetS in Caucasians and in Asians residing in Japan, Taiwan, Hong Kong, China, and Korea. In contrast, this association has not been replicated in Caucasian, Arabic, and Hispanic populations. Several meta-analysis studies have also suggested that the APOA5 rs662799 SNP is associated with an increased risk of developing MetS in Asians, but not in European populations.

Furthermore, the LIPA gene is located on chromosome 10q23 and encodes the lysosomal acid lipase enzyme, which functions in the lysosome of cells to hydrolyze cholesteryl esters and triglycerides and then to generate free cholesterol and free fatty acids. Several genome-wide association studies (GWAS) indicated that the rs1412444 SNP in the intron region of the LIPA gene was associated with CAD in Caucasian and Asian populations. Evidence has also been reported for an association of the rs1412444 SNP with CAD in Caucasian and Asian populations. In light of the aforementioned considerations, we thus assessed both the primary effects of single loci and multilocus interactions for an association of SNPs within these genes with the prevalence of MetS and its individual components in Taiwanese individuals. We also determined whether significant gene-environment interactions exist between SNPs within these genes and health-related behaviors.

### Results

Table 1 describes the demographic and clinical characteristics of the study population, including 533 MetS subjects and 2,467 non-MetS subjects. The MetS prevalence in our cohort was 17.8%. As shown in Table 1, the distribution of gender was well matched, and the distribution of age was not matched. Moreover, there was a significant difference in waist circumference, triglyceride, HDL, blood pressure, and fasting glucose between the MetS and non-MetS subjects (Table 1; all \( P < 0.0001 \) respectively). Furthermore, there was a significant difference in current alcohol consumption (\( P = 0.029 \)) and smoking status (\( P = 0.001 \)) between the MetS and non-MetS subjects. However, there were no significant differences found between participants with and without the MetS in level of physical activity.

Among the 82 SNPs investigated in this study (Supplementary Table S1), there were 19 SNPs showing an evidence of association (\( P < 0.05 \)) with MetS. However, none of the SNPs were significantly associated with MetS after Bonferroni correction (\( P < 0.05/82 = 0.0006 \)). We also calculated pairwise linkage disequilibrium (LD) between 82 SNPs, and Supplementary Table S2 shows a list of SNP pairs with strong LD (\( r^2 > 0.8 \)). As shown in Table 2, we then selected the five key SNPs (including APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444) with nominal evidence of association (\( P < 0.01 \)), which were further examined in the subsequent analyses. In addition, the genotype frequency distributions for the APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444 SNPs were in accordance with the Hardy–Weinberg equilibrium among the subjects (\( P = 0.595, 0.762, 0.692, 0.278, \) and 0.245, respectively).

### Table 1. Demographic and clinical characteristics of study subjects.

| Characteristic                        | Overall | MetS | No MetS | \( P \) value |
|--------------------------------------|---------|------|---------|--------------|
| No. of subjects, n                   | 3000    | 533  | 2467    |              |
| Mean age ± SD, years                 | 49.2 ± 11.0 | 53.3 ± 10.1 | 48.3 ± 10.9 | \(< 0.0001 \) |
| Male, n/Female, n                    | 1394/1606 | 257/276 | 1137/1330 | 0.371 |
| High waist circumference\( ^a \), n  | 1395    | 533  | 862     | \(< 0.0001 \) |
| High triglyceride\( ^b \), n         | 621     | 338  | 283     | \(< 0.0001 \) |
| Low HDL\( ^c \), n                   | 713     | 339  | 374     | \(< 0.0001 \) |
| High blood pressure\( ^d \), n       | 694     | 290  | 404     | \(< 0.0001 \) |
| High fasting glucose\( ^e \), n      | 732     | 345  | 387     | \(< 0.0001 \) |
| Current alcohol drinker, n           | 225     | 52   | 173     | 0.029 |
| Current smoker, n                    | 320     | 78   | 242     | 0.001 |
| Physical activity, n                 | 1759    | 309  | 1450    | 0.733 |

\( ^a \) Waist circumference \( \geq 90 \) cm in male subjects, waist circumference \( \geq 80 \) cm in female subjects. 
\( ^b \) Triglyceride \( \geq 150 \) mg/dl. 
\( ^c \) HDL \( < 40 \) mg/dl in male subjects, HDL \( < 50 \) mg/dl in female subjects. 
\( ^d \) Systolic blood pressure \( \geq 130 \) mmHg or diastolic blood pressure \( \geq 85 \) mmHg. 
\( ^e \) Fasting glucose \( \geq 100 \) mg/dl.
Moreover, the OR analysis showed risk genotypes of variants of APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444. Chr = chromosome, CI = confidence interval, MetS = metabolic syndrome, OR = odds ratio. Analysis was obtained after adjustment for covariates including age, gender, smoking, alcohol consumption, and physical activity. P values of <0.01 are shown in bold.

Table 2. Odds ratio analysis with odds ratios after adjustment for covariates between the MetS and five SNPs including APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444. Chr = chromosome, CI = confidence interval, MetS = metabolic syndrome, OR = odds ratio. Analysis was obtained after adjustment for covariates including age, gender, smoking, alcohol consumption, and physical activity. P values of <0.01 are shown in bold.

| Chr | Gene | SNP | Case Allele and Alleles | Control Allele and Alleles | Additive | Recessive | Dominant |
|-----|------|-----|-------------------------|----------------------------|----------|-----------|----------|
|     |      |     | Genotype                | Genotype                   | OR       | 95% CI     | P        | OR       | 95% CI     | P        | OR       | 95% CI     | P        |
| 11  | APOA5| rs662799| 325/741                  | 1303/3621                  | 1.26     | 1.06-1.49  | 0.0086   | 1.47     | 1.06-2.04  | 0.0229   | 1.25     | 1.03-1.52  | 0.0218   |
| 11  | BUD13| rs11216129| 240/822                  | 1319/3611                  | 0.81     | 0.66-1.00  | 0.0532   | 0.74     | 0.49-1.12  | 0.1492   | 0.74     | 0.61-0.90  | 0.0027   |
| 11  | BUD13| rs623908 | 295/767                  | 1546/3372                  | 0.90     | 0.75-1.06  | 0.2091   | 0.92     | 0.66-1.29  | 0.6357   | 0.75     | 0.61-0.90  | 0.0027   |
| 11  | CETP | rs820299| 472/590                  | 2020/2894                  | 1.17     | 1.02-1.34  | 0.0211   | 1.47     | 1.16-1.86  | 0.0015   | 1.01     | 0.82-1.24  | 0.9387   |
| 16  | LIPA | rs1412444| 381/683                  | 1577/3349                  | 1.22     | 1.05-1.42  | 0.0097   | 1.41     | 1.06-1.88  | 0.0171   | 1.19     | 0.98-1.44  | 0.0826   |

Moreover, the OR analysis showed risk genotypes of variants of APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444 after adjusting for covariates, indicating an increased MetS risk among the subjects (Table 2). As demonstrated in Table 2 for the CETP rs820299 SNP, there was an indication of an increased MetS risk among the MetS and non-MetS subjects after adjustment of covariates such as age, gender, smoking, alcohol consumption, and physical activity for genetic models, including the recessive model (OR = 1.47; 95% CI = 1.16–1.86; P = 0.0015) and additive model (OR = 1.17; 95% CI = 1.02–1.34; P = 0.0211). Similarly, there was an indication of an increased risk of MetS among the subjects after adjustment of covariates for genetic models in the APOA5 rs662799 (P [additive model] = 0.0086; P [recessive model] = 0.0229; P [dominant model] = 0.0218), BUD13 rs11216129 (P [dominant model] = 0.0027), and LIPA rs1412444 (P [additive model] = 0.0097; P [recessive model] = 0.0171) SNPs (Table 2). Additionally, there were still residual associations between MetS and APOA5 rs662799 (P = 0.0114) as well as between MetS and CETP rs820299 (P = 0.0399) after further accounting for triglyceride and HDL, suggesting an independent association of MetS with APOA5 rs662799 and CETP rs820299.

Next, Table 3 shows the analysis of the APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444 SNPs with the individual components of MetS (as quantitative measures) including waist circumference, triglyceride, HDL, systolic blood pressure, diastolic blood pressure, and fasting glucose. When we treated the phenotypes as quantitative measures rather than dichotomous ones, there was evidence of an association between these five SNPs and quantitative traits such as triglyceride, HDL, or fasting glucose (Table 3). As shown in Table 3 for the APOA5 rs662799, BUD13 rs11216129, and BUD13 rs623908 SNPs, there was a significant difference in triglyceride or HDL (after Bonferroni correction; P < 0.0006) among the subjects after adjustment of covariates for genetic models.

In addition, the GMDR analysis was used to assess the impacts of combinations between the five SNPs in MetS and its individual components including age, gender, smoking, alcohol consumption, and physical activity as covariates. Table 4 summarizes the results obtained from GMDR analysis for two-way up to five-way models with covariate adjustment. As shown in Table 4 for MetS, there was a significant two-way model involving CETP rs820299 and LIPA rs1412444 (P = 0.005), indicating a potential gene-gene interaction between CETP and LIPA in influencing MetS. The effect of CETP rs820299 and LIPA rs1412444 interaction remained significant after Bonferroni correction (P < 0.05/5 = 0.01). The CETP rs820299 and LIPA rs1412444 interaction was shown to be statistically significant (OR = 1.26; 95% CI = 1.02–1.54; P = 0.0282) in the subsequent logistic regression analysis, adjusted to age, gender, smoking, alcohol consumption, and physical activity. Further, our analysis suggested that the individuals carrying the risk allele for CETP rs820299 were more likely to also carry the risk alleles for LIPA rs1412444 (P = 0.05). Additionally, there were a three-way model involving BUD13 rs623908, CETP rs820299, and LIPA rs1412444 (P = 0.001) as well as a four-way model involving APOA5 rs662799, BUD13 rs623908, CETP rs820299, and LIPA rs1412444 (P = 0.012), indicating a potential gene-gene interaction among APOA5, BUD13, CETP, and LIPA in influencing MetS. The effect of the three-way model remained significant after Bonferroni correction (P < 0.01); however, the effect of the four-way model did not. Similarly, there were significant two-way up to four-way gene-gene interaction models (P < 0.001) in influencing individual components such as high triglyceride or low HDL, and the effect remained significant after Bonferroni correction (P < 0.01).

Moreover, Table 5 shows the GMDR analysis of gene-environment interaction models in MetS and its individual components using age and gender as covariates. As shown in Table 5 for MetS, there were a significant two-way model involving BUD13 rs623908 and smoking (P < 0.001), a three-way model involving BUD13 rs623908, CETP rs820299, and smoking (P < 0.001), a four-way model involving BUD13 rs623908, CETP rs820299, LIPA rs1412444, and smoking (P < 0.001), as well as a five-way model involving BUD13 rs623908, CETP rs820299, LIPA rs1412444, smoking, and physical activity (P < 0.001), indicating a potential gene-environment interaction among BUD13, CETP, LIPA, smoking, and physical activity in influencing MetS. The effect of these models remained significant after Bonferroni correction (P < 0.05/8 = 0.006). Similarly, there were significant two-way up
to five-way gene-environment interaction models in influencing individual components such as high triglyceride (P < 0.001) or low HDL (P < 0.001), and the effect remained significant after Bonferroni correction (P < 0.006).

Furthermore, we utilized multivariable logistic regression analysis with adjustment for age and gender to assess the two-way gene-environment interaction models selected by the GMDR method (Supplementary Table S3). Our analysis revealed that smokers with the G allele of BUD13 rs623908 had a 1.61-fold increased risk for MetS, compared to non-smokers with the AA genotype of BUD13 rs623908 (Supplementary Table S3). Similarly, smokers with the C allele of APOA5 rs662799 had a 3.42-fold increased risk for high triglyceride, compared to non-smokers with the TT genotype of APOA5 rs662799 (Supplementary Table S3). Additionally, smokers with the G allele of APOA5 rs662799 had a 2.62-fold increased risk for low HDL, compared to non-smokers with the TT genotype of APOA5 rs662799 (Supplementary Table S3). Moreover, individuals with the G allele of CETP rs820299 and low levels of physical activity had a 1.44-fold increased risk for high waist circumference, compared to those with the A allele of CETP rs820299 and high levels of physical activity (Supplementary Table S3).

Finally, statistical power analysis revealed that the present study had a 99.9% power to detect associations of APOA5 rs662799 (effect size = 1.26; minor allele frequency [MAF] = 27.2%), BUD13 rs11216129 (effect size = 0.74; MAF = 26.8%), BUD13 rs623908 (effect size = 0.75; MAF = 30.8%), CETP rs820299 (effect size = 1.47; MAF = 41.7%), or LIPA rs1412444 (effect size = 1.22; MAF = 32.7%) with MetS among the MetS and non-MetS subjects after applying Bonferroni correction (P < 0.0006).

Table 3. Clinical characteristics of study subjects by genotypes in the APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, and LIPA rs1412444 SNPs. HDL = high-density lipoprotein cholesterol. Analysis was obtained after adjustment for covariates including age, gender, smoking, alcohol consumption, and physical activity. P values of < 0.0006 (Bonferroni correction: 0.05/82) are shown in bold.
Table 4. Gene-gene interaction models identified by the GMDR method with adjustment for age, gender, smoking, alcohol consumption, and physical activity. GMDR = generalized multifactor dimensionality reduction, HDL = high-density lipoprotein cholesterol, MetS = metabolic syndrome. P value was based on 1,000 permutations. Analysis was obtained after adjustment for covariates including age, gender, smoking, alcohol consumption, and physical activity. P values of < 0.01 (Bonferroni correction: 0.05/5) are shown in bold.

| Phenotype                                                                 | Best interaction model                      | Testing accuracy (%) | P value |
|---------------------------------------------------------------------------|----------------------------------------------|----------------------|---------|
| (a) Two-way interaction models                                            |                                              |                      |         |
| MetS                                                                      | CETP rs820299, LIPA rs1412444                | 54.19                | 0.005   |
| High waist circumference²                                                 | BUD13 rs623908, CETP rs820299               | 51.96                | 0.056   |
| High triglyceride³                                                        | APOA5 rs662799, LIPA rs1412444              | 56.69                | < 0.001 |
| Low HDL³                                                                  | APOA5 rs662799, CETP rs820299               | 55.90                | < 0.001 |
| High blood pressure³                                                      | APOA5 rs662799, CETP rs820299               | 51.45                | 0.205   |
| High fasting glucose³                                                     | BUD13 rs11216129, LIPA rs1412444            | 53.31                | 0.007   |
| (b) Three-way interaction models                                          |                                              |                      |         |
| MetS                                                                      | BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 55.59                | 0.001   |
| High waist circumference²                                                 | BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 49.55                | 0.618   |
| High triglyceride³                                                        | APOA5 rs662799, BUD13 rs623908, LIPA rs1412444 | 59.10                | < 0.001 |
| Low HDL³                                                                  | APOA5 rs662799, CETP rs820299, LIPA rs1412444 | 54.84                | < 0.001 |
| High blood pressure³                                                      | APOA5 rs662799, CETP rs820299, LIPA rs1412444 | 51.74                | 0.167   |
| High fasting glucose³                                                     | BUD13 rs11216129, CETP rs820299, LIPA rs1412444 | 54.34                | 0.004   |
| (c) Four-way interaction models                                           |                                              |                      |         |
| MetS                                                                      | APOA5 rs662799, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 53.99                | 0.012   |
| High waist circumference²                                                 | APOA5 rs662799, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 50.49                | 0.374   |
| High triglyceride³                                                        | APOA5 rs662799, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 58.30                | < 0.001 |
| Low HDL³                                                                  | APOA5 rs662799, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 56.52                | < 0.001 |
| High blood pressure³                                                      | APOA5 rs662799, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 51.93                | 0.135   |
| High fasting glucose³                                                     | APOA5 rs662799, BUD13 rs11216129, CETP rs820299, LIPA rs1412444 | 51.50                | 0.195   |
| (d) Five-way interaction models                                           |                                              |                      |         |
| MetS                                                                      | APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 52.47                | 0.093   |
| High waist circumference²                                                 | APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 50.64                | 0.334   |
| High triglyceride³                                                        | APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 58.03                | < 0.001 |
| Low HDL³                                                                  | APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 55.53                | 0.001   |
| High blood pressure³                                                      | APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 50.94                | 0.294   |
| High fasting glucose³                                                     | APOA5 rs662799, BUD13 rs11216129, BUD13 rs623908, CETP rs820299, LIPA rs1412444 | 51.75                | 0.151   |

Discussion

Our replication study is the first study to date to examine whether the main effects of the APOA5, APOC1, BRAP, BUD13, CETP, LIPA, LPL, PLGC1, and ZPR1 genes are significantly associated with the risk of MetS and its individual components independently and/or through gene-gene interactions among Taiwanese individuals. We also investigated the relationship between these genes and health-related behaviors to examine whether these genes confer a risk of MetS according to its effect on gene-environment interactions. In this study, we found that APOA5 rs662799, BUD13 rs11216129, and BUD13 rs623908 were significantly associated with the individual components of MetS such as high triglyceride and low HDL (as well as with triglyceride and HDL levels). Our data also indicated that gene-gene interactions of APOA5, BUD13, CETP, and LIPA may contribute to the etiology of MetS. Finally, there was a significant gene-environment interaction between these four genes and health-related behaviors, such as alcohol consumption, smoking status, and physical activity.

Here, we report for the first time that the BUD13 rs11216129, BUD13 rs623908, and CETP rs820299 SNPs may play an important role in the modulation of MetS in a Taiwanese population. In addition, we observed that there were a significant association of BUD13 rs11216129 and BUD13 rs623908 with high triglyceride and low HDL as well as a significant association of both SNPs with triglyceride and HDL levels. Our data also suggested that CETP rs820299 was involved in high waist circumference, high triglyceride, and HDL levels. Similarly, previous studies reported that BUD13 rs10790162 and CETP rs173539 may contribute to the susceptibility for MetS in European subjects and Mexican women. However, we did not detect an association between BUD13 rs10790162 and MetS in the present study. Further, we did not test CETP rs173539 and CETP rs708272 due to lack of these two SNPs in the custom chip. Previously, the CETP gene has been reported in association with HDL levels in Caucasian and Asian Indian subjects as well as with higher triglyceride levels in Caucasian...
Additionally, BUD13 variants have been associated with triglyceride levels\(^{27,28}\), total cholesterol levels\(^{27}\), and hypercholesterolaemia\(^{27}\) in Chinese subjects.

Moreover, another intriguing finding was a positive association of LIPA rs1412444 with MetS, low HDL, high fasting glucose, HDL levels, and fasting glucose levels in a Taiwanese population. In line with our results, a previous study by Vargas-Alarcón \textit{et al.} demonstrated that the LIPA rs1412444 polymorphism was likely to influence MetS and hypertriglyceridaemia in a Mexican population\(^{19}\). It has also been suggested that the LIPA rs1412444 polymorphism was involved in CAD\(^{17-18}\), premature CAD\(^{19}\), and myocardial infarction\(^{20}\). Furthermore, Wild \textit{et al.} reported a strong association of the CAD risk allele (T) of LIPA rs1412444 with higher LIPA expression as well as an association of elevated LIPA expression with lower HDL levels and subclinical atherosclerotic disease\(^{30}\). Additionally, mutations in the LIPA gene are the cause of Wolman's Disease, Cholesteryl ester storage disease, hyperlipidaemia, premature cardiovascular disease, and increased risk for atherosclerosis\(^{31}\). Finally, it should be noted that the T allele frequency of LIPA rs1412444 varies considerably between different ethnic populations, ranging from 34% in European subjects\(^{17}\), 51% in South Asian subjects\(^{17}\), 49.1% in Mexican subjects\(^{19}\), 32% in Chinese subjects\(^{20}\), 32.5% in German subjects\(^{30}\), and 37.2% in the present Taiwanese population assessed in our study.

The APOA5 rs662799 polymorphism has been widely implicated to affect the MetS risk\(^{26}\), although genetic evidence of its effect on MetS has been inconsistent. In this study, we observed that there was an association of APOA5 rs662799 with MetS after covariate adjustment in OR analysis. Our results are in agreement with those of several other studies\(^{26-10}\). We also observed that there was a significant association of APOA5 rs662799 with high triglyceride and low HDL as well as with triglyceride and HDL levels. Xu \textit{et al.} performed a meta-analysis on data from 91 studies including 51,886 subjects in Asian, European, and other ethnic populations and detected a significant association of the G allele of APOA5 rs662799 with elevated triglyceride levels and decreased HDL levels\(^{9}\). In the subgroup analysis stratified by the ethnicity, this association was also detected in both Asian and European populations\(^{9}\). It is worth mentioning that the C allele frequency of APOA5 rs662799 varies considerably between different ethnic populations, ranging from 8.5% in Hungarian subjects\(^{3}\), 35.3% in Japanese subjects\(^{8}\), 28.6% in Hong Kong subjects\(^{8}\), 21.6% in Chinese subjects\(^{8}\), 7% in Germany subjects\(^{11}\), and 27.2% in the present Taiwanese population assessed in our study.

By using the GMDR approach, we further inferred the epistatic effects between APOA5, BUD13, CETP, and LIPA in influencing MetS and its individual components. To our knowledge, no other study has been conducted to evaluate gene–gene interactions between these genes. Although ZPR1 was not a key gene in the present study (that is, no association with MetS), Aung \textit{et al.} identified a potential gene–gene interaction between the BUD13 and ZPR1 genes on the risk of hypercholesterolaemia and hypertriglyceridaemia in Chinese subjects using GMDR analyses\(^{29}\). Another promising finding in the present study was an interaction between these genes and environmental factors in MetS and its individual components. In accordance with our analysis, Wu \textit{et al.} reported that APOA5 rs662799 had a positive interaction with environmental factors, such as tobacco use and alcohol consumption, on MetS with participations in China\(^{29}\). Likewise, a previous study by Hiramatsu \textit{et al.} found the synergistic effects of APOA5 rs662799 and the rs6929846 SNP of the butyrophilin subfamily 2 member A1 (BTN2A1) gene on the development of MetS in Japanese individuals\(^{51}\). Son \textit{et al.} also suggested an interaction between APOA5 rs662799 and alcohol drinking as well as an interaction between APOA5 rs662799 and physical activity in affecting triglyceride levels in Korean men, but no interaction between APOA5 rs662799 and smoking status\(^{24}\).

While our results showed that the individuals carrying the G allele of BUD13 rs623908 had a protective effect (OR = 0.75) for MetS (as compared to those carrying the AA genotype of BUD13 rs623908), the interaction effect between BUD13 rs623908 and smoking on MetS yielded an OR value of 1.61 when we compared smokers carrying the G allele of BUD13 rs623908 with non-smokers carrying the AA genotype of BUD13 rs623908. Our analysis also implicated the interaction effect between APOA5 rs662799 and smoking on high triglyceride (OR = 3.42) or low HDL (OR = 2.62) as well as the interaction effect between CETP rs820299 and physical activity on high waist circumference (OR = 1.44). According to our and previous results\(^{32}\), smoking seems to cause increased health risks, especially for the individuals with the CT and CC genotypes of APOA5 rs662799.

Besides the statistical significance, the potential biological mechanism under the interaction models was our concern. The functional relevance of the interactive effects of APOA5, BUD13, CETP, and LIPA on MetS remains to be elucidated. If there is a deficiency of lysosomal acid lipase encoded by the LIPA gene, lipids such as triglycerides and cholesteryl esters accumulate in the cell, resulting in pre-mature atherosclerosis\(^{32}\). It has also been suggested that LIPA rs1412444 is associated with increased LIPA expression, which is expected to enhance intracellular release of fatty acids and cholesterol via the lysosomal route\(^{33,34}\). Furthermore, the risk allele of LIPA rs1412444 may increase the generation of free cholesterol in the arterial intima and, likely as a consequence, may promote an inflammatory process and atherosclerotic plaque formation\(^{35}\). Likewise, it is speculated that APOA5 rs662799 may be involved in the regulation of gene transcription due to its location in the promoter region and thereby considerably impact serum apolipoprotein A5 levels\(^{9}\). Additionally, an animal study showed that overexpression of human APOA5 in mice is correlated with decreased plasma triglyceride levels\(^{36}\). Moreover, APOA5, BUD13, and CETP are known to play a key role in lipid metabolism\(^{21}\). Some speculate that the association of the BUD13 gene with serum lipid levels may be relevant to the nearby APOA5 gene because BUD13 is located in the downstream of APOA5\(^{39}\). Finally, CETP contributes to lower HDL since it enables the transfer of cholesteryl esters in HDL toward triglyceride-rich lipoproteins\(^{31}\).

This study has both strengths and limitations. The main weakness of this study is that our observations require much further validation to test whether the findings are replicated in various ethnic groups\(^{37,38}\). Second, to our knowledge, there are no viable molecular biological models that support the gene–gene and gene-environment interactions found in this study. In future work, prospective clinical trials with other ethnic populations are necessary to facilitate a thorough evaluation of the association and interactions of the investigated SNPs with MetS and its individual components\(^{39,40}\). On the other hand, an important strength of our study was the use
Table 5. Gene-environment interaction models identified by the GMDR method with adjustment for age and gender. GMDR = generalized multifactor dimensionality reduction, HDL = high-density lipoprotein cholesterol, MetS = metabolic syndrome. P value was based on 1,000 permutations. Analysis was obtained after adjustment for covariates including age and gender. P values of <0.006 (Bonferroni correction: 0.05/8) are shown in bold. \(^a\)Triglyceride \(\geq 150\) mg/dl. \(^b\)HDL < 40 mg/dl in male subjects, HDL < 50 mg/dl in female subjects. \(^c\)Systolic blood pressure \(\geq 130\) mmHg or diastolic blood pressure \(\geq 85\) mmHg. \(^d\)Fasting glucose \(\geq 100\) mg/dl.

### Materials and Methods

#### Study population.
This study incorporated subjects from the Taiwan Biobank\(^{41}\). The study cohort consisted of 3,000 participants. Recruitment and sample collection procedures were approved by the Internal Review Board of the Taiwan Biobank before conducting the study. Each subject signed the approved informed consent form. All experiments were performed in accordance with relevant guidelines and regulations.

Current alcohol drinker was defined as currently drinking 150 ml of alcohol per week for more than six months. Current smoker was defined as currently smoking for more than six months. Physical activity was defined by the amount of exercise activity for more than three times and more than 30 minutes each time in each week.

#### Metabolic Syndrome.
The MetS was diagnosed using the International Diabetes Federation (IDF) definition, which requires that the participant represented by central obesity (defined as waist circumference \(\geq 90\) cm in male subjects and \(\geq 80\) cm in female subjects) plus the presence of two or more of the following four components:

- High waist circumference\(^a\)
- High blood pressure\(^a\)
- Low HDL\(^c\)
- High triglyceride\(^b\)

Studies have shown that gene-gene and gene-environment interactions of the \(APOA5\), \(BUD13\), \(CETP\), and \(LIPA\) genes may affect the prevalence of MetS independently and/or through complex gene-gene and gene-environment interactions. Furthermore, the \(APOA5\) and \(BUD13\) genes are a determinant of MetS component factors, such as high triglyceride and low HDL. Independent replication studies with larger sample sizes will likely provide further insights into the role of the \(APOA5\), \(BUD13\), \(CETP\), and \(LIPA\) genes found in this study.
(1) triglycerides $\geq 150$ mg/dl; (2) HDL cholesterol <40 mg/dl in male subjects and <50 mg/dl in female subjects; (3) systolic blood pressure $\geq 130$ mmHg or diastolic blood pressure $\geq 85$ mmHg; and (4) fasting plasma glucose $\geq 100$ mg/dl. Blood pressure was based on the average of two measurements.

**Genotyping.** DNA was isolated from blood samples using a QIAamp DNA blood kit following the manufacturer’s instructions (Qiagen, Valencia, CA, USA). The quality of the isolated genomic DNA was evaluated using agarose gel electrophoresis, and the quantity was determined by spectrophotometry. SNP genotyping was carried out using the custom Taiwan BioBank chips and run on the Axiom Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA). The SNP panel consisted of variants from the following genes: APOA5, APOC1, BRAP, BUD13, CETP, LIPA, LPL, PLCG1, and ZPR1.

**Statistical analysis.** Categorical data were evaluated using the chi-square test. We conducted the Student’s t test to compare the difference in the means from two continuous variables. To estimate the association of the investigated SNP with MetS, we conducted a logistic regression analysis to evaluate the odds ratios (ORs) and their 95% confidence intervals (CIs), adjusting for covariates, including age, gender, smoking, alcohol consumption, and physical activity. Furthermore, we estimated the association of the investigated SNP with individual components of MetS (as quantitative measures) by using linear regression analysis, adjusting for age, gender, smoking, alcohol consumption, and physical activity. The genotype frequencies were assessed for Hardy-Weinberg equilibrium using a $\chi^2$ goodness-of-fit test with 1 degree of freedom (i.e. the number of genotypes minus the number of alleles). Multiple testing was adjusted by the Bonferroni correction. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as the mean $\pm$ standard deviation.

To investigate gene-gene and gene-environment interactions, we employed the generalized multifactor dimensionality reduction (GMDR) method. We tested two-way up to five-way interactions using 10-fold cross-validation. The GMDR software provides some output parameters, including the testing accuracy and empirical P values, to assess each selected interaction. Moreover, we provided age, gender, smoking, alcohol consumption, and physical activity as covariates for gene-gene interaction models in our interaction analyses. We also prepared gender and age as covariates for gene-environment interaction models. Permutation testing obtains empirical P values of prediction accuracy as a benchmark based on 1,000 shuffles. In order to correct for multiple testing, we applied a conservative Bonferroni correction factor for the number of SNPs and environmental factors employed in the GMDR analysis.

Based on the effect sizes in this study, the power to detect significant associations was evaluated by QUANTO software (http://biosofts.usc.edu/Quanto.html).

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