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*ADIPOQ, PPAR, LEP, TCF7L2, FTO*及*PTPN1*基因多型性與代謝症候群之關聯與基因-基因交互作用：社區為基礎之病例對照研究

Individual Association and Gene-Gene Interaction among Genetic Variants of *ADIPOQ, PPARG, LEP, TCF7L2, FTO* and *PTPN1* with Metabolic Syndrome in Taiwanese Adults: A Community-based Case-Control Study

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本論文係 陳瑩萍 君（R97842020）在國立臺灣大學流行病學研究所完成之碩士學位論文，於民國 99 年 6 月 21 日承下列考試委員審查通過及口試及格，特此證明

口試委員：

（簽名）

（指導教授）

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中文摘要

背景：偵測複雜性疾病的基因-基因交互作用為目前基因研究較受注目的部分。一般多使用以基因多型性為基礎之方法--generalized multifactor dimensionality reduction (GMDR) 偵測，其對高維度之基因-基因交互作用有較好的檢定力。但在同一基因上之基因多型性的相關功能性可能會導致使用 GMDR 時有偽陰性的結果產生。而使用以基因為基礎之方法可以捕捉到在同一個基因內的遺傳變異之交互作用。

目標：1）從六個與代謝症候群因子相關之基因（ADIPOQ, LEP, PPARG, FTO, TCF7L2, 及 PTPN1)的基因多型性中，尋找單獨基因多型性與代謝症候群之相關；2）並利用 GMDR 及以基因為基礎之方式尋找是否有基因-基因交互作用存在。

方法：樣本來自於桃園縣年度成人健康檢查收集而來的 611 名 40 歲以上之代謝症候群病例及 1117 名對照。我們選出 *ADIPOQ, PPARG, LEP, TCF7L2, FTO* 及 *PTPN1* 六個與代謝症候群危險因子相關之基因中的 19 個 SNPs, 尋找它們與代謝症候群之相關性，並使用 generalized multifactor dimensionality reduction (GMDR) 方法及透過(genotype-trait distortion score, GTD score)的計算並以基因為基礎的方式探討是否有基因-基因交互作用的存在。

結果：在獨立相關分析部分，沒有 SNP 與代謝症候群相關。使用以基因為基礎的方式分析基因-基因交互作用時，透過 GMDR 得到 *PTPN1* 與 *ADIPOQ* 及 *TCF7L2* 有交互作用(交互驗證一致性為 4/10，permutation P = 0.01)；而透過以基因為基礎之方法得到使用 mean-ratio 方法時，可看到 *PTPN1* 分別和 *FTO* 及 *ADIPOQ* 有交互作用；而 *PTPN1* 和 *FTO* 的交互作用情形也可在 quantile ratio 看到。

結論： *PTPN1* 在代謝症候群中是一個與 *ADIPOQ, TCF7L2* 和 *FTO* 有交互作用的重要因子，但這個潛在的生物機制仍須被探討並可能可以找出代謝症候群發生的新見解。

關鍵字：基因-基因交互作用、GMDR、以基因為基礎之方式、代謝症候群

ABSTRACT

Background: A growing focus in genetic studies of complex diseases is how to detect the joint effects of susceptibility genes. A common method is generalized multifactor dimensionality reduction (GMDR), which is Single Nucleotide Polymorphism (SNP)-based and has good power in identifying high-order gene-gene interactions. However, the functional relevance among the SNPs in the same gene may result in false negatives in using the GMDR. Instead, the gene-based method has been proposed to capture interactions that may involve such functional variants within a gene.

Objective: 1) To investigate whether there exist associations with the metabolic syndrome for individual SNPs in each of six genes that have been implicated because of their association with some components of the metabolic syndrome, including *ADIPOQ*, *LEP*, *PPARG*, *FTO*, *TCF7L2*, and *PTPN1*; and 2) to search for gene-gene interactions among these genetic loci on the metabolic syndrome by means of the GMDR as well as the gene-based analysis.

Methods: A case-control study among participants of health check-up, aged 40 years or above, was carried out in Northern Taiwan, with a total of 611 cases with metabolic syndrome and 1117 controls. Among 19 SNPs of the six genes selected for this study, multivariable logistic regression analyses were used to estimate the association between individual SNPs and metabolic syndrome with adjustment for sex and age. Gene-gene interactions were then investigated using both the GMDR method and a gene-based approach that utilities the genotype-trait distortion score.

Results: Individual SNPs did not exhibit significant association with metabolic syndrome. The gene-gene interaction analyses using the GMDR showed that a combination of SNPs in *ADIPOQ*, *TCF7L2*, and *PTPN1* was the best model, with a prediction accuracy of 0.54 and a cross-validation consistency of 4/10 (permutation P = 0.01). Meanwhile, the gene-based

analysis indicated that *PTPN1* interacted with *FTO*, regardless of the type of ratio statistics (mean-ratio or quantile ratio) or the method used in assessing significance level (curve method or rank method), or with *ADIPOQ* if the curve method was used for the quantile ratio. **Conclusions:** Our results suggest that *PTPN1* is an important gene that may interact with *FTO*, *ADIPOQ*, and *TCF7L2* in exerting their influence on the metabolic syndrome. The underlying mechanism of the interaction warrants further investigation and may lead to new insights about the occurrence of the metabolic syndrome.

Key words: gene-gene interaction, GMDR, gene-based approach, metabolic syndrome

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INTRODUCTION

The metabolic syndrome, which includes insulin resistance, glucose tolerance, dyslipidemia, central obesity, and hypertension, is an important and prevalent predictor of diabetes and cardiovascular diseases (Eckel et al. 2005; Mathieu et al. 2006). The prevalence of the metabolic syndrome has been found to range from 10% to 60% in different ethnic groups (Eckel et al. 2005). Although the underlying mechanism remains not fully understood, genetic factors have been indicated to have substantial contribution to the metabolic syndrome and its components, supported by heritability estimates from both family (h^2 ranging from 0.14 to 0.52) (Freeman et al. 2002; Austin et al. 2004) and twin (h^2 ranging from 0.45 to 0.84) (Schousboe et al. 2003) studies.

Obesity and insulin resistance have been considered the core pathophysiology of the metabolic syndrome (Eckel et al. 2005; Chuang 2008; Cornier et al. 2008), with many genetic variants involving these two components becoming the research focus in looking for its genetic underpinnings. Pertaining to obesity, the genetic variants in the fat mass and obesity-associated (*FTO*) gene has been strongly associated with obesity (Dina et al. 2007; Frayling et al. 2007), with one of its single nucleotide polymorphism (SNP) rs9939609 consistently replicated in different ethnic groups (odds ratio [OR] = 1.19 to 1.43) (Al-Attar et al. 2008; Chang et al. 2008). Genes involving the adipocyte differentiation have also attracted much attention. One of such genes is peroxisome proliferator-activated receptor γ *(PPARG)*, though the associations of its variant Pro12Ala (rs1801282) with body mass index (BMI) (Meirhaeghe et al. 2005a; Semple et al. 2006; Montagnana et al. 2008; Tellechea et al. 2009) or insulin sensitivity (Chuang et al. 2001) have been less consistent across studies. In addition, many genetic variants of adiponectin (*ADIPOQ*) gene, which is regulated by PPARG, have

been associated with obesity and insulin sensitivity, especially for SNPs rs182052, rs2241766, and rs1501299 (Yang and Chuang 2006; Yang et al. 2007). Another gene in adiposity sensing pathway, leptin (*LEP*), has SNPs in the 5' region that were associated with obesity (Jiang et al. 2004). With regard to the insulin signaling pathway, protein tyrosine phosphatase non-receptor type 1 (*PTPN1*) gene is involved in the decreasing of insulin action, and its SNPs have been associated with fasting glucose levels (Palmer et al. 2004; Florez et al. 2005; Spencer-Jones et al. 2005) as well as obesity and blood pressure (Olivier et al. 2004).

A recent strategy that was considered a powerful tool in searching for novel genetic variants associated with complex diseases, genome-wide association study, has been applied for the metabolic syndrome (Chuang 2008) and found that transcription factor 7-like 2 (*TCF7L2*) is associated with type 2 diabetes (Grant et al. 2006; Scott et al. 2007). Subsequently, SNPs at *TCF7L2* have been associated with blood lipid levels and blood pressure (Liu et al. 2009) as well as fasting glucose (Guo et al. 2007).

Despite our increasing understanding of the genetic susceptibility to individual components of the metabolic syndrome, studies searching for genetic variants for the syndrome as a whole have had limited success (Meirhaeghe et al. 2005a; Marzi et al. 2007; Al-Attar et al. 2008; Montagnana et al. 2008). Since individual genetic variants may influence more than one trait simultaneously, they might underlie the metabolic syndrome both independently and through more complex interactions (Groop 2000; Moore 2005). A traditional method used in detecting gene-gene interaction, logistic regression analysis, is limited by its dimensionality problems that may lead to inaccurate parameter estimates for interactive effects as the number of genes increases (Heidema et al. 2006). An alternative method is generalized multifactor dimensionality reduction (GMDR), which is SNP-based and has good statistical power in identifying

high-order gene-gene interactions (Lou et al. 2007). Because there may be functional relevance among the SNPs in the same gene, which may result in false negatives in using the GMDR (Huang et al. 2009), a gene-based method that utilizes the genotype-trait distortion (GTD) score (Zheng et al. 2006) was proposed to capture interactions that may involve such functional variants within a gene (Lo et al. 2008).

To date, most studies on the relations of genetic variants to the metabolic syndrome were from Caucasian populations (Florez et al. 2005; Meirhaeghe et al. 2005a; Heid et al. 2006; Marzi et al. 2007; Freathy et al. 2008), which may limit their generalizability since allele frequencies tend to differ in different ethnic populations. The few studies investigating such relations in non-Caucasian populations were of relatively small sample size (Al-Attar et al. 2008; Liu et al. 2008; Yang et al. 2009) or limited to clinical cases (Yang et al. 2009).

To fill in these gaps, we turned to a community-based case-control study of the metabolic syndrome among Han-Chinese population in northern Taiwan. The specific aims of this study were 1) to investigate whether there exist associations with the metabolic syndrome for individual SNPs in each of six genes that have been implicated because of their association with some components of the metabolic syndrome, including *ADIPOQ*, *LEP*, *PPARG*, *FTO*, *TCF7L2*, and *PTPN1*; and 2) to search for gene-gene interactions among these genetic loci on the metabolic syndrome by means of the SNP-based GMDR (Lou et al. 2007) as well as the gene-based GTD analysis (Lo et al. 2008).

METHODS

Subjects

Subjects in this study were from a survey that was collaborated between a research team of National Taiwan University and the Bureau of Health of Tao-Yuan County in Taiwan in recruiting community residents who participated in an annual adult health check-up program in 2004, which was covered by the National Health Insurance program. In this survey, all participants (n = 6,449) were older than 40 years and available for check-ups results with blood sample. All participants gave written informed consent after complete description. The study was approved by the Internal Review Board of National Taiwan University Hospital.

A case of the metabolic syndrome was defined in accordance with a modified Adult Treatment Panel III (ATP III) criteria (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults 2001) as the presence of three or more of the following metabolic abnormality: 1) body mass index (BMI) ≥ 27 kg/m^2, which was shown o be highly consistent with original ATP III criteria of waist circumference among Han Chinese in Taiwan (Chien et al. 2008); 2) triglycerides ≥150 mg/dL; 3) HDL < 40mg/dL in men and < 50 mg/dL in women; 4) blood pressure ≥ 130/85 mmHg or medication for hypertension; and 5) fasting glucose ≥ 110 mg/dL or medication for diabetes. In order to increase the efficiency, we matched each case with two controls, who had at most one metabolic abnormality, by age, sex, education, and ethnicity. However, 47 cases in our sample did not have any matched controls, and 11 cases had just one matched control. In total, there were 611 cases with the metabolic syndrome and 1,117 controls.

Measurements

Information about demographical features and substance use was obtained from self-reported questionnaires including sex, birthday, educational level, ethnicity, health and diet behavior (frequency of substance use, dietary habits, and exercise regimen), and disease history. The measures on substance use covered experiences of tobacco, alcohol, and betel nut. BMI was calculated using the weight and height for each person. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after the subject had been seated for at least 5 min.

The venous blood samples were collected in the morning after 8 hours or more overnight fast. Triglyceride and fasting glucose were determined using standard laboratory methods. After routine laboratory assay, the participants' blood sample would be stored in the 4°C refrigerator at check-up unit no more than one week. Then, the blood samples would be frozen on dry ice, sent to our laboratory, and were stored at -80°C. The high-density lipoproteins (HDL) cholesterol levels, which had not been covered by the National Health Insurance Check-Up Program, were measured using enzymatic methods with an automated biochemical analyzer (TBA-200FR, Toshiba, Tokyo, Japan).

Genotyping

DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, USA). A total of 19 SNPs were selected for this study on the basis of their associations with individual components of the metabolic syndrome, including three SNPs (rs182052, rs2241766, and rs1501299) in *ADIPOQ* (Heid et al. 2006; Yang and Chuang 2006), one SNP (rs1801282) in *PPARG* (Meirhaeghe et al. 2005b; Zeggini et al. 2005), one SNP (rs11770725) in *LEP* (Jiang et al. 2004), five

SNPs (rs7903146, rs7919409, rs10749127, rs290489, and 290487) in *TCF7L2* (Chang et al. 2007; Vaxillaire et al. 2008; Liu et al. 2009), three SNPs (rs1421085, rs17817449, and rs9939609) in *FTO* (Chang et al. 2008) , and 6 SNPs (rs6512651, rs6012953, rs2426156, rs16995294, rs2038526, and rs2282146) in *PTPN1* (Olivier et al. 2004; Palmer et al. 2004; Spencer-Jones et al. 2005). Primers and probes were designed by Applied Biosystems (Foster City, CA, USA). The PCR-reactions were performed according to the manufacturer's instructions and different genotypes were determined using an ABI PRISM® 7900HT Sequence Detector System (Applied Biosystems).

Statistical analysis

Single locus analysis

Hardy-Weinberg equilibrium (HWE) was tested for all the 19 SNPs using χ^2 tests and an empirical *P* value was obtained from 10,000 permutations among control subjects. Multivariable logistic regression analysis was used to estimate the potential association between gene polymorphisms and the metabolic syndrome, and odds ratios (ORs) were estimated after adjusting for potential confounders, including sex, age, education, and ethnicity. All statistical analyses were carried out using the SAS statistical software package (Version 9.1.3) and the SAS/Genetics module (SAS Institute, Cary, NC).

Haplotype analysis

We assessed the presence of linkage disequilibrium (LD) using D′ and r^2. The structure of the haplotype block was evaluated using the solid spine of LD method implemented in the HaploView program version 4.1 (Barrett et al. 2005). The relations of haplotypes to the metabolic syndrome were estimated using the THESIAS program (Tregouet and Garelle 2007) with adjustment for sex, age, education, and ethnicity.

Gene-gene interaction

Possible interactions among genetic variants were evaluated using two different approaches: the GMDR Software Beta version 0.7 (Lou et al. 2007) and the gene-based approach (Zheng et al. 2006; Lo et al. 2008). The GMDR, an extension of the multifactor dimensionality reduction method (Ritchie et al. 2001) that allows for adjustment for covariates, is a non-parametric, generalized linear method that reduces multiloci genotypes into high-risk and low-risk groups. It then uses cross-validation/permutation to minimize false-positive results. An empirical *P* value of prediction accuracy was obtained from 1,000 permutations. In comparing different interactive models, the 'best model' is usually selected on the basis of highest cross-validation consistency, followed by better predictive accuracy, and then the parsimony of parameter number.

For the gene-based analysis, a more detailed description is available elsewhere (Lo et al. 2008). In brief, several statistics are calculated for the gene-based approach, including the genotype trait distortion (GTD) score, the interaction genotype statistic, the M statistic, the R statistic, and the Q statistic. The GTD score, ν, measures the joint association of SNPs with the disease outcome and is defined as follows:

$$\nu = \sum_{s=1}^{3^k} \left(\frac{n_{D,s}}{n_D} - \frac{n_{U,s}}{n_U} \right)^2,$$

where 3^k is the number of genotypes constituted by k SNPs, $n_{D,s}$ and $n_{U,s}$ are the number of cases and controls having the genotype s, respectively, and n_D and n_U are the numbers of total cases and controls, respectively. Moreover, if the $k = 1$, we would obtain the marginal effect of single SNP; if the $k = 2$, we would obtain the pairwise interaction. Then, the interaction genotype statistic, r (i_d,j_e), is defined as follows:

$$r(i_d, j_e) = \frac{v_{i_d j_e} - v_{i_d} \vee v_{j_e}}{v_{i_d} \vee v_{j_e}},$$

where i_d is the d^{th} SNP of gene i, j_e is the e^{th} SNP of gene j, and the '$\vee$' means max (ν_{id}, ν_{je}).

Next, the 'M statistic' is defined as the 'average maximum marginal ν' in gene-level as follows:

$$M_{ij} = \frac{\sum_{d=1}^{m_i} \sum_{e=1}^{m_j} (v_{i_d} \vee v_{j_e})}{m_i m_j},$$

where m_i and m_j are the counts of SNPs in i^{th} and j^{th} gene, respectively. To capture 'mean interaction' in gene-level, the 'R statistic' is defined as follows:

$$R_{ij} = \frac{\sum_{d=1}^{m_i} \sum_{e=1}^{m_j} r(i_d, j_e)}{m_i m_j},$$

This way of expressing gene-gene interaction is called 'mean-ratio method.'

In addition to R statistic, a quantile-ratio based Q statistic has also been proposed to investigate the gene-gene interaction, especially for SNPs with small or negligible marginal effects that may lead to inflated r (i_d,j_e). In that case, one may obtain a high r (i_d,j_e) that have a large effect on the overall mean R_{ij}. To avoid this, the Q statistic is based only on those r (i_d,j_e) that are on the small end, e.g., the top 95^{th} quantile of the SNP-wise ratios. Since we included six genes in this study, there are 15 gene pairs to be considered for pair-wise interaction.

To evaluate the significance level of the gene-gene interaction finding, both the 'curve method' and the 'rank method' were used to summarize the 1,000 permutations over the diseased outcome. For the curve method, we used the 95^{th} percentile R (or Q) threshold curve conditioning on M as the significant level and plotted the (M, R)–plane (or (M, Q)–plane). An alternative 'rank method' was used to evaluate the statistical

significance because of its less assumption and better reliability than the curve method (Lo et al. 2008; Qiao et al. 2009). For each gene pair, the observed R (or Q) value corresponds to a rank value, and the R (or Q) value from permutation *p* receives a rank $T^{(p)}$. The proportion of $T^{(p)} \geq T$ is the *P* value of gene pairs. The software package, provided by the author of the gene-based analysis Prof. Lo SH, was executed using R program (version 2.8.1).

Because the gene-based approach cannot handle data with missing genotype, the genotype imputation was performed using Markov Chain Haplotyping software package MACH 1.0 (Li et al. 2006; Li et al. 2009; Li and GR 2006), which generates higher imputation accuracy rates and provides closest resemblance to those based on complete data than other software packages (Biernacka et al. 2009; Li et al. 2009; Nothnagel et al. 2009). In our data, all of the 19 SNPs had a missing rate of less than 5%. Totally we imputed 467 markers in 273 subjects.

RESULTS

The socio-demographical and clinical characteristics of individuals with and without the metabolic syndrome are shown in **Table 1**. There were no differences in age, sex, education, and ethnicity between cases and controls, reflecting the match design of the study. Although cases had a higher cigarette smoking rate than controls ($P = 0.0007$), tobacco smoking was not associated with any genetic variants genotyped in this study (all $P > 0.05$). Hence tobacco smoking was not considered as a confounder in the subsequent association analyses between genetic variants and the metabolic syndrome.

Table 2 shows the genetic information of the SNPs genotyped in this study. All the SNPs were in HWE ($P \geq 0.05$) among controls. The successful allele-calling rates of individual SNPs were above 96%.

The relations of each SNP to the metabolic syndrome are summarized in **Table 3**. No single SNP had a significant association with the metabolic syndrome with adjustment for sex, age, education, and ethnicity (*P* value ranging from 0.1 to 0.6).

Haplotype Analyses

Testing for LD showed that one LD block was identified in each of *ADIPOQ, FTO*, and *PTPN1* (D′ ranging from 0.97 to 0.99) but not in *TCF7L2* (**Figure 1**). The SNPs constituting these three blocks were rs2241766 and rs1501299 in *ADIPOQ*, rs1421085, rs17817449, and rs9939609 in *FTO*, and rs16995294, rs2038526, and rs2282146 in *PTPN1*. We then performed haplotype association analyses for these three haplotypes using THESIAS software. After adjusting for sex, age, education, and ethnicity, all of these haplotypes were not associated with the metabolic syndrome (**Table 4**).

Gene-Gene Interaction

The results on gene-gene interactions using the GMDR method are shown in **Table 5**. Among a variety of interactive models, the best model was the interactive combination of rs182052 in *ADIPOQ*, rs10749127 and rs290487 in *TCF7L2*, and rs6512651 in *PTPN1*, with the highest value in cross-validation consistency (4/10) and prediction accuracy (0.54) and a significant effect for the prediction accuracy (permutation-based $P = 0.01$) after adjusting for sex, age, education, and ethnicity.

For the gene-based approach, we sought for pairwise interactions among the 6 genes. By plotting the M statistics, representing the average marginal effect, versus the mean-ratio R statistics, representing mean interactive effect, one pair of gene interaction (*FTO, PTPN1*) exceeds the 95th percentile of R (**Figure 2**, panel a). Similarly, if we plotted M statistics versus the Q statistics, representing the top 95th quantile of the interaction genotype statistic, two pairs of gene interactions, (*FTO, PTPN1*) and (*ADIPOQ, PTPN1)*, exceeds the 95th percentile of Q (**Figure 2**, panel b). If the observed R (or Q) value was compared to 1000 permutated R (or Q) in ranking, one gene pair (*FTO, PTPN1*) had interaction effect on the metabolic syndrome at the significance level of 0.05 for both the mean-ratio R and quantile-ratio Q. **Table 6** summarizes the results of significant gene-gene interactions detected by means of the gene-based approach using both the curve method and the rank method for mean ratios as well as quantile ratios, in which the (*FTO, PTPN1*) pair was detected consistently in all methods.

DISSCUSION

In this community-based case-control study, we examined the relations of 19 SNPs from 6 genes to the metabolic syndrome. Despite the lack of association of individual SNPs with the metabolic syndrome, our gene-gene interaction analyses using both the SNP-based GMDR and the gene-based GTD analysis did show that there were possible interactive effects among these genetic variants on the metabolic syndrome. In particular, the gene-based approach is suitable for the detection of interactive effect in the absence of marginal main effect of individual genetic variants. To our knowledge, this is the first study investigating the gene-gene interaction on the metabolic syndrome using the gene-based approach. The results may provide new insights to the occurrence of the metabolic syndrome.

The lack of the association between individual SNPs and the metabolic syndrome in this study are in line with the majority of previous studies (Heid et al. 2006; Marzi et al. 2007; Al-Attar et al. 2008; Montagnana et al. 2008). Since the study sample was recruited from the community, the finding is unlikely to be tampered by some unknown clinical ascertainment process. In contrast, those studies that found an association of certain SNPs with the metabolic syndrome were either family-based samples (Yang et al. 2009; Henneman et al. 2010) or of different demographic features, such as elderly population (Yang et al. 2007), males only (Tellechea et al. 2009), or different ethnic groups (Al-Attar et al. 2008), in which the association was found in non-Caucasian minority subgroups but not in Chinese subgroup. Given the minor allele frequencies of the 19 SNPs examined in this study were more than 10% except two, and the study was matched in several demographic features, these factors might not have much influence on the lack of association of individual genetic variants with the metabolic syndrome.

Of the two approaches in detecting gene-gene interaction, the GMDR can adjust for the influence from covariates. Since this case-control study was designed to be matched by sex, age, education, and ethnicity, this feature allow us to control for these covariates in the association analyses to avoid potential selection bias built in for this design (Rothman et al. 2008). However, even the best interactive model detected using the GMDR only had a modest cross-validation consistency (4/10) and moderate prediction accuracy (0.54). One possibility is that the GMDR has low power when there is presence of the genetic heterogeneity (Ritchie et al. 2003), which is believed to be common for a complex disease like the metabolic syndrome. Another possibility is that the GMDR looks for the major signal in the variation and ignore minor signals (Lou et al. 2007), whereas in this study individual genetic variants failed to exhibit any meaningful association with the metabolic syndrome.

In contrast, one major strength of the gene-based method is that it can detect interactive effect among those individual genes that has little sign of marginal effects (Qiao et al. 2009), which fit this study quite well. In addition, the gene-based approach provides some protection against inflation of interactive genotype statistic due to small marginal effect, such as quantile-ratio based Q statistic and rank method in assessing the significance level. Regardless of the type of ratio statistics (mean-ratio or quantile ratio) or the method used in assessing significance level (curve method or rank method), the pairwise *FTO-PTPN1* interaction was consistently found. To some extent, this alludes to the robustness of the finding.

Among the genes implicated in the interactive effect on the metabolic syndrome, only *PTPN1* gene is uncovered by both the GMDR and the gene-based approaches. The *PTPN1* gene encodes the protein tyrosine phosphatase 1B, which suppresses the signaling pathway of insulin (McGuire et al. 1991; Spencer-Jones et al. 2005). The most

consistent gene-gene interaction involving *PTPN1* is *FTO*, which encodes a 2-oxoglutarate-dependent nucleic acid demethylase that is present in many tissues but most abundant in the hypothalamus (Gerken et al. 2007). In addition to *FTO*, *PTPN1* is also implicated by the gene-based approach in the pairwise interaction with the *ADIPOQ* gene, which encodes the protein adiponectin that increases insulin sensitivity through stimulating fatty acid oxidation (Vasseur et al. 2002; Nedvidkova et al. 2005; Vasseur et al. 2006). Finally, the results of the GMDR analyses indicate that *PTPN1* might be involved in a multi-way interaction with *ADIPOQ* and *TCF7L2*, which plays an important role in Wnt signaling pathway (Yi et al. 2005) that regulates adipogenesis (Ross et al. 2000; Gustafson and Smith 2006; Prestwich and Macdougald 2007). Further, the overexpression of Wnt signaling reduces adiposity and enhances insulin sensitivity *in vivo* (Wright et al. 2007). Taken together, the findings imply that the insulin signal may be an important factor underlying the interaction between *PTPN1* and other genes such as *ADIPOQ* and *TCF7L2* on the metabolic syndrome. However, there is no known mechanism underlying the interaction between *PTPN1* and *FTO*. Future investigation in this aspect is warranted.

In summary, the present study indicates that despite the lack of the association between individual SNPs and the metabolic syndrome, some potential gene-gene interactions on the metabolic syndrome were found using both the SNP-based GMDR and the gene-based GTD analysis, with the latter being suitable for the detection of interactive effect in the absence of marginal main effect of individual genetic variants. Our results suggest that *PTPN1* is an important gene that may interact with *FTO*, *ADIPOQ*, and *TCF7L2* in exerting their influence on the metabolic syndrome. The underlying mechanism of the interaction warrants further investigation and may lead to new insights about the occurrence of the metabolic syndrome.

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Table 1. Socio-demographical and clinical characteristics of subjects with and without the metabolic syndrome among adults aged 40 years or older in northern Taiwan.

Variables	Controls (n = 1117)	Cases (n = 611)	*P* value
	N (%)	N (%)	
Sex			ns
Male	451 (40.38)	246 (40.26)	
Female	666 (59.62)	365 (59.74)	
Ethnic group			ns
Min-nan	734 (65.7)	405 (66.2)	
Hakka	383 (34.3)	206 (33.8)	
Education			ns
Less than junior high school	704 (63.0)	381 (62.4)	
Junior high school	196 (17.6)	107 (17.5)	
High school or above	217 (19.4)	123 (20.1)	
Cigarette smoking, n (%)			0.0007
Yes	237 (21.22)	174 (28.48)	
No	880 (78.78)	437 (71.52)	
	Mean (SD)	Mean (SD)	
Age (years)	56.3 (7.9)	56.9 (7.9)	ns
BMI (kg/m^2)	23.5 (2.7)	27.9 (3.6)	< 0.0001
SBP (mmHg)	123.6 (17.8)	140.5 (18.1)	< 0.0001
DBP (mmHg)	76.6 (12.4)	87.1 (12.6)	< 0.0001
Fasting glucose (mg/dL)	87.5 (17.6)	123.8 (62.1)	< 0.0001
Triglyceride (mg/dL)	101.8 (46.7)	247.9 (184)	< 0.0001
HDL (mg/dL)	60.6 (12.8)	45.8 (9.7)	< 0.0001

ns = non-significant.

Table 2. The SNPs of candidate genes for the metabolic syndrome selected for this study.

#	SNP	Chr	Gene	Location	Call rate (%)	Minor/major Allele	MAF	*P* value for HWE
1	rs182052	3	*ADIPOQ*	Intron 1	99.9	A/G	0.42	0.67
2	rs2241766	3	*AIDPOQ*	Exon 2	100.0	G/T	0.30	0.57
3	rs1501299	3	*ADIPOQ*	Intron 2	96.1	T/G	0.28	0.29
4	rs11770725	7	*LEP*	5' flank	99.9	A/C	0.23	0.39
5	rs1801282	3	*PPARG*	Missense	99.9	G/C	0.04	0.26
6	rs7903146	10	*TCF7L2*	Intron 3	99.1	T/C	0.03	1.00
7	rs7919409	10	*TCF7L2*	Intron 4	100.0	C/T	0.26	0.94
8	rs10749127	10	*TCF7L2*	Intron 4	99.7	T/C	0.27	1.00
9	rs290489	10	*TCF7L2*	Intron 7	99.80	A/G	0.31	0.73
10	rs290487	10	*TCF7L2*	Intron 7	99.7	C/T	0.41	0.12
11	rs1421085	16	*FTO*	Intron	98.3	T/C	0.12	0.59
12	rs17817449	16	*FTO*	Intron	98.10	T/G	0.12	0.78
13	rs9939609	16	*FTO*	Intron	99.0	A/T	0.12	1.00
14	rs6512651	20	*PTPN1*	Intron	99.7	G/C	0.28	0.50
15	rs6012953	20	*PTPN1*	Intron	99.5	G/A	0.32	0.30
16	rs2426156	20	*PTPN1*	Intron 1	96.30	G/T	0.25	0.05
17	rs16995294	20	*PTPN1*	Intron 2	99.6	T/A	0.36	0.22
18	rs2038526	20	*PTPN1*	Intron 4	98.8	T/C	0.29	0.67
19	rs2282146	20	*PTPN1*	Exon 8	97.6	T/C	0.20	0.26

Note. Chr = chromosome; MAF = minor allele frequency in 1117 healthy controls; HWE *P*-value = empirical p-values of the χ^2 test for Hardy-Weinberg equilibrium in the control group.

Table 3. Association analysis of individual SNPs and the metabolic syndrome in the study of 611 cases and 1117 controls in northern Taiwan after adjustment for age, gender, education, and ethnicity.

#	SNP	Chr	Gene	Minor/major Allele	MAF	*P* value for aOR	aOR (95% CI)
1	rs182052	3	*ADIPOQ*	A/**G**	0.420	0.61	1.04 (0.90-1.20)
2	rs2241766	3	*ADIPOQ*	**G**/T	0.302	0.65	0.97 (0.83-1.12)
3	rs1501299	3	*ADIPOQ*	T/**G**	0.281	0.49	1.06 (0.90-1.24)
4	rs11770725	7	*LEP*	**A**/C	0.230	0.08	1.16 (0.98-1.36)
5	rs1801282	3	*PPARG*	G/**C**	0.041	0.52	1.13 (0.78-1.63)
6	rs7903146	10	*TCF7L2*	**T**/C	0.027	0.15	1.37 (0.90-2.11)
7	rs7919409	10	*TCF7L2*	C/**T**	0.264	0.23	1.10 (0.94-1.30)
8	rs10749127	10	*TCF7L2*	T/**C**	0.274	0.13	1.13 (0.96-1.33)
9	rs290489	10	*TCF7L2*	A/**G**	0.313	0.25	1.10 (0.94-1.28)
10	rs290487	10	*TCF7L2*	C/**T**	0.406	0.47	1.06 (0.91-1.22)
11	rs1421085	16	*FTO*	T/**C**	0.125	0.71	1.04 (0.84-1.30)
12	rs17817449	16	*FTO*	T/**G**	0.122	0.98	1.00 (0.81-1.25)
13	rs9939609	16	*FTO*	A/**T**	0.116	0.34	1.12 (0.89-1.42)
14	rs6512651	20	*PTPN1*	G/**C**	0.284	0.97	1.00 (0.86-1.17)
15	rs6012953	20	*PTPN1*	G/**A**	0.324	0.18	1.11 (0.96-1.29)
16	rs2426156	20	*PTPN1*	G/**T**	0.252	0.95	0.99 (0.85-1.17)
17	rs16995294	20	*PTPN1*	T/**A**	0.358	0.21	1.10 (0.95-1.27)
18	rs2038526	20	*PTPN1*	**T**/C	0.289	0.61	0.96 (0.82-1.12)
19	r2282146	20	*PTPN1*	**T**/C	0.203	0.83	0.98 (0.83-1.17)

Note. Bold font indicates the risk allele; MAF = minor allele frequency in 1117 healthy controls; aOR = the ORs adjusted for age, gender, education, and ethnicity in the multivariable logistic regression analysis.

Table 4. Association analysis of haplotypes and the metabolic syndrome in the study of 611 cases and 1117 controls in northern Taiwan after adjustment for age, gender, education, and ethnicity.

	Frequency	aOR	95% CI	*P* value
ADIPOQ				
rs2241766/rs1501299				
TG	0.42			
TT	0.28	0.93	[0.79 - 1.11]	0.44
GG	0.30	0.94	[0.80- 1.12]	0.50
FTO				
rs1421085/rs17817449/rs9939609				
CGT	0.88			
TTA	0.11	0.89	[0.70 - 1.13]	0.32
PTPN1				
rs16995294/rs2038526/rs2282146				
ACC	0.63			
TCC	0.08	0.80	[0.60 - 1.06]	0.11
TTC	0.08	0.92	[0.71 - 1.21]	0.56
TTT	0.20	0.96	[0.80- 1.14]	0.63

Note. Only those haplotypes with a frequency of ≥ 0.01 were included for the analysis; aOR = the ORs adjusted for age, gender, education, and ethnicity in the multivariable logistic regression analysis.

Table 5. Comparison of a variety of best models for single SNP, two-way, three-way, and four-way interactions among 19 SNPs from six genes in their associations with the metabolic syndrome using the GMDR in the study of 611 cases and 1117 controls in northern Taiwan after adjustment for age, gender, education, and ethnicity.

Best model	Test balanced accuracy	Cross-validation consistencies	*P* value
TCF7L2 (rs7919409)	0.5082	4/10	0.30
LEP (rs11770725), *TCF7L2* (rs10749127)	0.5369	3/10	0.01
ADIPOQ (rs182052), *TCF7L2* (rs290487), *PTPN1* (rs2426156)	0.5370	3/10	0.02
***ADIPOQ* (rs182052*), TCF7L2* (rs10749127), *TCF7L2* (rs290487), *PTPN1* (rs6512651)**	**0.5403**	**4/10**	**0.01**

Note. Test balanced accuracy is adjusted for the 1:2 ratio of cases versus controls in the original design; *P* value was derived from 1,000 permutations over the disease status; bold font indicates the best model among the four models listed in this table.

Table 6. Significant gene-gene interactions on the metabolic syndrome detected using different procedures of the gene-based approach in the study of 611 cases and 1117 controls in northern Taiwan.

Method	Mean ratio	Quantile ratio
Curve method	*FTO PTPN1*	*FTO PTPN1* *ADIPOQ PTPN1*
Rank method	*FTO PTPN1*	*FTO PTPN1*

(a) *ADIPOQ* (b) *FTO* (c) *PTPN1*

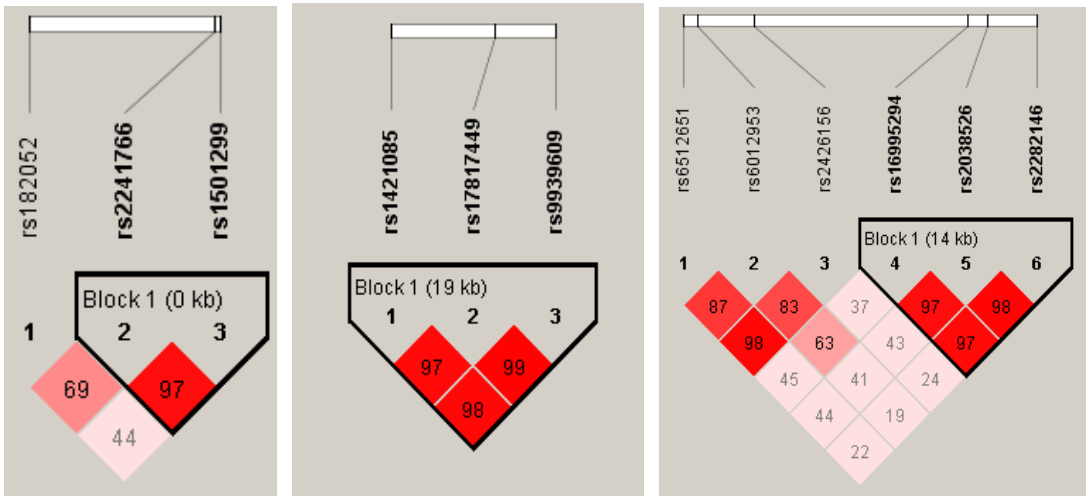
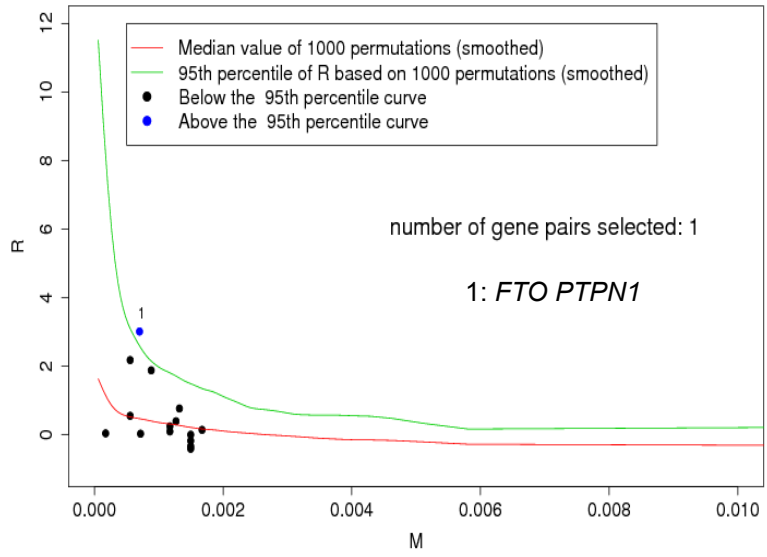


Figure 1. LD block structure for SNPs in (a) *ADIPOQ,* (b) *FTO*, and (c) *PTPN1* in 1117 healthy controls. The colors (white to red) represent the increasing strength of LD, and the values of D′ are shown within the corresponding cells.

a. (M, R)–plane, pairwise interaction



b. (M, Q)–plane, pairwise interaction

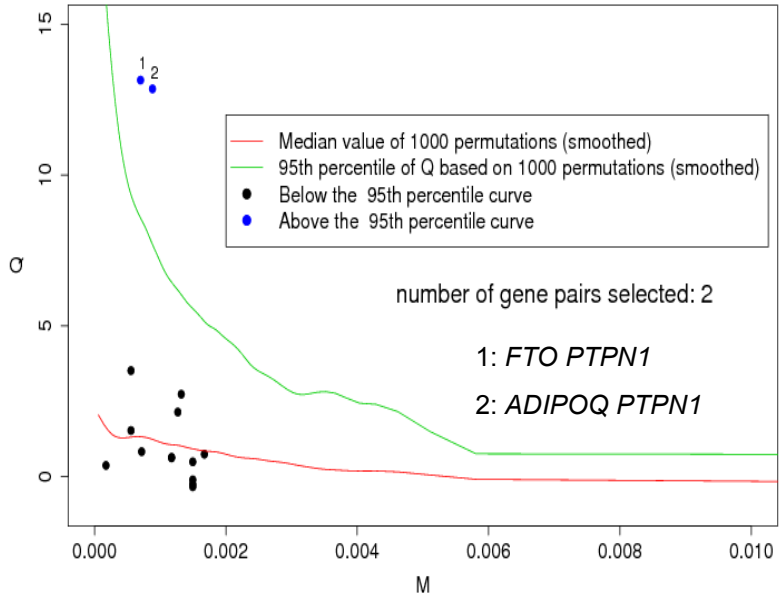


Figure 2. Selection of potential pair-wise gene-gene interaction using a) (M, R)–plane and b) (M, Q)–plane, in which the observed data and the 95th percentiles of R or Q statistics derived from 1000 permutations over the diseased status are displayed, in the study of 611 cases and 1117 controls in northern Taiwan.