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# Interactions of a PPARGC1A Variant and a PNPLA3 Variant Affect Nonalcoholic Steatohepatitis in Severely Obese Taiwanese Patients

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**Abstract:** The patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) variant is associated with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). However, the role of genetic variations of the peroxisome proliferator-activated receptor gamma coactivator-1-alpha gene (*PPARGC1A*) and glucokinase regulatory (*GCKR*) gene on NASH in obese patients remains unclear. We studied the effects and interaction of these genetic polymorphisms on NASH in severely obese Taiwanese patients.

The genotypes of *PPARGC1A* rs8192678, *PNPLA3* rs738409, and *GCKR* rs780094 were determined in 177 severely obese patients who underwent bariatric surgery. NASH was evaluated by liver histopathology.

Of 177 patients, 29 (16.4%), 57 (33.2%), and 91 (51.4%) were in the non-NAFLD, steatosis, and NASH groups, respectively. We found that the *PPARGC1A* and *PNPLA3* variants, but not the *GCKR* variant, were associated with NASH. The *PPARGC1A* rs8192678 GA/AA genotype was associated with higher steatosis grade and presence of ballooning degeneration. The *PNPLA3* rs738409 GG genotype was associated with higher severity in all histologic features except for fibrosis. In multivariate analysis, both the *PPARGC1A* rs8192678 GA/AA genotype (odds ratio [OR] 2.32; 95% confidence interval [CI] 1.08–4.98;

$P=0.031$ ) and the *PNPLA3* rs738409 GG genotype (OR 4.05; 95% CI 1.24–13.23;  $P=0.021$ ), and also body mass index were independent risk factors for NASH. Further, there was an additive effect of the *PPARGC1A* rs8192678 GA/AA genotype and the *PNPLA3* rs738409 GG genotype on the presence of NASH (OR 6.83; 95% CI 1.61–29.01;  $P=0.009$ ).

The *PPARGC1A* rs8192678 GA/AA genotype and the *PNPLA3* rs738409 GG genotype had an additive effect on NASH in severely obese Taiwanese patients.

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**Abbreviations:** ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, GCKR = glucokinase regulatory protein, GGT = gamma-glutamyl transpeptidase, GWAS = genome-wide association study, HOMA-IR = homeostasis model assessment of insulin resistance, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, PGC-1 $\alpha$  = proxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$ , PNPLA3 = patatin-like phospholipase domain-containing protein 3, PPAR $\gamma$  = proxisome proliferator-activated receptor  $\gamma$ , PPARGC1A = peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$  gene, PPP1R3B = protein phosphatase 1 regulatory subunit 3b, SNP = single-nucleotide polymorphism.

## INTRODUCTION

Along with the increase in the prevalence of obesity, the disease burden of nonalcoholic fatty liver disease (NAFLD) is increasing in both Western and Eastern countries.<sup>1–3</sup> Nonalcoholic steatohepatitis (NASH), an extreme form of NAFLD, is associated with progressive liver disease and can lead to cirrhosis.<sup>4</sup> Morbidly obese patients are especially at high risk for NAFLD and NASH. Prevalence rates are as high as 84% to 96% for NAFLD and 25% to 55% for NASH.<sup>5</sup>

Both genetic and environmental factors are implicated in the development of NASH.<sup>6</sup> Using genome-wide association studies (GWAS), Romeo et al first demonstrated that the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) rs738409 variant is associated with increased liver fat content, and subsequent studies confirmed the association between this single-nucleotide polymorphism (SNP) and NASH.<sup>7,8</sup> Recently, we also reported that the *PNPLA3* rs738409 GG genotype increases susceptibility to NASH in severely obese Taiwanese patients.<sup>9</sup>

Several SNPs have also been associated with NASH. A GWAS revealed that genetic variants in or near neurocan (*NCAN*), lysophospholipase-like 1 (*LYPLAL1*), glucokinase regulatory protein (*GCKR*), and protein phosphatase 1 regulatory subunit 3b (*PPP1R3B*) were associated with hepatic steatosis, lobular inflammation, or fibrosis in persons with

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European ancestry,<sup>10</sup> but inconsistent results were found in different ethnic groups.<sup>11,12</sup> In addition, only the genetic variant in *GCKR* was found to be associated with ultrasound-defined NAFLD in obese Taiwanese children.<sup>13</sup> The peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$  gene (*PPARGC1A*) rs8192678 variant was also found to be inconsistently associated with ultrasound-defined NAFLD.<sup>14,15</sup> However, no study reported the association between the *PPARGC1A* variant and biopsy-proven NASH.

Although the prevalence of NAFLD is high in morbidly obese patients, not all of them develop NASH. The role of genetic disposition in the pathogenesis of NASH remains not well established. Although the association between *PNPLA3* and NAFLD is consistent in most studies, the association between other SNPs and NAFLD is inconsistent. Furthermore, studies about the association between these SNPs and NASH are scarce. Because the allele frequency and genotype frequency of homozygous mutants in *NCAN*, *LYPLAL1*, and *PPP1R3B* are very low in the Taiwanese population,<sup>13</sup> we examined only the effects of the *PPARGC1A* variant and the *GCKR* variant on NASH in severely obese Taiwanese patients. We also attempted to elucidate the effects of these SNPs and their interactions with the *PNPLA3* variant on NASH.

## METHODS

### Eligible Patients

According to the recommendations of the Asia-Pacific consensus,<sup>16</sup> obese Taiwanese patients with a body mass index (BMI)  $\geq 37$  kg/m<sup>2</sup> or a BMI  $\geq 32$  kg/m<sup>2</sup> with obesity-related comorbidities were considered for bariatric surgery. The patients who underwent bariatric surgery and intraoperative liver biopsy in E-Da Hospital, Kaohsiung, Taiwan, were consecutively enrolled in the present study. The exclusion criteria included age  $< 20$  years, alcohol consumption  $> 140$  g/week, use of hepatotoxic drugs, presence of liver diseases (such as Wilson disease,  $\alpha 1$ -antitrypsin deficiency, hemochromatosis, or autoimmune hepatitis), and malignant diseases. All patients were negative for hepatitis B and C viral markers. To avoid the potential influence of medications on NAFLD, we also excluded patients on glucose-lowering drugs. Written informed consent was obtained from each participant, and the study was approved by the E-Da Hospital Ethical Committee.

### Clinical and Biochemical Characteristics

Preoperative data, including age, sex, coexisting medical diseases (eg, type 2 diabetes mellitus and hypertension), and anthropometric measurements (weight and height) were collected. The BMI was calculated as the weight in kilograms divided by the height in meters squared. Biochemical data included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), fasting blood glucose, fasting insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and uric acid levels. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using glucose and insulin levels in a fasting state. Briefly, the HOMA-IR was calculated as follows: (insulin [mU/mL])  $\times$  (glucose [mmol/L])/22.5.<sup>17</sup>

### Histologic Examination

Wedge liver biopsy specimens, about 2 cm  $\times$  2 cm, obtained at the beginning of bariatric surgery, were routinely

stained with hematoxylin and eosin, and Masson trichrome. All biopsy specimens were evaluated by an experienced pathologist (JC Hwang). According to the histologic results, patients were classified into one of 3 groups, as follows: non-NAFLD; steatosis; and NASH. The diagnosis of NASH was made according to the criteria of Brunt et al, and required the presence of steatosis, ballooning, and lobular inflammation.<sup>18,19</sup> Histologic features were assessed semiquantitatively.<sup>20</sup> Steatosis was graded on a scale of 0 to 3: 0—steatosis  $< 5\%$ ; 1—steatosis 5% to 33%; 2—steatosis  $> 33\%$  to 66%; and 3—steatosis  $> 66\%$ . Lobular inflammation was scored according to the number of foci of inflammatory infiltration per 200 $\times$  microscopic field, as follows: 0—no inflammation; 1— $< 2$  foci; 2—2 to 4 foci; and 3— $> 4$  foci. Ballooning degeneration was scored between 0 and 2 points as follows: 0—none; 1—few balloon cells; and 2—many balloon cells. Fibrosis was staged as follows: 0—no fibrosis; 1—perisinusoidal or periportal fibrosis; 2—perisinusoidal and portal/periportal fibrosis; 3—bridging fibrosis; and 4—cirrhosis. Portal inflammation was scored between 0 and 2 points as follows: 0—none; 1—mild; and 2— $>$  mild.

### DNA Extraction and SNP Genotyping

Genomic DNA was extracted from 3 mL of ethylenediaminetetraacetic acid whole blood samples using the salt fractionation method, which was standardized in the Puregene kit commercialized by Gentra (Research Triangle, NC) and was stored at  $-80^{\circ}\text{C}$ . Approximately 10 ng of DNA was used for genotyping by TaqMan polymerase chain reaction (Applied Biosystems, Foster City, CA) according to the manufacturer's directions. After thermal cycling ( $50^{\circ}\text{C}$  for 2 minutes,  $95^{\circ}\text{C}$  for 10 minutes, and then 40 cycles at  $95^{\circ}\text{C}$  for 15 seconds and at  $60^{\circ}\text{C}$  for 90 seconds), allelic discrimination for rs8192678 (cat.C\_1643192\_20) in *PPARGC1A*, rs738409 (cat.C\_7241\_10) in *PNPLA3*, and rs780094 (cat.C\_2862873\_10) in *GCKR* was performed by measuring the allele-specific fluorescence on an ABI Prism Sequence Detection System ABI 7900HT (Applied Biosystems).

### Statistical Analysis

Descriptive results regarding categorical variables were given as percentages, and continuous variables were presented as the mean and standard deviation (SD). Continuous variables, such as insulin, HOMA-IR, AST, ALT, GGT, and triglycerides were not normally distributed, and they were therefore logarithm-transformed to normality before use in statistical models. The Hardy–Weinberg equilibrium test was employed to assess the discrepancy between the observed and the expected SNP genotypic frequency. Data for continuous and categorical variables were compared using the chi-square test, Fisher exact test, or Student *t* test as appropriate. Multiple comparisons of means of continuous variables were analyzed by 1-way analysis of variance and were compared post hoc by the Bonferroni test. The association between SNPs and NAS was analyzed using the Cochran–Armitage test for linear trends. The odds ratio (OR) and corresponding 95% confidence intervals (CIs) calculated from logistic regression models were employed to evaluate the association between genotypic distribution (additive, dominant, or recessive model) with NASH. After adjustment for potential confounding variables contributing to the development of NASH, including age, sex, BMI, HOMA-IR, and triglycerides, we assessed the independent effect of the *PPARGC1A* rs8192678 and *PNPLA3* rs738409 genes on NASH using multiple logistic regression models. The potential gene–gene interaction was evaluated by fitting a multiplicative model with

cross-product terms representing the interaction between the 2 genes studied. The statistical analyses were performed using SAS (version 9.3; SAS Institute, Inc., Cary, NC).

**RESULTS**

**Clinical and Biochemical Characteristics in Patients With Non-NAFLD, Steatosis, and NASH**

Between November 2007 and August 2009, 243 obese patients were consecutively enrolled. Sixty-two patients were excluded from this study, including 40 patients with hepatitis B and/or hepatitis C, 12 patients because of alcohol consumption, and 10 patients on glucose-lowering drugs. In addition, 4 patients without available *PPARGC1A* rs8192678 variant results were excluded. Finally, a total of 177 obese patients (56 [31.6%] men and 121 [68.4%] women) were enrolled in the present study (Supplementary Figure 1, <http://links.lww.com/MD/A807>). The liver histology demonstrated that 29 (16.4%) patients were non-NAFLD, 57 (32.2%) patients were steatosis, and 91 (51.4%) patients were NASH. Compared with non-NAFLD patients, more NAFLD patients were male who had significantly higher BMIs and levels of all biochemical variables except for glucose, total cholesterol, and low-density lipoprotein cholesterol (Table 1). Among NAFLD patients, patients with NASH had significantly higher BMIs and levels of glucose, AST, ALT, GGT, and total cholesterol than those without (Table 1).

***PPARGC1A* rs8192678 and *PNPLA3* rs738409 Variants Associated With Nash**

None of the SNPs deviated from the Hardy–Weinberg equilibrium (all  $P > 0.05$ ). We found that the *PPARGC1A* and *PNPLA3* variants, but not the *GCKR* variant, were associated with NASH (Table 2). The distribution of the *PPARGC1A*

rs8192678 genotype was GG in 59 patients (33.3%), GA in 88 patients (49.7%), and AA in 30 patients (17.0%). Severely obese patients with the *PPARGC1A* rs8192678 GA genotype showed a significant association with NASH compared with patients having the *PPARGC1A* rs8192678 GG genotype (OR 2.26; 95% CI 1.07–4.78;  $P = 0.032$ ). Marginally significant association was found in patients with the *PPARGC1A* rs8192678 AA genotype ( $P = 0.082$ ). In addition, we found that patients with the *PPARGC1A* rs8192678 GA/AA genotype (dominant model) showed a significant association with NASH compared with patients having the *PPARGC1A* rs8192678 GG genotype (OR 2.31; 95% CI 1.14–4.68;  $P = 0.020$ ). However, patients with the *PNPLA3* rs738409 GG genotype (recessive model) had a significant association with NASH compared with patients having the *PNPLA3* rs738409 CC/CG genotype (OR 3.97; 95% CI 1.29–12.27;  $P = 0.016$ ).

**Clinical and Biochemical Characteristics Stratified by *PPARGC1A* rs8192678 Genotypes or *PNPLA3* rs738409 Genotypes**

We further investigated the association between genotypes, and clinical and biochemical characteristics (Table 3). No significant difference was found among the 3 *PPARGC1A* rs8192678 genotypes. However, patients with the *PNPLA3* rs738409 GG genotype had significantly higher levels of glucose, AST, and ALT compared with patients having the *PNPLA3* rs738409 CG or CC genotypes.

***PPARGC1A* rs8192678 and *PNPLA3* rs738409 Genotypes Associated With Histologic Severity**

All histologic features except for chronic portal inflammation were correlated with the diagnostic categories

**TABLE 1.** Clinical and Biochemical Characteristics in Patients With Non-NAFLD, Steatosis, and NASH

	Non-NAFLD (n = 29)	Steatosis (n = 57)	NASH (n = 91)	NASH vs Steatosis <i>P</i>	NAFLD* vs Non-NAFLD <i>P</i>
Age (SD), years, mean	29.3 (9.7)	29.8 (9.8)	30.9 (9.6)	0.489	0.594
Males, n (%)	0 (0.0)	20 (35.1)	36 (39.6)	0.585	<0.001
BMI (SD), kg/m <sup>2</sup>	37.2 (4.3)	41.2 (7.6)	43.8 (7.5)	0.039	<0.001
Glucose (SD), mg/dL	98.3 (27.2)	96.4 (15.6)	113.4 (37.4)	0.001	0.173
Insulin (SD), μU/mL	16.5 (9.6)	28.5 (26.9)	28.1 (20.9)	0.622	0.001
HOMA-IR	3.8 (1.9)	6.7 (6.2)	7.4 (5.3)	0.099	<0.001
AST (SD), IU/L	22.7 (6.6)	40.7 (36.7)	56.1 (41.9)	0.002	<0.001
ALT (SD), IU/L	20.8 (11.7)	60.8 (61.4)	81.2 (62.9)	0.002	<0.001
GGT (SD), IU/L	20.0 (9.4)	44.1 (31.8)	57.4 (57.1)	0.049	<0.001
Triglycerides (SD), mg/dL	104.0 (50.1)	147.5 (99.3)	165.7 (113.4)	0.139	<0.001
Total cholesterol (SD), mg/dL	189.0 (27.1)	190.1 (34.9)	203.9 (43.5)	0.047	0.227
HDL-cholesterol (SD), mg/dL	53.0 (14.3)	45.6 (10.2)	45.5 (9.3)	0.935	<0.001
LDL-cholesterol (SD), mg/dL	120.2 (23.6)	128.2 (30.4)	138.5 (41.5)	0.108	0.050
Uric acid (SD), mg/day	5.8 (1.2)	6.9 (1.8)	7.2 (1.9)	0.368	0.001
Hypertension, n (%)	14 (48.3)	34 (59.6)	60 (65.9)	0.440	0.124
Diabetes mellitus, n (%)	4 (13.8)	5 (8.8)	15 (16.5)	0.182	0.969

Continuous variables were presented as means and standard deviations, and categorical variables were presented as proportions. Student *t* test was used to compare continuous variables, and chi-square tests or Fisher exact test was used to compare categorical variables, as appropriate.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, GGT = gamma glutamyl transpeptidase, HDL = high-density lipoprotein, HOMA-IR = homeostasis model assessment of insulin resistance, LDL = low-density lipoprotein, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, SD = standard deviation.

\*NAFLD, including subjects with steatosis and NASH.

**TABLE 2.** Association of *PPARGC1A* rs8192678, *PNPLA3* rs738409, and *GCKR* rs780094 With NAFLD and NASH

	Non-NAFLD (n = 29)	Steatosis (n = 57)	NASH (n = 91)	NASH vs Steatosis OR (95% CI)	P	NAFLD* vs Non-NAFLD OR (95% CI)	P
<i>PPARGC1A</i> rs8192678							
GG, n (%)	11 (37.9)	25 (43.9)	23 (25.3)	1.00		1.00	
GA, n (%)	14 (48.3)	24 (42.1)	50 (54.9)	2.26 (1.07–4.78)	0.032	1.21 (0.51–2.89)	0.666
AA, n (%)	4 (13.8)	8 (14.0)	18 (19.8)	2.45 (0.89–6.69)	0.082	1.49 (0.43–5.15)	0.529
Per A				1.69 (1.03–2.78)	0.039	1.22 (0.68–2.19)	0.509
GG, n (%)	11 (37.9)	25 (43.9)	23 (25.3)	1.00		1.00	
GA/AA, n (%)	18 (62.1)	32 (56.1)	68 (74.7)	2.31 (1.14–4.68)	0.020	1.27 (0.56–2.90)	0.566
GG/GA, n (%)	25 (86.2)	49 (86.0)	73 (80.2)	1.00		1.00	
AA, n (%)	4 (13.8)	8 (14.0)	18 (19.8)	1.51 (0.61–3.74)	0.374	1.33 (0.43–4.15)	0.621
<i>PNPLA3</i> rs738409							
CC, n (%)	12 (41.4)	31 (54.4)	34 (37.4)	1.00		1.00	
CG, n (%)	15 (51.7)	22 (38.6)	36 (39.6)	1.49 (0.73–3.06)	0.276	0.71 (0.31–1.65)	0.430
GG, n (%)	2 (6.9)	4 (7.0)	21 (23.1)	4.79 (1.48–15.5)	0.009	2.31 (0.48–11.0)	0.295
Per G				1.93 (1.18–3.15)	0.009	1.16 (0.66–2.05)	0.607
CC, n (%)	12 (41.4)	31 (54.4)	34 (37.4)	1.00		1.00	
CG/GG, n (%)	17 (58.6)	26 (45.6)	57 (62.6)	2.00 (1.02–3.92)	0.044	0.90 (0.40–2.02)	0.801
CC/CG, n (%)	27 (93.1)	53 (93.0)	70 (66.9)	1.00		1.00	
GG, n (%)	2 (6.9)	4 (7.0)	21 (23.1)	3.97 (1.29–12.27)	0.016	2.74 (0.61–12.29)	0.187
<i>GCKR</i> rs780094							
CC, n (%)	9 (31.0)	13 (22.8)	25 (27.5)	1.00		1.00	
CT, n (%)	14 (48.3)	30 (52.6)	42 (46.2)	0.73 (0.32–1.65)	0.447	1.22 (0.48–3.07)	0.676
TT, n (%)	6 (20.7)	14 (24.6)	24 (26.4)	0.89 (0.35–2.28)	0.811	1.50 (0.49–4.63)	0.481
Per T				0.95 (0.60–1.50)	0.814	1.22 (0.70–2.14)	0.478
CC, n (%)	9 (31.0)	13 (22.8)	25 (27.5)	1.00		1.00	
CT/TT, n (%)	20 (69.0)	44 (77.2)	66 (72.5)	0.78 (0.36–1.69)	0.528	1.30 (0.55–3.11)	0.551
CC/CT, n (%)	23 (79.3)	43 (75.4)	67 (73.5)	1.00		1.00	
TT, n (%)	6 (20.7)	14 (24.6)	24 (26.4)	1.10 (0.51–2.36)	0.806	1.32 (0.50–3.50)	0.571

OR and their P values were estimated using a logistic regression model.

CI = confidence interval, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio.

\*NAFLD, including subjects with steatosis and NASH.

( $P < 0.001$  for all) (Table 4). We further investigated the association between genotypes and histologic severity (Table 5). Patients with *PPARGC1A* rs8192678 GA/AA genotype had higher steatosis grade and presence of ballooning degeneration compared with patients having the *PPARGC1A* rs8192678 GG genotype. Compared with patients with the *PNPLA3* rs738409 CG/CC genotype, patients with the *PNPLA3* rs738409 GG genotype had higher severity in all histologic features except for fibrosis.

### Interaction Between the *PPARGC1A* rs8192678 Variant and the *PNPLA3* rs738409 Variant on NASH in NAFLD Patients

After adjustment for potential confounders contributing to the development of NASH, including age, sex, BMI, HOMA-IR, and triglycerides, we determined that both the *PPARGC1A* rs8192678 GA/AA genotype (OR 2.32; 95% CI 1.08–4.98;  $P = 0.031$ ) and the *PNPLA3* rs738409 GG genotype (OR 4.05; 95% CI 1.24–13.23  $P = 0.021$ ) were independent risk factors for NASH (Table 6). As shown in Table 7, there was an additive significant effect of the combined *PPARGC1A* rs8192678 GA/AA and *PNPLA3* rs738409 CC/CG genotypes on the presence of NASH (OR 2.72; 95% CI 1.21–6.09;  $P = 0.015$ ). Of note, among severely obese patients, a much higher risk of NASH

was observed with the combined *PPARGC1A* rs8192678 GA/AA and the *PNPLA3* rs738409 GG genotypes (OR 6.83; 95% CI 1.61–29.01;  $P = 0.009$ ). No such effect was observed for the combined *PPARGC1A* rs8192678 GG and *PNPLA3* rs738409 GG genotypes (Table 7).

### DISCUSSION

In the current study, we reported that the *PPARGC1A* and *PNPLA3* variants, but not the *GCKR* variant, were associated with NASH in severely obese Taiwanese patients. Patients with the *PPARGC1A* rs8192678 GA/AA genotype showed increased susceptibility for NASH, which seemed to be independent of the *PNPLA3* rs738409 GG genotype. The combination of the *PPARGC1A* rs8192678 GA/AA genotype and the *PNPLA3* rs738409 GG genotype increased the risk of NASH (OR 6.83; 95% CI 1.61–29.01;  $P = 0.009$ ).

Reported associations between the *PPARGC1A* rs8192678 variant and ultrasound-defined NAFLD have been inconsistent. Hui et al<sup>14</sup> reported no association between the *PPARGC1A* rs8192678 variant and ultrasound-defined NAFLD in Chinese adults. In contrast, Lin et al<sup>15</sup> suggested an association between the *PPARGC1A* rs8192678 variant and ultrasound-defined NAFLD in obese Taiwanese children. These contradictory conclusions may be due to the different ultrasonographic

**TABLE 3.** Clinical and Biochemical Characteristics Stratified by *PPARGC1A* rs8192678 Genotypes or *PNPLA3* rs738409 Genotypes

	<i>PPARGC1A</i> rs8192678			<i>P</i>	<i>PNPLA3</i> rs738409			<i>P</i>
	GG (n = 59)	GA (n = 88)	AA (n = 30)		CC (n = 77)	CG (n = 73)	GG (n = 27)	
Age (SD), years, mean	29.8 (9.6)	29.7 (8.7)	33.1 (12.3)	0.226	29.5 (9.8)	32.1 (10.4)	27.8 (6.5)	0.921
Male, n (%)	20 (33.9)	26 (29.5)	10 (33.3)	0.836	24 (31.2)	22 (30.1)	10 (37.0)	0.799
BMI (SD), kg/m <sup>2</sup>	40.6 (7.9)	42.5 (7.4)	42.6 (7.0)	0.292	41.6 (7.7)	41.1 (7.2)	44.7 (7.5)	0.177
Glucose (SD), mg/dL	108.3 (34.9)	101.3 (26.9)	112.2 (34.6)	0.175	98.7 (17.9)	105.6 (34.0)	124.4 (44.5)	<0.001
Insulin (SD), μU/mL	24.7 (20.6)	29.5 (25.1)	19.9 (11.6)	0.093	27.3 (22.4)	24.2 (20.4)	29.2 (25.5)	0.9827
HOMA-IR	6.3 (5.3)	7.3 (6.0)	5.2 (2.8)	0.159	6.5 (5.0)	6.1 (5.1)	8.2 (6.9)	0.3602
AST (SD), IU/L	41.3 (33.0)	46.7 (42.0)	51.3 (38.1)	0.488	38.8 (31.8)	38.0 (21.1)	86.1 (62.0)	<0.001
ALT (SD), IU/L	56.3 (50.7)	68.4 (70.4)	70.4 (47.4)	0.426	50.8 (43.6)	54.7 (39.8)	131.6 (98.1)	<0.001
GGT (SD), IU/L	47.6 (58.8)	48.6 (43.6)	40.9 (24.2)	0.730	43.0 (45.9)	44.5 (30.2)	65.0 (75.4)	0.074
Triglycerides (SD), mg/dL	158.6 (126.2)	143.6 (95.6)	150.2 (68.8)	0.688	156.0 (109.6)	147.2 (102.6)	138.7 (84.6)	0.426
Total cholesterol (SD), mg/dL	191.6 (35.6)	198.6 (43.2)	203.5 (31.5)	0.351	194.9 (38.9)	197.4 (41.6)	202.4 (32.4)	0.400
HDL-cholesterol (SD), mg/dL	45.0 (10.0)	48.0 (11.7)	46.8 (9.6)	0.249	47.1 (11.0)	47.3 (11.6)	44.6 (8.2)	0.430
LDL-cholesterol (SD), mg/dL	127.6 (31.6)	133.0 (39.7)	138.9 (34.2)	0.372	128.7 (34.2)	133.1 (40.6)	139.8 (28.4)	0.166
Uric acid (SD), mg/dL	7.1 (1.7)	6.7 (1.8)	7.0 (1.9)	0.385	6.8 (1.7)	6.8 (1.8)	7.2 (2.1)	0.335
Hypertension, n (%)	33 (55.9)	55 (62.5)	20 (66.7)	0.570	46 (59.7)	47 (64.4)	15 (55.6)	0.691
Diabetes mellitus, n (%)	10 (16.9)	7 (8.0)	7 (23.3)	0.068	7 (9.1)	10 (13.7)	7 (25.9)	0.089

Continuous variables were presented as means and SD and categorical variables were presented as percentages.

One-way analysis of variance was used to compare continuous variables and the chi-square test or Fisher exact test was used to compare categorical variables.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, GGT = gamma glutamyl transpeptidase, HDL = high-density lipoprotein, HOMA-IR = homeostasis model assessment of insulin resistance, LDL = low-density lipoprotein, SD = standard deviation.

**TABLE 4.** Histologic Features in Patients With Non-NAFLD, Steatosis, and NASH

Histology variables	Non-NAFLD (n = 29)	Steatosis (n = 57)	NASH (n = 91)	<i>P</i>
Steatosis grade, n (%)				<0.001
0—<5%	29 (100.0)	0 (0.0)	0 (0.0)	
1—5%–33%	0 (0.0)	34 (59.6)	12 (13.2)	
2—>33%–66%	0 (0.0)	21 (36.8)	56 (61.5)	
3—>66%	0 (0.0)	2 (3.5)	23 (25.3)	
Lobular inflammation, n (%)				<0.001
0—none	1 (3.4)	4 (7.0)	0 (0.0)	
1—<2 foci	25 (86.2)	35 (61.4)	29 (31.9)	
2—2–4 foci	3 (10.3)	17 (29.8)	46 (50.5)	
3—>4 foci	0 (0.0)	1 (1.8)	16 (17.6)	
Ballooning, n (%)				<0.001
0—none	29 (100.0)	57 (100.0)	0 (0.0)	
1—few	0 (0.0)	0 (0.0)	55 (60.4)	
2—many	0 (0.0)	0 (0.0)	36 (39.6)	
Chronic portal inflammation, n (%)				0.335
0—none	18 (62.1)	36 (63.2)	43 (47.3)	
1—mild	10 (34.5)	19 (33.3)	44 (48.4)	
2—> mild	1 (3.4)	2 (3.5)	4 (4.4)	
Fibrosis, n (%)				<0.001
0—none	29 (100.0)	49 (86.0)	56 (61.5)	
1	0 (0.0)	7 (12.3)	24 (26.4)	
2	0 (0.0)	1 (1.8)	9 (9.9)	
3	0 (0.0)	0 (0.0)	2 (2.2)	
4	0 (0.0)	0 (0.0)	0 (0.0)	

The Fisher exact test was used to compare categorical variables.

NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis.

**TABLE 5.** PPARGC1A rs8192678 and PNPLA3 rs738409 Genotypes Associated With Histologic Severity

	Steatosis Grade			Lobular Inflammation			Ballooning			Chronic Portal Inflammation			Fibrosis		
	>33%	≤33%	P	≥2 Foci	<2 Foci	P	≥Few	None	P	≥Mild	None	P	2–4	0–1	P
<i>PPARGC1A</i> rs8192678															
GG	27 (26.5)	32 (42.7)		24 (28.9)	35 (37.2)		23 (25.3)	36 (41.9)		30 (37.5)	29 (29.9)		5 (41.7)	54 (32.7)	
GA	53 (52.0)	35 (46.7)		45 (54.2)	43 (45.7)		50 (54.9)	38 (44.2)		40 (50.0)	48 (49.5)		4 (33.3)	84 (50.9)	
AA	22 (21.6)	8 (10.7)	0.037	14 (16.9)	16 (17.0)	0.475	18 (19.8)	12 (14.0)	0.062	10 (12.5)	20 (20.6)	0.294	3 (25.0)	27 (16.4)	0.438
GG	27 (26.5)	32 (42.7)		24 (28.9)	35 (37.2)		23 (25.3)	36 (41.9)		30 (37.5)	29 (29.9)		5 (41.7)	54 (32.7)	
GA/AA	75 (73.5)	43 (57.3)	0.036	59 (71.1)	59 (62.8)	0.266	68 (74.7)	50 (58.1)	0.025	50 (62.5)	68 (70.1)	0.337	7 (58.3)	111 (67.3)	0.538
<i>PNPLA3</i> rs738409															
CC	38 (37.3)	39 (52.0)		34 (41.0)	43 (45.7)		34 (37.4)	43 (50.0)		39 (48.8)	38 (39.2)		7 (58.3)	70 (42.4)	
CG	42 (41.2)	31 (41.3)		30 (36.1)	43 (45.7)		36 (39.6)	37 (43.0)		23 (28.8)	50 (51.5)		2 (16.7)	71 (43.0)	
GG	22 (21.6)	5 (6.7)	0.012	19 (22.9)	8 (8.5)	0.029	21 (23.1)	6 (7.0)	0.009	18 (22.5)	9 (9.3)	0.003	3 (25.0)	24 (14.5)	0.133
CC/CG	80 (78.4)	70 (93.3)		64 (77.1)	86 (91.5)		70 (76.9)	80 (93.0)		62 (77.5)	88 (90.7)		9 (75.0)	141 (85.5)	
GG	22 (21.6)	5 (6.7)	0.006	19 (22.9)	8 (8.5)	0.011	21 (23.1)	6 (7.0)	0.003	18 (22.5)	9 (9.3)	0.020	3 (25.0)	24 (14.5)	0.397

The Fisher exact test was used to compare categorical variables.

criteria for the definition of NAFLD and the age ranges of subjects in these studies.<sup>15</sup> In the current study, we reported the first association study of *PPARGC1A* rs8192678 variant with the presence of NASH by using the diagnostic gold standard for NASH, liver histopathology, to avoid the potential image bias in severely obese Asian patients.

Proximosome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) encoded by the *PPARGC1A* gene is a transcriptional coactivator of the nuclear receptor PPAR $\gamma$  that exerts powerful effects on hepatic glucose and fat metabolism and may be involved in the development of NAFLD.<sup>21</sup> The role of PGC-1 $\alpha$  in protecting against hepatic steatosis has been found in both in vivo and in vitro studies.<sup>21,22</sup> In addition, Chen et al<sup>22</sup> also reported in an in vivo study that the *PPARGC1A* rs8192678 mutation can impair the activities of

PGC-1 $\alpha$  in decreasing fat deposition in cultured hepatocytes and can result in steatosis.

Mitochondrial dysfunction that promotes reactive oxygen species production and lipid peroxidation plays a key role in the pathophysiology of NASH.<sup>23</sup> PGC-1 $\alpha$  orchestrates mitochondrial biogenesis and functional capacity, and abnormal PGC-1 $\alpha$  activities may result in mitochondrial dysfunction and thereby may lead to the development of NASH.<sup>24</sup> However, although the *PPARGC1A* rs8192678 mutation may affect the activities of PGC-1 $\alpha$ , further studies are needed to elucidate whether the *PPARGC1A* rs8192678 mutation contributes to the development of NASH.

Moreover, we also found an additive effect of the *PPARGC1A* rs8192678 variant and the *PNPLA3* rs738409 variant on NASH in severely obese patients. The mechanism by which the *PNPLA3* rs738409 variant results in steatosis was hypothesized to involve promoting triglyceride accumulation by limiting triglyceride hydrolysis.<sup>25–29</sup> Although the mechanism by which the *PNPLA3* rs738409 variant results in NASH is still elusive, the additive effect of these 2 SNPs on NASH noted in the current study suggests that there might be different mechanisms for the *PPARGC1A* rs8192678 and *PNPLA3* rs738409 variants on the susceptibility of NASH.

Significant associations between the *GCKR* rs780094 variant and NAFLD were found in several studies, including a meta-analysis.<sup>30</sup> However, studies about the association between the *GCKR* rs780094 variant and NASH are scarce and inconsistent. Although Tan et al<sup>11</sup> reported a significant association between the *GCKR* rs780094 variant and NASH, Gorden et al<sup>31</sup> reported that the *GCKR* rs780094 variant did not increase the risk for NASH in morbidly obese patients. In agreement with a previous study,<sup>31</sup> we also found that the *GCKR* rs780094 variant was not associated with NASH in severely obese Taiwanese patients. However, further studies that enroll more patients from diverse ethnic groups are needed to confirm this finding.

The novelty of the present study is that we used biopsy-proven NASH to confirm that the *PPARGC1A* rs8192678 variant is associated with NASH. However, our study has some limitations. First, there might exist selection bias in the current

**TABLE 6.** The *PPARGC1A* rs8192678 Variant and *PNPLA3* rs738409 Variant Predict the Risk of NASH in NAFLD Patients

	NASH vs Steatosis	
	Adjusted OR (95% CI)	P
<i>PPARGC1A</i> rs8192678 genotype		
GA/AA vs GG	2.32 (1.08–4.98)	0.031
<i>PNPLA3</i> rs738409 genotype		
GG vs CC/CG	4.05 (1.24–13.23)	0.021
Age	1.03 (0.99–1.07)	0.186
Sex, male	1.05 (0.48–2.28)	0.909
BMI, kg/m <sup>2</sup>	1.06 (1.01–1.12)	0.024
Log (HOMA-IR)	1.38 (0.81–2.34)	0.236
Log triglycerides, mg/dL	2.11 (0.98–4.56)	0.058

Adjusted ORs and their P values were estimated using a multiple logistic regression model.

BMI = body mass index, HOMA-IR = homeostasis model assessment of insulin resistance, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio.

**TABLE 7.** Interaction Between the *PPARGC1A* rs8192678 Variant and the *PNPLA3* rs738409 Variant on NASH in NAFLD Patients

<i>PPARGC1A</i> rs8192678	<i>PNPLA3</i> rs738409	NASH, n (%)	Steatosis, n (%)	Adjusted OR (95% CI)	<i>P</i>
GG	CC/CG	16 (17.6)	24 (42.1)	1.00	
GA/AA	CC/CG	54 (59.3)	29 (50.9)	2.72 (1.21–6.09)	0.015
GG	GG	7 (7.7)	1 (1.8)	8.50 (0.88–81.88)	0.064
GA/AA	GG	14 (15.4)	3 (5.3)	6.83 (1.61–29.01)	0.009

Adjusted ORs and their *P* values were estimated after adjustment for age, sex, body mass index, log (homeostasis model assessment of insulin resistance), and log (triglycerides) using a multiple logistic regression model.

CI = confidence interval, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio.

study, because we enrolled only patients undergoing bariatric surgery, and overall they were young and predominantly female. Further studies are needed to elucidate whether our findings can be applied either to the general population and specific ethnic populations. Second, the association between NASH and the *PPARGC1A* rs8192678 AA genotype was only marginally significant (OR 2.45; 95% CI 0.89–6.69; *P* = 0.082). This could result from a limited number of patients with the *PPARGC1A* rs8192678 AA genotype. Therefore, further studies with larger sample sizes are needed to clarify the issue.

In conclusion, we reported that the *PPARGC1A* rs8192678 GA/AA genotype and the *PNPLA3* rs738409 GG genotype had an additive effect on NASH in severely obese Taiwanese patients.

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