A common variant in the peroxisome proliferator–activated receptor-γ coactivator-1α gene is associated with nonalcoholic fatty liver disease in obese children¹–³

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ABSTRACT

Background: The single nucleotide polymorphism in the peroxisome proliferator–activated receptor-γ coactivator (PGC)-1α gene (PPARGC1A) was identified to be associated with nonalcoholic fatty liver disease (NAFLD) in adults. The PPARGC1A gene encodes PGC-1α, which regulates cellular energy metabolism.

Objective: We aimed to test the hypothesis that the PPARGC1A rs8192678 risk A allele would influence the risk of NAFLD in obese children.

Design: We genotyped PPARGC1A rs8192678 and PNPLA3 rs738409 in 781 obese children aged 7–18 y. NAFLD was determined by ultrasonography. We evaluated the independent influence of the PPARGC1A rs8192678 risk A allele on pediatric NAFLD after control for the effect of the PNPLA3 rs738409 polymorphism.

Results: A total of 23.3% of the recruited obese children had NAFLD. PNPLA3 rs738409 increased the OR of NAFLD by 1.622 (95% CI: 1.071, 2.457; \( P = 0.023 \)) in subjects with GC alleles and 2.659 (95% CI: 1.509, 4.686; \( P < 0.001 \)) for GG alleles, as compared with CC alleles. After control for the effects of age- and sex-adjusted BMI, sex, and PNPLA3 rs738409 polymorphism, the PPARGC1A rs8192678 risk A allele was an independent risk factor for developing NAFLD, with an OR of 1.740 (95% CI: 1.149, 2.637; \( P = 0.009 \)). Subjects with the PPARGC1A rs8192678 risk A allele had an increase in the mean serum alanine aminotransferase concentration of 5.2 IU/L, as compared with subjects without this allele (\( P = 0.011 \)).

Conclusion: The PPARGC1A rs8192678 risk A allele is associated with an increased risk of NAFLD, independent of the effect of the PNPLA3 rs738409 polymorphism in our population of obese Taiwanese children. This project was registered at clinicaltrials.gov as NCT00274183.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD)⁴ represents a spectrum of conditions ranging from simple hepatic steatosis to potentially fatal nonalcoholic steatohepatitis and cirrhosis (1). Concurrent with the epidemic of childhood obesity, pediatric NAFLD is a growing global problem (2).

The pathogenesis of NAFLD is multifactorial; it is strongly associated with obesity and insulin resistance (3, 4). Not all obese children develop NAFLD, which suggests that environmental and genetic factors influence the susceptibility in each obese individual. Recently, the rs738409 (G/C) single nucleotide polymorphism (SNP) in PNPLA3, causing Ile148Met, has been identified as a strong genetic determinant of liver fat (5). We recently showed that the PNPLA3 rs738409 SNP was strongly associated with NAFLD in obese Taiwanese children (6).

Several other genes have been reported to contribute to the NAFLD susceptibility or progression (7–11). The peroxisome proliferator–activated receptor-γ coactivator (PGC)-1α gene (PPARGC1A) is an interesting candidate. The protein PGC-1α, encoded by the PPARGC1A gene, regulates mitochondrial biogenesis and function, oxidative stress, gluconeogenesis, and lipogenesis, all of which are key factors in the development of NAFLD (12). The most frequently studied PPARGC1A polymorphism is Gly482Ser (Ref SNP ID: rs8192678). This SNP has been associated with several human diseases, including type 2 diabetes, hypertension, and obesity (13–15). Recent evidence also indicates that the PPARGC1A sequence variant increased NAFLD susceptibility in adults (16).

Herein we tested the hypothesis that the PPARGC1A rs8192678 risk A allele is associated with NAFLD in obese children. Because PNPLA3 rs738409 SNP is known to confer the susceptibility to NAFLD, we assessed the independent influence of the PPARGC1A rs8192678 risk A allele on pediatric NAFLD after controlling for the effect of PNPLA3 rs738409 SNP.

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²Supported by research grants from Far Eastern Memorial Hospital (FEMH-99-C-026, FEMH-2011-C-002, and FEMH-2012-C-013) and the National Science Council, Taiwan (NSC-98-2628-B-002-006-MY3).
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⁴Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease; PGC, peroxisome proliferator–activated receptor-γ coactivator; SNP, single nucleotide polymorphism.

Received July 6, 2012. Accepted for publication November 12, 2012.
First published online December 26, 2012; doi: 10.3945/ajcn.112.046417.
SUBJECTS AND METHODS

Subjects
From October 2006 to June 2011, 781 obese children aged from 7 to 18 y were enrolled from public schools in Taipei, Taiwan. Obesity was defined as a BMI greater than the 95th percentile by different age and sex groups according to the standards of the Department of Health in Taiwan (17). All of the participants’ parents gave their informed consent, and the study was approved by the ethics committee of the Far Eastern Memorial Hospital and National Taiwan University Hospital.

To learn the population frequency of PPARGC1A rs8192678 SNP in Taiwan, we randomly selected 120 DNA samples from a study cohort of exclusively breastfed newborns in Far Eastern Memorial Hospital as the “healthy control” group (18).

Data collection
The following data were obtained for each subject: age, sex, BMI, and waist and hip circumferences. BMI was calculated as body weight (kg)/height (m)^2. The ratio between waist and hip circumferences was calculated and referred to as the waist-to-hip ratio.

In fasting venous blood samples, we measured total serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), y-glutamyltransferase, fasting glucose, triglycerides, total cholesterol, and HDL. Insulin concentrations were measured by using a chemiluminescence immunoassay (Diagnostic Products Corporation). Insulin resistance was measured by HOMA-IR and was calculated as follows: HOMA-IR = [fasting insulin (µU/mL) × fasting glucose (mmol/L)/22.5] (19).

Liver ultrasonography
All participants underwent an ultrasonographic study of the liver performed by one operator. The hand-carried machine used (TITAN; SonoSite Ltd) was equipped with a 2–5-MHz convex probe.

NAFLD was defined as the presence of an ultrasonographic pattern consistent with the following criteria: liver-kidney echo discrepancy, attenuated echo penetration and visibility of diaphragm, and obscure hepatic vessel structures. The aforementioned ultrasonographic pattern was scored as described by Chan et al (20). The child was considered to have mild, moderate, or severe steatosis if the overall score was 1–3, 4–6, or 7–9, respectively. Because ultrasonography is much more accurate for the detection of moderate to severe steatosis than for mild steatosis (21), a score ≥4 was used as the diagnostic criterion for pediatric NAFLD in this study.

Genotype extraction
Genomic DNA was extracted from venous blood from each participant by using a commercial kit (Puregene; Gentra Systems) according to the manufacturer’s instructions. After extraction, the genomic DNA was immediately stored at -80°C.

The TaqMan SNP genotyping assays C_7241_10 for PNPLA3 rs738409 and C_1643192_20 for PPARGC1A rs8192678 SNPs (Applied Biosystems) were performed on ABI 7300 Real-Time PCR System (Applied Biosystems).

Statistical analysis
Statistical analysis was performed by using SAS software (version 9.1.3; SAS Institute Inc). A 2-sided P value ≤0.05 was considered statistically significant. Standardized BMI z scores were not available for children in Taiwan. To adjust for the intrinsic effects of age and sex on BMI, we derived the variable “adjusted BMI” by subtracting the Taiwanese children’s age- and sex-specific population median value of BMI from each subject’s BMI value.

In a univariate analysis, the chi-square test and Student’s t test were used to compare categorical and continuous variables. Next, a multivariate analysis was conducted to identify predictive factors of pediatric NAFLD and serum ALT concentrations by fitting multiple logistic and linear regression models, respectively. To ensure the quality of the analytic results, we used standard model-fitting techniques for variable selection, goodness-of-fit assessment, and regression diagnostics in our regression analyses. The best final regression model was identified manually by reducing the significance levels to 0.05 corresponding to the chosen a level.

RESULTS

Subject characteristics
The basic characteristics of the study subjects are summarized in Table 1. The study subjects were divided into 2 groups: 532 subjects carrying the risk A allele (combined AA and AG genotypes) and 249 noncarriers (GG genotype, the major allele homozygote). We found statistically significant differences in AST, ALT, insulin concentration, and HOMA-IR. The mean serum ALT concentration was 28.2 ± 31.1 IU/L in subjects carrying the risk A allele compared with 22.8 ± 22.2 IU/L in noncarriers (P = 0.006). The prevalence of an elevated ALT concentration, defined as >30 IU/L in males and >19 IU/L in females (22), was 31.3% in subjects carrying the risk A allele compared with 23.4% in noncarriers (P = 0.023). The mean HOMA-IR concentration was 3.97 ± 5.44 in subjects carrying the risk A allele compared with 3.27 ± 2.79 in noncarriers (P = 0.019).

Similarly, we found statistically significant differences in serum ALT, AST, and y-glutamyltransferase concentrations among the 3 PNPLA3 rs738409 genotypes (data not shown). The mean serum ALT concentration was 33.7 IU/L in those with the GG genotype (highest), 27.7 IU/L in those with the GC genotype, and 22.1 IU/L in those with the CC genotype (lowest) (P < 0.001).

Genotype distribution of PPARGC1A rs8192678 and PNPLA3 rs738409
Among the 781 enrolled obese subjects, 182 (23.3%) had an ultrasonographic score ≥4 and thus received a diagnosis of pediatric NAFLD. The genotype frequencies of PPARGC1A rs8192678 and PNPLA3 rs738409 between the NAFLD and non-NAFLD groups are shown in Table 2. The percentage of PPARGC1A rs8192678 AG genotype was significantly higher.
To investigate whether the variant PPARGC1A allele is a risk factor for obesity in addition to NAFLD, we compared the genotype distribution of PPARGC1A rs8192678 in the healthy control group and our obese subjects as a whole. As mentioned above, the healthy control group was randomly selected from a cohort of exclusively breastfed newborns. The percentage of study subjects carrying the PPARGC1A rs8192678 risk A allele was 68.1% and of the healthy control group was 65%. No difference in the genotype distribution was found ($P = 0.497$), which suggests that the positive association between NAFLD and the variant PPARGC1A allele in our study was not confounded by obesity.

### Multivariate analysis of predicting factors for pediatric NAFLD

As shown in Table 3, the fitted final multiple logistic regression model showed that the PPARGC1A rs8192678 risk A allele was an independent risk factor for pediatric NAFLD after control for the effects of sex, adjusted BMI, and PNPLA3 rs738409 polymorphism.

This final multiple logistic regression model was obtained after age, sex, adjusted BMI, waist circumference, HOMA-IR, PNPLA3 rs738409 polymorphism, and the PPARGC1A rs8192678 risk A allele were considered in the stepwise variable selection procedure. The PPARGC1A rs8192678 risk A allele was coded as a categorical variable as follows: 0 = noncarriers (GG genotype, the reference group) and 1 = the subjects carrying the PPARGC1A rs8192678 risk A allele (combined GA and AA genotypes).

Specifically, as compared with the noncarriers, the subjects carrying the PPARGC1A rs8192678 risk A allele would have an OR of 1.740 (95% CI: 1.149, 2.637; $P = 0.009$) times that of those with pediatric NAFLD, whereas the values of the other covariates were held fixed. The goodness-of-fit measures and tests listed below Table 3 indicate that the final multiple logistic regression model fitted the observed binary data well.

### Table 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NAFLD (n = 182)</th>
<th>Non-NAFLD (n = 599)</th>
<th>$P^2$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARGC1A rs8192678$^1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>44 (24.2)</td>
<td>205 (34.2)</td>
<td>0.008</td>
<td>1.699 (1.149, 2.515)</td>
</tr>
<tr>
<td>AG</td>
<td>108 (59.3)</td>
<td>296 (49.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>30 (16.5)</td>
<td>98 (16.4)</td>
<td>0.182</td>
<td>1.426 (0.848, 2.398)</td>
</tr>
<tr>
<td>PNPLA3 rs738409$^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>54 (29.7)</td>
<td>237 (39.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>93 (51.1)</td>
<td>288 (48.1)</td>
<td>0.069</td>
<td>1.417 (0.973, 2.063)</td>
</tr>
<tr>
<td>GG</td>
<td>35 (19.2)</td>
<td>74 (12.5)</td>
<td>0.004</td>
<td>2.076 (1.264, 3.412)</td>
</tr>
</tbody>
</table>

1. NAFLD, nonalcoholic fatty liver disease.
2. As compared with the reference genotype group, the differences in genotype frequencies were analyzed by using a chi-square test. $P < 0.05$ indicates significance.
3. The PNPLA3 rs738409 SNP has GG, GC, and CC genotypes, and the CC genotype is the wild type.
4. The PPARGC1A rs8192678 SNP has AA, AG, and GG genotypes, and the GG genotype is the wild type.
PPARGC1A IN PEDIATRIC NAFLD

Multiple logistic regression analysis of predictive factors for pediatric NAFLD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>SE</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.833</td>
<td>0.224</td>
<td>2.301 (1.483, 3.570)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted BMI</td>
<td>0.244</td>
<td>0.027</td>
<td>1.276 (1.210, 1.346)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PNPLA3 rs738409 GC genotype</td>
<td>0.484</td>
<td>0.212</td>
<td>1.622 (1.071, 2.457)</td>
<td>0.023</td>
</tr>
<tr>
<td>PNPLA3 rs738409 GG genotype</td>
<td>0.978</td>
<td>0.289</td>
<td>2.659 (1.509, 4.686)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carrier of the PPARGC1A rs8192678 risk A allele</td>
<td>0.554</td>
<td>0.212</td>
<td>1.740 (1.149, 2.637)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

1 Logistic regression model: n = 778, percentage of concordant pairs = 76.9%, percentage of discordant pairs = 22.8%, adjusted generalized $R^2 = 0.231$, deviance goodness-of-fit test $P = 0.943 > 0.05$ (df = 703), Pearson goodness-of-fit test $P = 0.897 > 0.05$ (df = 703), and Hosmer-Lemeshow goodness-of-fit test $P = 0.296 > 0.05$ (df = 8). NAFLD, nonalcoholic fatty liver disease.

2 Adjusted BMI = (BMI of the case) – (age- and sex-specific median value of BMI in Taiwan).

3 The PNPLA3 rs738409 SNP has GG, GC, and CC genotypes, and the CC genotype is the wild type and was considered the reference group.

4 Subjects with PPARGC1A rs8192678 AA or AG genotypes were referred to as carriers of the PPARGC1A rs8192678 risk A allele. The GG genotype was the wild type and was considered the reference group.

DISCUSSION

The PPARGC1A rs8192678 risk A allele confers a genetic susceptibility to NAFLD independent of the effect of PNPLA3 rs738409 polymorphism (Table 4). The mean ALT concentration of the subjects carrying the PPARGC1A rs8192678 risk A allele was higher than that of noncarriers by 5.2 IU/L ($P = 0.011$).

Similarly, the fitted final linear regression model showed a significant association between the PPARGC1A rs8192678 risk A allele and serum ALT concentration after control for the effect of the PNPLA3 rs738409 polymorphism (Table 4). The mean ALT concentration of the subjects carrying the PPARGC1A rs8192678 risk A allele was higher than that of noncarriers by 5.2 IU/L ($P = 0.011$).

TABLE 4
Multiple linear regression analysis of predictive factors for serum ALT concentration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient estimate</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7.073</td>
<td>2.168</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>0.527</td>
<td>0.096</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>0.162</td>
<td>0.031</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>0.056</td>
<td>0.021</td>
<td>0.009</td>
</tr>
<tr>
<td>PNPLA3 rs738409 GC genotype</td>
<td>6.451</td>
<td>2.096</td>
<td>0.002</td>
</tr>
<tr>
<td>PNPLA3 rs738409 GG genotype</td>
<td>13.834</td>
<td>3.045</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carrying PPARGC1A rs8192678 risk A allele</td>
<td>5.243</td>
<td>2.070</td>
<td>0.011</td>
</tr>
</tbody>
</table>

1 Linear regression model: $n = 772$, $R^2 = 0.142$. ALT, alanine aminotransferase.

2 The PNPLA3 rs738409 SNP has GG, GC, and CC genotypes, and the CC genotype is the wild type and was considered the reference group.

3 Subjects with the PPARGC1A rs8192678 AA or AG genotype were referred to as carriers of the PPARGC1A rs8192678 risk A allele. The GG genotype was the wild type and was considered the reference group.
nonsignificance could be due to sample variation and inadequate statistical power. Second, the cellular effects of PGC-1α are not simple. The exact mechanisms by which PGC-1α influences the pathogenesis of fatty liver remain unclear. PGC-1α may be involved in different pathways that either increase or reduce fat accumulation. In this case, it is possible that the effect of homozygous mutants may not be higher than that of heterozygotes.

We recognize the lack of identification of the PPARGCA rs8192678 SNP in genome-wide association studies evaluating the risk of hepatic steatosis (5, 11). It is expected because of different characteristics of the study population, such as those that follow. First, we studied obese children and adolescents, instead of general population, used in previous genome-wide association studies. The effect of PPARGCA may be more evident in the obese population. Second, this study was conducted in the Han Chinese population. According to the National Center for Biotechnology Information human SNP database, the frequency of the PPARGCA rs8192678 A allele is 0.453 in Han Chinese, 0.350 in Europeans, and 0.040 in Africans (32). From this perspective, we estimate that the PPARGCA rs8192678 risk A allele will confer a higher genetic susceptibility to NAFLD in Asians than in Europeans and Africans.

The PPARGCA rs8192678 SNP is a known risk factor for obesity and insulin resistance (29, 31). To control confounding, we adopted 2 approaches. First, we selected 120 newborns as healthy controls and found no difference in genotype frequencies in our obese subjects and controls. Second, the rs8192678 risk A allele remains an independent predictor after control for BMI in the multivariate analysis. Although HOMA-IR is statistically different in the univariate analysis, it is no longer significant in the fitted regression model. Even if HOMA-IR is forced in the model, the rs8192678 risk A allele remains significant (P = 0.011). Therefore, it is unlikely that this issue would bias our positive findings.

Our diagnostic modality for NAFLD was liver ultrasonography. A recent meta-analysis has shown that the diagnostic accuracy and reliability of ultrasonography for the detection of moderate to severe fatty liver are fairly good (21). In addition, we selected a relatively strict ultrasonographic score of ≥4 as the cutoff for pediatric NAFLD. Thus, some individuals with mild NAFLD might be categorized as non-NAFLD. Such categorization would generally decrease the power to detect differences rather than bias toward a significant difference.

To evaluate the influence of different ultrasonographic criteria of NAFLD, we used several different cutoff values to validate our positive association results. Our findings remained the same when a high cutoff (≥7) was used. In contrast, no difference was found when a low cutoff (≥1) was used. Because ultrasonography underestimates hepatic steatosis when there is <20% fat (38), we believe that the lack of association with NAFLD defined by a low cutoff does not invalidate the inferences reported in our study.

In conclusion, we showed that the PPARGCA rs8192678 risk A allele significantly increased the risk of pediatric NAFLD independent of the effect of PNPLA3 rs738409 polymorphism in our population of obese Taiwanese children. This finding has important implications, because the PPARGCA rs8192678 polymorphism is common in the general population, and PGC-1α is central to the regulation of metabolic homeostasis in the liver. Future studies should examine the PPARGCA rs8192678 polymorphism among other ethnic groups to confirm the current findings.

We are indebted to Kevin Liu for his laboratory work, to Li-Chin Fan and Ro-Lan Han for their help with data collection, and to Fu-Chang Hu for his excellent assistance with the statistical analysis. The authors’ responsibilities were as follows—Y-CL and Y-HN: designed and conducted the research; Y-CL and P-FC: analyzed the data; Y-CL, M-HC, and Y-HN: wrote the manuscript; and Y-HN: had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript. None of the authors had a conflict of interest.

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