Genetic Variation in the NOC Gene Is Associated with Body Mass Index in Chinese Subjects

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Abstract

Circadian clock genes are critical regulators of energy homeostasis and metabolism. However, whether variation in the circadian genes is associated with metabolic phenotypes in humans remains to be explored. In this study, we systematically genotyped 20 tag single nucleotide polymorphisms (SNPs) in 8 candidate genes involved in circadian clock, including CLOCK, BMAL1, PER1, PER2, CRY1, CRY2, CSNK1E, and NOC(CCRN4L) in 1,510 non-diabetic Chinese subjects in Taipei and Yunlin populations in Taiwan. Their associations with metabolic phenotypes were analyzed. We found that genetic variation in the NOC gene, rs9684900 was associated with body mass index (BMI) (P=0.0016, Bonferroni corrected P=0.032). Another variant, rs135764 in the CSNK1E gene was associated with fasting glucose (P=0.0023, Bonferroni corrected P=0.046). These associations were consistent in both Taipei and Yunlin populations. Significant epistatic and joint effects between SNPs on BMI and related phenotypes were observed. Furthermore, NOC mRNA levels in human abdominal adipose tissue were significantly increased in obese subjects compared to non-obese controls.

Conclusion: Genetic variation in the NOC gene is associated with BMI in Chinese subjects.

Introduction

The rotation of the earth around its axis generates the dark and light cycles, and organism living on earth developed circadian rhythms to adapt their activities to the dark and light cycles. In mammals, the central clock is located in the suprachiasmatic nucleus (SCN). The SCN clock is composed of single-cell neuronal oscillators that generate a coordinated output, which controls the systemic circadian rhythm. SCN neurons respond to external stimuli such as light or nutrients by adjusting their oscillatory activity [1–3]. In addition to external stimuli, the central circadian rhythm is also controlled by a intrinsic transcriptional auto-regulatory feedback loop. This feedback loop is composed of the transcriptional activators CLOCK and BMAL1 and their target genes PERs and CRYs. The CLOCK gene encodes a transcription factor, CLOCK, which dimerizes with BMAL1 to activate downstream genes, including PERs and CRYs [1–3]. PERs and CRYs oligomerize and translocate to the nucleus to inhibit CLOCK-BMAL1-mediated transactivation, thereby forming a negatively feedback loop. This autoregulatory loop is post-translationally regulated by the casein kinases, which target the PER proteins for degradation [1–3]. Another important circadian gene is the NOC gene, which encodes a deadenylase, nocturnin, that removes the 3’ adenosine residue from the mRNA transcripts of target genes at a post-transcriptional level to regulate gene function [4].

Several clinical studies have shown association between disturbed circadian rhythm and adverse metabolic consequence [5,6]. Furthermore, mouse models with a targeted ablation of circadian genes also displayed abnormal energy metabolism. For example, Clock mutant mice developed hyperphagia, obesity, hyperlipidemia, and hyperglycemia [7]. Per2 knockout mice showed an alteration in lipid metabolism, with a decrease in levels of triglyceride and free fatty acid [8]. Nocturnin knockout mice exhibited a resistance to diet-induced obesity, decreased triglycerides levels and improved insulin sensitivity [9]. These data indicate a critical role of circadian genes in energy homeostasis, glucose, and lipid metabolism.

In this present study, we systematically analyzed the relationship between variations in circadian genes and metabolic phenotypes in general populations in Taiwan. We found that genetic variation in the NOC gene was associated with body mass index (BMI). NOC...
expression in adipose tissue was increased in obese subjects. These data suggested a potential role for NOC in obesity.

**Results**

The clinical and biochemical characteristics of study participants are summarized in Table 1. The nucleotide composition, chromosomal position, and minor allele frequencies for all genotyped SNP are summarized in Table 2. On average, the genotype call rate was 97.6%. All SNP were in Hardy-Weinberg equilibrium. The LD patterns between SNPs are shown in Figure 1.

**SNP association with metabolic phenotypes**

Single-locus SNP associations with metabolic phenotypes are summarized in Table 3. Among the 20 genotyped SNPs, the A allele at rs9684900 in the NOC gene was associated with higher BMI ($P=0.0016$, Bonferroni adjusted $P=0.032$) (Table 4). The estimated effect size associated with each A allele was 0.46 kg/m². This direction associations are consistent in both Taipei and Yunlin populations ($P=0.0045$ and $P=0.091$, respectively) (Table S1). We also observed nominal associations of rs17050679 in the NOC gene with BMI ($P=0.0057$, Bonferroni corrected $P=0.074$). The directions of association with BMI ($P=0.021$ in Taipei and $P=0.074$ in Yunlin population) and fasting plasma triglycerides ($P=0.026$ in Taipei and $P=0.046$ in Yunlin population) are also consistent in both study populations (Table S1).

Another variant, the A allele at rs135764 in the CSNK1E gene was significantly associated with fasting plasma glucose ($P=0.0023$, Bonferroni corrected $P=0.096$) (Table 5) with concordant directions of association in Taipei and Yunlin populations ($P=0.058$ and $P=0.021$, respectively) (Table S1).

**The effect of multi-locus interaction between SNPs on metabolic phenotypes**

We next evaluated the effect of interaction between SNPs on metabolic phenotypes. Results of the multilocus interaction analyses using GMDR are summarized in Table 6. The most significant interactions with a good cross-validation consistency (CVC) were found between NOC rs17050679 and NOC rs9684900 ($P<0.001$, CVC: 8/10) for BMI and between NOC rs9684900 and BMAL1 rs2290035 ($P=0.001$, CVC: 7/10) for fasting glucose level.

**Joint effects of risk alleles on metabolic phenotypes**

We further evaluated the joint effects of risk alleles with nominal association ($P<0.1$) with metabolic phenotypes. The distribution of
BMI, blood pressures, fasting triglycerides, and fasting glucose according to different number of risk alleles is shown in Figure 2. Increasing risk alleles were significantly associated with higher BMI ($P = 0.0001$), systolic blood pressure (SBP) ($P = 0.0041$), DBP ($P = 0.031$), fasting triglycerides ($P = 0.0033$) and fasting glucose levels ($P = 0.00027$).

**NOC expression in adipose tissue between obese and non-obese subjects**

Nocturnin was previously reported to be a regulator of adipogenesis highly expressed in adipocytes [10]. We next explored whether NOC expression in adipose tissue was associated with obesity. We found that NOC expression was higher in obese subjects in either subcutaneous or visceral adipose tissue ($P < 0.001$) (Figure 3).

**Discussion**

In this present study, we systemically analyzed the association of tag SNPs in 8 circadian genes with metabolic phenotypes in 1,510 otherwise healthy Taiwanese individuals. We found that variation in the NOC gene was associated with BMI. Interestingly, NOC expression in abdominal adipose tissue was positively associated with obesity. Consistent with our findings, nocturnin has been shown to regulate adipogenesis by stimulating nuclear translocation of PPARγ [11] or by modulating mitotic clonal expansion during early adipogenesis [10]. Importantly, Nocturnin knockout mice were protected from diet-induced obesity [9]. These findings

### Table 1. Characteristics of study participants.

|                  | Taipei participants | Yunlin participants | Total   |
|------------------|---------------------|---------------------|---------|
| Number           | 760                 | 750                 | 1,510   |
| Age              | 64.52 (13.64)       | 47.51 (12.39)       | 56.06 (15.56) |
| Male sex (%)     | 56.6 (%)            | 44.53 (%)           | 50.06%  |
| Obesity (%)      | 12.8 (%)            | 26.28 (%)           | 19.4%   |
| Body mass index (kg/m2) | 23.64 (3.14)      | 24.89 (3.89)        | 24.25 (3.58) |
| Systolic blood pressure (mmHg) | 127.62 (16.98)   | 128.25 (16.93)      | 127.9 (16.95) |
| Diastolic blood pressure (mmHg) | 76.15 (10.1)      | 84.25 (10.94)       | 80.1 (11.27) |
| Fasting serum triglyceride (mg/dL) | 112.3 (63.83)   | 119.58 (90.24)      | 115.9 (78.14) |
| Fasting plasma glucose (mg/dL) | 96.84 (13.11)     | 90.93 (20.26)       | 93.90 (17.28) |

Data are presented as mean (S.D.) or percentage.

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### Table 2. SNP information.

| Gene  | SNP name  | Chr. | Position(kb) | Major/minor Allele | Minor allele frequency | HW $P$-value |
|-------|-----------|------|--------------|---------------------|------------------------|--------------|
| PER2  | rs2304676 | 2    | 238844643    | C/T                 | 0.23                   | 0.71         |
| PER2  | rs11892306| 2    | 238853208    | G/T                 | 0.32                   | 1            |
| CLOCK | rs3736544 | 4    | 56004739     | G/A                 | 0.35                   | 0.74         |
| CLOCK | rs12504300| 4    | 56043274     | C/G                 | 0.41                   | 0.53         |
| NOC   | rs9684900 | 4    | 140160847    | G/A                 | 0.27                   | 0.48         |
| NOC   | rs17050679| 4    | 140165667    | G/C                 | 0.43                   | 0.27         |
| NOC   | rs1112828 | 4    | 140176410    | T/G                 | 0.4                    | 0.35         |
| BMAL1 | rs6486120 | 11   | 13280708     | T/G                 | 0.48                   | 0.76         |
| BMAL1 | rs7396943 | 11   | 13285545     | C/G                 | 0.4                    | 0.28         |
| BMAL1 | rs11022769| 11   | 13308971     | A/C                 | 0.45                   | 0.68         |
| BMAL1 | rs2278749 | 11   | 13354444     | G/A                 | 0.14                   | 0.45         |
| BMAL1 | rs2290035 | 11   | 13364337     | T/A                 | 0.25                   | 0.94         |
| CRY2  | rs4756034 | 11   | 45832521     | A/G                 | 0.39                   | 0.6          |
| CRY2  | rs7945565 | 11   | 45835558     | A/G                 | 0.26                   | 0.43         |
| CRY2  | rs17787136| 11   | 45851202     | C/G                 | 0.12                   | 0.8          |
| CRY1  | rs11829762| 12   | 105912668    | C/A                 | 0.22                   | 0.83         |
| CRY1  | rs11113181| 12   | 105992381    | A/G                 | 0.3                    | 0.95         |
| PER1  | rs2304911 | 17   | 7991694      | T/C                 | 0.22                   | 0.32         |
| CSNK1E | rs135764 | 22   | 37040348     | G/A                 | 0.16                   | 0.85         |

HW $P$-values, empirical $P$-values of the $\chi^2$ test for Hardy-Weinberg equilibrium.

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dependent degradation. In yeasts, glucose-dependent activation of variant.
that the functional causative variant is in LD with this associated
gene influences phenotype is currently not known. It is most likely

| SNP       | Gene | Estimate | P   | Estimated | P   | Estimated | P   | Estimated | P   |
|-----------|------|----------|-----|-----------|-----|-----------|-----|-----------|-----|
| rs934945  | PER2 | -0.004   | 0.52| 0.0069    | 0.19| 0.0007    | 0.91| -0.025    | 0.26|
| rs2304676 | PER2 | 0.0056   | 0.38| 0.012     | 0.036| 0.0557    | 0.35| 0.02      | 0.39|
| rs11892306| PER2 | -0.0013  | 0.82| 0.00066   | 0.9 | 0.0064    | 0.25| 0.0081    | 0.7 |
| rs3736544 | CLOCK| -0.002   | 0.76| -0.0036   | 0.52| 0.0037    | 0.53| 0.011     | 0.66|
| rs12504300| CLOCK| -0.0332  | 0.56| -0.007    | 0.14| -0.0022   | 0.67| -0.0019   | 0.92|
| rs9684900 | NOC  | 0.019    | 0.0016| 0.001     | 0.85| -0.00054  | 0.93| 0.042     | 0.055|
| rs17050679| NOC  | -0.015   | 0.0057| 0.0057   | 0.22| -0.0074   | 0.15| -0.056    | 0.0037|
| rs1112828 | NOC  | 0.0045   | 0.4  | -0.0027   | 0.56| -0.0061   | 0.24| 0.043     | 0.029|
| rs6486120 | BMAL1| 0.005    | 0.35| 0.006     | 0.2 | 0.0056    | 0.27| 0.016     | 0.41|
| rs7396943 | BMAL1| 0.011    | 0.047| 0.008     | 0.93| 0.0082    | 0.12| 0.0074    | 0.71|
| rs11022769| BMAL1| -0.0005  | 0.92| -0.00703  | 0.13| -0.0062   | 0.24| 0.0049    | 0.81|
| rs2278749 | BMAL1| 0.01     | 0.17| 0.0054    | 0.42| 0.0022    | 0.76| 0.052     | 0.067|
| rs2290035 | BMAL1| -0.0024  | 0.0049| 0.36     | 0.003 | 0.003    | 0.62| 0.023     | 0.31|
| rs4756034 | CRY2  | -0.0038  | 0.56| 0.0046    | 0.41| 0.0088    | 0.13| 0.022     | 0.35|
| rs7945565 | CRY2  | -0.0076  | 0.2  | 0.0034    | 0.51| 0.0015    | 0.8 | -0.002    | 0.93|
| rs17787136| CRY2  | 0.0043   | 0.61| -0.0012   | 0.87| 0.0096    | 0.24| 0.0347    | 0.26|
| rs11829762| CRY1  | -0.01    | 0.036| -0.007    | 0.21| -0.0085   | 0.16| -0.0185   | 0.43|
| rs1113181 | CRY1  | 0.014    | 0.014| 0.0069    | 0.17| 0.007     | 0.21| 0.0022    | 0.92|
| rs2304911 | PER1  | -0.0043  | 0.5  | 0.0119    | 0.031| 0.013    | 0.031| 0.017     | 0.47|
| rs135764  | CSNK1E| 0.01     | 0.18| 0.004     | 0.53| 0.0057    | 0.42| -0.013    | 0.64|

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TG: triglycerides; Bold indicates P<0.05. Data are presented as mean (S.D.).

indicate nocturnin is an important regulator of adiposity. The molecular mechanism by which an intronic variant in the NOC gene influences phenotype is currently not known. It is most likely that the functional causative variant is in LD with this associated variant.

We also found a significant association between CSNK1E genetic variant and fasting glucose. The CSNK1E encodes for the casin kinase 1 epsilon, a serine/threonine kinase that phosphorylates PER proteins in the cytoplasm and triggers their proteasome-dependent degradation. In yeasts, glucose-dependent activation of casin kinase I catalyzes phosphorylation of Mth1, a glucose transporter gene (HXT) repressor, triggering degradation of Mth1 by the proteasome and leads to de-repression of HXT gene expression [12,13]. These data suggest a potential role of casin kinase in glucose homeostasis.

Interestingly, we also found that an interaction between NOC and BMAL1 genetic variants may affect fasting glucose levels. Loss of Bmal1 in mice has been shown to cause hyperglycemia due to impairment in β-cells function while deletion of NOC in mice causes higher fasting glucose. Although no main effect of BMAL1 and NOC genetic variants on fasting glucose was observed, fasting glucose were significantly elevated in the presence of both genetic

| Phenotypes | GG | GA | AA | P  |
|------------|----|----|----|----|
| BMI (kg/m2)| 24.01| 24.48| 24.93| 0.0016|
| SBP (mmHg)| 128.06| 127.45| 129.92| 0.85|
| DBP (mmHg)| 80.24| 79.95| 80.81| 0.93|
| Fasting TG (mg/dL)| 113.02| 112.44| 112.27| 0.055|
| Fasting glucose (mg/dL)| 93.58| 94.09| 90.64| 0.38|

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TG: triglycerides; Bold indicates P<0.05. Data are presented as mean (S.D.).

Table 5. Metabolic phenotypes according to CSNK1E rs135764 genotypes.

| Phenotypes | GG | GA | AA | P  |
|------------|----|----|----|----|
| BMI (kg/m2)| 24.16| 24.49| 24.54| 0.0023|
| SBP (mmHg)| 127.81| 128.17| 127.45| 0.53|
| DBP (mmHg)| 80.01| 80.39| 81.47| 0.42|
| Fasting TG (mg/dL)| 116.02| 113.59| 133.63| 0.64|
| Fasting glucose (mg/dL)| 93.25| 94.22| 99.66| 0.0023|

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TG: triglycerides; Bold indicates P<0.05. Data are presented as mean (S.D.).

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variants. These data indicate that a clinically evident phenotype may require a close interaction between 2 or more genetic factors. However, our results were not consistent with a previous genome-wide association analysis reporting an association between CRY2 variant and fasting glucose [14]. In our study, all tag SNPs in the CRY2 gene were not associated with fasting plasma glucose. Our results are also not consistent with previous candidate-gene association studies reporting significant associations between CLOCK genetic variation and obesity [15–17]/metabolic syndrome [18], PER2 genetic variation and obesity [19]/fasting plasma glucose [20], and BMAL1 genetic variation and type 2 diabetes/hypertension [21]. Nevertheless, since we did not genotype the same SNPs (or tagSNPs) with previous studies, a direct comparison between studies is not possible. Heterogeneity between different ethnic groups may also confound the association.

The major limitation of this study is the lack of replication. However, the directions of association between NOC variant and BMI and between CSNK1E variant and fasting glucose are the same in both Taipei and Yunlin populations, indicating consistency in different populations. Further study is still warranted to confirm the association.

In conclusion, we systematically analyzed the association of tag SNPs in circadian genes with metabolic phenotypes. We identified associations of NOC genetic variation with BMI. A strong correlation of NOC expression in human adipose tissue with obesity was also observed. These data, together with previous animal models, suggest a substantial role of nocturnin in obesity.

### Materials and Methods

#### Subjects

The studied population comprised 760 subjects recruited from the Health Management Center of National Taiwan University Hospital (NTUH) in Taipei and 750 subjects recruited from a community-based screening program in the Yunlin County in southern Taiwan. The detailed description of study subjects have been described previously [22,23]. Patients with a history of

### Table 6. Multi-locus interaction on metabolic phenotypes.

| Traits                  | # Loci | Polymorphism in model | Cross-validation consistency | P*  |
|-------------------------|--------|-----------------------|------------------------------|-----|
| Body mass index         | 2      | NOC rs17050679, NOC rs9684900 | 8/10                         | <0.001 |
| Diastolic blood pressure| 3      | PER2 rs11892306, PER1 rs2304911, CK1 rs2075983 | 5/10                      | 0.003  |
| Fasting triglycerides   | 2      | BMAL1 rs2278749, CRY2 rs7945565 | 5/10                      | 0.012  |
| Fasting glucose         | 2      | NOC rs9684900, BMAL1 rs2290035 | 7/10                      | 0.001  |

*permutated for 1,000 times and adjusted for age and sex.

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![Figure 2. Distribution of body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP), fasting triglycerides, and fasting plasma glucose according to number of risk alleles.](doi:10.1371/journal.pone.0069622.g002)
Genotyping based on this platform was 99.62%. The raw genotype accompanying SNPstream software suite. The concordance rate of genotyping platform (Beckman Coulter, Brea CA, USA) and its Genotyping was performed using the GenomeLab SNPstream platform between obese and non-obese subjects. SNP genotyping tissue between obese and non-obese subjects.

Subjects for measurement of gene expression in adipose tissue

We recruited 60 non-diabetic subjects undergoing bariatric surgery or elective abdominal surgery such as cholecystectomy or partial hepatectomy at the Ming-Sheng General Hospital and the Yuulin branch of NTUH in Taiwan. Abdominal subcutaneous adipose tissues were sampled from patients in a fasting state prior to surgery and were immediately placed in liquid nitrogen until processing. The study was approved by the National Taiwan University Hospital Research Ethics Committee. Written informed consent was obtained from each participant.

SNP genotyping

The Tagger program implemented in Haploview version 4.0 software (http://www.broad.mit.edu/mpg/haploview/) [24] was used to select tag SNPs (with a minor allele frequency threshold of 0.1) from the HapMap Chinese Beijing (CHB) databank (Rel 24/ phase II). In total, 20 tag SNPs were selected to capture the genetic variation of 8 genes involved in circadian clock, including CLOCK (clock circadian regulator, Gene ID: 9575), BMAL1 (ARNTL, aryl hydrocarbon receptor nuclear translocator-like, Gene ID: 406) PER1 (period circadian clock 1, Gene ID: 5187), PER2 (period circadian clock 2, Gene ID: 8364), CRC (cryptochrome 1, Gene ID: 1407), CRC2 (cryptochrome 2, Gene ID: 1408), CNNK1E (casein kinase 1, epsilon, Gene ID: 1454), and NOC (CGRN4L, CCR4 carbon catabolite repression 4-like, Gene ID: 25819). These 20 tag SNPs capture average 71.4% of all SNPs in these 8 genes with a minor allele frequency greater than 0.1 at $\chi^2$ of 0.7. Genotyping was performed using the GenomeLab SNPStream genotyping platform (Beckman Coulter, Brea CA, USA) and its accompanying SNPStream software suite. The concordance rate of genotyping based on this platform was 99.62%. The raw genotype and phenotype data were deposited as Data S1.

Reverse transcription and quantitative real-time PCR

Total RNA was isolated using RNeasy© C&T Reagent (Promega, Taipei, Taiwan) and reverse transcribed with Superscript III kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. PCR amplification was performed using LightCycler FastStart DNA master Plus SYBR (Roche, Mannheim, Germany). Each sample was analyzed in duplicate and the gene expression was normalized to that of the PPIA (cyclophilin A), a housekeeping gene. The primers used for NOC were PPH23949A (SA BioScience, Frederick, MD, USA). The primers used for PPLA (cyclophilin A) were forward: 5′-GCATACGGGTCCTGG-CATCTTGTC-3′ and reverse: 5′-ATGGT-GATCTTTCTTGTGGTCTGGTC-3′, respectively. The correlation ($R^2$) between Ct value and log cDNA input was 0.99 for NOC primers and 0.99 for PPLA primers (Figure S1A&S1B). The efficiency of primers for NOC and PPLA are 0.99 and 0.93, respectively. The slope between delta Ct (Ct of PPLA minus Ct of NOC) and log cDNA input was 17.1% (Figure S1C). There is no difference of Ct of PPLA between obese and non-obese subjects in either subcutaneous or visceral fat (Figure S1D).

Statistical analyses

Data that were not normally distributed were logarithmically transformed to approximate normal distribution. A Hardy-Weinberg equilibrium (HWE) test was performed for each sequence variant before marker-trait association analysis was estimated by using the Haplovie softare. Linear regression with an additive genetic model was used to analyze SNP association with quantitative traits. Inter-marker linkage disequilibrium (LD) was measured by pairwise $D'$ and $\chi^2$. Multi-locus interaction between SNPs on metabolic phenotypes was analyzed using the Generalized Multifactor Dimensionality Reduction (GMDR) computing package (http://www.ssg.uab.edu/gmdr/) with adjustment for age and sex. GMDR is a model-free combinatorial approach for detecting multi-locus interactions [25]. The combinations of all factors were classified into high- or low-risk groups based on the score-based statistic. The best n-factor model that yielded a minimum misclassification error was chosen ($n = 1, 2, 3$ in this study). The significance of the best n-factor model was assessed based on 1,000 permutations. To test for cumulative effects of genetic variants on the individual phenotypes, a subset of SNPs that had nominal associations ($P < 0.1$) with metabolic phenotypes were collected as a risk-SNP set. Consequently, an individual can carry the number of risk alleles ranging from 0 to 2 times of the total number of risk SNPs. For instance, one individual can carry 0 up to 10 risk alleles for BMI, 0 up to 6 risk alleles for SBP or DBP, 0 up to 8 risk alleles for fasting serum triglycerides, and 0 up to 8 risk alleles for fasting plasma glucose. Subjects were then divided into 4, 5 or 6 groups based on the number of risk alleles for each phenotype. An ordinal variable coded as 1–4 for the 4 groups (or 1–5 for the 5 groups; 1–6 for 6 groups) was used as a covariate for testing the trend effect from the cumulative risk alleles on the individual phenotype using a linear regression with adjustment for age and sex.

Supporting Information

Figure S1 Correlation between Ct value and log (cDNA input) for (A) NOC primers and (B) PPLA primers. (C) slope (dotted line) between delta Ct (Ct of PPLA minus Ct of NOC) and log (cDNA input) (D) Ct of PPLA (internal control) in subcutaneous and visceral fat in obese and non-obese subjects. (TIF)
Table S1  SNP association with metabolic phenotypes according to study populations.

Data S1  (XLSX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: YCC YFC LMC. Performed the experiments: YCC HYK SWH. Analyzed the data: PHL. Contributed reagents/materials/analysis tools: TJC YDJ WJL PCL JJH. Wrote the paper: YCC LMC.

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