Comprehensive evaluation of the neuropeptide-y gene variants in risk of obesity: A case-control study and meta-analysis

Short title: Meta-analysis of NPY variants and obesity

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Abstract

**Background** Orexigenic actions mediated by neuropeptide-y (NPY) promote body weight regulation. Genetic variations in the *NPY* gene could therefore influence susceptibility to obesity, but results have been conflicting. In this study, we performed for the first time a case-control study to examine the effect of *NPY* rs16147 and rs5574 variants with obesity risk in Asians, and also a meta-analysis to summarize the effect of these variants including that of the widely studied rs16139.

**Material and methods** Genotypes and biochemistry data were determined for 942 children (262 cases and 680 controls) recruited from 23 randomly selected schools in Malaysia. Relevant articles were identified from Pubmed, Embase, Web of Science and Google Scholar. Data were extracted and summary estimates of the association between the *NPY* variants and obesity were examined.

**Results** The frequency of rs16147 T-allele was significantly higher in the cases than controls (OR 1.27, 95% CI 1.04-1.55, P = 0.022), while rs5574 T-allele was significantly higher in the controls (OR 0.76, 95% CI 0.61-0.96, P = 0.020). In addition, *NPY* rs16147 was significantly correlated with obesity parameters including BMI, waist circumference, triglyceride and body fat percentage (P < 0.05). Meta-analysis including nine case-control studies further confirmed the findings of the association of the two variants with obesity risk and also found that rs16139 was associated with increased risk.

**Conclusion** This study suggests that *NPY* rs16147 T and rs16139 C minor alleles are associated with increased risk, while minor allele T of the rs5574 is associated with reduced risk of obesity.

**Keywords:** BMI, genetic variation, neuropeptide y, NPY, obesity, polymorphism, SNP
**Introduction**

Obesity poses a serious socioeconomic and health-related burden worldwide [1]. It is an exaggeration of normal adiposity and may cause a broad range of health problems such as diabetes mellitus, hypertension and cardiovascular disease [2-4]. These health problems have been traditionally considered to affect the adults, but it is now also found to affect younger age groups as a result of the increasing incidence of childhood obesity [5]. Genetic factor explains 40-70% of the inter-individual variability in body mass index (BMI), the most commonly used measure for obesity [6-7]. In a recent large-scale genome-wide association study (GWAS) that involved more than 330,000 individuals, about 500 gene sets were significantly enriched for genes in BMI-associated loci, one of which is neuropeptide-y (NPY) gene that is mapped to the 7p15.1 chromosome in humans [8].

Neuropeptide-y, a peptide derived from both the brain and sympathetic nerves, exerts its orexigenic activity following a carbohydrate-rich food intake [9]. This activity is mediated through NPY receptors. In the animal model of feeding behaviour, NPY reduces the latency to eat, increases motivation to eat and delays satiety [10]. Conversely, the absence of NPY through the knockdown of the NPY receptors promotes anti-adipogenic, hence reducing abdominal obesity [11]. NPY locus has also been discovered to be associated with non-alcoholic fatty liver disease (NAFLD), a hepatic manifestation of the metabolic syndrome [12]. Epidemiological studies have described the positive relationship between genetic variants of NPY and obesity [13-14].

Much attention has been given to investigate the role of the functional NPY rs16139 (Leu7Pro) variant in obesity and obesity-related parameters. This variant has also been linked to several conditions such as diabetes and cardiovascular disease [15], as well as with progression of carotid atherosclerosis, increased blood pressure and raised serum lipids [16]. Other important NPY variants that have been studied and could be functional are rs16147 and
rs5574. The rs16147 is located on the promoter region, whereas rs5574 is located on exon 3 of the NPY gene. Both variants have been associated with early-onset atherosclerosis [17] and antipsychotic-induced weight gain in schizophrenia patients [18]. Additionally, NPY rs16147 is reported to be associated with metabolic syndrome [19], ischemic stroke [20], tobacco smoking and anxiety [21]. The association of the two variants with obesity is evidenced in several reports [14, 22-23]. However, there are no data on the Asian population.

The aim of this study is to perform a case-control study to investigate the relationship between the two variants (rs16147 and rs5574) of the NPY gene and obesity risk. In addition, we aimed to conduct a meta-analysis to quantitatively assess the effect of both variants on the risk of obesity. The meta-analysis will also include the widely studied rs16139 variant. To our knowledge, a meta-analysis evaluating the relationships between these NPY variants and obesity has not been previously conducted, and results of such evaluations could provide new insights into the relationships.

Methods

Case-control

Study population

From December 2011 until July 2012, the Cohort of Pediatric Obesity Working Research Group (CO-POWR) initiated a longitudinal study that aimed to investigate the epidemiology of paediatric obesity and cardiometabolic risks among children in Kuala Lumpur, Malaysia. The cohort recruited 1113 apparently healthy children aged 13 years, selected via multi-stage sampling from 23 randomly selected national secondary schools in the urban setting of Kuala Lumpur. Ethics clearance was obtained from the Medical Ethics Committee of the University of Malaya Medical Centre (UMMC) (Reference Number: MEC 896.123). Written
approval to conduct the study was obtained from the Ministry of Education, the Federal Territory of Kuala Lumpur Education Department and the respective school principals. Participants were provided with a briefing by the clinicians involved and an information sheet detailing the study. Written informed consent was obtained from the parents or legal guardians prior to participation in the study.

Anthropometric and biochemical measurement

The anthropometric measurements were taken by trained enumerators following a standard protocol. Body weight was measured in light clothing and with shoes removed, to the nearest 0.01 kg using a digital calibrated floor scale (SECA 813, Hamburg, Germany) and the weight of the clothing subtracted from the observed weight. Height was measured without shoes to the nearest 0.1 cm with a portable stadiometer (SECA 813, Hamburg, Germany). BMI was calculated as baseline weight divided by the squared height (kg/m^2) and was converted to standardised z-scores, adjusted for gender and age based on the International Obesity Task Force (IOTF) cut-off points [24]. Participants were categorized as overweight when BMI lies between 85th to 95th percentile for age and gender and as obese when BMI > 95th percentile for age and gender. Waist circumference (WC) was measured using an inelastic measuring tape to the nearest 0.1 cm at the umbilicus, between the tenth rib and the iliac crest as per the WHO STEPS protocol [25]. Waist circumference was classified according to waist circumference percentile values for age and sex for Malaysian children [26]. Blood pressure was obtained three times by the same examiner with participants sitting in a chair with arm resting at the heart level. Blood samples were drawn and the biochemical parameters such as glucose, insulin, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, triglyceride, aspartate transaminase (AST) enzyme, alanine
aminotransferase (ALT) enzyme, and C-reactive protein were determined according to standard clinical laboratory methods carried out in an accredited laboratory at UMMC.

Measures of body fatness

Body fat percentage was measured using the validated portable body composition analyzer (Inbody 230, Biospace Co. Ltd, Korea) as per instruction manual.

Genotyping

Genomic DNA was extracted using the QiAamp DNA Mini Kit (Qiagen, Hilden, German) and the quality was checked to meet absorbance ratios of ≥ 1.8 for 260/280 and 260/230. The sample DNA was diluted to 10 ng/µL and 20 ng/µL for the duplicate sample and placed in the well. Each amplification reaction contained 1µL of DNA. The NPY rs16147 and rs5574 were genotyped at the University of Hong Kong, Genome Research Centre using the Sequenom MassARRAY technology platform with the iPLEX GOLD chemistry (Sequenom, San Diego, CA) according to the manufacturer’s protocols. Briefly, the specific assays with proximal variants filtering were designed using MassARRAY AssayDesign software package (v4.0). The quality of the PCR fragment amplification and extension primer specificity was checked prior to running the reaction. Residual nucleotides were dephosphorylated prior to the iPLEX Gold reaction. Following a single-base extension, reaction products were desalted with SpectroClean resin (Sequenom, San Diego, CA) and 10 nL was spotted onto the SpectroCHIP using the MassARRAY Nanodispenser. MassARRAY Analyzer Compact MALDI-TOF mass spectrometer was used to determine the mass. For proper data acquisition and analysis, the MassARRAY Typer 4.0 software was used. Genotypes were called after cluster analysis using the default setting of Gaussian mixture model. Cluster inspection was
done to ensure a clear cluster separation with good signal to noise cut-off, followed by a manual review to further clarify uncertain genotype calls. Assay with less than 80% call rate within the same SpectroChip was considered to have failed. Quality controls included a blank and five duplicates. SpectroChip with more than 25% call rate in the blank control or with less than 99.5% concordance in duplicate checks along with more than 10% call rate in blank check were considered to have failed and would be required to be repeated.

Statistical analysis

For the case-control study, genotype distribution was assessed for Hardy–Weinberg equilibrium (HWE) using chi-square ($\chi^2$) test. A P-value of more than 0.05 indicates an agreement with HWE. Prior to analysis, all variables were tested for normality using Kolmogorov-Smirnov test. The demographic data between cases and controls were compared using $\chi^2$ test. The clinical data between groups were assessed by Analysis of Covariance (ANCOVA) using the general linear model. Comparisons of clinical data among genotypes were analysed using general linear model with gender and ethnicity as covariates. Genotype was coded with 0, 1, or 2 corresponding to the number of minor alleles carried by each individual. The association between the variants and obesity risk was evaluated using logistic regression derived from log-additive genetic model. Linear regression for log-transformed variables, where appropriate, was performed to evaluate the correlation between the variant and obesity parameters. Statistical analyses were performed using SPSS 22.0 (Chicago, IL) with a two-sided P < 0.05 considered as statistically significant. Linkage disequilibrium analysis was performed using Haploview 4.2 program.
Meta-analysis

Search strategy

A comprehensive search of medical databases including Pubmed, Embase, Web of Science and Google Scholar was conducted to identify relevant studies with the last update being on March 25, 2015. The search terms used were according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement [27] with the following keyword combinations: “(neuropeptide y or npy) and (snps or polymorphisms or genetic variants) and (obesity or BMI)”. References of retrieved articles, including review articles related to this topic were also screened for potential articles that possibly have been missed in the initial search. The search was also expanded to different terminology used for the variants investigated: (rs16147 or -399T>C) and (rs5574) and (rs16139 or +1128T>C or Leu7Pro).

Inclusion and exclusion criteria

Only published articles with the following inclusion criteria were included in this study: (i) full text published in English (ii) obesity as the outcome of the study; (iii) evaluated the association between NPY rs16147/rs5574/rs16139 with obesity; (iv) case-control study, and; (v) provided sufficient data with an odds ratio (OR) and 95% confidence intervals (CIs) under an additive model or at least allowed for calculation of this estimate. Studies that were not in accordance with these criteria were excluded.
Data extraction and quality score assessment

Data from eligible articles were extracted in duplicate by two independent authors using standard protocol. The same two authors reviewed the titles and abstracts of potential articles to gather valuable information. Full texts were retrieved when the relevance of the articles could not be determined by the title or abstract alone. In the event of conflict, disagreements were resolved through discussion among all authors, and a consensus decision was reached. Corresponding authors of studies were contacted for missing data. Included studies were also subjected to quality assessment by the same two authors. The quality of observational studies which included three aspects (selection, comparability and exposure) was evaluated by Newcastle-Ottawa Scale (NOS) [28]. The NOS score ranges from 0-9, in which studies with a score of ≥ 7 were considered to be of high quality. Details of the PRISMA criteria used are available in Supplemental Digital Content 1 and 2, which provide the PRISMA checklist and flow diagram of the included studies.

Statistical analysis

The primary outcome of this meta-analysis was to assess the pooled ORs with 95% CIs to determine the strength of association. Allele frequencies were determined using the allele counting method, otherwise provided in the studies. Hardy-Weinberg equilibrium (HWE) was checked for each study using $\chi^2$-test and deviations were those with $P < 0.05$. The following outcomes of interest were assessed from the OR of each individual study: additive (B vs. A), dominant (AB + BB vs. AA), recessive (BB vs. AA + AB), homozygote (BB vs. AA) and the heterozygote (AB vs. AA) genetic models of inheritance. Mantel-Haenszel test was performed to estimate the pooled ORs and corresponding 95% CIs by assuming either fixed or random effect meta-analysis, where appropriate. Visual inspection of the forest plot
was first carried out, followed by $\chi^2$-test of heterogeneity (a test of significance for heterogeneity) and inconsistency index $I^2$ (for the magnitude of heterogeneity). Heterogeneity was deemed when $\chi^2$-test gave a $P < 0.10$ and $I^2$ values > 50%. A fixed effect model was used in the presence of little or no heterogeneity, otherwise random effect model was adopted [29]. Begg’s funnel plot asymmetry and Egger’s correlation tests were used to evaluate the risk of publication bias [30-31]. Subgroup analysis of different covariates was performed in consideration of the influence of the covariates. The influence of each study in the pooled estimates was investigated in a sensitivity test. Meta-analysis was conducted using the Review Manager (RevMan 5.3) of the Cochrane Collaboration.

**Results**

**Case-control**

Subject characteristics

For the purpose of this study, 942 participants (28% male, 74% of Malay ethnic, 13% Chinese, 10% Indian and 3% others) were available for blood sampling and were considered eligible for inclusion into the study. In summary, 262 participants were classified as cases (overweight plus obese) and 680 as controls. The baseline characteristics of the study participants are shown in Table 1. Significantly higher levels of anthropometric parameters (weight, height, BMI, BMI z-score, and waist circumference) as well as other metabolic and clinical parameters (LDL cholesterol, triglyceride, insulin, body fat percentage, systolic and diastolic blood pressure, ALT, and C-reactive protein) were observed in the cases compared to controls. HDL cholesterol levels were found to be significantly lower in the cases.
Association of NPY variants with obesity and obesity-related parameters

Table 2 shows the association analysis of NPY variants and obesity risk. Genotype distributions for each variant followed HWE. The frequency of rs16147 T allele was significantly higher in the overweight plus the obese group when compared to the C allele (P = 0.04) and remained significant following adjustment for gender and ethnicity (adjusted OR 1.46, 95% CI 1.02-2.07, P = 0.036). In contrast, the T allele frequency for rs5574 was significantly higher in the controls (adjusted OR 0.63, 95% CI 0.46-0.86, P = 0.02). Both variants were in strong linkage disequilibrium (D’ = 0.97). We further evaluated the association of the variants with obesity-related parameters (Table 3). We found that rs16147 homozygous TT was associated with increased levels of BMI, waist circumference, triglyceride and body fat percentage (P < 0.05), however, we observed no significant association of rs5574 with these parameters. To investigate the correlation between the rs16147 and these associated parameters, we performed linear regression analyses of log-transformed variables. Analyses showed positive significant correlations between rs16147 T allele and obesity parameters (P < 0.05), indicating an increased level of obesity parameters in those harbouring the T allele. These results thus support and suggest the significant impact of the NPY variants with obesity.

Meta-analysis

Study characteristics

The study selection procedure is detailed in Figure 1. Overall, 84 articles met the search terms, of which 27 were considered potentially relevant and underwent a careful examination. After reading the full texts, 18 articles were subsequently excluded due to reasons presented in Figure 1. Finally, four studies investigating the rs16147 [14, 22-23], two studies
investigating the rs5574 [14], and six studies investigating the rs16139 [13-14, 32-35] were incorporated in the meta-analysis.

Table 4 shows the characteristics of the studies. In the present study and eight other articles published between 1998 and 2015, the study size ranged from 263 to 3,339, with a total of 8,895 participants. The majority of the studies was carried out in Caucasians and involved adults [13-14, 22, 33-35], while there were only two studies in Asians [32] and involved children [23]. All of the studies scored well on quality assessment that includes selection criteria, comparability of cases and controls in terms of design and analysis, and availability of genetic data except for Mutschler et al. [22] which was of moderate quality (Supplemental Digital Content 3, which demonstrates assessment score for study quality).

Quantitative synthesis

rs16147

Four case-control studies [14, 22-23] from nine reports, involving a total of 5,706 subjects (1,021 cases and 4,685 controls) evaluated the association between rs16147 and obesity. Fixed effect models were assumed in the analyses due to the absence of significant heterogeneity as assessed by Q statistic (P > 0.10) and $I^2$ values (< 50%) for all genetic models. Meta-analysis of the pooled studies revealed a significant risk of obesity associated with rs16147 (OR 1.20, 95% CI 1.08-1.33, P = 0.0005) (Figure 2). Likewise, the association was seen in the pooled estimates when assuming alternative genetic models (Table 5) except in the heterozygote. After stratification by ethnicity, significant associations were observed in Caucasians (OR 1.18, 95% CI 1.05-1.33, P = 0.006) [14, 22-23] and Asians (OR 1.26, 95% CI 1.03-1.55, P = 0.02) with no difference in ORs being observed. This association was also
significant in the children (OR 1.31, 95% CI 1.12-1.53, P = 0.007) [23] but not in adults [14, 22]. The association was also not affected when we analysed according to the NOS score.

\textit{rs5574}

There were only two studies [14] that investigated the association between rs5574 and obesity, with a total of 4,125 subjects (626 cases and 3,499 controls). Fixed effect models were assumed for all genetic models. Unlike rs16147, the minor allele T of the rs5574 was associated with reduced risk of obesity (OR 0.84, 95% CI 0.74-0.96, P = 0.008) (Figure 2). This significant association was replicated in all other genetic models except for the recessive model (Table 5).

\textit{rs16139}

Meta-analysis on the relationship between rs16139 and obesity risk included six independent studies [13-14, 32-35] containing data from 6,148 subjects (1,367 cases and 4,781 controls). We adopted fixed effect models in additive, dominant and heterozygote models. The recessive and homozygote models were not applied in the analysis due to the rare presence of a Pro-allele in the populations that would not allow estimation of the pooled. Associations with risk of obesity were seen in the additive (OR 1.32, 95% CI 1.08-1.60, P = 0.006), dominant (OR 1.36, 95% CI 1.10-1.67, P = 0.004) and the heterozygote (OR 1.36, 95% CI 1.10-1.68, P = 0.004) models (Figure 2 and Table 5). We also analysed the results in random effect models as the Q statistic and $I^2$ values were moderate, analysis however, confirmed our primary results (P < 0.01). The availability of data from various subgroups has made possible to carry out different comparisons. Results indicated statistically significant association in both genders (Male: OR 2.32, 95% CI 1.33-4.06, P = 0.003, and female: OR 1.58, 95% CI
1.10-2.28, P = 0.01) [13-14] and Caucasians (OR 1.49, 95% CI 1.20-1.86, P = 0.0003) [13-14, 33-35].

Sensitivity analysis

We performed a sensitivity analysis by sequential removal of the included studies in order to assess the stability of the results. A slight quantitative alternation of the estimates was observed when we removed the study by Yeung et al. [14], although it did not affect the homogeneity of the studies. This could be due to the deviation from HWE observed in the study controls of Yeung et al. [14]. This deviation is likely due to the rare existence of the rs16139 risk allele. In addition, the controls have relatively lower risk allele in nature as would have been expected, in contrast to the cases that are enriched for that risk allele.

Publication bias

No evidence of publication bias was observed across studies as indicated by the symmetry visual inspection of the funnel plot for all variants (Supplemental Digital Content 4, which shows funnel plot of each variant under additive model). The subjective visual interpretation of the plot was overcome by the Egger’s test that provided further supportive statistical evidence for the absence of significant publication bias (P > 0.05).

Discussion

This case-control study evaluated the association between NPY gene variants (rs16147 and rs5574) with risk of obesity in children from a multi-school cohort study in Malaysia and found that the rs16147 T-allele contributed to an increased risk of obesity, while the rs5574 T-allele conferred reduced risk. The meta-analysis further explored these relationships, and
by summarizing the evidences from several independent studies, the results confirmed the association of both variants with obesity. In addition to the investigated variants, meta-analysis had also revealed that rs16139 was also associated with increased susceptibility to obesity.

Evidence linking NPY rs16147 and rs5574 with obesity outcome has been demonstrated in several studies, although this has not been extensively studied. Three studies reported the association of rs16147 with obesity [14, 22-23], however the results are incompatible. A study by Olza et al. [23] showed a significant association of rs16147 with obesity, but no significant association was reported by Yeung et al. [14]. Although Mutschler et al. [22] failed to identify significant association of rs16147 with obesity, this variant was however found to be significantly associated with lower WHRs and higher serum leptin levels in women. In our case-control study, we replicated the results of Olza et al. [23] by showing significant association of rs16147 with increased risk of obesity. NPY rs16147 is a functional variant in which the C-allele is associated with a decreased expression level of NPY [36]. As NPY stimulates fat growth, an increase in BMI is therefore expected with homozygosity for T allele, a trend that was significantly observed in this study. Support for the finding that rs16147 as a risk variant for obesity comes from a study by Hohman et al. [37], in which the authors showed that carriers for homozygote C allele exhibit significantly lower BMI scores when compared to non-carriers. This notion is further strengthened by our correlation models in which rs16147 T allele was significantly correlated with increased levels of BMI, body fat percentage, waist circumference and triglyceride. As for rs5574, this variant is significantly associated with reduced risk of obesity and decreased levels of BMI in our study, but did not corroborate with the earlier report by Yeung et al. [14] which found no significant association. In fact, we showed that rs5574 is in strong linkage disequilibrium with rs16147, which was supported by the finding by Yeung et al. [14]. In addition,
haplotype TTCC inferred from four SNP (rs17149106, rs16147, rs16139, and rs5574) appears with twice the risk of obesity when compared to the common haplotype GCTT [14].

Meta-analysis has been regarded as the best tool to summarize the findings from different independent studies into one estimate that could act as a representative value for overall findings and to overcome the limitation in sample size and statistical power [38]. In order to quantitatively examine the potential effect of both variants rs16147 and rs5574 with obesity, we performed a meta-analysis based on several case-control studies. Our pooled estimates showed that rs16147 T allele was significantly associated with increased risk of obesity while significantly reduced risk was observed for rs5574 T allele. In addition, rs16147 was positively associated for dominant, recessive and homozygote models, and in the case of rs5574, it was associated for all genetic models. There also seemed to be a homogenous association of the rs16147 with obesity risk among Caucasian and Asian, with no difference in the observed ORs. Thus, the results of these meta-analyses further supported our case-control findings. This meta-analysis also included the rs16139, a widely studied variant of NPY, and its relationship with various metabolic conditions, including impaired glucose tolerance and type 2 diabetes [39], hypertension [16], as well as BMI changes [13, 33]. We are able to show that this variant is significantly associated with increased risk of obesity. Subgroup analyses also indicated that there was no variation in the results when interpreted in a different set of comparisons, indicating a robust estimate without other contributing factors.

Nonetheless, the findings from the present meta-analysis should be interpreted within the context of several limitations. First, the relatively small number of included studies, particularly for the rs16147 and rs5574 may affect the precision of the overall estimates and subsequently the results of the subgroup comparisons. Second, different studies used different set of BMI range to define the obese group from the controls and thus may affect the true
findings. For example, three studies [14, 22, 33] adopted cases as BMI $\geq$ 30 kg/m$^2$ and controls as BMI $< 30$ kg/m$^2$, three [13, 32, 35] adopted cases as BMI $\geq 25$ kg/m$^2$ and controls as BMI $< 25$ kg/m$^2$, and one [34] adopted cases as BMI $\geq 28$ kg/m$^2$ and controls as BMI $< 27$ kg/m$^2$. Third, most of the studies are from Caucasian ethnic origins, thus the results from the Asian studies would not have truly represented the Asians, given that the effective allele frequency between Asians and Caucasians varies. Fourth, the overall outcomes were estimated based on case-control studies without adjustments. A nested case-control that is matched by age, gender and ethnicity from multi-ethnic prospective studies appear to be an optimal design to assess the relationship between $NPY$ variants and obesity. Fifth, detailed information such as gender is not easily obtainable from published studies therefore limiting the gender comparison. Notwithstanding, our meta-analysis possesses some strengths. The results of this meta-analysis support most of the previously reported studies of significant association between $NPY$ variants and obesity. The homogeneity among studies has enabled the conclusion to be drawn with more confidence. Furthermore, we explored the relationship between these variants and obesity in different genetic models.

In conclusion, we present the first report in Asian population and confirmed the association of $NPY$ rs16147 and rs5574 with obesity. Our meta-analysis further supports the results for these variants and also revealed that a non-synonymous variant rs16139 is associated with increased risk of obesity. To our knowledge, this is the first meta-analysis to provide evidence of association between $NPY$ variants and obesity. Further studies with larger sample size in diverse populations are required to further confirm this association. In light of this finding, it is also important to dissect the role of other variants that are in close proximity to these investigated variants.
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Conflict of interest

The authors declare no conflict of interest.
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**Figure legends**

**Figure 1** Flow chart showing the study selection procedure

**Figure 2** Association analyses between *NPY* variants and obesity. Estimations of odds ratios (OR) and 95% confidence intervals (CI) in each study are displayed as closed squares and horizontal lines, respectively. The size of the black squares reflects the weight of the study in the meta-analysis. The diamond represents the combined OR, calculated using a fixed effect model, with its 95% CI. M-H, Mantel-Haenszel.