Association between patatin-like phospholipase domain-containing 3 gene (PNPLA3) polymorphism and overweight/obesity: a meta-analysis

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Abstract: Background: The patatin-like phospholipase domain-containing 3 (PNPLA3, rs738409) gene polymorphism has been implicated in susceptibility to overweight and obesity, but study results are still controversial. Objective: The present meta-analysis was performed to evaluate the relationship between PNPLA3 polymorphism and overweight/obesity among adults, children or adolescents. Methods: A literature search was conducted in PubMed, Medline, Embase, EBSCO and Web of Science databases up to December 2015. Statistical analysis of qualified studies was performed with Stata 12.0 software. Results: Ten studies comprising 14,220 participants (7,120 cases and 7,100 controls) were included in the analysis. A total of 6,963 patients had the rs738409 GG+CG genotype, whereas 7,257 patients had the CC genotype. No evidence of an association between the rs738409 polymorphism and overweight/obesity risk was observed in incorporated patients (OR = 0.929, 95% CI: 0.863-1.000; P = 0.051). Ethnicity stratification analyses revealed that the G allele (GG+CG) did not show an unfavorable effect compared to CC genotype in Caucasians (OR = 0.960, 95% CI: 0.885-1.042; P = 0.327) and Asians (OR = 0.707, 95% CI: 0.575-0.869; P = 0.001). Also, no significant association was found between the PNPLA3 gene polymorphism and the risk of overweight/obesity when the population was stratified by age (children or adolescents, adults) and non-alcoholic fatty liver disease (NAFLD). Conclusion: Our meta-analysis revealed that the PNPLA3 gene rs738409 polymorphism was not associated with an enhanced risk of overweight/obesity among Caucasians, neither adults nor children or adolescents. This conclusion accredits confirmation by more case-control and cohort studies.

Keywords: Obesity, overweight, PNPLA3, gene polymorphism, meta-analysis

Introduction

Currently, an estimation of at least 300 million people worldwide are obese [1], which poses a global health problem among children and adolescents, caused a broad range of disorders such as diabetes mellitus, hypertension, cardiovascular disease and cancer [2]. Pathogenesis of overweight or obesity is multifactorial and involves the environment, genetic predisposition, and human behavior [3, 4]. The genetic pathways indicate a significant involvement in the occurrence and development of obesity [5, 6]. However, the genetic pathways underlying obesity still remain elusive.

One variant has garnered much research attention in the past few years. Early in 2008, Romeo and colleagues [7] discovered a novel single nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing 3 (PNPLA3, rs738409) localized on human chromosome 22, which was designated the PNPLA3 protein (also known as adiponutrin) [8]. The substitution from cytosine to guanine in rs738409 C>G caused an amino acid change from isoleucine (I) to methionine (M) at residue 148 (I148M) of the protein, the G allele frequently to be adverse allele, was reported to be associated with an increased liver fat content. In addition, the variant polymorphism exhibits lipase activity against triglycerides and acylglycerol transacylase activity, and its expression delivers highly responsive in energy mobilization and the storage of lipid droplets [9], which regulates weight gain and obesity. Subsequently, studies have identified an association of this
SNP with overweight and obese patients [10-12]. Therefore, this SNP seems not only to be a risk factor in the development of liver fat content but also overweight/obesity. However, the association between PNPLA3 and overweight/obesity is still less; indeed, the influence of PNPLA3 variation on obesity is limited by sample size.

Meta-analyses of genetic association studies have the benefit to overcome the limitations and evaluate the strength of an association [13]. Therefore, we performed a meta-analysis to analyze the influence of this polymorphism on overweight/obese patients, and to estimate the strength of this association.

Materials and methods

Search strategy

The initial search was done on December 8, 2015 and no language or time restrictions were applied. Relevant studies were identified by a PubMed, Medline, Embase, EBSCO and Web of Science literature search with the following terms: (obesity or adipose tissue or overweight or weight or body mass index or BMI) and (adiponutrin, human or PNPLA3 or patatin-like phospholipase domain containing 3 or rs738409). Additionally, references of eligible studies were also screened for any potential studies.

Study selection

Inclusion and exclusion criteria were established prior to initiation of the literature search.

Inclusion criteria were as follows: (1) case-control and cohort studies; (2) studies evaluating the relationship between rs738409 polymorphism and the risk of overweight or obesity; (3) studies including overweight or obesity as the cases versus groups of controls; (4) studies that included cases or controls with different phenotypes had to provide separate genotype information; (5) studies have been published as a whole-length article.

Exclusion criteria were as follows: (1) studies that do not meet the inclusion criteria; (2) dis-
## Table 1. Characteristics of the 10 studies included in meta-analysis

<table>
<thead>
<tr>
<th>Subgroup ID</th>
<th>First author</th>
<th>Year</th>
<th>Ethnicity (region)</th>
<th>Age groups</th>
<th>Genotype method</th>
<th>Criteria for selection of cases</th>
<th>Total</th>
<th>Males</th>
<th>Population types</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nikolaj Thure Krarup</td>
<td>2012</td>
<td>Caucasian</td>
<td>Adults</td>
<td>KASPar SNP Genotyping</td>
<td>BMI $\geq$30 kg/m$^2$ or a waist-to-hip ratio $\geq$0.9 (men) and 0.85 (women)</td>
<td>4328</td>
<td>2285</td>
<td>Healthy Population</td>
<td>T</td>
</tr>
<tr>
<td>2</td>
<td>Elena Larrieta-Carrasco</td>
<td>2013</td>
<td>Mexican Mestizo (Mexico)</td>
<td>Children</td>
<td>TaqMan SNP assays</td>
<td>BMI percentile $\geq$85th</td>
<td>1037</td>
<td>493</td>
<td>Healthy Population</td>
<td>T</td>
</tr>
<tr>
<td>3</td>
<td>S. Lallukka</td>
<td>2013</td>
<td>Caucasian</td>
<td>Adults</td>
<td>Real-time PCR</td>
<td>BMI SD values</td>
<td>82</td>
<td>19</td>
<td>NAFLD Population</td>
<td>F</td>
</tr>
<tr>
<td>4</td>
<td>A. Viitasalo</td>
<td>2014</td>
<td>Caucasian</td>
<td>Children</td>
<td>TaqMan SNP assays and Real-time PCR</td>
<td>The age- and sex- specific BMI cut-offs of the IOTF</td>
<td>481</td>
<td>254</td>
<td>Healthy Population</td>
<td>T</td>
</tr>
<tr>
<td>5</td>
<td>Elena Larrieta-Carrasco</td>
<td>2014</td>
<td>Mexican Mestizo (Mexico)</td>
<td>Adults</td>
<td>TaqMan SNP assays</td>
<td>BMI $\geq$25 kg/m$^2$</td>
<td>529</td>
<td>144</td>
<td>Healthy Population</td>
<td>T</td>
</tr>
<tr>
<td>6</td>
<td>Ivana A. Stojkovic</td>
<td>2014</td>
<td>Caucasian</td>
<td>Adults</td>
<td>TaqMan SNP assays</td>
<td>BMI $\geq$25 kg/m$^2$</td>
<td>4824</td>
<td>-</td>
<td>Healthy Population</td>
<td>T</td>
</tr>
<tr>
<td>7</td>
<td>H. Mangge</td>
<td>2015</td>
<td>Caucasian</td>
<td>Children/Adults</td>
<td>TaqMan SNP assays</td>
<td>BMI percentile $\geq$85th (Youth), BMI $\geq$25 kg/m$^2$ (Adults:)</td>
<td>510</td>
<td>309</td>
<td>Healthy Population</td>
<td>F</td>
</tr>
<tr>
<td>8</td>
<td>Kenichi Nishioji</td>
<td>2015</td>
<td>Asian (Japan)</td>
<td>Adults</td>
<td>TaqMan SNP assays</td>
<td>BMI $\geq$25 kg/m$^2$</td>
<td>824</td>
<td>548</td>
<td>NAFLD Population</td>
<td>T</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Kentaro Oniki</td>
<td>2015</td>
<td>Asian (Japan)</td>
<td>Adults</td>
<td>Real-time PCR</td>
<td>BMI $\geq$25 kg/m$^2$</td>
<td>740</td>
<td>478</td>
<td>Healthy Population</td>
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<tr>
<td>11</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Xiao-Rui Shang</td>
<td>2015</td>
<td>Asian (China)</td>
<td>Children/Adolescents</td>
<td>MassARRAY System</td>
<td>The age- and sex- specific BMI cut-offs of the IOTF and BMI percentile $\geq$95th</td>
<td>1027</td>
<td>574</td>
<td>Healthy Population</td>
<td>T</td>
</tr>
</tbody>
</table>

Note: BMI, Body Mass Index; SD, Standard Deviation; NAFLD, Non Alcoholic Fatty Liver Disease; IOTF, International Obesity Task Force.
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Table 2. Distributions of PNPLA3 rs738409 genotypes of eligible studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Subgroup ID</th>
<th>First author</th>
<th>Population types</th>
<th>Sample size</th>
<th>Genotype in cases</th>
<th>Genotype in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nikolaj Thure Krarup</td>
<td>Healthy Population</td>
<td>2637</td>
<td>1691 1597 912</td>
<td>1010 593 88</td>
</tr>
<tr>
<td>2</td>
<td>Elena Larrieta-Carrasco</td>
<td>Healthy Population</td>
<td>486</td>
<td>551 231 175</td>
<td>84 248 219</td>
</tr>
<tr>
<td>3</td>
<td>S. Lallukka</td>
<td>NAFLD Population</td>
<td>41</td>
<td>41 29</td>
<td>19 26 8</td>
</tr>
<tr>
<td>4</td>
<td>A. Vitasalo</td>
<td>Healthy Population</td>
<td>58</td>
<td>423 36</td>
<td>22 247 176</td>
</tr>
<tr>
<td>5</td>
<td>Elena Larrieta-Carrasco</td>
<td>Healthy Population</td>
<td>334</td>
<td>195 143 169</td>
<td>22 71 102</td>
</tr>
<tr>
<td>6</td>
<td>Ivana A. Stojkovic</td>
<td>Healthy Population</td>
<td>2478</td>
<td>2361 824</td>
<td>93 1465 780</td>
</tr>
<tr>
<td>7</td>
<td>H. Mange</td>
<td>Healthy Population</td>
<td>288</td>
<td>209 173 96</td>
<td>19 117 78</td>
</tr>
<tr>
<td>8</td>
<td>Kenichi Nishioji</td>
<td>Healthy Population</td>
<td>156</td>
<td>396 55 27</td>
<td>27 115 210</td>
</tr>
<tr>
<td>9</td>
<td>Elena Larrieta-Carrasco</td>
<td>Healthy Population</td>
<td>212</td>
<td>60 47 109</td>
<td>56 7 33 20</td>
</tr>
<tr>
<td>10</td>
<td>Kentaro Oniki</td>
<td>Healthy Population</td>
<td>70</td>
<td>402 27 34</td>
<td>9 116 215</td>
</tr>
<tr>
<td>11</td>
<td>Ivana A. Stojkovic</td>
<td>Healthy Population</td>
<td>64</td>
<td>55 14 35</td>
<td>9 34 12</td>
</tr>
<tr>
<td>12</td>
<td>Xiao-Rui Shang</td>
<td>Healthy Population</td>
<td>175</td>
<td>690 79 81</td>
<td>15 259 337</td>
</tr>
<tr>
<td>13</td>
<td>Xiao-Rui Shang</td>
<td>NAFLD Population</td>
<td>121</td>
<td>41 46 55</td>
<td>20 14 19 8</td>
</tr>
</tbody>
</table>

Figure 2. Forest plot for overall studies evaluating the association between overweight/obesity and the PNPLA3 rs738409 polymorphism.

Data extraction and quality assessment

Data were systematically extracted by two independent investigators (CW, QW) using a standard protocol. When duplicate studies existed, only the most extensive data were taken into account. When several subgroups existed in a
The association of rs738409 polymorphism with the risk of overweight/obesity was obtained by pooling odds ratio (OR) and 95% confidence intervals (CI). The significance of the pooled OR and 95% CI were calculated by the Z-test, and a P-value <0.05 was considered statistically significant. We assessed study heterogeneity basing on the Cochran's Q statistic and I² statistic [15]. Heterogeneity was considered significant when a Q statistic P<0.1 or I²≥50%. Therefore, the fixed effects model (Mantel and Haenszel method [16]) was selected for homogeneous outcomes (P≥0.1 or I²<50%) and the random effects model (DerSimonian and Laird method [17]) was applied for heterogeneous outcomes (P<0.1 or I²≥50%). The potential outliers of the heterogeneity were detected by Galbraith plot [18]. This graphical method enabled us to check those studies which could have biased the combined estimate. In addition, the sensitivity analysis was conducted to evaluate the stability of results and to precisely investigate the heterogeneity. By the way, publication bias was observed by Begg's funnel plot and the Egger's linear regression test [18].

For rs738409, a recessive model was applied (GG+CG versus CC). Separate analyses were performed to reduce the potential heterogeneity. Therefore, summarized ORs were stratified by ethnicity (Caucasian, Asian and Mexican Mestizo), age (children or adolescents, adults), and non-alcoholic fatty liver disease (NAFLD). Statistical analysis was performed with Stata software (v. 12.0; Stata, College Station, TX), and a two tailed P value less than 0.05 was considered to be statistically significant.
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Results

Search results

The initial search yielded 1,185 entries for possible inclusion in our meta-analysis. Figure 1 presents a flow chart of retrieved and excluded studies. Six hundred and ninety-four articles were excluded because of duplication. After screening the titles and abstracts, 448 articles were excluded because they were inconsistent with the inclusion criteria. This step left 43 references that were considered to have latent value, and the full texts were evaluated carefully. Among these, 33 studies were excluded because they lacked a case and/or control group. Three additional studies were excluded for participants with a variety of diseases such as diabetes. One more study was excluded because we were unable to compare rs738409 polymorphism and the risk of overweight/obesity. Finally, the article by Nishioji et al. [19], Oniki et al. [20], and Shang et al. [21] were considered two separate studies respectively, as the authors reported information from two different populations (with or without NAFLD). Thus, a total of 10 studies including 13 subgroups were included in this meta-analysis.

The characteristics of included studies are shown in Table 1, and the distribution of the rs738409 genotypes and alleles is presented in Table 2. According to quality assessment, these studies were relatively well-designed, and the quality assessments were at a high level. These studies (published from 2012 to 2015) comprising 14,220 participants (7,120 cases and 7,100 controls) were selected for the present study. Of all of the studies included, 5 studies involved Caucasians, 3 studies investigated Asians, and 2 studies researched Mexican Mestizo. In addition, 4 studies enrolled children or both children and adolescents, and 7 investigated adult patients. Three subgroups patients carried NAFLD.

Meta-analyses results

Ten studies stratifying into 13 subgroups were included in the analysis [10, 19-27]. A total of
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6,963 patients had the rs738409 GG+CG genotype, whereas 7,257 patients had the CC genotype. For PNPLA3 rs738409, the between-study heterogeneity was not significant when all 10 studies (13 subgroups) were pooled for analysis ($I^2 = 26.9\%, P = 0.173$); thus, we selected the fixed effect model. Mantel and Haenszel method was used.

No evidence of an association between the rs738409 polymorphism and obesity risk was observed in incorporated patients (OR = 0.929, 95% CI: 0.863-1.000; P = 0.051 (Figure 2). Furthermore, three subgroup analyses were additionally carried out by ethnicity (Caucasian, Asian and Mexican Mestizo), age (children or adolescents, adults), and NAFLD (with or without).

Figure 5. Forest plot of the association between overweight/obesity and the PNPLA3 rs738409 polymorphism stratified by non-alcoholic fatty liver disease.

Figure 6. Sensitivity analysis showing the odds ratio and its 95% CI after sequentially omitting each study.
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When combined data from Caucasians, the G allele, consisted of GG+CG, did not show an unfavorable effect compared to CC genotype (OR = 0.960, 95% CI: 0.885-1.042; P = 0.327). When combined data from Asian, the G allele was observed to have a favorable effect (OR = 0.707, 95% CI: 0.575-0.869; P = 0.001). Since only two studies referred to Mexican Mestizo, ethnicity stratification analyses were not established (Figure 3).

For adults group, six studies including 8 subgroups evaluated the association between rs738409 polymorphism and overweight/obesity risk. Since one study [2] was unavailable to separate children or adults from the population, it was not included in the comparison. However, no significant association between rs738409 and the risk of obesity was identified in adults (OR = 0.950, 95% CI: 0.877-1.030; P = 0.215). For children or adolescents group, similar results were observed from three studies including 4 subgroups (OR = 0.825, 95% CI: 0.669-1.017; P = 0.072) (Figure 4).

Among 10 incorporate studies of this meta-analysis, four of them including 4 subgroups were NAFLD patients. The risk of overweight/obesity was not related to PNPLA3 polymorphism both in patients with NAFLD or without NAFLD (OR = 0.665, 95% CI: 0.436-1.014; P = 0.058; OR = 0.940, 95% CI: 0.872-1.013; P = 0.103, respectively) (Figure 5).

Heterogeneity analysis

There was no significant heterogeneity for the pooled analysis (n = 10, subgroups = 13). In the subgroup analyses, heterogeneity was not significant for the risk of overweight/obesity in Caucasians (P = 0.893, I^2 = 0) and Asians (P = 0.924, I^2 = 0). In addition, the between-study heterogeneity was not significant in adults (P = 0.126, I^2 = 38.1%) and children or adolescents (P = 0.829, I^2 = 0). Furthermore, no significant heterogeneity was observed between studies in NAFLD patients (P = 0.771, I^2 = 0) and healthy patients (P = 0.221, I^2 = 25.1%).

Sensitivity analysis

The influence of a single study on the overall meta-analysis was performed by sequential omission of every study respectively. No significant difference was showed when omitted any study; thus, our results were reliable (Figure 6).

Publication bias

Publication bias was examined by using Begg’s test and Egger’s test respectively. The funnel plot did not reveal any evidence of an obvious asymmetry (Figure 7). The P values of Begg’s test and Egger’s test were 0.200 and 0.087, respectively. No significant publication bias was detected in our meta-analysis.

Discussion

The PNPLA3 gene (rs738409) has garnered much research attention recently. This SNP exhibits a C-to-G transition generating an amino acid substitution of isoleucine to methionine, was initially one of the potential candidate genes related to NAFLD susceptibility in patients with G allele [7]. Several replication studies have also replicated an association between the rs738409 polymorphism and NAFLD risk [28-30]. It is strongly associated with factors such as aging, obesities, and diabetes [31]. Consistent with this result, some following studies also demonstrated an association between the rs738409 polymorphism and obesity or overweight and less clearly with insulin resistance in the general population [7, 22, 32]. Also, in liver transplant recipients, PNPLA3 non-CC genotype was independently associ-
ated with obesity at 3 years post-transplant [12]. Therefore, this SNP seems not only to be a risk factor in the development of liver fat content but also overweight/obesity.

In the present study, we performed a meta-analysis included 10 studies involving 14,220 patients to evaluate the association of the PNPLA3 gene rs738409 polymorphism with overweight/obese patients. In this meta-analysis, we found no evidence that PNPLA3 rs738409 the G allele was associated with risk of overweight/obesity in either adults or children/adolescents, despite the fact that whether patients carried NAFLD or not. Ethnicity stratification analyses revealed that rs738409G allele was not associated with overweight/obesity in Caucasians. However, in our Asian-based samples of 798 cases and 1,644 controls, we observed that rs738409G allele had a favorable effect on lower risk of overweight/obesity. It was inconsistent with previous studies for rs738409 G allele (GG+GC) frequently to be adverse genotypes in metabolic disorders [32]. Results could be explained by several factors. On the one hand, the etiology of overweight/obesity is complex and the rs738409 in I148M may interact with other risk factors, such as differences in diet and physical activity (PA) behaviors, not assessed in these studies. On the other hand, the polymorphisms in PNPLA3 may have a complex interactive effect to increase both adiponutrin activity and overweight/obesity risk.

Some limitations of this study should be discussed. First, meta-analysis reflects the methodological problems of the included studies. Second, only published studies were included in the present meta-analysis, which may bring publication bias although Begg’s test and Egger’s test of publication bias turned out to be acceptable. Furthermore, overweight/obesity would be interpreted for multiple influence variables known or unknown. In this analysis, we could not adjust several potential factors for very limited data were reported and not available to compare.

In conclusion, the present meta-analysis, which included 10 independent studies containing 13 subgroups, has shown that the PNPLA3 gene rs738409 polymorphism was not associated with an enhanced risk of overweight/obesity among Caucasians, neither adults nor children or adolescents. However, there is still a need for further research on this topic, including screening for etiological relationships between the other potential factors, and the susceptibility toward overweight/obesity.

Disclosure of conflict of interest

None.

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[29] Santoro N, Kursawe R, D’Adamo E, Dykas DJ, Zhang CK, Bale AE, Cali AM, Narayan D, Shaw MM and Pierpont B. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is
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