Association between rs4730153 Gene SNP and Fasting Glucose, Triglyceride, HDL and Body Mass Index Levels in Overweight Brazilian Adults

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Abstract

**Background:** Metabolic syndrome is an obesity-related comorbidity with increasing worldwide occurrence. In addition to environmental factors, it is speculated that the variables included in the metabolic syndrome (waist, fasting glycemia, HDL, triglycerides and blood pressure values) can be modulated by genetic variants. It has recently been reported that some polymorphisms of the pre-beta cell growth factor gene may play a modulatory role in glucose and lipid metabolism, as well as influence body mass index.

**Objective:** To investigate the influence of the rs4730153 polymorphism of the pre-beta cell growth factor gene on the levels of glycemia, triglycerides, HDL and body mass index of overweight and sedentary individuals.

**Methods:** Brazilian men and women with a body mass index >24.9 kg/m², over 18 years of age, who did not use medication for glycemia or hypercholesterolemia were included. The rs4730153 polymorphism was amplified by real-time polymerase chain reaction using a genotyping kit. Genotypes AA, AG and GG were evaluated separately.

**Results:** A total of 112 individuals with a mean age of 40.52 ± 10.30 years were included, of which 77% were women. Genotype frequency was 29.5, 41.0 and 29.5% (AA, GA and GG, respectively). No association was observed between glycemia, triglycerides, HDL and body mass index in the different alleles of the studied SNP.

**Conclusions:** Despite reports of studies in other ethnicities, in the present study no association was found between the rs4730153 polymorphism of the pre-beta cell growth factor gene and serum levels of glycemia, triglycerides, HDL and body mass index in a sample of the Brazilian population with overweight/obesity.

(Keywords: Metabolic Syndrome; Hyperlipidemias; Polymorphism, Genetic; Hypertension; Obesity; Sedentary Lifestyle.)

Introduction

Metabolic Syndrome (MS) is a sum of risk factors for cardiovascular diseases that affect a large part of the Brazilian population. Several criteria are used to characterize MS and those by the International Diabetes Federation (IDF) are widely applied worldwide and have been recently recommended for use in the Brazilian population. Among the risk factors for MS are serum fasting blood glucose levels, considered by some as the main factor of this comorbidity, as well as triglycerides (TG) and abdominal obesity.

The variables considered for the MS criteria are affected by environmental, socioeconomic, behavioral and genetic factors. Among the genetic factors, one particular gene is highlighted due to the recent investigation of its influence on lipid and glucose metabolism, in addition to its association with abdominal fat. The PBEF (pre-beta cell growth factor) gene was discovered in 1994 and renamed “visfatin” by Japanese researchers in 2005, after observing its association to abdominal fat - the name is a combination of the words visceral and fat. In their studies, these researchers observed that visfatin had a mimetic effect of insulin, binding to the same receptor, but at different points.

Over the years, several researchers have studied the association between visfatin gene expression and central obesity and several polymorphisms have started to appear as candidates for risk factors for diabetes and serum TG elevation. Currently there are 52 known polymorphisms of the human visfatin gene, some of which seem to be related to...
alterations in glucose and lipid metabolism. One of these polymorphisms is the Single-Nucleotide Polymorphism (SNP) rs4730153, which consists of an AG transversion within intronic region, and its influence on glucose / lipid metabolism is still being disputed. However, Lai et al. suggested that the G-948T SNP has an influence on serum glycemia and TG. As these SNPs are in binding disequilibrium, that is, they segregate together, it has not been possible yet to evaluate the direct influence of each on the phenotypes of interest, thus making studies in both SNPs relevant ones.

In the Genome-Wide Association Study (GWAS), four genes were considered the best candidates for analysis and among them, the most often studied is G-948T, considered a factor that influences diastolic blood pressure, High Density Lipoprotein (HDL) and glucose and lipid metabolism. Only five studies analyzed the rs4730153 (A-87G) SNP, with different objectives related to obesity and cardiovascular risk factors.

Among these, Lai et al. raised the hypothesis that the A allele has an influence on the basal levels of TG in Chinese individuals, increasing them. This association was also analyzed by studies in the Scandinavian population, in which there was an interest for the possible association with HDL values. Another study, also in the Chinese population, assessed the influence of this SNP on BMI values.

Although several studies focused on the different mutations of the visfatin gene and the possible effects of these mutations on different variables, it was not possible to find any in the Brazilian population. Additionally, studies focusing on the rs4730153 SNP are rare and show different and inconclusive results, thus further studies are needed to elucidate the influence of this SNP on the variables proposed by previous studies.

Thus, the present study aims to analyze the possible effects of the rs4730153 (A-87G) SNP on blood variables of MS (fasting glycemia, TG and HDL levels) and BMI of healthy overweight / obese individuals in a sample of the Brazilian population.

Methods

A total of 112 individuals participated in this study, of which 81 were women (72%), aged >18 years and BMI > 24.9 kg/m², who did not use drugs for glycemia or hypercholesterolemia. The sample size was obtained by convenience, encompassing as many people as possible throughout the year of 2015. The research was performed according to the Helsinki Declaration and was approved by the Research Ethics Committee of Escola de Educação Física e Esporte de Ribeirão Preto of Universidade de São Paulo (CAAE 37573114.6.0000.5659). All participants signed the Free and Informed Consent form.

The evaluations were performed on two distinct days, with blood collection being carried out on one and anthropometric measurements (mass, height, waist circumference and blood pressure) on another day. For blood glucose and TG analysis, 5 mL of blood were collected in a dry tube with separator gel (BD, Franklin Lakes, United States) after a 12-hour fasting period. Blood analysis was performed in the laboratory of clinical analyses of Faculdade de Ciências Farmacêuticas de Ribeirão Preto, using an enzymatic analysis kit (Wiener Lab, Argentina) in an automatic device (Konelab 600i, Wiener Lab, Argentina).

Intervening variables that could influence the variables of interest were evaluated by questionnaires, such as physical activity level and eating habits.

To evaluate the level of physical activity, the short version of the International Physical Activity Questionnaire (IPAQ) was applied as an interview. The questionnaire classifies individuals into five categories - very active (1); active (2); irregularly active A (3); irregularly active B (4); and sedentary (5). The Food Intake Markers (FIM) Form was used to identify the individuals’ eating habits. The foods were grouped into "healthy" and “unhealthy”, and the weekly consumption of each category was evaluated.

DNA extraction was carried out in 4 mL of total peripheral blood using the adapted salting out method. After collection, the blood was transferred to a tube containing a solution that disrupts cell membranes (0.3 M sucrose, 10 mM Tris-HCl - pH 7.5, 5 mM MgCl₂·6 H₂O and 1% Triton X). The obtained solution was centrifuged at 3,300 revolutions per minute (rpm) for 5 minutes, at 4 °C. The supernatant was discarded and the precipitate was suspended in a solution to rupture the nuclear membrane (0.075 M NaCl, 0.024 M EDTA) and 1.1 mL of 5M sodium perchlorate, 1.25 μL of 10% SDS and stirred vigorously for 15 seconds. Then, 2 mL of 6M NaCl (saturated solution) was added and the solution was vigorously stirred for another 15 seconds. The supernatant was collected in a sterile tube and 7 mL of isopropyl alcohol was added. The pellet was collected and transferred to a 1.5 mL tube and washed with 70% ethyl alcohol three times.
After drying, the pellet was diluted in 200 μl ultrapure water (Invitrogen, California, USA).

Amplification of the fragment of interest was performed in qPCR (Step One-Plus, Thermo Fisher Scientific, Massachusetts, USA) using an assay for SNP genotyping (C_2673294_10, Life Technologies, California, USA). The running configuration used was the one recommended by the manufacturer.

For the statistical analysis, the normality of data was assessed by the Shapiro-Wilks test, and a non-normal distribution was verified. The Kruskal-Wallis test for independent samples (non-parametric data) was used to compare the values of fasting glucose, HDL, TG and BMI between genotypes, with genotypes being analyzed as independent variables. The Hardy-Weinberg equilibrium was assessed using the chi-square test. The values of the variables were expressed as mean and standard deviation. The level of significance was set at 5%. All analyzes were performed using the IBM Statistical Package for Social Sciences (SPSS), version 21 (IBM, New York, USA).

**Results**

The total values of age, body mass, BMI and waist circumference of the individuals can be observed in table 1. Differences in the sample number are related to the non-attendance of some participants on the second day of evaluations.

When analyzing lifestyle variables that could influence the possible differences in anthropometric, body composition and blood biochemistry values, it was observed that the groups did not differ in relation to physical activity level and eating habits. Table 2 shows that most individuals were sedentary or little active, and maintained a similar weekly intake volume of healthy and unhealthy foods.

The genotypic frequency observed was 29.5% for AA, 41.0% for AG and 29.5% for GG and these data differed from those obtained in studies in the Scandinavian and Chinese populations. Despite the difference, the data obtained are in Hardy-Weinberg equilibrium (p > 0.05).

Table 3 shows the allele and genotype frequencies observed in the sample.

Data on BMI, serum glucose, HDL and TG according to the genotypes are shown in Table 4. No statistically significant differences (p > 0.05) were observed regarding the analyzed variables.

**Discussion**

Since 2005, following the reports by Fukuhara et al. on the association between levels of visfatin and central obesity, in addition to the possible mimetic effects of insulin exerted by this new adipokine, studies with this molecule have increased. However, after the inability to reproduce the results

<table>
<thead>
<tr>
<th>Table 1 – Total sample characterization</th>
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<tbody>
<tr>
<td>Age*, years</td>
</tr>
<tr>
<td>Body mass†, kg</td>
</tr>
<tr>
<td>BMI†, kg/m²</td>
</tr>
<tr>
<td>WC†, cm</td>
</tr>
<tr>
<td>Total cholesterol†, mg/dL</td>
</tr>
<tr>
<td>HDL-c†, mg/dL</td>
</tr>
<tr>
<td>LDL-c†, mg/dL</td>
</tr>
<tr>
<td>Triglycerides†, mg/dL</td>
</tr>
<tr>
<td>Fasting glucose†, mg/dL</td>
</tr>
<tr>
<td>SBP†, mmHg</td>
</tr>
<tr>
<td>DBP†, mmHg</td>
</tr>
</tbody>
</table>

* n = 112; † n = 93. Data expressed as mean (± standard deviation). BMI: body mass index; WC: waist circumference; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Table 2 – Level of physical activity, Food Intake Markers (FIM) Form and socioeconomic characterization - visfatin

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPAQ*</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>Healthy FIMF†</td>
<td>22</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Non-healthy FIMF</td>
<td>14</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

*Socioeconomic characterization shown in number of individuals; † shown as mean weekly consumption of healthy and unhealthy foods. IPAQ: International Questionnaire of Physical Activity.

Table 3 – Allele and genotype frequencies

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>n (%)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>112 (50.0)</td>
</tr>
<tr>
<td>G</td>
<td>112 (50.0)</td>
</tr>
<tr>
<td>AA</td>
<td>33 (29.5)</td>
</tr>
<tr>
<td>AG</td>
<td>46 (41.0)</td>
</tr>
<tr>
<td>GG</td>
<td>33 (29.5)</td>
</tr>
</tbody>
</table>

Table 4 – Glucose, triglycerides, high density lipoprotein (HDL) levels and body mass index (BMI) in the different genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.274</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>0.706</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.390</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>0.386</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test for independent samples. Data expressed as mean ± standard deviation. P value refers to the difference between groups.

obtained by the Japanese investigators, researchers started to discuss whether the newly baptized visfatin would actually have any association to visceral obesity and whether its insulinomimetic effect could be considered relevant. Three years after the initial article, the authors withdrew the article, but in a
even after the original article was withdrawn, several researchers continued their research with visfatin and obtained varied results.

The interest in the visfatin gene regulation led to studies that initiated the analysis of its mutations. Currently, 52 single-nucleotide polymorphisms (SNPs) are known for this gene, located on chromosome 7, a chromosome known for its possible association with BMI and susceptibility to obesity. 14

Among the known SNPs, two are considered candidates for lipid metabolism and glycemia regulators 11,14,15 and others were analyzed for their possible relation with BMI. 12 SNP rs4730153 (A-87G) has been studied by a few researchers in the Scandinavian 10,11,15 and Chinese 12,14 populations, but the analysis of this SNP is unheard of in the Brazilian population.

The allelic frequency found in the present study was similar to that observed in a Scandinavian population sample, 10,11 but different from results found in Asian individuals, where the A allele had a frequency of approximately 8%, 14 and 11%. 12

The study by Bottcher et al., 15 carried out in individuals with and without type 2 diabetes, assessed the association between SNP rs4730153 (A-87G) and diabetes. The genotypic frequencies were not different between the groups. In the end, the authors concluded there was no influence of this polymorphism on glucose metabolism. The authors also failed to demonstrate any association between the SNP and gene expression.

In an expression analysis, Johansson et al. 11 did not observe any difference in the visfatin gene expression in visceral and subcutaneous fat in different SNPs. The messenger RNA (mRNA) expression increases during the development of obesity, and its plasma levels are strongly correlated with the amount of visceral fat, but it seems that the SNPs have no effect on the modulation of this expression. In this study, in addition to gene expression, BMI, insulin, glycemia, HDL and TG values were analyzed. The authors did not observe any association between the SNP rs4730153 (A-87G) and the analyzed variables.

Among the studies that evaluated the expression of visfatin, the only one that found a difference between the genotypes was carried out in Chinese children and adolescents. 14 The authors divided individuals in two groups according to their genotypes (AG and GG). No assessed individual had the AA genotype. The authors reported differences in basal serum TG levels between the AG and GG genotypes, but reservations should be made regarding the methods of the aforementioned article: the sample was small (88 individuals); the percentage of the minor allele frequency (MAF) very low (~8%), generating the need for a larger sample 22; in addition to the fact that the study had only two genotypes.

Confronting the data by Lai et al., 14 the study carried out by Körner et al. 10 with a sample of 898 individuals in a MAF of 42% (allele A) observed no association between SNP rs4730153 and glucose metabolism (fasting glycemia, oral tolerance test, plasma insulin and HOMA), HDL and TG.

Our analysis is, to the best of our knowledge, the first to be carried out with the Brazilian population. The PBEF / NAMPT gene was renamed “visfatin” a little over a decade ago and research on the constituent variables in obesity and associated problems is still scarce and divergent. Such studies are of great importance. If associations between genetic variants and characteristics of obesity are found, functional gene studies can be performed to develop a drug to treat this disease, of which prevalence is increasingly higher.

Limitations

The limitation of this study is its sample size, which can be considered small for genetic analysis of only one SNP. However, the present study had more than 100 volunteers and methodological care was taken to control possible intervening variables (food intake and physical activity).

Conclusions

Despite reports in studies carried out in other ethnicities, the present study disclosed no association between rs4730153 polymorphism of the PBEF gene and serum levels of glycemia, triglycerides, HDL and body mass index in a sample of the Brazilian population with body mass index >24.9 kg/m². However, more studies are required with this population to verify whether there is any influence of the SNPs of the VISFATIN gene on these and other variables related to obesity.

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Author contributions

Conception and design of the research and Analysis and interpretation of the data: Ferrari GD, Rodrigues JAL, Bueno Júnior CR; Acquisition of data: Ferrari GD, Rodrigues JAL, Fernandes IA; Statistical analysis:Ferrari GD, Bueno Júnior CR; Obtaining financing: Bueno Júnior CR; Writing of the manuscript: Ferrari GD; Critical revision of the manuscript for intellectual content: Ferrari GD, Rodrigues JAL, Fernandes IA, Bueno Júnior CR.

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