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Trabajo Original

Obesidad y síndrome metabólico

Prevalence of the genetic variant rs61330082 and serum levels of the visfatin gene in Mexican individuals with metabolic syndrome: a clinical and bioinformatics approach

Prevalencia de la variante genética rs61330082 y niveles séricos del gen de la visfatina en individuos mexicanos con síndrome metabólico: una aproximación clínica y bioinformática

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Abstract

Background: metabolic syndrome (MetS) is a group of clinical anomalies that share an inflammatory component of multifactorial etiology.

Objectives: the present study aims to relate the genetic variant (rs61330082 C/T) with dietary patterns in the presence of MetS and the application of molecular docking according to the genotype and associated transcription factors.

Methods: 197 individuals aged 18 to 65 were included, from whom anthropometric measurements were taken, and a blood sample from the forearm. DNA extraction and enzymatic digestion were performed to determine the genotype of each participant by PCR-RFLP. Dietary patterns were analyzed using a nutritional questionnaire validated for the Mexican population. Serum levels of the protein visfatin were assessed by ELISA. Finally, bioinformatics tools were used for molecular docking to infer the binding of transcriptional factors in the polymorphic region.

Results: the TT genotype was present in only 10 % of the population. Women carrying the CT+TT genotype, according to the dominant genetic model, had higher serum levels of triglycerides and VDLD-C. Statistical analysis did not show a significant association between the presence of MetS and the dominant CT+TT model (OR = 1.41, 95 % CI = 0.61-3.44, $p = 0.53$). We identified PAX5 as a transcription factor binding to the polymorphic site of this genetic variant.

Conclusions: this study demonstrated a significant association between the genetic variant (rs61330082 C/T) and lipid parameters. Women carrying the T allele have a higher risk of high triglyceride levels, a criterion for metabolic syndrome.

Keywords:

Metabolic syndrome. Genetic variant. *Visfatin*. Dietary patterns. Bioinformatics.

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Abstract

Objetivos: el presente estudio pretende relacionar la variante genética (rs61330082 C/T) con los patrones dietéticos en presencia de SM y la aplicación del acoplamiento molecular según el genotipo y los factores de transcripción asociados.

Métodos: se incluyeron 197 individuos de entre 18 y 65 años, a los que se tomaron medidas antropométricas y una muestra de sangre del antebrazo. Se realizó una extracción de ADN y una digestión enzimática para determinar el genotipo de cada participante mediante PCR-RFLP. Los patrones dietéticos se analizaron mediante un cuestionario nutricional validado para la población mexicana. Los niveles séricos de la proteína visfatina se evaluaron mediante ELISA. Finalmente, se utilizaron herramientas bioinformáticas de acoplamiento molecular para inferir la unión de factores transcripcionales en la región polimórfica.

Resultados: el genotipo TT solo estaba presente en el 10 % de la población. Las mujeres portadoras del genotipo CT+TT, según el modelo genético dominante, presentaban niveles séricos más elevados de triglicéridos y VDLD-C. El análisis estadístico no mostró una asociación significativa entre la presencia de MetS. y el modelo dominante CT+TT (OR = 1,41; IC 95 % = 0,61-3,44; $p = 0,53$). Identificamos PAX5 como un factor de transcripción que se une al sitio polimórfico de esta variante genética.

Conclusiones: este estudio demostró una asociación significativa entre la variante genética (rs61330082 C/T) y los parámetros lipídicos. Las mujeres portadoras del alelo T presentan un mayor riesgo de niveles elevados de triglicéridos, un criterio de síndrome metabólico.

Palabras clave:

Síndrome metabólico. Variante genética. Visfatina. Patrones dietéticos. Bioinformática.

INTRODUCTION

Metabolic syndrome (MetS) is a severe health problem worldwide, with a global prevalence of 37.4 % (1). In 2020, Mexico reported a 41.7 % prevalence of MetS in a population of subjects aged 35-70 years (2). The diagnosis of MetS is defined by the presence of three or more metabolic alterations, such as high concentrations of cholesterol, triglycerides, decreased HDL cholesterol, glucose intolerance, and the presence of visceral obesity (3-5), the latter being an essential factor, since it predisposes to arterial disease, diabetes-related complications and various types of cancer (3,4).

The pathophysiology of MetS is not fully described, but it is known to be influenced by environmental (such as dietary habits) and genetic variables. However, how these factors interact is not fully understood since MetS is significantly affected by chronic low-grade inflammation (5). Adipose tissue is an organ with important endocrine functions, including the stimulation of anti-inflammatory cytokines such as IL-10 and IL-5 and proinflammatory cytokines such as IL-32 and visfatin secreted by monocytes, and therefore actively participates during MetS progression (6,7).

Nicotinamide phosphoribosyltransferase, also known as visfatin/NAMPT/PBEF, has been recognized as an adipokine, cytokine, and NAD+ precursor enzyme and plays an important role in metabolism (8,9). This adipokine is mainly expressed in adipose tissue but is also secreted in other tissues such as skeletal muscle, spleen, testes, and bone marrow (10,11). Visfatin can increase cytokine synthesis in monocytes, so elevated levels of these proinflammatory markers can trigger different systemic signaling cascades in organs and tissues (12).

The *visfatin* gene, located on chromosome 7q22.2.2, consists of 11 exons and ten introns, has a length of 36,908 bp, which translates into a protein of 491 amino acids that varies in weight from 52 to 57 kDa, and has a promoter region, in which some genetic variants have been described, including -1535C/T (rs61330082), which has been related to alterations in the transcriptional rate of visfatin (13,14). This genetic variant has been associated with effects on tumorigenesis and chronic inflammation, which increases the risk of different types of cancer and diabetes *mellitus* (15).

As visfatin is secreted in various target tissues and affects rather complex biological pathways, it isn't easy to understand its role in the development of MetS (16). Bioinformatics is fundamental to researching and understanding metabolic syndrome. These analysis offers tools and methods to analyze large amounts of biological data due to its ability to interact between biology and informatics, considering sciences such as genomics, transcriptomics, and metabolomics, which is essential for deciphering the mechanisms underlying the pathogenesis of MetS. Bioinformatics can identify potential biomarkers, understand molecular interactions, and design more precise therapeutic strategies through data integration and biological network analysis (17).

Due to the epidemiological burden of MS in Mexico, intervention through prevention and control studies is crucial (18); therefore, the present study evaluates for the first time in a grouped manner the genetic, biochemical and pathophysiological parameters of MS and visfatin in a Mexican population of young adults.

The study aimed to examine the impact of the NAMPT rs61330082 genetic variant and serum levels of this adipokine on risk factors for metabolic syndrome, as well as the interaction of molecular docking according to genotype and associated transcription factors.

SUBJECTS AND METHODS

STUDY POPULATION

This was a cross-sectional study with subject selection centered on non-probabilistic sampling; 197 individuals participated, who met the inclusion criteria (147 women and 50 men), aged between 18 and 65 years, who were previously informed about the implications of their participation before signing the informed consent. The study was conducted at the Centro de Atención Médica Integral, under the Centro Universitario de Los Altos, with the full approval of the institutional ethics committee under number (CEI-04/2022-08), complying with the guidelines established by the general health law on research and the declaration of Helsinki. Participants with autoimmune diseases, consumption of anti-inflammatory drugs, and pregnant women were excluded.

METABOLIC SYNDROME PARAMETERS

Anthropometric analysis was performed by determining weight and height with the Tanita BC-533. Each subject presented at least three of the five established risk criteria: fasting triglycerides ≥ 150 mg/dL, HDL cholesterol (HDL-c) < 40/50 mg/dL in men/women, increased waist circumference ≥ 102/88 cm, men/women, blood pressure ≥ 130/85 mmHg, systolic and diastolic, respectively or antihypertensive treatment, as well as elevated fasting glucose ≥ 110mg/dL predetermined from the MetS. Patients without MetS did not present the characteristics described by ATP III (19).

GENOTYPING

According to the provider's guidelines, DNA was extracted from peripheral blood leukocytes using the PureLink™ Genomic DNA Mini Kit (K182002). Genotyping of the rs61330082genetic variant was performed by polymerase chain reaction-restriction fragment length (PCR-RFLP), with the conditions described in a previous study (20). A 283-bp fragment was amplified using primers specific for the rs61330082 fragment of the *visfatin* gene, which had the following sequence 5'TGTTTCAAACCTCGTTGTTGCTGA-3 'and 5'AGTGATGGTGGTGGTGGTGGTA-3'. The PCR cycling conditions usings Applied Biosystems™, SimpliAmp™ Thermocycler (A24811) were as follows: initial denaturation at 95 °C for 5 min, followed by 32 cycles of denaturation at 95 °C for 30 s; subsequently, alignment was carried out at a temperature of 60 °C for 45 s, culminating in an extension at 72 °C for 1 min and an extension at 72 °C for 10 min. Once the fragment of interest was amplified, enzymatic digestion was performed using the *BstN1* enzyme, and the products were visualized in 6.0 % polyacrylamide gel stained with silver nitrate. Three genotypes could be characterized: homozygous polymorphic TT (218 and 65 bp), heterozygous CT (283, 218, and 65 bp), and homozygous wild-type CC (283 bp).

SERUM VISFATIN MEASUREMENTS AND BIOCHEMICAL PARAMETERS

Visfatin levels were measured by enzyme-linked immunosorbent assay (ELISA) using the commercial Human Visfatin/PBEF ELISA kit, R&D Systems, catalog number DY4335-05. A microplate reader determined the absorbance at 450 and 570 nm (Multiskan GO, Thermo Scientific). All participants had a peripheral blood sample taken after fasting for 8 hours. For serum collection, tubes were centrifuged for 20 minutes at 3500 rpm. Laboratory analyses included the following biochemical parameters: serum glucose, triglycerides (TG), total cholesterol (CHOL), and high-density lipoprotein cholesterol (HDL-C) levels, which were performed with an Abbott Aeroset automatic analyzer. The Friedewald equation determined low-density lipoprotein cholesterol (LDL-C) levels. Finally, to obtain the very low-density lipoprotein cholesterol (VLDL-C) values, total TG (mg/dL) was divided among five.

EVALUATION AND APPLICATION OF MINI-ECCA

The dietary pattern survey of the study population was applied at the same time as the anthropometric measurements; these data were evaluated using the Mini-ECCA questionnaire, which was previously developed and validated by Bernal et al. (21). The Mini-ECCA includes 12 items based on Mexican and international guidelines on food and non-alcoholic beverage consumption, using images to estimate portions. Each item receives a score of 0 (unhealthy) or 1 (healthy), and according to the total score, each participant is classified into a group according to dietary quality. The Mini-ECCA presented significant reproducibility ($\rho = 0.713$, $p < 0.001$) and high precision concordance (ICC = 0.841, 95 % CI: 0.779-0.885). These results indicate that this survey is a highly reliable tool, appropriate for dietary assessment and orientation in the university population as well as in young adults (22,23).

BIOINFORMATICS ANALYSIS

We searched for possible transcription factors that bind to the region of the genetic variant using the HaploReg v4.2 websites (24). The 3D DNA structure was then generated using BIOVIA Discovery Studio V21.1.0.20298 software. The sequences used were 5´AAAGATCATGGAAGTGGAAGGTATCACCACGCACTCACCAATGTAGTAAATACTAGTAC3´ and 5´AAAGATCATGGAAGTGGAAGGTATCACCATGCACTCACCAATGTAGTAAATACTAGTAC3´. The protein structures of the transcription factors were obtained from the Alphafold website (25). DNA-protein docking was performed with pyDockDNA server software (26), with standard parameters, and the results obtained were visualized in BIOVIA Discovery Studio V21.1.0.20298 software.

STATISTICAL ANALYSIS

The patients' characteristics were reported in frequency, percentages, mean, and standard deviation. The SPSS v.22 statistical program was used, taking into account a significance level of 0.05. The inheritance model of the allelic and genotypic frequencies of the variant rs61330082 was analyzed by chi-square test, previously confirming the Hardy-Weinberg equilibrium. Disease risk was estimated using the odds ratio, with a 95 % confidence interval. After the corresponding normality tests, the association between quantitative study variables was analyzed using the Mann-Whitney U test or student's t-test. The sample size was determined by considering the global allele frequencies according to the National Center for Biotechnology Information (NCBI) database of the rs61330082 variant of the NAMPT gene.

To calculate the sample size, the estimation formula for a proportion was used considering the following parameters: a confidence level of 95 %, precision of 5 %, and an expected proportion of the variant of 15 %, resulting in a sample size of 196 individuals.

RESULTS

One hundred ninety-seven patients were recruited for the present research, classified as patients with metabolic syndrome ($n = 31$) and controls ($n = 166$). These current data represent a prevalence of 15 % in the population studied, as shown in table I. As expected, the five diagnostic criteria considered for the presence of MetS were significantly different from the control group. The control group had slightly higher visfatin levels but did not represent a significant difference between groups. After analyzing the dietary pattern data acquired from the Mini-ECA, no significant variations in food consumption were found between the control group and the people with MetS (Table I).

Table II shows the distribution of the rs61330082 genetic variant in the study, according to the presence or absence or Mets. The genotypic frequencies in the total population studied were as follows: the CC genotype 72 (36 %), CT 107 (54 %), and TT 19 (10 %), the frequency for the C allele was 62 %, and for the T allele it was 38 %. Likewise, we used the dominant pattern genetic model (CC vs CT+TT) as well as the recessive model (TT vs CC+CT) to determine the risk of presenting metabolic syndrome according to the five criteria evaluated by the ATP III, which showed no statistically significant association between the control and MetS groups.

Likewise, we found that within the diagnostic criteria for MetS, the female carriers of the CT+TT genotype (dominant genetic pattern) had higher levels of TG and VLDL-C ($p = 0.006$ and $p = 0.026$, respectively), as shown in table III. This same result was also observed in the whole sample analyzed but not in the group of men. We also evaluated the association between inheritance patterns and serum visfatin levels, which did not show a statistically significant difference between the study groups. In addition, according to mini-ECCA, dietary habits showed no association when analyzed by any of the three inheritance models (data not shown).

Finally, we decided to explore the effect of this genetic variant on the binding of some transcription factors using a bioinformatics approach. We infer three transcription factors that probably bind to the site of this genetic variant: NRSF, NRSF, and SIX5. We then verified these results by DNA-protein docking, the results of which are shown in figure 1. The polymorphic variant does not appear to affect the binding energetics of the NRSF and SIX5 transcription factors. However, it does affect the binding of PAX5, as a significant change in pyDockDNA scoring is observed. pyDockDNA scoring represents the total binding energy between transcription factors and DNA from electrostatic, solvation, and Van der Waals energies between protein and DNA.

Table I. Anthropometrics, criteria MetS, nutritional and biochemical parameters of the study population

Criteria MetS*	MetS ($n = 166$)	Controls ($n = 31$)	p
Age (years)	37.60 ± 14.03	25.77 ± 9.44	< 0.001
SBP (mmHg)	129.13 ± 20.52	111.77 ± 12.47	< 0.001
DBP (mmHg)	86.47 ± 13.25	75.89 ± 8.50	< 0.001
Fasting glucose (mg/dl)	114.26 ± 31.23	92.58 ± 23.71	< 0.001
Total cholesterol (mg/dl)	193.83 ± 70.74	169.41 ± 76.73	0.106
Triglycerides (mg/dl)	196.62 ± 89.92	114.78 ± 55.22	< 0.001
HDL-cholesterol (mg/dl)	40.89 ± 19.30	46.54 ± 20.16	0.156
Waist circumference (cm)	95.55 ± 14.38	78.20 ± 12.28	< 0.001
Visfatin (ng/mL)	16.32 ± 19.72	31.61 ± 50.14	0.102
Sex			
Male *n* (%)	12 (40.0)	38 (22.8)	0.066
Female *n* (%)	19 (60.0)	128 (77.2)	
Dietary patterns Mini-ECCA			
Unhealthy food intake *n* (%)	18 (58.1)	88 (48.9)	0.543
Habits in need of improvement *n* (%)	2 (6.5)	29 (16.1)	
Unhealthy eating habits *n* (%)	8 (25.8)	47 (26.1)	
Healthy food intake *n* (%)	3 (9.7)	16 (8.9)	

**Quantitative variables are presented as mean ± SD.*

Table II. Genotype visfatin rs61330082 C > T and allele distributions in MetS patients and control subjects

Models and alleles	MetS, *n* (%)	Control, *n* (%)	*p*	OR, 95 % CI
Codominant				
CC	9 (30.0)	63 (37.5)	Reference	
CT	19 (63.3)	88 (52.4)	0.344	0.66 (0.28-1.55)
TT	2 (6.7)	17 (10.1)	0.814	1.21 (0.24-6.15)
Dominant				
CC	9 (30)	63 (37.8)	Reference	
CT + TT	21 (70)	104 (62.3)	0.530	1.41 (0.61-3.44)
Recessive				
CC + CT	28 (89.82)	150 (93.33)	Reference	
TT	2 (10.18)	17 (6.67)	0.740	0.63 (0.13-2.51)
Alleles				
C	37 (61.7)	214 (64)	Reference	
T	23 (38.3)	122 (36)	0.720	0.90 (0.51-1.61)

Categorical variables were compared using the χ^2 test. Odds ratios (OR) and 95 % confidence intervals (CI) were used for the assessment of risk factors. Significance level: $p < 0.05$.

Table III. Analysis of biochemical parameters according to the dominant model of inheritance in women

Variable	CC, *n* = 52	CT + TT, *n* = 95	*p*
SBP (mmHg)	110.04 ± 14.28	113.48 ± 14.64	0.142
DBP (mmHg)	77.38 ± 10.66	77.22 ± 10.09	0.781
Fasting glucose (mg/dL)	92.33 ± 16.20	91.81 ± 12.02	0.415
Total cholesterol (mg/dL)	173.50 ± 88.19	176.39 ± 79.58	0.417
Triglycerides (mg/dL)	103.90 ± 52.00	131.20 ± 63.18	*0.006*
HDL-cholesterol (mg/dL)	42.78 ± 19.74	46.96 ± 19.65	0.200
VLDL-cholesterol (mg/dL)	19.53 ± 10.85	24.38 ± 13.17	*0.026*
Waist circumference (cm)	76.90 ± 10.69	77.37 ± 12.76	0.697
Visfatin (ng/mL)	20.10 ± 23.40	25.78 ± 37.35	0.759

**Quantitative variables are presented as mean ± SD.*

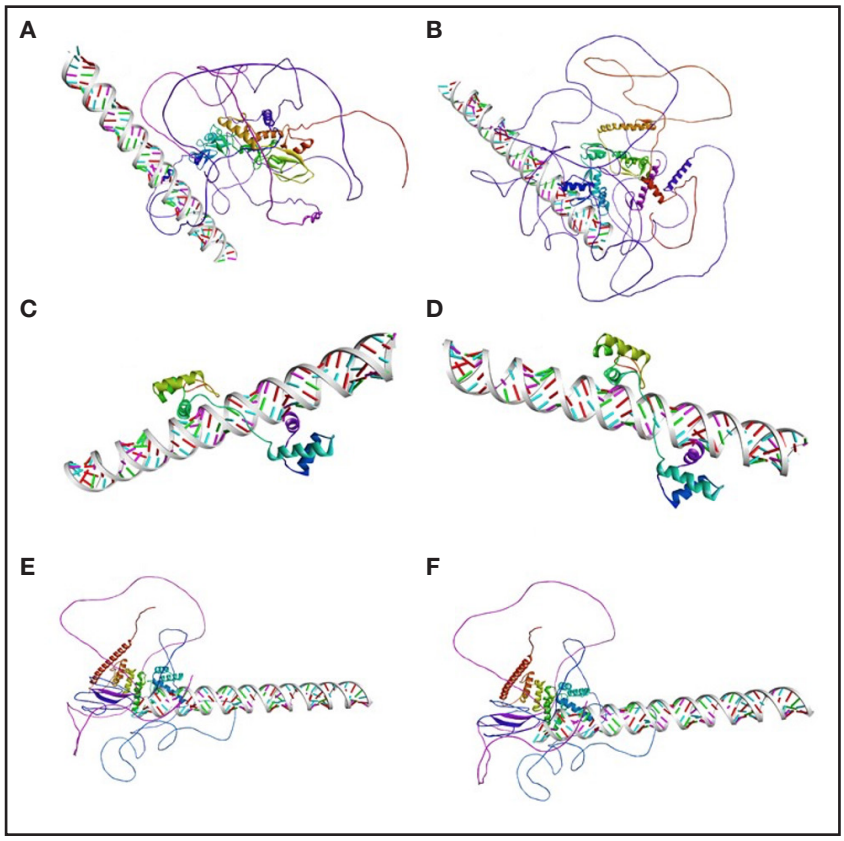

Figure 1.

Docking protein-DNA analysis between the gene region where the gene variant is located and the inferred transcription factors. A. Docking wild variant-NRSF, pyDockDNA scoring -115.02. B. Docking polymorphic variant-NRSF, pyDockDNA scoring -159.26. C. Docking wild variant-PAX5, pyDockDNA scoring -192.66. D. Docking polymorphic variant-PAX5, pyDockDNA scoring -192.47. E. Docking wild variant-SIX5, pyDockDNA scoring -58.21. F. Docking polymorphic variant-SIX5, pyDockDNA scoring -58.20.

DISCUSSION

One of the most significant results in the present study is the association between the presence of the genotypes CT+TT of the rs61330082 genetic variant and the increase in triglyceride levels, as well as VLDL in women from western Mexico diagnosed with metabolic syndrome.

It is important to consider each component of MetS, as TG levels in the Mexican population have been associated with an increased risk of coronary artery disease (2). Studies in different populations worldwide have evaluated a series of genetic variants in the promoter region of the *visfatin* gene. The results show an association with metabolic abnormalities associated with the diagnostic criteria for MetS (27–29). Ooi et al. reported for the first time that the genetic variant -3187 G>A is in linkage disequilibrium with the variant rs61330082, which indicates that the alleles -3187 A/-1535 T behave the same, so therefore our results coincide with this author regarding the increase of triglycerides in women carriers of the dominant model CT+TT (30).

The rs61330082 variant found in the promoter region can transcriptionally influence variations promoted by the genotype (27). Pleiotropic capacities of visfatin have been observed, so alterations in the transcriptional rate of visfatin promote changes in NAD+ cosubstrate levels, including at the intracellular level (iNAMPT). It has been described that iNAMPT catalyzes the rate-limiting step in the NAD+ biosynthesis pathway from nicotinamide. Therefore, NAD+ insufficiency contributes to various diseases, such as metabolic disorders, cancer, aging, and inflammation, by producing TNF-α and IL-6. When NAD+ concentrations are decreased, there is an energy imbalance between NADH/NADPH, leading to a more significant amount of oxidative stress due to the activation of the HIF1-α pathway under hypoxic conditions, which plays a crucial role in positively regulating NAMPT, influencing lipid processing, glucose homeostasis and dietary intake (31). It has also been shown that an adequate amount of NAD+ activates SIRT3, which promotes an improvement in oxidative metabolism and, in turn, a decrease in lipid abnormalities, such as elevated triglyceride levels (32).

In our study population, no difference was found between subjects with and without MetS concerning serum visfatin levels. However, Masood et al. investigated the association of other visfatin genotypes with MetS in the Pakistani population, finding significant differences in serum visfatin levels in subjects who presented MetS *vs* controls (33). In a study conducted on Mexican women with COPD, a decrease in serum visfatin levels was observed in the control group (34). These data are relevant since our population was predominantly female and without MetS. eNAMPT has not been associated with sustained viral response (15). Still, as an adipocytokine generated mainly through visceral adipose tissue, it impacts glucose and lipid metabolism while attenuating chronic inflammation associated with obesity. The connection between visfatin and metabolic abnormalities in patients with obesity or insulin resistance is still debated.

In our study, we evaluated the rs61330082 genotype and the dietary patterns associated with the development of MS in the population of western Mexico for the first time. However, no associations were found between the dietary component, the genetic variant of *visfatin*, and the risk of developing MetS. Some authors have been able to show that there is a positive association, which has an essential influence on the increase in susceptibility in the biochemical alterations of the components of MetS, among which is the change in eating habits, lifestyle, and the genetic factor of the Mexican population (35,36). Therefore, the associations not found in our study could be due to the characteristics of the study population, which has a mean age of 27 years. Higgins et al. showed that subjects in the university stage are less likely to develop metabolic alterations related to MetS criteria (37).

The evaluation of dietary patterns, assessed using the Mini-ECCA, suitable for the Mexican population, showed a greater orientation towards an intake of unhealthy foods. Still, no significant differences were obtained between MetS and dietary patterns. On the other hand, considering the genetic variant (rs61330082) and dietary patterns, it has been shown that other factors, considering the microbiota, circadian cycles, regular exercise, and mental health in the Latino population, could delay the development of MetS (37-39).

The presumed promoter region before the *visfatin* gene has been confirmed with multiple binding sites for transcription factors. Therefore, in this study, we performed a molecular docking test with a computational approach based on structures that predict the interactions between ligands and targets, generate the binding mode, and estimate the corresponding affinity. Another important finding of this study highlights the impact on transcriptional regulation. Our bioinformatics analyses show the strong coupling effect of the transcription factor PAX 5 with the polymorphic genetic variant, so we suggest that there is an influence on transcriptional regulation. When a protein-ligand complex's binding free energy (ΔG) is lower, the complex is more stable (40). In previous studies of biological process analysis, the role of PAX5 in transcriptional processes and the regulation of proinflammatory genes has been identified (41).

Finally, we are aware that our work has some limitations. This study is cross-sectional. Therefore, it is impossible to infer cause and effect in the development of MetS from personal genetic variations with nutritional interactions. Consequently, further studies on MetS are needed to understand the complexity of the factors involved in its pathogenesis. Therefore, the application of a longitudinal study that considers nutritional and genetic variables of visfatin and its involvement in systemic inflammation could explain the development of MetS and thus promote personalized nutrition and precision treatment with the aim of preventing comorbidities such as cardiometabolic risks.

An important limitation of the study was the lack of an instrument to assess caloric intake and the food groups from which daily calories were obtained, which prevented an adequate analysis of the caloric intake of the study subjects. In addition, the absence of an instrument to assess physical activity limited the ability to assess sedentary lifestyle and energy expenditure. Another limitation of this study is the low prevalence of MetS in the study subjects since we cannot deduce the incidence in the general population. However, more participants covering a broader age spectrum are required. Lastly, it is necessary to support the results of the *in silico* analysis with laboratory tests of an experimental nature.

CONCLUSION

This study found an association of the rs61330082 genetic variant in women carrying the CT+TT genotype with higher levels of TG and VLDL-C. However, no significant association was found between genotype and MetS development or serum visfatin levels. It should be noted that our bioinformatics study showed the interaction of the genotype with the transcription factor PAX5. Through the results obtained from this work, it is important to encourage translational studies with a dietary, genetic, inflammatory, and bioinformatics approach so that prevalent pathologies in the world population can be addressed in a preventive manner due to the tremendous epidemiological burden in health that it is currently facing.

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