ORIGINAL ARTICLE

Prevalence of Early B-Cell Factor 1 Gene rs4704963 Single Nucleotide Polymorphism (T>C) in a Population of Type 2 Diabetic Patients with Obesity

Prevalencia del polimorfismo del nucleótido simple rs4704963 (T>C) del gen Early B-Cell Factor 1 en una población de pacientes diabéticos tipo 2 con obesidad

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ABSTRACT

Background: Previous studies established that in a population with exacerbation of type 2 diabetes with obesity (DBT+Ob) suffering from stress, the prevalence of early B-Cell Factor 1 (EBF1) gene rs4704963 single nucleotide polymorphism (SNP) (T>C) is 16.5%.
Objectives: The aim of this study was to determine the prevalence of this SNP in patients with DBT+Ob attending Hospital Ramos Mejía of the Autonomous City of Buenos Aires and to ascertain whether this polymorphism is associated with stress or acute coronary events.
Methods: An observational, prospective study on the prevalence of rs4704963 SNP was performed in 53 patients with DBT+Ob and body mass index between 28 and 41, seen in Hospital Ramos Mejía for a period of 15 months. Each patient was evaluated with a stressful life events scale and a perceived stress scale.
The chi-square test and odds ratio (OR) were used for statistical analysis.
Results: A total of 53 patients were included in the study. Mean population age was 60.2±9.77 years and 47.2% were men. Among these patients, 8 (15.1%) presented SNP and all were heterozygous. Fifteen patients (28.3%) had acute ischemic syndrome (AIS), and among these, only one (6.6%) had SNP. No statistically significant relationship was found between the presence of SNP and AIS (p=0.282). Fourteen patients (26.4%) presented moderate or severe chronic stress, and there was no relationship between this finding and the presence of SNP (p=0.979).
Conclusions: The prevalence of EBF1 gene rs4704963 SNP (T>C) in the DBT+Ob population was 15.1%. No statistically significant association was found between SNP and stress or AIS.

Key words: Diabetes-Obesity- EBF1 gene – Polymorphism - Acute ischemic syndrome – Stress

RESUMEN

Introducción: En estudios previos, se determinó para una población con agravamiento de la diabetes tipo 2 con obesidad (DBT+Ob) que sufría estrés una prevalencia del polimorfismo de nucleótido único (SNP) rs4704963 (T>C) del gen Early B-Cell Factor 1 (EBF1) del 16,5%.
Objetivos: Determinar la prevalencia de este SNP en pacientes con DBT+Ob que acuden al Hospital Ramos Mejía de la Ciudad Autónoma de Buenos .Aires y establecer si dicho polimorfismo se asocia con el estrés o la ocurrencia de eventos coronarios agudos.
Material y métodos: Se llevó a cabo un estudio observacional, prospectivo, sobre prevalencia del polimorfismo en 53 pacientes con DBT+Ob e índice de masa corporal (IMC) entre 28 y 41, atendidos en el citado hospital en un período de 15 meses. A cada paciente se le computó una escala de estrés percibido, además de evaluarlo mediante la escala de acontecimientos vitales estresantes.
Para el análisis estadístico, se realizaron las pruebas de Chi cuadrado y se calcularon los odds ratio (OR).
Resultados: La población evaluada (53 pacientes) tuvo una media de edad de 60,2 ±9,77 años; 47,2% fueron hombres. De ellos, 8 individuos (15,1%) presentaron el SNP y todos fueron heterocigotas. Quince sujetos (28,3%) tuvieron síndrome isquémico agudo (SIA) y de estos solo uno (6,6%) tenía el SNP. No se halló relación estadísticamente significativa entre la presencia del SNP y la aparición de SIA (p=0,282). Catorce pacientes (26,4%) presentaron estrés crónico moderado o grave, y no hubo relación entre este hallazgo y la presencia del SNP (p=0,979).
Conclusiones: La prevalencia del SNP rs4704963 (T>C) del gen EBF1 en la población de DBT+Ob estudiada fue del 15,1% y no se halló relación estadísticamente significativa del SNP con el estrés ni con el SIA.

Palabras clave: Diabetes – Obesidad - Gen EBF1 – Polimorfismo - Síndrome isquémico agudo – Estrés

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Abbreviations

AIS	Acute ischemic syndrome	**EBF1**	Early B-Cell Factor 1
BMI	Body mass index	**Ob**	Obesity
DBT	Type 2 diabetes	**SNP**	Single nucleotide polymorphism

INTRODUCTION

Cardiovascular diseases are a first order public health problem and represent the main cause of death in our country. Hypertension along with obesity, sedentary lifestyle, dyslipidemia, smoking and type 2 diabetes are risk factors associated with cardiovascular death. In 75% of cases coronary artery disease is explained by the presence of these factors (1).

There is currently an increase in the prevalence of obesity and type 2 diabetes favored by lifestyle changes such as hypercaloric feeding and decreased physical activity.

The relationship between type 2 diabetes and obesity with the development of vascular disease is well known (2). The increase of these two factors can potentially generate a new epidemic of cardiovascular diseases not only in Argentina but also in the rest of the world.

In recent years the focus has also been placed on the importance of stress and its association with acute ischemic syndromes. Of importance, the results of the INTERHEART study confirm stress as another coronary risk factor. (3).

Although psychosocial risk factors may not have per se a direct effect on the development of cardiovascular disease, they may contribute indirectly through mechanisms that involve various biological pathways. (4) Thus, for example, repeated exposure to psychosocial stress has been related to the development of visceral obesity. (5)

In turn, stressful events can alter gene expression through epigenetic modifications such as methylation of gene promoter regions. (6) These findings support the need to understand the relationship between genetic susceptibility, psychosocial stress and metabolic factors that act in combination to increase cardiovascular risk.

One of the genes that plays an important role in the risk of cardiovascular disease is that of the transcription factor Early B-Cell Factor 1 (EBF1). (7) This factor participates in the development of the immune system, (8) adipogenesis (9, 10), and acts as an important regulator of adipose morphology, lipolysis and the development of insulin resistance.

A familial genetic link study established the association between individuals having the EBF1 gene single nucleotide polymorphism (SNP) [rs4704963: change of T by C (T>C)], located in the intronic region, with the early onset of coronary heart disease. (7, 11)

Likewise, the study of Singh et al. showed that patients of Caucasian origin living in the USA, who suffered stress and had SNP, had a higher incidence of central obesity measured by hip and waist circumference. However, these authors did not evaluate the incidence of these mutations in patients with the same conditions of obesity and type 2 diabetes who had suffered acute coronary events. (12) It should be noted that there are no previous studies that have considered this relationship in other populations of the continent, including South America.

Therefore, the aim of this research was to analyze the prevalence of the EBF1 gene rs4704963 SNP (T>C) in the population attending Hospital Ramos Mejía with DBT+Ob and establish whether there is a relationship with the presence of stress and acute coronary events.

METHODS

An observational, prospective study was carried out on the prevalence of EBF1 polymorphism in a population diagnosed with type 2 diabetes and body mass index (BMI) between 28 and 41, with presence or absence of acute ischemic syndrome (AIS). The study included 53 consecutive patients seen at Hospital General de Agudos JM Ramos Mejía of the Autonomous City of Buenos Aires, from December 2015 to March 2017. All patients were treated with metformin.

The research was conducted according to the Ministry of Health Resolution No. 1490/07 that approved the internationally recognized "Guide to Good Clinical Research Practice in Human Beings" and the International Conference on Harmonization "Good Clinical Research Practices" (ICH-GCP), the letter and spirit of the declarations of Nüremberg, Helsinki and their amendments, including those of Washington 2002, Tokyo 2004, Seoul 2008 and Fortaleza 2013, and Law 3301 on the "Protection of Subjects Participating in Clinical Research" of the Government of the City of Buenos Aires.

The study, together with the informed consent form and the information for patient sheet (ICF) was approved by the Research Ethics Committee of Hospital J.M. Ramos Mejía. Patients were invited to sign the ICF, and upon agreeing kept the original document. All patients were identified by their initials, a number and the group they belonged to. Their real identity was only known by the physician participating in the project, who is observant of the legislation on medical secrecy and Personal Data Protection Law 25,326.

The 53 patients enrolled in the study were over 18 years, of both genders and classified as follows:

A- Patients with AIS within the last 12 months and who at the time of the event had type 2 diabetes and BMI between 28 and 41.

B- Patients who presented BMI between 28 and 41 and diagnosis of type 2 diabetes, who had not suffered coronary events.

Patients who refused signing the informed consent, pre-

sented with known severe pathologies (excluding cardiovascular disease), had a life expectancy of less than one year, were participating in research protocols 30 days prior to blood test, and those in whom contact could not be ensured (personal or by telephone) were not included in the project. Subjects with type 1 diabetes, alcohol or drug abuse in the last 6 months, with indication for heart transplantation, with BMI below 28 or greater than 41, with clinical signs of liver or kidney failure (creatinine level at admission >2 mg/dl) were also excluded.

For the diagnosis of type 2 diabetes, the following indicators were considered:

a) Symptoms of diabetes (polyuria, polydipsia, weight loss) with random blood glucose levels (at any time of the day) >200 mg/dl.
b) Fasting blood glucose level (8 hours) >126 mg/dl.
c) Blood glucose level after glucose overload (75g) >200mg/dl at 2 hours.
d) Glycosylated hemoglobin HbA1c ≥6.5.

PROCEDURES

The Mental Health Team of Hospital General de Agudos J.M. Ramos Mejía was in charge of the application of the following scales to evaluate stress characteristics: the perceived stress scale (13-15) and the stressful life events scale (16) The perceived stress (acute) scale and the stressful life (chronic) events scale were positive for values ≥28 and ≥200, respectively. The study considered that the patient suffered stress when at least one of the two tests was positive.

The genetic analysis was based on DNA extracted from blood samples using the commercial Wizard® Genomic DNA Purification (Promega) kit. The concentration was evaluated measuring its absorption in a Nanodrop (De novix) spectrophotometer at 260 nm wavelength.

This DNA was used to amplify the segment of interest using nested polymerase chain reaction (nested PCR), in order to increase the specificity and lower the sequencing cost. For the first PCR reaction the following primers: 5`AGGAGAACATGCTTTGCCGT3` (forward) and 5`AGACACTTCAGGCTGACACA3` (reverse) were used, both produced by Genbiotech, and the PCR reaction was performed under the following conditions: a 2 min initial denaturation at 94°C, 2-35 cycles of 15 s denaturation at 92°C, 15 s pairing at 55°C, 70 s extension at 68°C; and a 5 min final extension at 68°C.

For the second PCR reaction the following primers: 5`GCCAGGATTCACTATCTTTGGAC3` (forward) and 5`ACAGCTCTAAGCTTCCTCCC3` (reverse), also from Genbiotech, were used, and the PCR reaction consisted in a 2 min-initial denaturation at 94°C, 35 cycles of 15 s-denaturation at 92°C, 15 s-pairing at 53°C, 30 s-extension at 68°C; and a 5 min-final extension at 68°C.

The amplicon was sequenced by Macrogen. The presence of the polymorphism was evaluated from the results obtained, using the DNA Baser program.

Patients were followed-up by the project medical team to evaluate the occurrence of an adverse event, and when necessary, other studies were requested according to their current pathology. Patients who refused this possibility were invited to attend the hospital once a year to identify the events they might have suffered and those who refused to attend were contacted by telephone by the team of professionals. Patients were also free to refuse telephone calls.

Statistical analysis

SPSS, version 22.0 (SPSS Inc., Chicago, IL, USA) statistical package was used to analyze the data. Continuous variables were described through central trend and dispersion measurements, while absolute and relative frequencies were used for categorical variables.

To test an association between polymorphism and the presence of AIS, the chi square test was used and the odds ratio (OR) was calculated with a 95% confidence interval (CI). A p value ≤ 0.05 was considered statistically significant.

Ethical considerations

The protocol was evaluated and approved by the Institutional Ethics Committee.

RESULTS

Baseline characteristics and risk factors according to the presence or not of AIS are described in Table 1.

Mean population age was 60.2±9.77 and 47.2% were men. Average BMI was 32.4±3.53, 45.3% were smokers, 60.4% hypertensive and 56.6% had dyslipidemia.

Fifteen patients of the total population (28.3%) had AIS. Of these, 9 (17%) presented AIS with persistent ST-segment elevation and 6 (11.3%) without persistent ST-segment elevation. The remaining 38 patients did not present any coronary event.

Among the total number of patients, 8 (15.1%) presented heterozygous SNP (Figure 1). The homozygous form was not found in this population

Among the 15 patients with AIS, only one (6.6%) presented the polymorphism, while there were 7 patients (18.4%) with SNP who did not present coronary events (Figure 2).

When the relationship between the presence of SNP and the appearance of AIS considering the domi-

Table 1. Population characteristics. Selected variables according to the presence of acute ischemic syndrome

Variables	DBT patients n=53	Acute ischemic syndrome	
		Without AIS n=38	With AIS n=15
Age (Mean, SD)	60.2±9.77	59.8±10.25	61.3±8.66
Male sex (%)	47.2	26.4	20.8
BMI (kg/m2)	32.4±3.53	33.1±3.41	30.6±3.29
Smoking, n (%)	24 (45.3)	15 (28)	9 (17)
HTN, n (%)	32 (60.4)	19 (35.8)	13 (24.5)
Dyslipidemia, n (%)	30 (56.6)	21 (39.6)	9 (17)

DBT: Diabetes; AIS: Acute ischemic syndrome; SD: Standard deviation; BMI: Body mass index; HTN: Hypertension.

nant genetic model was analyzed, no association was found between them. The OR was 3.161, and no statistical significance was found with the Cochran test (p=0.282).

When assessing the level of stress in patients according to whether or not they had AIS, no statistically significant differences were observed. In both groups approximately 74% of patients presented mild stress, while around 26% registered moderate or severe stress (p=0.979) (Figure 3).

When the population with stress and SNP is considered, no statistically significant differences are observed among patients with or without AIS (p=0.837).

DISCUSSION

This aim of this study was to establish the prevalence of EBF1 gene rs4704963 SNP (T>C) in patients presenting DBT+Ob and its relationship with stress and acute coronary events, considering a dominant genetic model.

Results showed a significant 15.1% prevalence of this SNP in our population, similar to that found by Singh et al. in the Caucasian USA population. (12) All patients presenting this SNP had overweight or grade I or moderate obesity according to the World Health Organization classification. No patient with SNP presented with severe obesity.

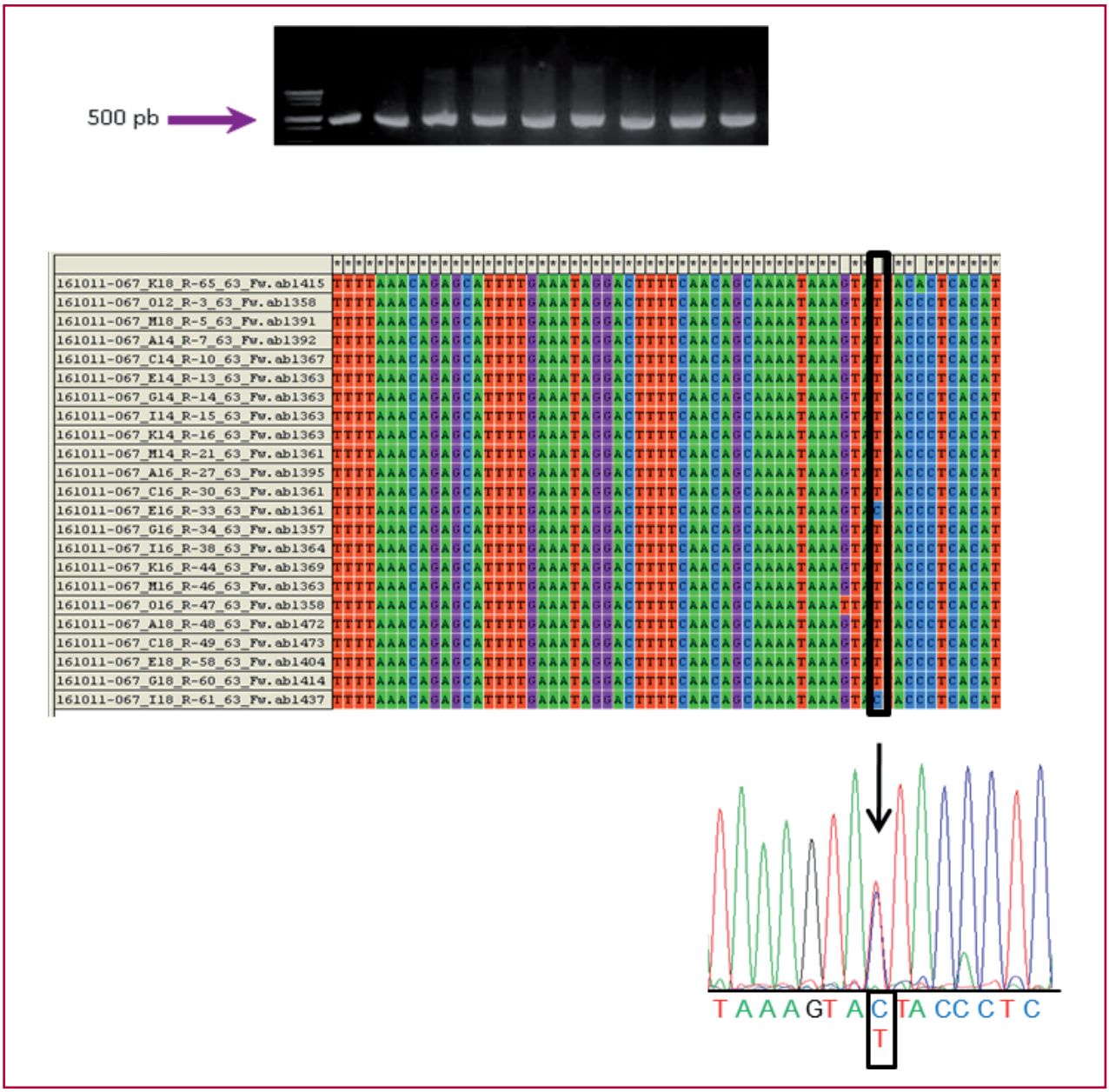

Fig. 1. Representative electrophoretic image of amplicons obtained by nested PCR belonging to different patients. Analysis of different patient EBF1 gene sequence homology. The inset shows an electropherogram corresponding to a C/T heterozygous patient for the SNP analyzed.

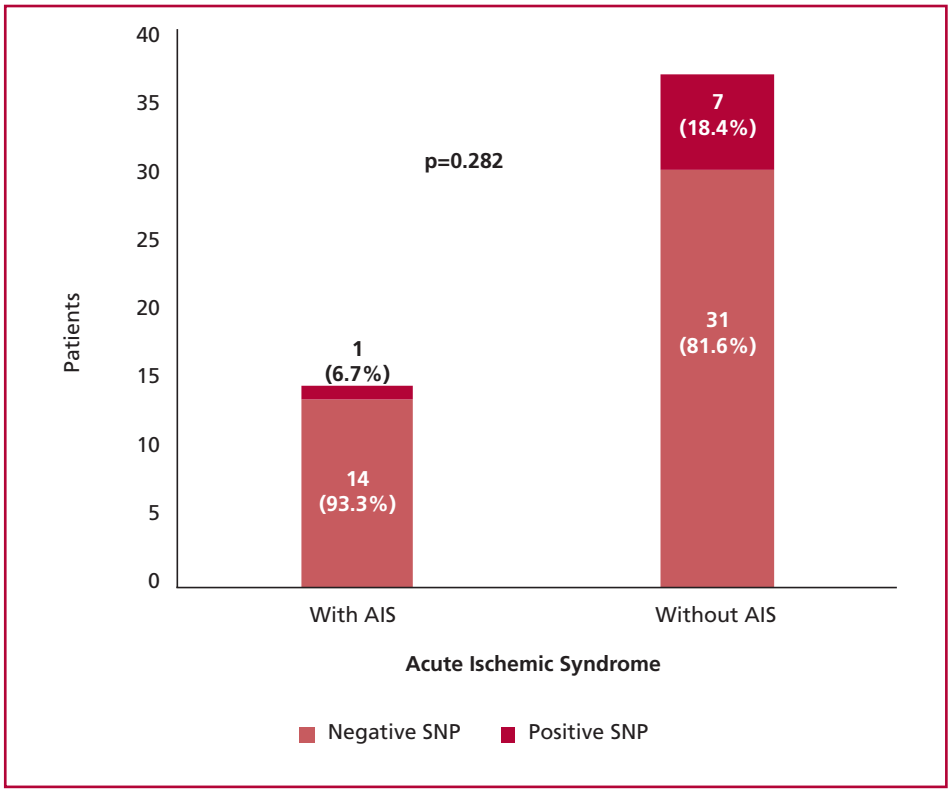

Fig. 2. Prevalence of single nucleotid polymorphism (SNP) in patients with and without acute ischemic syndrome (AIS).

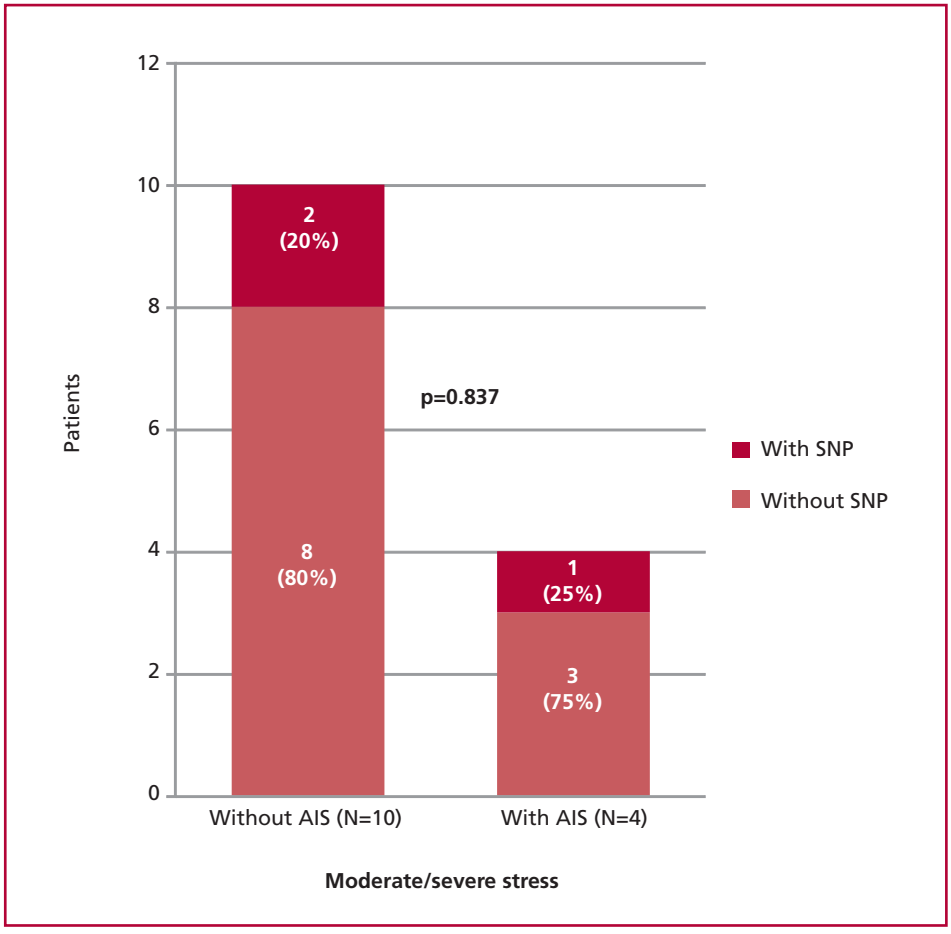

Fig. 3. Prevalence of chronic stress and single nucleotid polymorphism (SNP) in patients with and without acute ischemic syndrome (AIS). No significant differences were observed between presence of stress and SNP (p=0.837 between patients with or without AIS.

When analyzing patients with positive test for stressful life events, which means moderate or severe stress of several months duration, 21.4% had SNP, though this did not mean a statistically significant relationship between stress and the presence of SNP in the population sample.

EBF1 is a member of the EBF gene family that is widely expressed in many tissues as lymphocytes B, bone marrow, olfactory neurons and adipocytes. Specifically, EBF1 plays an important role in the promotion of adipogenesis. (18) In addition, it has been shown that leptin concentrations are significantly reduced in knockout mice for EBF1 (19) Adipocyte-generated leptin is an essential hormone to prevent obesity, as it regulates energy expenditure, appetite and metabolism, and this hormone deficit results in obesity. It should also be mentioned that in general, serum leptin levels increase with emotional stress. (20) However, at the same time, stress, promotes glucocorticoid release, inducing leptin resistance. (21) In summary, psychosocial stress may develop obesity despite the increase in leptin levels by inducing the resistance to this protein. On the other hand, EBF1 polymorphisms could contribute to the process of central obesity triggered by stress due decreased leptin levels.

Coronary heart disease is highly associated with hypercholesterolemia, obesity and type 2 diabetes, and has a hereditary component, with the role of genetic factors increasingly becoming more evident in the early stages of the disease. (22, 23)

Shah et al. studied families with high LDL-cholesterol levels and saw that this was related with variations in the long arm of chromosome 5 (5q). (24) Later, Nolan et al. demonstrated that variations in genes located in a portion of chromosome 5 (5q31-33), among them the EBF1, were associated with the early development of cardiovascular disease. Moreover, the rs4704963 SNP (T>C) studied in our work is one of the polymorphisms related with atherosclerosis and cardiovascular disease. (25)

Although previous studies have associated rs4704963 SNP with the development of vascular disease, this study is the first that has investigated whether there is a relationship between this polymorphism and the occurrence of AIS in a population with high prevalence of polymorphism, as that of DBT+non-morbid Ob patients. Results could not establish this relationship.

CONCLUSIONS

This genomics work is the first analyzing the prevalence of EBF1 gene rs4704963 SNP (T>C) in a population sample of DBT+Ob in South America. The prevalence found in this study is 15.1%. No statistically significant relationship between stressful life events and presence of SNP was found in this population, and neither greater incidence of major acute ischemic events

Future investigations approaching this topic should increase sample size, to verify whether a larger sample modifies the results of statistical tests.

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/ Supplementary material)

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