ARTÍCULO ORIGINAL

Gac Méd Caracas 2020;128(3):360-372
DOI: 10.47307/GMC.2020.128.3.9

The A54T polymorphism in the FABP2 gene and its relationship with obesity

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SUMMARY

Introduction: *Obesity is a complex, multifactorial, and mostly preventable disease affecting, along with overweight, more than a third of today's world population. Variations in the nucleotide sequence of both metabolic and appetite control genes have been counted among these non-modifiable factors and are associated with BMI, lipidic profile, and abdominal circumference alterations.* **Methods:** *An analytical, non-experimental, and transversal research was done with the purpose to assess the presence of A54T polymorphism in the FABP gene in a sub-sample from the Maracaibo City Metabolic Syndrome Prevalence Study.* **Results:** *154 individuals eight subjects were carriers of the A54T polymorphism, namely, a genotypic frequency of 5.19 %, with a sex distribution of 50 % for women (n = 4) and 50 % (n = 4) for men. In respect of alleles similarity degree, 75 % (n = 6) were homozygous, and 25 % (n = 2) were heterozygous. Obesity diagnosis throughout BMI was only present in 12.50 % (n = 1) of the A54T carriers. Conversely, 25 % (n = 2) of the carriers were overweighed; 50 % (n = 4) were presented as normal-weight people; and only 12.50 % (n = 1), in one underweighted person.* **Conclusion:** *As in many other studies, we do not find an association between Ala54Thr polymorphism and obesity. This result reinforces the fact of the multifactorial character of these diseases and a carrier state of this polymorphism is not necessarily to experience a higher obesity risk, at least, in our environment.*

Key words: *Obesity, polymorphism, mutations, cardiovascular diseases, Type 2 Diabetes mellitus, FABP2*

DOI: https://doi.org/10.47307/GMC.2020.128.3.9

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RESUMEN

Introducción: *La obesidad es una enfermedad compleja, multifactorial y en su mayor parte prevenible que afecta, a más de un tercio de la población mundial actual. Las variaciones en la secuencia de nucleótidos de los genes de control del metabolismo y del apetito se consideran hasta ahora como factores no modificables y se asocian con alteraciones del IMC, del perfil lipídico y de la circunferencia abdominal.* **Métodos:** *Se realizó una investigación analítica, no experimental y transversal con el propósito de evaluar la presencia del polimorfismo A54T en el gen FABP en una submuestra del Estudio de Prevalencia del Síndrome Metabólico de la Ciudad de Maracaibo.* **Resultados:** *De los 154 individuos de la submuestra ocho sujetos fueron portadores del polimorfismo A54T, es decir, una frecuencia genotípica de 5,19 %, con una distribución por sexo de 50 % para las mujeres (n=4) y 50 % (n=4) para los hombres. Con respecto al grado de similitud de los alelos, el 75 % (n=6) eran homocigotos y el 25 % (n=2) heterocigotos. El diagnóstico de obesidad a lo largo del IMC sólo estuvo presente en el 12,50 % (n=1) de los portadores de A54T. Por el contrario, el 25 % (n=2) de los portadores tenían sobrepeso; el 50 % (n=4) se presentaron como personas de peso normal; y sólo el 12,50 % (n=1) en la categoría de peso insuficiente.* **Conclusión:** *No se encontró una asociación entre el polimorfismo de Ala54Thr y la obesidad. Este resultado refuerza el carácter multifactorial de estas enfermedades y que un estado portador de este polimorfismo no es causa necesaria para padecer obesidad, al menos, en nuestro medio.*

Palabras clave: *Obesidad, polimorfismo, mutaciones, enfermedades cardiovasculares, Diabetes mellitus de tipo 2, FABP2*

INTRODUCTION

Obesity is a complex, multifactorial, and mostly preventable disease (1) affecting, along with overweight, more than a third of today's world population (2). If the tendencies continue, it is estimated by 2030 that 38 % of the adult world population will be overweight, and another 20 % will be obese (2). Today's situation in Venezuela is similar to that in other parts of the world. In a study conducted by Bermúdez et al (3) in Maracaibo, obesity exhibited a prevalence of 33.3 %, with 32.4 % among women and 34.2 % among men.

Even if a significant part of the component of the obesity pathogenesis has been related to modifiable factors such as eating habits, physical activity, and the environmental influence, somewhere between 40 % and 70 % have been attributed to genetic and other non-modifiable factors (4,5). Thus, variations in the nucleotide sequence of both metabolic and appetite control genes have been counted among these non-modifiable factors and are associated with BMI, lipidic profile, and abdominal circumference alterations (6). One of these factors is the A54T (rs1799883) polymorphism in the *FABP2* gene, a genetic variation associated with obesity (7–10). FABP2 protein transports intestinal fatty acids, regulating the intestinal absorption of fatty acids (11-13). Notably, the genetic variant A54T (rs1799883), which corresponds to alanine for threonine change in the amino acid 54 of the transcript has been related to a higher affinity for long-chain fatty acids with a corresponding increased risk for obesity and metabolic syndrome (14).

In physiological conditions, this protein is involved in long-chain fatty acids (LCFA) transportation from the intestinal lumen to the enterocyte, a fundamental step to metabolize LCFA as both energy source and membranes biosynthesis (15). *FABP2* gene polymorphisms are relatively frequent, with genotypic frequencies ranging between 30 % to 40 %, and depending on the studied populations it has been associated with obesity, type 2 diabetes, and insulin resistance (9). Phenotypic features in lipidic profile and both fasting and postprandial glycemic patterns associated with the A54T are different, maybe due to several factors like methodological or genetic/environmental interactions, two relevant points to consider when studying the causes of obesity and overweight (7). In this research, sequence polymorphism A54T in exon 2 of FABP2 was studied because of the scarce information regarding neither the prevalence nor the association with obesity, and possible confounder effect of other cardiovascular risk variables (16,17).

MATERIALS AND METHODS

Population, sample size calculation, and sampling technique

An analytical, non-experimental, and transversal (18) research was done with the purpose to assess the presence of A54T polymorphism in FABP gen located in exon 2 in obese individuals. The original sample was conformed by 2 230 individuals from the Maracaibo City Metabolic Syndrome Prevalence Study [3]. Subsequently, the sample was stratified in two groups: People with a BMI below 30 kg/m^2 (controls), and those with a BMI equal or over to 30 kg/m^2 (cases). As a result of this process, 33.50 % (n=747) were classified as obese, and the remaining 66.50 % (n=1 483) were classified as non-obese individuals. Of these subpopulations, a sub-sample was extracted through a stratified random sampling method. A 95 %confidence level and an 80 %test power were assumed. The exposure proportion between cases was calculated at a 30 % level, OR detection limits of 4 and a case-control ratio of 1:3. The assigned value for was assumed by the literature review, which reflects similar data as used here (8). For calculation procedure, the program Epidat (4.2 version for Windows 64 bits) was used, obtaining a sample size of 144 individuals.

Clinical evaluation and standard laboratory workup

Clinical evaluation, anthropometric assessment, and laboratory workout are published elsewhere. However, in brief, a full clinical history was made for each patient (19), in which the registration of anthropometric measurements like weight, height, and abdominal circumference was done. The waist circumference cut-off points for abdominal obesity diagnosis were the one published for Maracaibo city by our group, 91.50 cm for women, and 98.15 cm for men (20). Blood pressure quantitation was assessed by a calibrated sphygmomanometer, with the patient previously rested (for a minimum of 15 minutes) in a sitting position with both feet touching the floor. The arm was positioned at the heart level, and an appropriately sized cuff was employed for the procedure. Systolic blood pressure was determined at the first Korotkoff sound, whereas diastolic blood pressure was determined at the fifth Korotkoff sound. Blood pressure values were determined twice, with an interval of at least 15 minutes, and the results were averaged. Blood pressure classification was completed using the criteria proposed in the VII Joint National Committee (JNC-7). Overnight fasting determination of glucose, total cholesterol, triglycerides, and HDL-C was done with an automated analyzer (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany); the intra-assay variation coefficient for the total cholesterol, TAG, and HDL-C was 3 %, 5 %, and 5 %, respectively. LDL-C and VLDL-C levels were calculated applying the Friedewald' sequations only if triglycerides were below 400 mg/dL. If LDL-C was above this cut-off point, LDL-C concentration was quantified through lipoprotein electrophoresis and densitometry with an optical densitometer (GS-800 densitometer BioRad, USA) (21). Insulin was determined using an ultrasensitive ELISA double-sandwich method (DRG Instruments GmbH, Germany, Inc.). Homeostasis Model Assessment (HOMA-IR) calculation for insulin sensitivity by the equation proposed by Matthews et al. (22). The cut-off point ≥ 3.03 was selected from those previously published for Maracaibo city population (23).

DNA extraction and polymorphism identification

Genomic DNA extraction was done according to the combined DNA "Saltingout" extraction technique (24). The integrity of the extracted DNA was evaluated through 1 % agarose gel electrophoresis with TBE 1X buffer (100 mm Tris-HCl, 2mm EDTA, 100 mm boric acid) dyed with ethidium bromide (0,5 μg/mL), allowing the direct observation of the total DNA. After the run, an UV light transilluminator (Mini Digidoc UVP System) was employed to visualize the DNA. The exon 2 of the *FABP2* gene was amplified using the PCR protocol proposed by Miller et al (25). A single-strand DNA polymorphism conformational analysis (SSCP) technique, as proposed by Baier et al (26) was applied to evidence sequence alterations at exon 2 in the *FABP2* gene. The SSCP identify a single nucleotide variation in a segment of DNA, typically between 150 to 200 nucleotides long. The technique is simple, versatile, and affordable, but demands optimizing parameters in gel composition for each fragment to be evaluated. PCR products showing PCR-SSCP altered migration patterns were selected for sequencing

through an automatized sequencing protocol in an ABI PRIMS 310 sequencer (Applied Biosystem, CA, USA). A sequencing kit Big Dye® Terminator v3.1 (Thermo Fisher Scientific, MA, USA) was used to obtain the sequence of nucleotides, which were interpreted in the Sequencing Analysis Software v5.3 (Thermo Fisher Scientific, MA USA) and aligned with reference sequences in BLAST to detect changes in the gene's sequence studied.

Statistics analysis

Univariate normality assumption was verified through both Kolmogorov-Smirnov's and Geary's tests. The chi-square independence test was employed to assess the association between qualitative variables, while proportions were compared through the Z test. A binary logistic regression model was built, calculating both raw and adjusted OR for the presence or absence of obesity and adjusted according to metabolic syndrome diagnosis, age, sex, and ethnicity. Arithmetic means were contrasted by the independent-samples Student's t-test and unifactorial variance analysis test. Tukey's model was used as a post-hoc analysis. Moreover, both the Mann-Whitney´s U test and the Kruskal-Wallis H test were used to assess multiple group's means comparisons when these groups exhibited a non-normal distribution pattern. The size of the effect in these cases was calculated only when significant differences were found using the Cohen's "d" for the parametrical contrasts and the approximation method based on the relative asymptotic efficiency for the non-parametrical. Likewise, the test power was estimated from the observed difference or the detected effect only when the contrast was significant. The data was processed in SPSS (version 25), Epidat (version 4.2), and G-Power (version 3.1.9.2). Test results were considered statistically significant when the P-value was ≤ 0.05.

RESULTS

The biochemical, clinical, and anthropometrical variables in obese and non-obese individuals are described in Table 1. As expected, significant differences were found between cases and controls in variables like BMI (25.60 kg/m^2 vs 35.71 kg/m^2, t=-9.06, $P=1.22\times10^{-19}$) and abdominal circumference (88.47 ± 11.42 cm vs 113.66 ± 12.28 cm, t=-9.06, $P=1.22\times10^{-19}$), and in biochemical variables like insulin (13.21 ± 7.05 μU/mL vs 21.05 ± 8.04 μU/mL, t=-5.43, $P=2.36\times10^{-7}$), HOMA (2.06 ± 1.08 vs 3.18 ± 1.24, t=-5.03, $P=1.44\times10^{-6}$), and HDL concentration (46.54 ± 14.59 mg/dL vs 36.83 ± 11.77 mg/dL, t=3.64, $P=3.67\times10^{-4}$). The same pattern was observed in the systolic blood pressure (117.50 mmHg vs 129.50 mmHg, z=-3.61, $P=3.01\times10^{-4}$) and diastolic blood pressure (80 mmHg vs 84 mmHg, z=-3.56, $P=3.58\times10^{-4}$). It must be noticed that our sample size allowed to reach a high power in all contrasts, detecting effects of considerable magnitude in BMI (TE=2.97, 1-β=1.000), abdominal circumference (TE=2.17, 1-β=1.000), insulin (TE=1.08, 1-β=0.999), and HOMA (TE=1.01, 1-β=0.999). Furthermore, the differences in HDL (TE=0.69, 1-β=0.952), systolic pressure (TE=2.97, 1-β=0.853), and diastolic pressure (TE=2.97, 1-β=0.957) were moderate.

From 154 individuals eight subjects were carriers of A54T polymorphism, namely, a genotypic frequency of 5.19 %, with a sex distribution of 50 % for women (n=4) and 50 % (n=4) for men. In respect of alleles similarity degree, 75 % (n=6) were homozygous, and 25 % (n=2) were heterozygous. Obesity diagnosis throughout BMI was only present in 12.50 % (n=1) of the A54T carriers. Conversely, 25 % (n=2) of the carriers were overweighed; 50 % (n=4) were presented as normal-weight people; and only 12.50 % (n=1), in one underweighted person.

Table 3 depicts the relevant variables in the homozygous and heterozygous groups. Aside from using the usual summary stats, the contrast was used through the t-Student test to identify significant differences. Hence, the behavior of the biochemical, clinical, and anthropometrical variables was quite similar between homozygous and heterozygous. Differences between the descriptive terms are appreciated, but for the magnitude of these discrepancies, they are not large enough to be declared statistically significant. Only in the case of the HDL concentration, a statistical significance was

Table 1

Biochemical, clinical, and anthropometric variables in obese and non-obese individuals

Variables	Obesity diagnosis according to BMI (cases-controls)			Est. (p)	TE (1-β)
	Total (n=154)	Cases (n=118)	Controls (n=36)		
Parametrical contrasts					
Age (years)	44.01 (16.59)	43.29 (17.58)	46.36 (12.76)	-1.14 (.255)	----
Abdominal circumference (cm)	94.36 (15.77)	88.47 (11.42)	113.66 (12.28)	-11.37 (4.35×10-22)	2.17 (1.000)*
Insulin (μU/dL)	15.11 (8.02)	13.21 (7.05)	21.05 (8.04)	-5.43 (2.36×10-7)	1.08 (0.999)
HOMA-IR	2.33 (1.22)	2.06 (1.08)	3.18 (1.24)	-5.03 (1.44×10-6)	1.01 (0.999)
Triglycerides (mg/dL)	153.06 (102.31)	147.19 (110.64)	172.27 (65.91)	-1.29 (.198)	----
Total cholesterol (mg/dL)	199.51 (48.34)	199.48 (49.74)	199.61 (44.13)	-0.01 (.988)	----
VLDL (mg/dL)	30.61 (20.46)	29.43 (22.12)	34.45 (13.18)	-1.29 (.198)	----
HDL (mg/dL)	44.27 (14.54)	46.54 (14.59)	36.83 (11.77)	3.64 (3.67×10-4)	0.69 (0.952)
Non-parametrical contrasts					
BMI (Kg/m^2)	26.82 (30.90)	25.60 (13.22)	35.71 (17.35)	-9.06 (1.22×10-19)	2.97 (1.000)*
Fasting glycaemia (mg/dL)	97.00 (291.00)	97.00 (291.00)	100.50 (156.00)	-1.40 (.160)	----
LDL (mg/dL)	121.43 (313.70)	119.60 (309.70)	124.70 (222.80)	-0.75 (.449)	----
Systolic pressure (mmHg)	120.00 (100.00)	117.50 (100.00)	129.50 (90.00)	-3.61 (3.01×10-4)	0.59 (.853)
Diastolic pressure (mmHg)	80.00 (60.00)	80.00 (40.00)	84.00 (50.00)	-3.56 (3.58×10-4)	0.72 (.957)

Both arithmetic's mean and standard deviation for normal-distributed variables are shown. Median and range were employed for those variables that did not follow the normal distribution. For both arithmetic means and medians comparison, the t-Student or U of Mann-Whitney tests were used respectively. The size of the effect has been calculated only when significant differences were found, by the Cohen's d statistical for parametrical contrasts and the method of approximation based on the relative asymptotic efficiency for the non-parametrical. Moreover, the test power was calculated from the observed difference or the detected effect, only when the contrast was significant. The asterisk represents the test power when asymptotically tends to one.

registered (t=-2.72, P=0.035, TE=2.06, 1-β=0.562), finding more elevated values in the group of the heterozygote (69.00 ± 15.55) than in the group of the homozygote (41.16 ± 11.82).

The results examining the possible association between polymorphism and obesity are shown in Table 3. The independence test did not result in an association between these two variables (X^2=0.56, P= 0.682). If the conditional probabilities are contrasted to a total of 36 selected obesity cases, only 2.78 % (1/36) has the wild form of the FABP2 gene. Of the total 118 chosen controls, 5.93 % (7/118) had polymorphism. The Z contrast conducted over these proportions was not significant (z=-0.75, P=0.456).

An in-deep analysis from a multivariant perspective, Table 5 shows the construction of a model of logistic binary multiple regression model, which response is derived from the obesity diagnostic, and which predictor's responds to the presence or absence of mutation. Sex, age, race, and metabolic syndrome were considered as adjustment variables, without finding important

The marginal probabilities and the conditions inside the cases and controls are shown. The conditional proportions with different sub-indexes differ between them at P<0.05.

differences between raw and adjusted OR. Interaction or confusion effects were not found, nor a significant association between obesity and polymorphism was found (raw-OR=2.21, P=0.466, ICB 95 %: 0.26 – 18.56, adjusted-OR=2.46, P=0.423, ICB 95 %: 0.27 – 22.19). Table 5 amplifies these comments.

Table 2

Description of the relevant variables for each of the subjects with A54T polymorphism in the FABP2 gene

Variables	Patients with polymorphism identified through electronic medical record codes							
	JV143	JV892	JV927	JV1238	JV450	JV449	JV1833	JV181
Sex	Female	Male	Female	Male	Male	Male	Female	Female
Type of polymorphism	Homozygous	Homozygous	Heterozygous	Heterozygous	Homozygous	Homozygous	Homozygous	Homozygous
Age (years)	21.00	33.00	20.00	29.00	49.00	52.00	35.00	55.00
BMI (kg/m^2)	16.60	22.06	21.37	22.47	24.39	29.80	29.12	45.34
Abdominal circ. (cm)	63.00	86.00	78.00	79.00	102.00	106.00	92.00	120.00
Basal glucose (mg/dL)	97.00	88.00	78.00	92.00	243.00	107.00	78.00	97.00
Insulin (μU/dL)	13.60	6.50	11.50	NI	5.80	38.50	NI	28.60
HOMA-IR	2.00	1.00	1.60	NI	1.30	5.60	NI	4.10
Triglyceride (mg/dL)	111.00	64.14	71.26	40.00	86.00	266.00	160.00	107.00
Total cholesterol (mg/dL)	158.00	143.00	159.00	188.00	130.00	207.00	219.00	184.00
VLDL (mg/dL)	22.20	12.83	14.25	8.00	17.20	53.20	32.00	21.40
LDL (mg/dL)	81.80	75.17	86.75	100.00	78.80	128.80	144.00	126.60
HDL (mg/dL)	54.00	55.00	58.00	80.00	34.00	25.00	43.00	36.00
Systolic blood pressure (mmHg)	110.00	110.00	110.00	100.00	130.00	110.00	111.00	124.00
Diastolic blood pressure (mmHg)	70.00	64.00	65.00	70.00	80.00	70.00	80.00	85.00

The specific values of each subject are shown in each cell. NI means "no information."

Table 3

Description of the relevant variables in the homozygous and heterozygous subjects

Variables	A54T Polymorphism			Est. (p)	TE (1-β)
	Total (n=154)	Homoz. (n=6)	Heteroz. (n=2)		
Age (years)	36.75 (13.73)	40.83 (13.27)	24.50 (6.36)	1.61 (.150)	----
BMI (kg/m^2)	26.39 (8.76)	27.88 (9.83)	21.92 (0.77)	0.81 (.447)	----
Abdominal circ. (cm)	90.75 (18.16)	94.83 (19.53)	78.50 (0.70)	1.12 (.305)	----
Glicemia basal (mg/dL)	110.00 (54.63)	118.33 (61.85)	85.00 (9.89)	0.72 (.498)	----
Insulin (μU/dL)	19.31 (11.81)	19.17 (12.97)	19.72 (11.63)	-0.05 (.960)	----
HOMA-IR	2.85 (1.63)	2.85 (1.77)	2.82 (1.73)	0.02 (.985)	----
Triglycerides(mg/dL)	113.17 (71.55)	132.35 (72.83)	55.63 (22.10)	1.40 (.211)	----
Totalcholesterol (mg/dL)	173.50 (31.13)	173.50 (35.68)	173.50 (20.50)	0.00 (1.000)	----
VLDL (mg/dL)	22.63 (14.31)	26.47 (14.56)	11.12 (4.42)	1.40 (.211)	----
LDL (mg/dL)	102.74 (26.68)	105.86 (30.54)	93.37 (9.37)	0.88 (.411)	----
HDL (mg/dL)	48.12 (17.33)	41.16 (11.82)	69.00 (15.55)	-2.72 (.035)	2.06 (0.562)
Systolic blood pressure (mmHg)	113.12 (9.40)	115.83 (8.86)	105.00 (7.07)	1.54 (.173)	----
Diastolicblood pressure (mmHg)	73.00 (7.69)	74.83 (8.01)	67.50 (3.53)	1.21 (.274)	----

Only the average and the standard deviation are shown because all characteristics were normally-distributed in the group with polymorphism. For comparison, the t-Student test was used. The size of the effect was calculated only when significant differences were found using Cohen's statistical d. Likewise, the test power was calculated from the observed difference or the desired effect, only when the contrast was significant.

Table 4

Independence chi-square test for obesity and A54Tpolymorphism in the FABP2 gene

Polymorphism	Obese (cases)			Non-obese (controls)			Est. (p)
	n	% inside the total	% inside the cases	n	% inside the total	% inside the controls	
Yes	1	0.65	2.78a	7	4.54	5.93a	0.56 (0.682)
No	35	22.73	97.22[a]	111	72.08	94.07a	
Total	36	23.38	100.00	118	76.62	100.00	

Table 5.

Binary logistic regression for obesity and A54T polymorphism in the FABP2 gene

Variable	Raw			Adjusted		
	OR	P-value	CI 95 %	OR	P-value	CI 95 %
Predictive variable						
FABP2 (present against absent)	2.21	.466	0.26 – 18.56	2.46	0.423	0.27 – 22.19
Adjustment variable						
Age	1.01	.332	0.99 – 1.03	1.01	0.332	0.99 – 1.04
Sex (masculine against feminine)	2.98	.006	1.37 – 6.46	3.13	0.005	1.40 – 6.96
Metabolic syndrome (yes against no)	1.74	.342	0.55 – 5.48	1.34	0.656	0.37 – 4.80
Race (Hispanic whites against mixed-race)	0.89	.812	0.35 – 2.29	1.03	0.960	0.38 – 2.78
Race (afro-Venezuelan against mixed-race)	1.27	.780	0.23 – 6.95	1.10	0.922	0.18 – 6.81

Logistic regression model according to the presence or absence of obesity. The odds raw and adjusted rations are presented according to age, sex, presence or absence of metabolic syndrome, and race. The possible interaction or confusion was investigated without finding any indications of it.

DISCUSSION

From multicellular organisms such as nematodes to *Homo sapiens*, all animal species store excess energy in the form of fat for their energy and lipidic synthesis needs. Nematodes such as C. elegans store fat in the intestine (27,28), while cartilaginous fish like sharks, store fat in the liver (29,30). Nevertheless, in most animal species, fat is stored in the white adipose tissue (WAT) to supply the energy demands when these exceed those provided by the food (31). The location of the WAT varies depending on the species studied. For example, in most amphibians and reptiles the WAT is found in the intra-abdominal region; in almost all mammals, except pinnipeds (seals) and some cetaceans (whales and dolphins), and in many birds, the adipose tissue is divided into a dozen or more deposits widely distributed around the body (6).

In the humans, the distribution, physiology, and dysfunction of body fat play an essential role in the risk of developing some pathological entities, since the increase of intra-abdominal/ visceral fat promotes micro-inflammation, diabetes and other metabolic diseases (20,32,33). Many studies have shown that both fat storage and distribution are related to age, sex, hormonal environment, fat synthesis and transport, catabolism of triacylglycerides, among other less

classic factors that have been elucidated in the last 20 years (20,34-38). Among these factors, the *fatty acid-binding proteins* (FABP) are critical mediators for the storage and distribution of triacylglycerides (39-43).

FABPs are a family of small cytosolic proteins that were first identified in the early 1970s. Initially, they were thought to bind almost exclusively to long-chain ASFs, but we now know that their specificity for ligands extends to many other hydrophobic molecules, including endocannabinoids and lipophilic drugs. In general, FABPs are proteins of 14 to 15 KDa and a primary structure of 126 to 134 amino acids, sharing 20 % to 70 % similarity in their amino acid sequence, with multiple isoforms depending on the tissue in which they have been isolated (44-46). The hallmarks of the secondary and tertiary structural organization of this protein superfamily are the presence of a central barrel-shaped cavity β consisting of 10 antiparallel beta leaves and two alpha (46,47). The interior of the barrel contains water molecules but is also lined with hydrophobic amino acid side chains that can accommodate ligands, such as long-chain fatty acids. The distinguishing characteristics of different members of this family are the width and volume of the binding cavity and the positioning and sequence of amino acids in the alpha-helix that is part of the "lid" of the beta-barrel. In fact, it has been shown that differences in the domain of the helical cap are reflected in striking differences in the mechanism of ligand transfer to and from the membranes (46,47). Variations in FABP structures are believed to modulate the stoichiometry of ligand binding, as well as ligand specificity.

To date, 12 FABPs are known in vertebrates and invertebrates and in mammals, nine different FABPs with very-specific distributions have been identified (43,49). The systemic effects of FABPs may be secondary to the actions on tissues in which they are expressed, or possibly through their presence in circulation. The circulating levels of many of the FABPs are considered diagnostic markers of the physiological state of their source tissues. The primary function of all members of the FABP family is the regulation of fatty acid absorption and intracellular transport. Therefore, we wonder how many FABP genes exist in species with different fat tissue deposits to regulate fat transport and storage.

In humans, the FABP-2 gene (IFABP) is located in the chromosomal region 4q28-4q31 and it is only expressed in the small intestine. Because of its specific tissue location, the potential of IFABP as a biomarker of intestinal damage has been explored under a variety of conditions, including celiac disease and necrotizing enterocolitis in premature infants (50–52). Similarly, the IFABP has been associated with higher intestinal permeability in patients with T2DM, along with the lipopolysaccharide (LPS) and the LPS binding protein (52). In line with the idea that IFABP is an essential marker of intestinal integrity, it has recently been discovered that mice without IFABP have decreased intestinal villus length, altered goblet cell density, and increased intestinal permeability (54).

While their physiological role is not yet fully clarified, hypotheses on the functions of FABPs include the neutralization of GAs in the cytosol, minimizing their toxic effects on the cell by avoiding interactions with cell membranes or solutes. Besides, these hypotheses say they would regulate lipid synthesis and secretion in intestinal cells, probably influencing intracellular transport of GA to cellular organelles. On the other hand, another possible functional role in gene modulation through lipid transduction signals. FABPs, as cellular transporters, can carry lipids with transcription regulatory function to the nucleus, influencing PPAR (55-59). Long-chain polyunsaturated AGs act as ligands of the PPARγ2 and other receptors of the PPAR family; these bonds can induce or reduce differentiation of adipocytes. Finally, differential GA absorption can influence the phospholipidic composition of the cell membrane, contributing to the state of mild chronic inflammation that accompanies insulin resistance and related pathologies (55-57).

In this regard, it is of interest that one of the most common polymorphisms in this protein that occurs in codon 54 of exon 2 of the human FABP2 gene exchanges an alanine (Ala) in the small helical region of the protein for threonine (Thr). It has been speculated that in humans, the binding of fatty acids to IFABP produces a conformational change that is expressed in the establishment of a closed spin containing the amino acid residues 54 and 55 of this protein.

These residues change position when long-chain fatty acids bind to the protein, so even a subtle change in its amino acid sequence could affect the structural properties of IFABP in such a way that it may alter its affinity for the ligand (60).

Different studies suggest that the substitution of Ala by Thr is, in fact, a functional mutation (26,61) because many of them have shown that the IFABP Thr54 allele is associated with a larger concentration of total cholesterol, with a significant incidence of strokes (62), an increase in fasting and postprandial triglycerides (63), insulin resistance (59) and greater concentrations of non-esterified fatty acids (NEFA) (64). Nevertheless, many contradicting studies have not been able to find a significative association with these variables (65-68). In light of the possible physiological role of FABP2 polymorphism, we assessed the frequency of the local population of the Thr54 allele and analyzed its possible associations with five selected markers: Glycaemia, Total Cholesterol, Body Mass Index (BMI), Hypertension and Cardiovascular Risk Index (CVR).

In the present study, Ala54Thr polymorphism was detected in the FABP2 gene in only eight subjects out of 154, representing a genotypic frequency of 5.19 % (Homozygote 3.89 %; n=6 and Heterozygote 1.29 %; n=2) and whose distribution according to sex was equal with 50 % (n=4) in men and 50 % (n=4) in women. In this sense, the frequency of FABP2 Ala54Thr polymorphism found in our study is lower than that found in other countries, where it fluctuates between 37 % and 45 %, such as in the United States (36.93 %), Sweden (41.9 %), Japan (40.62 %), Great Britain (44.77 %), Korea (41.66 %), Egypt (43.3 %) and India (42.04 %) (69,70). In contrast, studies in other ethnic settings have shown a lower frequency in this polymorphism, such as one conducted in Canadian aborigines that reported an overall genotypic frequency of 26.1 % (23.9 % for heterozygous and 2.35 % for homozygous) (71).

In Latin America, the genotypic frequency of the Ala54Thr variant is equally variable according to the population studied, as it is in the rest of the world. For example, in Chilean aboriginal populations, an allele frequency of 18.2 % in Aymaras and 31.9 % in Mapuches has been found (16). Similarly, in Argentina, a recent study revealed an allelic frequency of 0.277 (95 % confidence limits 0.234-0.323) and genotypic frequencies of 40 % for heterozygous and 7.42 % for homozygous (17). On the other hand, a genotypical frequency of 54.8 % for Ala54Thr was reported (10) in Mexico. These differences can be explained by the most diverse ethnic context of the studied populations, as well as the methodology and sample size used in each study.

In this research, no statistically significant association was observed between obesity and the presence of the Ala54Thr polymorphism of the FABP2 gene, which includes this study in a long list of works with conflicting results of this polymorphism and its association with type 2 diabetes, obesity, and metabolic syndrome. However, these results contrast with those obtained in a recent case-control study conducted by Liu et al. in Hubei province. The association between this polymorphism and the presence of T2DM (235 cases/431 controls), obesity (377 cases/431 controls), and metabolic syndrome (315 cases/323 controls) were investigated and found to be associated with the presence of obesity (TA vs. AA: OR = 2. 633, 95 % CI = 1.065-6.663, P = 0.036; TT vs. AA: OR = 4.160, 95 %CI = 1.609-10.757, P = 0.003) and with the diagnosis of metabolic syndrome (TT vs. AA: OR = 2.273, 95 %CI = 1.242-4.156, P = 0.008), after adjustment for covariates and possible confounding elements.

However, the study also concludes that no association was found between this polymorphism and the presence of T2DM. Interestingly, this study complements the analysis of its local data with a meta-analysis in which 24 studies were included that analyzed the association between the polymorphism and T2DM (4 517 cases, 5 224 controls), nine studies with obesity (949 cases, 2 002 controls), and six studies related to metabolic syndrome (2 194 cases, 3 282 controls). In this case, the study revealed significant association between polymorphism and the diagnosis of metabolic syndrome (T allele: OR = 1.179, 95 % CI = 1.015-1.362, P = 0.031) and T2DM (T allele: OR = 1.160, 95 % CI = 1.08-1.24, P < 0.001), without association with obesity (T allele: OR = 1.069, 95 % CI = 0.925-1.235, P = 0.367). These differences, placed

in the context of our study, could be due to the smaller number of subjects studied, which could affect the statistical power and consequently, the detection of a greater number of cases that allow the observation of differences between the metabolic phenotypes studied (Obesity, Diabetes, SM). Among the reasons that may explain these differences, we can also mention the different ethnic origin of the populations studied, the research methodology and the real prevalence of this polymorphism in our population, which is ultimately one of the most important variables to calculate the statistical power and the sample size to be used.

CONCLUSIONS

This research investigated and its relationship with obesity. However, as in many other studies, we do not found an association between Ala54Thr polymorphism and this condition. This result reinforces the fact of the multifactorial character of these diseases and that carrying this polymorphism is not necessarily to experience a higher obesity risk, at least, in our environment. Likewise, our findings suggest that, unlike other populations, the genotypic frequency of this polymorphism is low in our population.

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