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RESEARCH ARTICLE

Frequency of ADIPOQ 276 and ADIPOQ 45 Polymorphisms in Obese and Eutrophic Adolescents with and without Asthma and their Relationship with Serum Adiponectin Levels

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ABSTRACT

Background: Asthma is a chronic allergic disease characterized by variable airflow limitation; Obesity is a chronic disease that has reached epidemic proportions globally. Both are diseases with a significant inflammatory component, and their relationship suggests being weight dependent. Adiponectin (ADIPOQ) is the main adipokine secreted by white adipose tissue, it is an insulin synthesizer and regulator of energy homeostasis, and its plasma levels are inversely correlated with obesity and asthma. The effect of genetic factors in both diseases has been investigated, and haplotypes of the ADIPOQ 45 T/G (rs2241766) and ADIPOQ 276 G/T (rs1501299) polymorphisms have been related.

Aims: To know de polymorphisms frequency of ADIPOQ 45 and ADIPO 276 in obese and eutrophic adolescents with and without asthma, likewise, link the adiponectin levels with the presence of such polymorphisms.

Methods: An observational, analytical, and cross-sectional study in which 169 Mexican adolescents were recruited. Thirty mL of blood was taken from each individual; genomic DNA was extracted using the saline expulsion technique and quantified by spectrophotometry; two polymorphisms located in the promoter region were studied: ADIPOQ 45 and ADIPOQ 276; the determination of the different polymorphisms was carried out using TaqMan probes using real-time PCR (qPCR) using the commercial kit TaqMan One Step RT-PCR mastermix, the RNA extraction was carried out with Trizol Ls (Invitrogen), and the fluorescence was quantified employing the 7900HT ABI PRISM real-time computer SDS software.

Results: There were no statistically significant differences between ADIPOQ 276 and ADIPOQ 45 polymorphisms in asthmatic and obese patients. Compared to the control group, a negative correlation was observed between adiponectin plasmatic levels in obese and asthmatic individuals.

Conclusion: The ADIPOQ 276 and ADIPOQ 45 polymorphisms do not seem to be associated with asthma and obesity in the Mexican population. It is necessary to continue studying these polymorphisms and consider larger populations.

Keywords: adiponectin; asthma; obesity; polymorphisms; ADIPOQ 276; ADIPOQ 45

Introduction

Asthma is a chronic allergic disease characterized by variable airflow limitation due to narrowing, hypersensitivity, hypersecretion, and thickening of the inferior airway wall.

Different studies have confirmed the coexistence of obesity and asthma.[1,2] Obesity is a chronic inflammatory disease that has reached epidemic proportions worldwide. The excessive weight gain prevalence has doubled globally since 1980, and it is associated with a higher mortality risk; excessive visceral adiposity is associated with a higher risk of metabolic complications.[3,4] Obesity induces low-grade systemic inflammation in the adipose tissue (meta-inflammation), which is mainly mediated by macrophages; as the adipose tissue expands, the distance between the adipocytes increases, triggering hypoxic death to some adipocytes. In response to the adipocyte death, M1 macrophages remove the debris from the damaged area, producing inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1-β (IL-1β), and Monocyte chemoattractant protein-1 (MCP-1).[2]

White adipose tissue is an endocrine organ that secretes adipokines, which are physiologically involved in the energetic homeostasis and, pathologically, in typical alterations of obesity, such as immunological and inflammatory reactions. The main adipokines are leptin, adiponectin, TNF-α, IL-6, transforming growth factor β (TGF-β), plasminogen activator inhibitor-1 (PAI-1), angiotensin, adipsin, resistin and acylation stimulating protein. Adiponectin is the main adipokine expressed by adipocytes, it is an insulin synthesizer and regulator of energy homeostasis as it increases glucose utilization and fatty acid oxidation.[1,4] The adiponectin gene is apM1, it is located on chromosome 3q27.[4] In macrophages, adiponectin prevents M2 polarization, amplifies the response to IL-4, and inhibits the production of inflammatory cytokines such as TNF-α, IL-6, IFN-γ and activates IL-10, which is anti-inflammatory.[1, 2, 5]

Adiponectin inhibits apoptosis after cell injury and promotes the proliferation and repair of basal bronchial epithelial cells. Similarly, it decreases the production of chemokines from mast cells (CXCL1) and macrophages (CCL2), as well as the output of TNF-α, which limits inflammation and suppresses the production of metalloprotease 12 (MM-12), which mediates degradation of the extracellular matrix and is associated with greater hyperreactivity and severity of asthma. In airway smooth muscle, adiponectin stimulates AMP-activated kinase (AMPK), inhibits TGF-β-mediated smooth muscle proliferation, and interferes with mucus secretion by inhibiting omentin and MUC5AC.[1,6]

The relationship between asthma and obesity suggests being weight-dependent; a low concentration of adiponectin has been associated with a higher incidence of asthma and has been negatively correlated with severity. In addition, adiponectin levels are inversely proportional to body mass index (BMI), where it has been shown that adiponectin concentrations in people with anorexia nervosa are significantly higher than in their healthy counterparts. Similarly, it has been observed that adiponectin returns to normal levels after weight loss, which correlates with improvement in asthmatic symptoms.[2, 7, 8, 9]

The effect of genetic factors on obesity and asthma has been investigated, and haplotypes of the ADIPOQ 45 T/G (rs2241766) and ADIPOQ 276 G/T (rs1501299) polymorphisms have been related. Specifically, in a meta-analysis, it was observed that a subgroup in China with ADIPOQ 45 T/G polymorphisms with the GG genotype had a 1.54-fold increased risk of obesity compared to the population with the TT genotype.[10] On the other hand, a study in India found that ADIPOQ 276 G/T polymorphisms with the TT genotype were associated with increased hip circumference, serum cholesterol, LDL-C, triglycerides, and insulin during fasting, as well as low levels of HDL-C.[11]

Another study conducted in China demonstrated that ADIPOQ gene polymorphisms are associated with asthma, where the TT genotype of ADIPOQ 276 G/T polymorphism and single nucleotide polymorphisms (SNPs) of both ADIPOQ 276 G/T and ADIPOQ 45 T/G were associated with an increased risk of developing asthma and having an acute exacerbation.[12] The study of adiponectin polymorphisms and the relationship between these and serum levels of this protein can contribute to the understanding of the phenomenon that occurs between obesity and asthma and with this to be able to identify individuals in the long term susceptible and insist on their early treatment.

As far as we know, only one study in Mexico related to the polymorphisms mentioned above. This study demonstrates the relationship between overweight/obesity and leptin (rs7799039) and ADIPOQ 276 and 45 polymorphisms with a decreased response to breast cancer treatment. breast in a sample of 177 Mexican women.[13]

Therefore, considering the role of adipokines on lung function, and the importance of

treatment strategies to control obesity and respiratory conditions, the primary objective of the present study was to determine the frequency of ADIPOQ 45 and ADIPOQ 276 polymorphisms in obese and eutrophic adolescents with and without asthma and to relate adiponectin levels and the presence of these polymorphisms in adolescents in obese and eutrophic adolescents with and without asthma.

Methods

An observational, cross-sectional, and analytical study was conducted in collaboration with the National Institute of Cardiology in Mexico, in which 169 adolescents were recruited from the obesity clinic and secondary schools near the hospital. Once the consent form was signed, a complete clinical history was taken, the determination of anthropometric measurements and body composition (weight, height, waist circumference, BMI, and electrical bioimpedance), as well as peripheral blood extraction. The inclusion criteria were the following: adolescents aged 11-16 years, men and women, BMI above the 95th percentile of the CDC tables according to weight and age, eutrophic adolescents with a BMI of 50-85% of the CDC tables, and asthmatics controlled without inhaled steroids according to the GINA 2018 classification.

To ensure that the individuals belong to the Mexican population, each one of them was asked if their last two generations were born in Mexico. In the same way, ancestry genes were determined using 15 STRs markers (AmpfIstr identifiler, Applied Biosystems); this will be done by capillary electrophoresis in a 4-capillary 3130 AB automated sequencer, using interpreted fragment analysis in Genemapper V.4.0 software. Previous data on STR frequencies in Spanish from Andalucia, East Africans, and Amerindians from Hidalgo were used; these populations are used as ancestral populations in trihybrid models for admixture estimation. The STR markers determined are D8S1179, D21S11, D7S80, CSF1PO, DS1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWa, TPOX, D18S51, D5S818, and FGA. The combined discrimination power of the 15 STRs was 0.999999999, and thus the genetic admixture estimates reveal 69% Amerindian, 26% European, and 5% African.[14]

Individuals were classified into four groups based on BMI and whether they have asthma. Without asthma: non-morbidly obese with BMI >95% and eutrophic with BMI 50-84%; and with asthma: non-morbidly obese with BMI >95% and eutrophic with BMI 50-84%.

Molecular studies were carried out in the Department of Molecular Biology (National Institute of Cardiology, Mexico). 30 mL of blood were taken from each individual in Vacutainer tubes with EDTA as anticoagulant, of which 10 mL were used for the extraction of DNA necessary to analyze the polymorphisms and the remaining 20 mL were necessary to separate the monocytes since they are required 30 million cells with purity above 65% to carry out the gene expression assays of said adipokines. The plasma obtained was used to count adiponectin in it by ELISA.

Genomic DNA was extracted from this sample using the salting out procedure.[15] The integrity of the DNA was verified in 1% agarose gels stained with ethidium bromide, and the DNA obtained was quantified by spectrophotometry.

The frequency of the polymorphisms to be studied in other populations was reviewed in the Hap-Map, and only those polymorphisms whose minor allele frequency was greater than 5% were included; Therefore, two polymorphisms located in the promoter region were studied: ADIPOQ 45 and ADIPOQ 276.

The different polymorphisms were determined using TaqMan probes in a qPCR kit using the TaqMan One Step RT-PCR mastermix commercial kit. The alleles were assigned employing an allelic discrimination program included in the qPCR equipment.

Monocytes were isolated from individuals using the technique reported by Almeida et al.[16] The separation protocol included two steps: first, a Ficoll-Hypaque gradient was carried out on 20 mL of blood obtained from each individual; then, the buffy coat was passed through a gradient of hyperosmotic Percoll. The percentage of monocytes was evaluated in a flow cytometer using an anti-CD4 antibody. Only cultures with monocyte purity greater than 65% were used.

RNA extraction was performed with Trizol Ls (Invitrogen). 0.75 mL trizol was used per 5-10x^{10} cells and homogenized by pipetting at least ten times to lyse the cells. Homogenized samples were incubated for 5 min at 25°C to allow complete dissociation of the nucleoprotein complexes. Subsequently, 0.2 mL of chloroform were added for every 0.75 mL of Trizol Ls, vigorously shaken for 15 seconds, incubated for 15 minutes at 25°C and centrifuged at 12,000 x g for 15 minutes at 8°C. After centrifugation, the aqueous phase was

extracted and the RNA was precipitated by adding 0.5 mL of isopropanol for every 0.75 mL of Trizol LS and incubated at 20°C for 10 minutes, then centrifuged at 12,000 x g for 10 minutes at 8°C. RNA was precipitated, the RNA button was washed once with 75% ethanol, adding at least 1 ml of ethanol for every 0.75 ml of Trizol Ls. The sample was mixed vigorously and centrifuged at 7,500 x g for 5 minutes at 8°C. At the end of the procedure, the RNA button was dried for 5-10 minutes, and the RNA was dissolved in RNase-free water or a 0.5% SDS solution and incubated for 10 minutes at 55-60°C.

Once the polymorphisms of the different regions of the genes associated with the disease were identified, tests related to the effect of the polymorphism on gene expression were carried out. Titration curves of some healthy individuals were made to standardize the method, and constitutively expressed genes were used as positive controls. Fluorescence was quantified using the 7900HT ABI PRISM real-time instrument SDS software.

Results

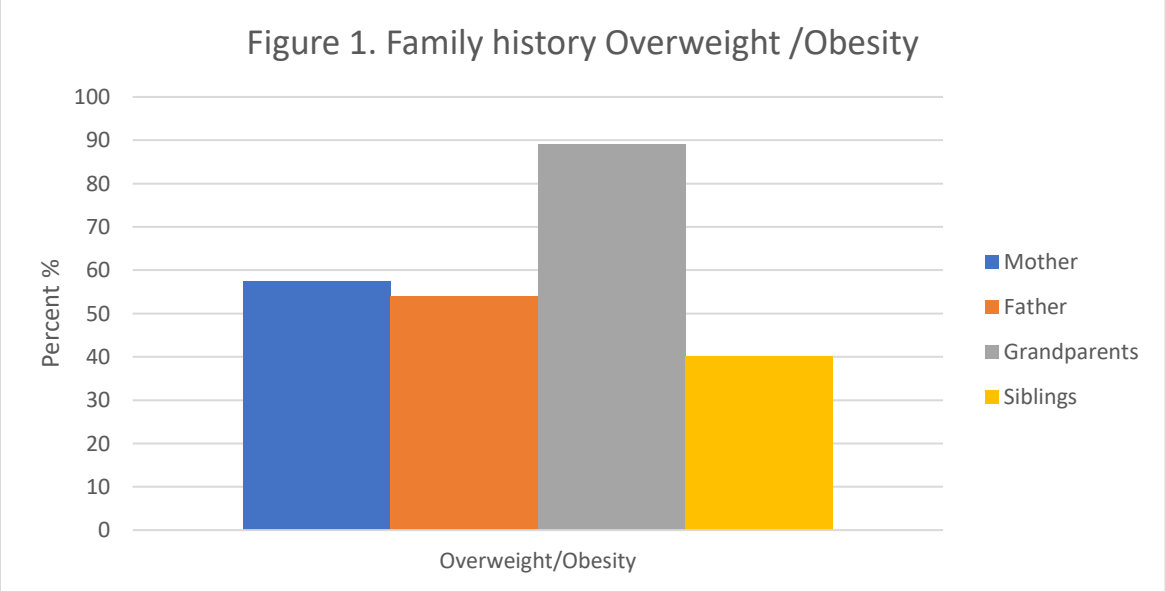
Of 169 patients, 55% were men and 45% were women. Taking into consideration BMI, it was found that the mean was 22.41 with a standard deviation of 5.01 with a minimum of 14.00 and a maximum of 37.70; with an average age of 12.6 years, a standard deviation of 1.5 years, a minimum of 10 and a maximum of 16.9 years (Tab. 1).

Table 1. Characteristics of population

	Individuals	Mean	SD
Age (months)	169	152.82	19.11
BMI (kg/m²)	169	22.42	5.01

BMI – body mass index. SD- standard deviation

It was found that the history of overweight/obesity in the mother was 57.4%, in the father 59%, grandparents 84%, and siblings 40% (Fig. 1)



The frequency of homozygote polymorphism for ADIPOQ 276 was 56.8% and heterozygote 42% (Tab. 2). In the different groups, the homozygote polymorphism of the group was 38 (62.29%), group 2 was 25 (60.97%), group 3 was 18 (43.90%), and group 4 was 15 (62.5%); while the heterozygote polymorphism was 23 (37.70%), 15 (39.02%), 23 (50.09%) and 9 (37.5%) respectively; with a p= 0.24 (Tab. 3).

Table 2. Frequency of polymorphisms of ADIPOQ 276 & ADIPOQ 45			
		Frequency	Percent %
ADIPOQ 276			
	Homozygote	97	56.8
	Heterozygote	72	43
ADIPOQ 45			
	Homozygote	123	72.8
	Heterozygote	44	26

Instead, the frequency of homozygote polymorphism for ADIPOQ 45 was 72.8% and heterozygote 26% (Tab. 2). In the different groups, the homozygote polymorphism of group 1 was 45 (73.77%), group 2 was 25 (60.97%), group 3 was 35 (85.36%), and group 4 was 18 (75%); while the heterozygote polymorphism was 16 (26.22%), 16 (39.02%), 6 (14.63%) and 6 (25%) respectively; with a $p = 0.09$ (Tab. 3).

Table 3. Distribution of ADIPOQ 276 and ADIPOQ 45 polymorphisms according to each group						
	ADIPOQ 276			**ADIPOQ 45**		
	A	B	Total	A	B	Total
1	38	23	61	45	16	61
2	25	16	42	25	16	41
3	18	23	42	35	6	41
4	15	9	24	18	6	24
Total	95	71	167	123	44	167
A – homozygote, B – heterozygote, 1- eutrophic without asthma, 2 – eutrophic with asthma, 3 – obese without asthma, 4 – obese with asthma.						

The mean of the adiponectin levels in the homozygote group of the ADIPOQ 276 polymorphism was 15.80, while in the heterozygote group, it was 15.42; with a $p=0.76$, while the mean of the adiponectin levels in the homozygous group of the ADIPO 45 polymorphism was 15.96 and in the heterozygous group it was 14.75 with a $p= 0.38$ (Tab. 4).

Table 4. Serum adiponectin levels (mg/ml) according to polymorphisms			
		Serum adiponectin levels	
	n	**Mean**	**SD**
ADIPOQ 276			
Homozygote	96	15.80	9.48
Heterozygote	71	14.42	5.41
ADIPOQ 45			
Homozygote	123	15.96	8.65
Heterozygote	44	14.97	5.71

Discussion

The social, environmental, cultural, and genetic determinants of obesity are responsible for the increase in prevalence. Our study showed that the genetic family history of overweight or obesity was high, which showed that at least one of the relatives living in the same household as the individual is overweight or obese. In a study by *Ma C. et al.*, they showed an inverse correlation between BMI and serum adiponectin levels, which is consistent with our study since adiponectin levels were lower in obese patients compared to the control group of eutrophic patients.[8] In complex genetic diseases such as obesity, many genes are involved, and unclear inheritance patterns are present.

Previous studies have shown the important role of multidisciplinary therapies in treating obesity [17-19]. However, the effects of this type of treatment on lung function, asthma-related symptoms, depending on the degree of weight loss, have not been studied. Obesity negatively affects several respiratory parameters such as compliance, lung volume and airway responsiveness [20]. The mechanisms underlying these effects are likely to be complex and may involve the mechanical effects of

central fat deposition on the diaphragm and chest wall, causing a reduction in diaphragm excursion and, consequently, it causes reduced thoracic compliance and lung volume, limiting lung expansion [21,22] and inflammatory processes associated with the release of adipokines from adipose tissue. Adiponectin, or pro-inflammatory properties such as leptin [23,24].

Similarly, we found that eutrophic patients with asthma had even lower adiponectin levels than the group of eutrophic individuals without asthma; this agrees with other authors, where an inverse correlation of plasma adiponectin was observed in asthmatic individuals in the Chinese population with acute exacerbation.[12] However, in this study, an association of the ADIPOQ polymorphism between the TT genotype and acute exacerbation in asthmatics was demonstrated; this was not observed in our study since individuals without asthma presented a greater number of polymorphisms than their asthmatic counterparts.

Ramya K. et al. reported an independent association of the ADIPOQ 276 G/T polymorphism in individuals with obesity, which is consistent with our study since eutrophic individuals compared to obese individuals had a greater number of polymorphisms.[11] Similarly, an association of the ADIPOQ 45 T/G polymorphism with central obesity in the Iranian population has not been found.[25]

Different studies on the Indian population and Caucasians have shown that the ADIPOQ 276 and ADIPOQ 45 polymorphisms are associated with a higher risk of obesity. This did not occur in our study, which could be due to the type of population.[11,26]

If there is an effect of leptin (and other adipokines) on asthma, it is difficult to assess their independent role due to their high correlation with obesity. Obesity is called a "leptin-resistant" state, and leptin is produced by fat cells (thus, higher BMI equals higher leptin levels). However, leptin is also part of the negative feedback. A loop that suppresses appetite and increases metabolic rate (thus leptin levels affect his BMI). It is widely used in the study of metabolic disorders, although it is not entirely clear whether it is appropriate to match one to the other when fat content and adipokines are measured at one time point. Epidemiologists who have studied associations between breast cancer [27], hypertension [28] and diabetes [29] and obesity and adipokines have also commented on the difficulty of elucidating their effects. This complicates causal conclusions and fails to provide evidence that adipokines influence the development of asthma or atopy [30].

Our study has several limitations. These types of studies are usually planned to be carried out in large populations, it is a difficult task to recruit individuals with this type of specific characteristics, and the availability of such patients is limited. The population size was decided considering previous studies of this type and our financial and technical capabilities; however, the number of individuals was sufficient to determine statistically significant serum levels of adiponectin.

Conclusions

In this study, we were able to confirm that family history is important since approximately 50% of adolescents have at least one relative who is overweight or obese.

It has been observed that obese individuals have decreased levels of adiponectin; although we would expect to find even lower levels of adiponectin in obese individuals with asthma, this was not the case.

The polymorphisms of the ADIPO 276 and 45 heterozygous genes studied have been related to obesity in some population groups, while others have no relationship. It is essential to continue studying these polymorphisms, both in these genes and in other related ones, since knowledge of the variants in these groups leads to identifying genetic factors associated with risk or protection.

Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

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To incredible staff of the Department of Molecular Biology of the National Institute of Cardiology, Mexico

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