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***ADIPOQ* and *LEP* variants on asthma and atopy: genetic association modified by overweight**

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RSC performed experiments and data analyses, explained the main results and wrote the final version. APCM reviewed the literature and contributed to data analysis and discussion. HSS performed experiments, analyzed genescore and helped with data interpretation. RCRS drafted the manuscript and contributed with the methodology nutrition design, SMAM revised the manuscript and contributed with the methodology nutrition design, MLB

designed the SCAALA, collected and created the database, and reviewed the manuscript. NMAN designed the SCAALA, collected and created the database, and reviewed the manuscript. CAVF contributed to the conception and design of this study, drafted, wrote and verified the manuscript. RdSC designed this study, and drafted, wrote and verified the manuscript analyzing results according to the immunogenetic. All authors approve the final version and they assume responsibility related to its accuracy and integrity.

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Abstract

Background

Asthma and atopy are considered condition associated with obesity, being affected by genetic and environmental factors. The LEP and ADIPOQ genes, responsible for the expression and secretion of leptin and adiponectin, respectively, and polymorphisms in such genes have been linked to both diseases, independently, and also with the obesity-associated asthma phenotype in populations with high European ancestry and high-income countries. However, in mixed populations, there are few studies evaluating the impact of these variants in genes associated with the phenotype of asthma and obesity. Thus, the aim of this study was to

investigate variants in LEP and ADIPOQ associated with asthma and atopy, and whether overweight modifies that effect.

Methods

The study involved 203 asthmatics children and 813 control subjects (between 5 and 11 years old), with or without overweight, from the SCAALA (Asthma and Allergy Social Changes in Latin America) program. Among them, 831 had data for allergy markers, being 258 atopic and 573 non-atopic. Genotyping was performed using a commercial panel *Omnium Illumina* 2.5. Logistic regression was performed to identify associations expected by using PLINK 1.09 and three genetic models: additive, dominant and recessive adjusted for sex, age, helminth infection, BMI and Principal Components (PC) 1 and 2, for ancestry, in order to control the confounding factor by population structure.

Results

For asthma, G allele of rs822396, in ADIPOQ, was positively associated in additive model (OR 1.4, 95% CI 1.08-1.83) and T allele of rs1063537 in dominant model (OR 1.52, 95% CI 1.01-2.30). In LEP, rs11763517 (C allele) and rs11760956 (A allele) were both negatively associated with asthma in the additive model (OR 0.70, 95% CI 0.54-0.91; OR 0.66, 95% CI 0.50-0.89) respectively, and the A allele of rs2167270 in dominant model (OR 0.71, 95% CI 0.51-0.98). The G allele of rs12706832 showed a positive association with asthma in the recessive model (OR 1.66, 95% CI 1.06-2.61). When the population was stratified by the BMI / Age Z-Score, the protection observed for asthma between the variants rs11760956, rs11763517 and rs2167270 was lost overweight individuals; The protection observed for

atopy was lost in all variants (rs16861205, rs2167270 and rs17151919) in the overweight group.

Conclusion

These results suggest that SNPs on the LEP and ADIPOQ genes may have an impact on atopy and asthma. Furthermore, we also show that the asthma and atopy protection attributed to variants on LEP and ADIPOQ genes is lost in individuals exposed to overweight.

Keywords: Adipokines; Asthma; Atopy; Genetic; Overweight.

1. Introduction

Asthma is described as a chronic disease that involves inflammation, high mucus production, obstruction, hyperresponsiveness and narrowing of the low airways (Azizpour et al., 2018; Lambrecht and Hammad, 2015). It has a complex etiology and high heterogeneity, which determines a wide variety of phenotypes, including obesity-associated asthma (Froidure et al., 2016; Moraes et al., 2018; Periyalil et al., 2013). According to Rastogi and Holguin (Rastogi and Holguin, 2017), this phenotype is associated with a greater burden of disease, reduced lung function and suboptimal response to medications in children.

The worldwide incidence of asthma has been increasing in developing countries (Stern et al., 2020). According to GINA (2020) (Global Initiative for Asthma., 2020), the disease affects around 358 million individuals, with approximately 40 million in Latin America, and represents one of the most common chronic disease in children and adolescents (Cruz et al., 2018; Pitchon et al., 2019; Solé et al., 2017). Obesity follows the same pattern of increasing prevalence in children and adolescents, actually, doubled in children and

quadrupled in adolescents in recent years (Azizpour et al., 2018). In Brazil, 47.8% of children and 25.7% of adolescents were overweight/obese, in the last population-based survey recorded (IBGE. Instituto Brasileiro de Geografia e Estatística and Coordenação de Trabalho e Rendimento, 2011).

Epidemiological studies have shown associations between asthma and obesity, with the obesity rate in asthmatic individuals being approximately 50% (Mukadam et al., 2018; Silva et al., 2013). For childhood asthma, obesity is recognized as an independent risk factor (Vijayakanthi et al., 2016). The relationship between obesity and pediatric asthma was analyzed in a meta-analysis study, with mixed populations and involving Brazilian studies (Deng et al., 2019). The authors corroborate the results of the meta-analysis by Chen et al. (2013), by demonstrating a high risk of developing asthma in overweight and obese children.

In a recent study of GWAS (genome-wide association study) in individuals of European descent, a positive genetic correlation was observed between characteristics of obesity and late onset and non-atopic asthma (Zhu et al., 2020). However, the correlation between childhood obesity and early-onset asthma still requires further investigation.

Inflammatory, genetic, epigenetic and mechanical factors may contribute to the asthma/obesity phenotype. It has been observed that changes in the secretion of adipokines, such as leptin and adiponectin, which are common in obesity, contribute to the development of adult asthma, which have been also investigated in the clinical manifestations of other respiratory disease as well as obesity in children (Magrone and Jirillo, 2015; Maniscalco et al., 2017).

Leptin and adiponectin can modify the natural history of childhood asthma (Kim et al., 2008). Increased leptin/adiponectin ratio, a condition commonly observed in

overweight and also more pronounced in asthmatic children, unbalances the regulation of inflammation by decreasing regulatory T cells and IL-10 secretion, inducing hyperreactivity in the immune system (Yuksel et al., 2012).

Leptin serum levels increases T_H1 immune response (IFN- γ production), increasing airway inflammation in children with asthma and overweight (Youssef et al., 2013). Adiponectin inhibits secretion of proinflammatory cytokines, such as IL-6 and TNF- α , and is positively associated with asthma control (de Lima Azambuja et al., 2015; Jensen et al., 2012). Thus, genetic variations in *LEP* and *ADIPOQ* might affect asthma and/or overweight in pediatric population (Yuksel et al., 2012). However, association studies exploring the variants in such genes with both asthma and overweight are scarce, especially in mixed populations.

In summary polymorphisms in adipokine genes may constitute risk factors for asthma and atopy, and this may be influenced by body adiposity. Therefore, the aim of this study was to investigate the association of variants in *LEP* and *ADIPOQ* genes with asthma and atopy, as well as to evaluate if overweight modifies this association in a population-based sample of children from Salvador, BA, Brazil.

2. Methods

2.1 Population and Data Collection

This research is a cross-sectional, population-based study, nested within a prospective cohort, with records in the SCAALA database (Social Changes, Asthma and Allergy in Latin America), in 2005, in the city of Salvador, Bahia, Brazil (Barreto et al., 2006; D'Innocenzo et al., 2014).

In this study were included 1016 unrelated individuals (where 203 of them are asthmatics and 258 are atopics), aged between 5 and 11 years. The characteristics of the studied population are presented in Table 1.

The initial research (see ALCANTARA-NEVES et al, 2012 (Alcantara-Neves et al., 2012); and D'INNOCENZO et al., 2014 (D'Innocenzo et al., 2014), for details) included information on asthma status of participants, with information on allergic diseases, wheezing, and risk factors, through to phase II of International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, as well as blood samples (evaluation of infection, total and specific IgE, immunological markers) and stool tests (detection of parasitic infections).

For the anthropometric measurements, electronic scale (Filizola, model E-150/3P, São Paulo, Brazil) and portable stadiometer (*Leicester height measure*), periodically calibrated, were used. Two measures were taken, and the mean value was considered; variations of 100g for weight and 0.1 cm for height/length were allowed (Matos et al., 2011). The measurements were performed according to WHO (1995) (WORLD HEALTH ORGANIZATION, 1995).

Ethical approval was obtained from the National Ethics Committee of Brazil in 2005 and the Ethics Committee of the Institute of Collective Health from Federal University of Bahia (Number: 003-05). Informed written consent was obtained from the legal guardian or parents of each individual. It details the entire procedure adopted throughout the project.

2.2 Definition of asthma and atopy

The definition of asthma was based on the information reported by the children's parents through ISAAC phase II questionnaire, adapted to the Portuguese language, and at least the presence of one of the following criteria: (1) diagnosis of asthma by a physician; (2) wheezing with exercise in the last 12 months; (3) 4 or more episodes of wheezing in the last 12 months; and (4) waking up at night because of wheezing in the last 12 months (Alcantara-Neves et al., 2012).

Atopy was defined when the individual was positive for the skin prick test (SPT) for at least one aeroallergen (reaction whose papule size was at least 3 mm larger than that caused by the negative control) plus a positive specific IgE (≥ 0.7 kU/L was considered positive) using ImmunoCAP system for at least one of the tested allergens. The aeroallergen tested by SPT were *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Blattella germanica*, *Periplaneta americana*, dog and cat epithelia, and fungi (*Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium brevicompactum*, *Penicillium expansum*, *Penicillium notatum*, *Penicillium roqueforti*, *Cladosporium fulvum*, and *Cladosporium herbarum*). It was measured the specific IgE (sIgE) anti-mite (*D. pteronyssinus* and *B. tropicalis*) and anti-cockroaches (*P. americana* and *B. germanica*) (Alcantara-Neves et al., 2012; Barreto et al., 2006).

2.3 Anthropometric assessment

The classification of nutritional status was determined by the BMI-Z score by age, where children with $\geq +1$ standard deviation of the median were considered overweight

and those with values below 1, non-overweight, according to the reference pattern curves of the World Health Organization (WHO) (Onis et al., 2007).

2.4 DNA extraction and genotyping

The DNA was extracted from the peripheral blood of the individuals, using the Flexigene QIAGEN kit following a protocol manufacturer's recommendations. The samples were genotyped by Illumina using the *Omnium Illumina* 2.5 chip, which is a large scanning platform with 2.5 million genotyped markers throughout genome. For this study, the genetic information was extracted from 186560463 to 186576252, positions located on chromosome 3 (*ADIPOQ*); and from 127881241 to 127897682, positions located on chromosome 7 (*LEP*), genome version: GRCh37.

2.5 Statistical analysis

Normality test (Kolmogorov-Sminov) and then association test (Chi-Square) with covariates were performed to define adjustments for logistic regression. The primary (Asthma) and secondary (Atopy) outcome were analyzed in three genetic models (additive, dominant and recessive) for each SNP.

PLINK software, version 1.09, was used to perform quality control of the SNPs, according to the following parameters: Hardy-Weinberg Equilibrium (HWE) deviation, with a p value $< 10^{-3}$; minor allele frequency (MAF) $< 1\%$; and absent loci, greater than 1%. After the quality control, 7 SNPs were excluded from the 31 that were typed for Leptin, and 6 out of 25 genotyped for Adiponectin were also excluded. The association analyzes were also performed in PLINK (Purcell et al., 2007).

Multivariate logistic regression analyzes for SNPs that passed the quality control were done using three genetic models: additive, dominant and recessive adjusted for sex, age, helminth infection, BMI and Principal Components (PC) 1 and 2, for ancestry, in order to control the confounding factor by population structure (Costa et al., 2015). The association analyzes were also performed in PLINK (Purcell et al., 2007). The asymptotic p-value was observed while the odds ratio (OR) was estimated. In addition, permutation procedures (Perm) were calculated to provide a computationally intensive approach to generate levels of significance empirically (Purcell et al., 2007).

Finally, linkage disequilibrium (LD) analysis of the observed SNPs was also performed. LD measures the non-random association of alleles at different loci (Fox et al., 2019). The observed associations may be affected by mutation, recombination, gene conversion, selection, genetic drift or demographic factors, such as inbreeding, migration and population structure (Wang et al., 2006). Thus, the LD standards are used to infer genetic parameters of a population (Tenesa et al., 2007). The degree of confidence in the R² value was calculated using the software Haploview 4.2. All statistical tests were two-tailed, and the significance level was defined as 5 %.

2.6 *In Silico* analyses

Functional information of the SNPs analyzed in this study was obtained through the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov), RegulomeDB (www.regulomedb.org) and Ensembl (www.ensembl.org).

RegulomeDB presents a range score, from 1 to 7, without qualified identification of the regulatory and functional potential of the variants, with an inversely attributed level of evidence (Boyle et al., 2012).

For the analysis of the impact of the missense variant, the SIFT (<https://sift.bii.a-star.edu.sg/>) and PolyPhen 2 (<http://genetics.bwh.harvard.edu/pph2/>) platforms were used. In the SIFT it predicts whether the amino acid substitution is damaging (≤ 0.05) or tolerable (> 0.05). While PolyPhen 2 classifies as damaging, probably damaging or benign, on a scale from 0 (benign) to 1 (damaging) (Nailwal and Chauhan, 2017).

For gene score analysis, we used the alleles associated with asthma risk in our population for the ADIPOQ and LEP genes. We assigned a score for the number of alleles and considered the presence of ≤ 2 risk alleles as the reference group. We then proceeded with logistic regression to compare the other groups with the reference. We adopted $p < 0.05$ as significant.

3. Results

From the 1016 individuals, 203 (20%) were asthmatic and 258 (25%) were atopic; 58% of individuals with asthma are younger than 6 years of age, while most atopics (53.9%) are older than 6 years. Overweight individuals, among asthmatics, are 32 (15.7%) and 52 (20.2%), among atopics. The population is comprised of the majority over the age of 6, eutrophic, male and non-atopic subjects (Table 01).

Table 1. Characteristics of the SCAALA population according to asthma status and variables included in the study.

Variables/Categories	Asthma (n=1.016)				P Value	Atopy (n=831)				P Value
	Non-asthmatic (n= 813)		Asthmatic (n=203)			Non-atopic (n=573)		Atopic (n=258)		
	n	%	N	(%)		n	(%)	n	(%)	
Age					0.002*					0.048*
< 6 (n=497)	378	46.49	119	58.62		269	46.9	119	46.1	
≥ 6 (n=519)	435	53.51	84	41.38		304	53.1	139	53.9	
Sex					0.568					0.000*
Male (n=552)	438	53.87	114	56.16		287	50.1	165	64.0	
Female (n=464)	375	46.13	89	43.84		286	49.9	93	36.0	
BMI/Age Z-Score					0.714					0.040*
Non-Overweight (n=861)	690	84.9	171	84.24		490	85.5	206	79.8	
Overweight (n=155)	123	15.1	32	15.76		83	14.5	52	20.2	
Atopy					0.001*					-
Yes (n=258)	192	28.5	66	41.8		-	-	-	-	
No (n=573)	481	71.5	92	58.2						

Data were analyzed using the chi-squared test. * p-value <0.05.

3.1 Description of the *ADIPOQ* and *LEP* polymorphisms

Table 2 presents the 26 significant SNPs associated with at least one of the outcomes studied. Most SNPs are intronic, and only one in the leptin gene exhibits missense function.

Table 2. Description of the *ADIPOQ* and *LEP* polymorphisms. Variants, position, alleles, minor allele frequency (MAF), function and regulome DB score.

SNP	Position	Alleles ^a	MAF	Function ^b	Score Regulome DB
ADIPONECTIN					
rs62292784	186843108	T/C	0.185	intron variant	4
rs16861205	186843845	A/G	0.174	intron variant	5
rs822395	186849018	C/A	0.389	intron variant	5

rs822396	rs822396	G/A	0.202	intron variant	5
rs36219755	186850989	T/C	0.074	intron variant	6
rs2241766	186853103	G/T	0.084	nc transcript variant, synonymous codon	5
rs1501299	186853334	A/C	0.347	intron variant	6
rs3821799	186853697	C/T	0.450	intron variant	no data
rs3774262	186854025	A/G	0.083	intron variant	5
rs6444172	186855282	C/A	0.018	nc transcript variant, utr variant 3 prime	5
rs6773957	186855916	G/A	0.467	nc transcript variant, utr variant 3 prime	no data
rs1063537	186856286	T/C	0.074	intron variant, utr variant 3 prime	no data
rs1063538	186856394	C/T	0.468	intron variant, utr variant 3 prime	no data
LEPTIN					
rs2167270	128241296	A/G	0.391	utr variant 5 prime	3a
rs2278815	128241798	A/G	0.310	intron variant	5
rs12706832	128247086	G/A	0.356	intron variant	no data
rs11763517	128250009	C/T	0.308	intron variant	no data
rs7795794	128250098	A/G	0.096	intron variant	5
rs11760956	128251034	A/G	0.243	intron variant	3 a
rs3828942	128254252	A/G	0.304	intron variant	5
rs17151919	128254539	A/G	0.059	missense	no data
rs28954118	128255129	T/A	0.032	utr variant 3 prime	5
rs17151922	128255163	T/G	0.173	utr variant 3 prime	5
rs6966536	128256006	G/A	0.061	utr variant 3 prime	no data
rs11761556	128257016	A/C	0.328	utr variant 3 prime	6
rs28959474	128257173	A/G	0.075	utr variant 3 prime	no data

^a First is the alternative allele and the second is the reference allele (1/2).

^b NCBI (National Center for Biotechnology Information).

3.2 Associations of studied SNPs with asthma

Table 3 summarizes the significant associations of variants on *ADIPOQ* and *LEP* with asthma symptoms. In *ADIPOQ*, two SNPs were positively associated with asthma. The rs822396 (G allele) was associated to asthma in the two genetic models evaluated, additive (OR 1.4, 95% CI 1.08-1.83) and dominant (OR 1.44, CI 95% 1.05-2.0); while T allele, of rs1063537, in dominant model (OR 1.52; CI 95% 1.01-2.30), only. In *LEP*, two SNPs (rs11763517 and rs11760956) (C and A alleles, respectively) were negatively associated with asthma in the additive (OR 0.70, 95% CI 0.54-0.91; OR 0.66, 95% CI 0.50-0.89) and dominant model (OR 0.67; 95% CI 0.49-0.93; OR 0.60, 95% CI 0.43-0.84), respectively.

Besides a SNP (rs2167270, A allele), also associated negatively, only in the dominant model (OR 0.71; 95% CI 0.51-0.98). Whereas the SNP rs12706832 showed a positive association in recessive model (OR 1.66; 95% CI 1.06-2.61).

Table 3. Significant associations between *ADIPOQ* and *LEP* SNPs with asthma analyzed by logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2.

SNP	Model	GENO	Non-Asthmatic n (%)	Asthmatic n (%)	OR	95% CI	P Value	P Perm
Adiponectin								
rs822396	ADD	AA	495 (64.3%)	105 (55.3%)	1.4	1.08-1.83	0.013*	0.011*
		GA	245 (31.9%)	72 (37.9%)				
		GG	29 (3.8%)	13 (6.8%)				
	DOM	AA	495 (64.3%)	105 (55.3%)	1.44	1.05-2.0	0.026*	0.049*
		GG+GA	274 (35.7%)	85 (44.7%)				
	REC	AA+GA	740 (96.2%)	177 (93.2%)	1.85	0.93-3.67	0.078	0.071
	GG	29 (3.8%)	13 (6.8%)					
rs1063537	ADD	CC	659 (85.4%)	153 (80.1%)	1.46	0.99-2.17	0.005*	0.065
		TC	109 (14.1%)	37 (19.4%)				
		TT	4 (0.5%)	1 (0.5%)				
	DOM	CC	659 (85.4%)	153 (80.1%)	1.52	1.01-2.30	0.046*	0.049*
		TT+TC	113 (14.6%)	38 (19.9%)				
	REC	CC+TC	768 (99.5%)	190 (99.5%)	1.11	0.12-10.09	0.928	0.444
	TT	4 (0.5%)	1 (0.5%)					
Leptin								
rs11763517	ADD	TT	360 (46.6%)	108 (56.5%)	0.70	0.54-0.91	0.007*	0.007*
		CT	336 (43.5%)	73 (38.2%)				
		CC	76 (9.9%)	10 (5.2%)				
	DOM	TT	360 (46.6%)	108 (56.5%)	0.67	0.49-0.93	0.016*	0.015*

rs11760956	REC	CC + CT	412 (53.4%)	83 (43.4%)	0.52	0.26-1.04	0.063	0.066
		TT + CT	696 (90.1%)	181 (94.7%)				
		CC	76 (9.9%)	10 (5.2%)				
	ADD	GG	430 (55.7%)	130 (68.0%)	0.66	0.50-0.89	0.005*	0.005*
		AG	297 (38.5%)	53 (27.8%)				
		AA	45 (5.8%)	8 (4.2%)				
	DOM	GG	430 (55.7%)	130 (68.0%)	0.60	0.43-0.84	0.003*	0.004*
		AA + AG	342 (44.3%)	61 (32.0%)				
	REC	GG + AG	727 (94.2%)	183 (95.8%)	0.72	0.33-1.57	0.412	0.667
		AA	45 (5.8%)	8 (4.2%)				
rs2167270	ADD	GG	267 (35.1%)	83 (43.9%)	0.74	0.58-0.95	0.018*	0.020*
		AG	378 (49.7%)	87 (46.0%)				
		AA	115 (15.2%)	19 (10.1%)				
	DOM	GG	267 (35.1%)	83 (43.9%)	0.71	0.51-0.98	0.036*	0.027*
		AA + AG	493 (64.9%)	106 (56.1%)				
	REC	GG + AG	645 (84.8%)	170 (89.9%)	0.64	0.38-1.07	0.088	0.090
		AA	115 (15.2%)	19 (10.1%)				
	ADD	AA	313 (40.5%)	75 (39.3%)	1.17	0.92-1.49	0.195	0.266
		GA	376 (48.7%)	85 (44.5%)				
		GG	83 (10.8)	31 (16.2%)				
rs12706832	DOM	AA	313 (40.5%)	75 (39.3%)	1.05	0.76-1.45	0.774	0.667
		GG+GA	459 (59.5%)	116 (60.7%)				
	REC	AA+GA	689 (89.2%)	160 (83.8%)	1.66	1.06-2.61	0.027*	0.028*
		GG	83 (10.8)	31 (16.2%)				

* p-value <0.05.

To verify if the increasing in the number of risk allele, on both genes, is associated with a higher risk of asthma, we conducted a risk scoring analysis including the G allele of rs822396 (*ADIPOQ*), T allele of rs1063537 (*ADIPOQ*), T allele of rs11763517 (*LEP*) and G allele of rs11760956, rs2167270 and rs12706832 (*LEP*). We observed a higher risk of asthma when the number of alleles was equal or greater than 7 (Table 4).

Table 4. Genetic risk score with the G allele of rs822396 (*ADIPOQ*), T allele of rs1063537 (*ADIPOQ*), T allele of rs11763517 (*LEP*), G allele of rs11760956 (*LEP*), G allele of rs2167270 (*LEP*) and G allele of rs12706832 (*LEP*)

Risk allele	Asthma		OR† / 95% CI	P-value
	Controls	Cases		
≤ 2	64 (8.8%)	10 (5.5%)	1	---
3	85 (11.7%)	13 (7.1%)	0.96	0.928
4	113 (15.6%)	16 (8.7%)	0.91	0.825
5	100 (13.8%)	23 (12.6%)	1.44	0.381
6	118 (16.2%)	31 (16.9%)	1.68	0.190
≥ 7	246 (33.9%)	90 (49.2%)	2.29	0.022*

† Adjusted for sex, age, helminth infections, and principal components 1 and 2. * p-value <0.05.

3.3 Associations with atopy

Only one SNP (rs16861205; A allele), in *ADIPOQ* gene, was negatively associated with atopy in additive (OR 0.71; 95% CI 0.52-0.96) and dominant model (OR 0.68; 95% CI 0.49-0.96). Whereas T allele of rs62292784 was positively association (OR 2.44, 95% CI 1.04-

5.72) in recessive model. For the *LEP* gene, three SNPs were positively associations to atopy, rs12706832 (OR 1.69; 95% CI 1.07-2.07), rs3828942 (OR 1.91; 95% CI 1.11-3.30), rs2278815 (OR 1.81; 95% CI 1.10-2.98) in recessive model (G, A and A alleles, respectively). Also in the *LEP* gene, three SNPs were negatively associated with atopy: rs17151919 in additive (OR 0.45; 95% CI 0.26-0.80) and dominant model (OR 0.51; 95% CI 0.23-0.76); rs2167270 in additive (OR 0.69; 95% CI 0.54-0.88) and dominant models (OR 0.72; 95% CI 0.52-1.00) (A allele, both); and rs11760956 (A allele) only in recessive model (OR 0.41; 95% CI 0.17-0.95) as shown in Table 5.

Table 5. Significant association between *LEP* and *ADIPOQ* SNPs and atopy, analyzed by logistic regression adjusted for sex, age, helminth infections, BMI, and principal components 1 and 2.

SNP	Model	GENO	Non-atopic (%)	Atopic n (%)	OR	95% CI	P Value	P Perm
Adiponectin								
rs1686120 5	ADD	GG	341 (65.3%)	173 (72.1%)	0.71	0.52-0.96	0.028*	0.025*
		AG	164 (31.4%)	62 (25.8%)				
		AA	17 (3.3%)	5 (2.1%)				
	DOM	GG	341 (65.3%)	173 (72.1%)	0.68	0.49-0.96	0.029*	0.026*
		AA + AG	181 (34.7%)	67 (27.9%)				
	REC	GG + AG	505 (96.7%)	235 (97.9%)	0.63	0.22-1.75	0.374	0.750
AA	17 (3.3%)	5 (2.1%)						
rs6229278 4	ADD	CC	11 (2.1%)	156 (65.0%)	1.27	0.96-1.69	0.096	0.098
		TC	156 (29.9%)	72 (30.0%)				
		TT	355 (68.0%)	12 (5.0%)				
	DOM	CC	11 (2.1%)	156 (65.0%)	1.21	0.87-1.69	0.252	0.306
		TT + TC	511 (97.9%)	84 (35.0%)				
	REC	CC + TC	167 (32.0%)	228 (95.0%)	2.44	1.04-5.72	0.040*	0.033*
TT	355 (68.0%)	12 (5.0%)						
Leptin								
rs1715191 9	ADD	GG	455 (87.2%)	226 (94.2%)	0.45	0.26-0.80	0.006*	0.004*
		AG	64 (12.3%)	13 (5.4%)				
		AA	3 (0.5%)	1 (0.4%)				

rs2167270	DOM	GG	455 (87.2%)	226 (94.2%)	0.41	0.23-0.76	0.004*	0.004*
		AA + AG	67 (12.8%)	14 (5.8%)				
	REC	GG + AG	519 (99.5%)	239 (99.6%)	0.51	0.05-5.00	0.561	0.406
		AA	3 (0.5%)	1 (0.4%)				
	ADD	GG	176 (34.3%)	19 (8.1%)	0.69	0.54-0.88	0.003*	0.003*
		AG	255 (49.7%)	120 (50.8%)				
		AA	82 (16.0%)	97 (41.1%)				
	DOM	GG	176 (34.3%)	19 (8.1%)	0.72	0.52-1.00	0.049*	0.048*
		AA + AG	337 (65.7%)	217 (91.9%)				
	REC	GG + AG	431 (84.0%)	139 (58.9%)	0.43	0.25-0.74	0.002*	0.002*
		AA	82 (16.0%)	97 (41.1%)				
rs1270683 2	ADD	AA	207 (39.7%)	95 (39.6%)	1.13	0.90-1.43	0.301	0.500
		GA	263 (50.4%)	106 (44.2%)				
		GG	52 (9.9%)	39 (16.2%)				
	DOM	AA	207 (39.7%)	95 (39.6%)	0.98	0.71-1.35	0.903	0.857
		GG + GA	315 (60.3%)	145 (60.4%)				
	REC	AA + GA	470 (90.1%)	201 (83.8%)	1.69	1.07-2.67	0.023*	0.024*
		GG	52 (9.9%)	39 (16.2%)				
	ADD	GG	303 (58.0%)	137 (57.1%)	0.90	0.69-1.17	0.433	0.727
		AG	186 (35.6%)	96 (40.0%)				
		AA	33 (6.3%)	7 (2.9%)				
rs1176095 6	DOM	GG	303 (58.0%)	137 (57.1%)	1.00	0.73-1.38	0.981	1.000
		AA + AG	219 (41.9%)	103 (42.9%)				
	REC	GG + AG	489 (93.6%)	233 (97.1%)	0.41	0.17-0.95	0.037*	0.036*
		AA	33 (6.3%)	7 (2.9%)				
	ADD	GG	249 (47.7%)	108 (45.0%)	1.23	0.96-1.57	0.096	0.082
		AG	241 (46.2%)	104 (43.3%)				
		AA	32 (6.1%)	28 (11.7%)				
	DOM	GG	249 (47.7%)	108 (45.0%)	1.14	0.83-1.56	0.409	0.778
		AA + AG	273 (52.3%)	132 (55.0%)				
rs3828942	REC	GG + AG	490 (93.9%)	212 (88.3%)	1.91	1.11-3.30	0.020*	0.022*
		AA	32 (6.1%)	28 (11.7%)				
	ADD	GG	255 (48.8%)	106 (44.2%)	1.26	1.00-1.60	0.054	0.049*
		AG	227 (43.5%)	101 (42.1%)				
		AA	40 (7.7%)	33 (13.7%)				
	DOM	GG	255 (48.8%)	106 (44.2%)	1.20	0.87-1.64	0.262	0.366
		AA + AG	267 (51.1%)	134 (55.8%)				
	REC	GG + AG	482 (92.3%)	207 (86.3%)	1.81	1.10-2.98	0.019*	0.020*

* p-value < 0.05.

3.4 Effect modifying of body mass index on the association between variants in *ADIPOQ* and *LEP* and asthma

Associations with asthma occurred in three variants in the *LEP* gene, modified in groups stratified by BMI / Age Z-score, as seen in figure 01 (A, B and C). The associations observed in eutrophic individuals (non-overweight group) are reflected in the general group. Three SNPs (rs11760956, rs11763517 and rs2167270) were negatively associated in the non-overweight group. When only overweight individuals were analyzed, there was no significant association between variants and asthma, using an additive model.

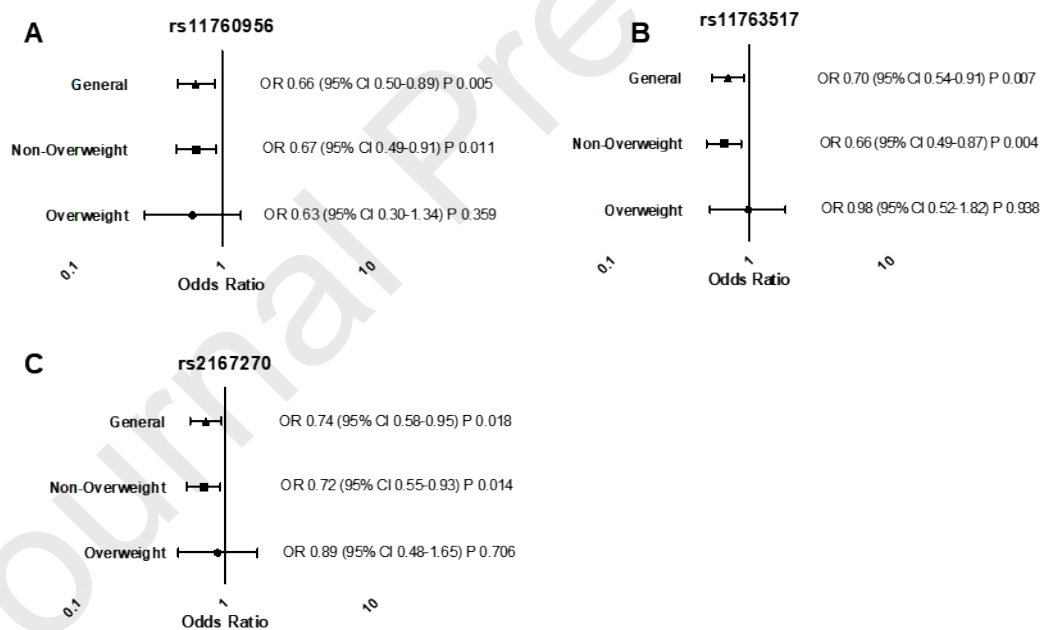


Figure 01. Association between variants in *LEP* and asthma in non-overweight individuals, overweight individuals, or in whole population analyzed by logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2. (A) rs11760956 (A allele), (B) rs11763517 (C allele) and (C) rs2167270 (A allele) on *LEP*.

3.5 Associations with atopy modified by body mass classification

The rs16861205 (A allele), in *ADIPOQ*; rs2167270 (A allele) and rs17151919 (A allele), in *LEP*, showed associations to atopy modified by BMI. When overweight individuals were analyzed, no significant association was observed between those variants and atopy in both genes. However, when stratified in non-overweight group, all SNPs were negatively association (Figure 02 - A, B, C).

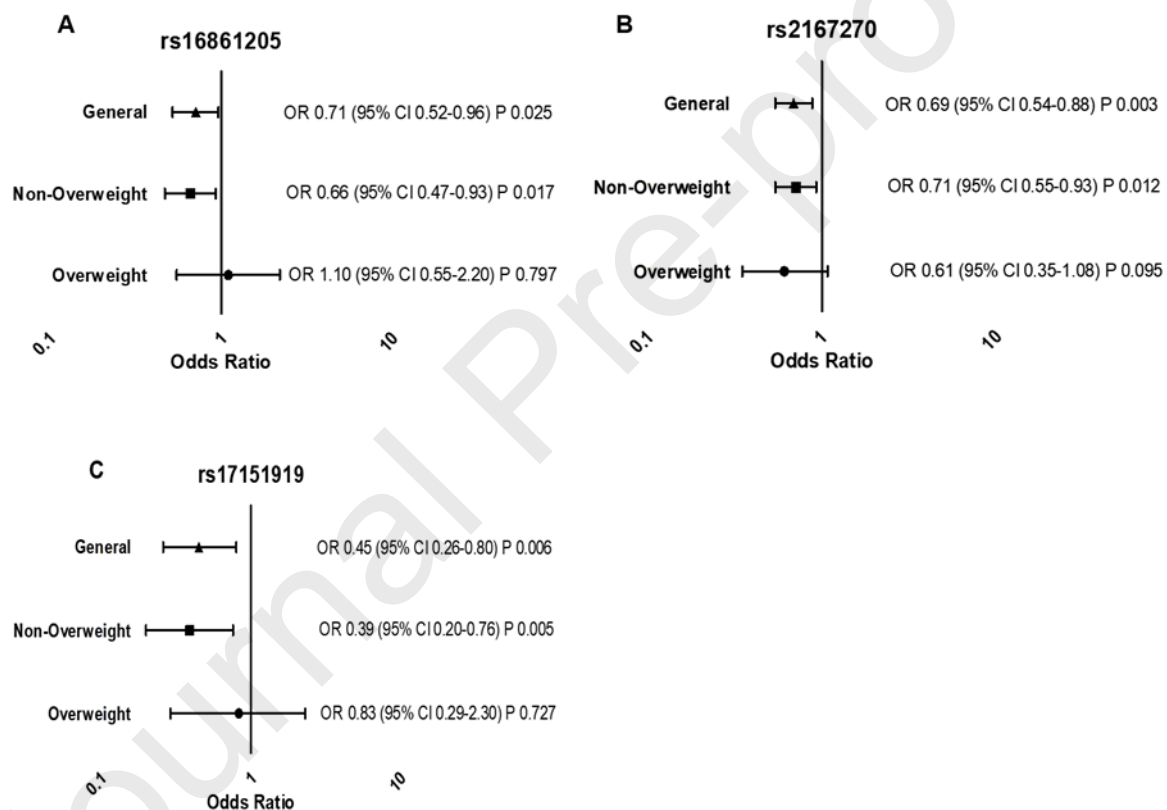


Figure 02. Association between variants in *ADIPOQ* and *LEP* and atopy in non-overweight individuals, overweight individuals or in whole population analyzed by logistic regression adjusted for sex, age, helminth infections, and principal components 1 and 2. (A) rs16861205 (A allele) on *ADIPOQ*, (B) rs2167270 (A allele) and (C) rs17151919 (A allele) on *LEP*.

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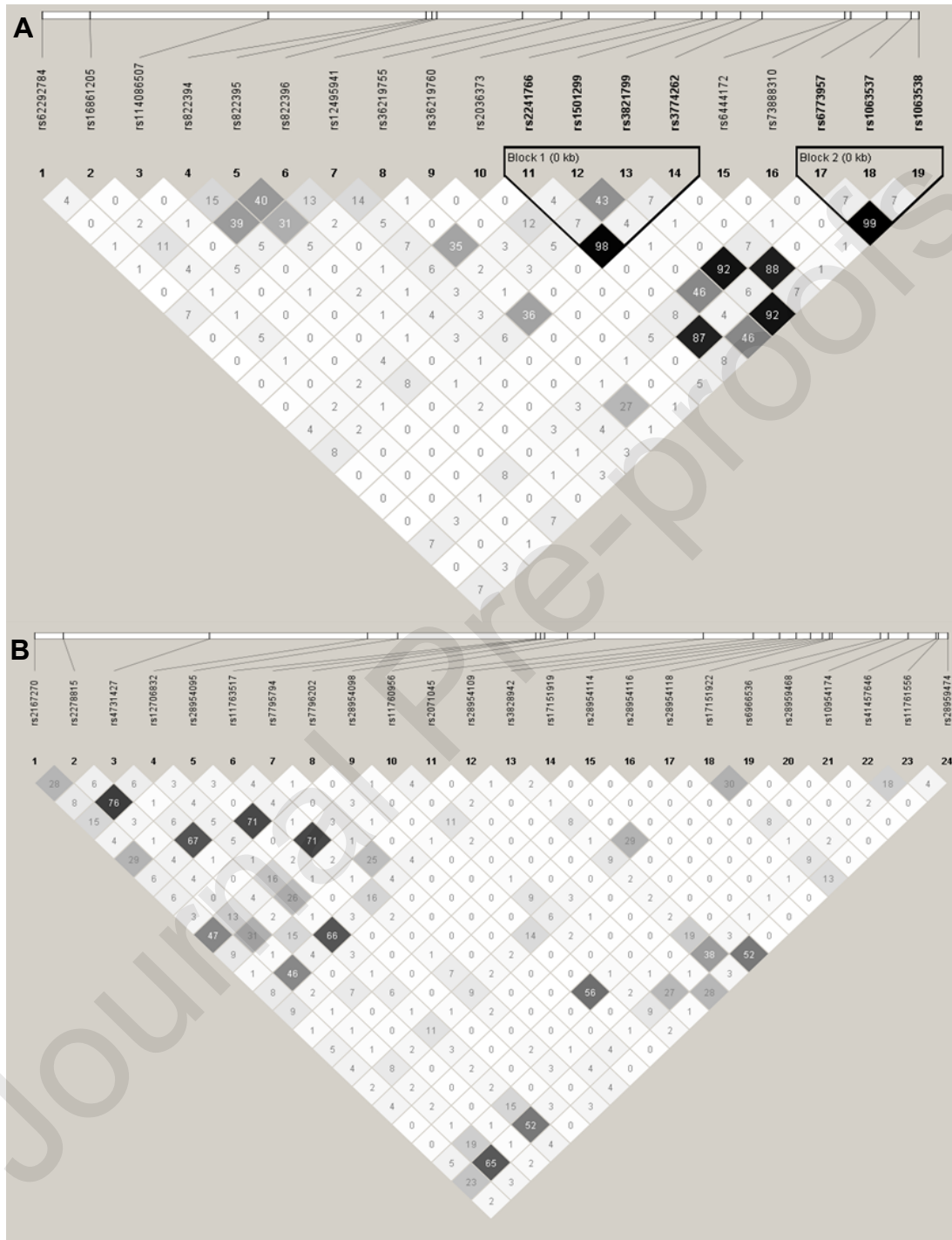
313 3.6 Linkage Disequilibrium (LD)

314 The results of LD among SNPs on *ADIPOQ* (Figure 3A) and *LEP* (Figure 3B)
315 are shown in percentage terms. Outcomes higher than 80% in the r^2 analysis are considered
316 to have a strong linkage disequilibrium. We have highlighted the lack of linkage
317 disequilibrium among all SNPs in *LEP* studied herein.

318 **Figure 03.** Pairwise LD within Haploview using the R^2 . Color intensity and value in square
319 indicate the degree of confidence in the r^2 value and how much the SNPs are in LD. (A)
320 *ADIPOQ*. (B) *LEP*.

321

322



4. Discussion

An asthma phenotype induced by childhood obesity, characterized by primary atopic asthma, was already proposed (Leiria et al., 2015). Recently, the Th1 phenotype has also been demonstrated in obese asthmatic children, compared to eutrophic children (Nyambuya et al., 2020), in a systematic review. Obesity seems to play a substantial risk factor to asthma due to the imbalance of mediators secreted by adipose tissue that exert effect on the lung (Leiria et al., 2015; Muc et al., 2016; Silva et al., 2013). In general, leptin playing a critical role on inflammation and airway responsiveness (Muc et al., 2016), is produced in excess in obese individuals; meanwhile, adiponectin is reduced. In our study we investigated the association of polymorphisms on the *ADIPOQ* and *LEP* genes with asthma and atopy.

On *ADIPOQ*, the G allele of rs822396 was positively associated with asthma in this study. The SNP is in noncoding region and potentially can modify the gene expression according to Ramya et al (Ramya et al., 2013). Changes in the serum level of adiponectin or *ADIPOQ* transcription rates, related to rs822396, are discussed by Rubio-Chavez et al. (Rubio-Chavez et al., 2020), as a result of the presence of intronic amplifiers, miRNAs or alternative splices. Ding et al. (Ding, 2015) and Nagel et al. (Nagel et al., 2009) argue that adiponectin may act as a protective agent on asthma. There is an inverse correlation observed between disease exacerbation and adipokine plasma concentration due to its regulatory role in the immune system, suppressing inflammatory responses associated with asthma.

Ramya et al (Ramya et al., 2013). describe association between rs822396 (allele A) and low adiponectin serum levels. Therefore, it is suggested that the association in our study (where the G allele was risk factor to asthma), is based on the regulation of

inflammatory effects in the airways, generally observed in elevated serum adiponectin levels (Szymczak-Pajor and Pawliczak, 2017).

The rs1063537 (T allele), located in the 3'UTR region and impact modifier, was associated with the risk of asthma in our population. Kyriakou et al. (Kyriakou et al., 2008) evaluated the association of the same allele with the level of fasting serum adiponectin in two female populations, caucasian and adult, but obtained conflicting results. In one cohort, the T allele was significantly associated with a higher level of serum adiponectin, however, the same result was not replicated in the second cohort. Our research, in addition to not comparing serum levels of adiponectin, is composed of a mixed and infantile population that approaches both genders, which is very different from Kyriakou's sample.

Subsequently, this SNP was also investigated for COPD (chronic obstructive pulmonary disease) by Yuan et al. (Yuan et al., 2012), but significant association was not observed. Although the 3'UTR region of *ADIPOQ* has already been studied in associations with metabolic risk diseases (Kanu et al., 2016; Muiya et al., 2013), we have not found, in the literature, studies with asthma using the variant described in our research.

Two variant in the *ADIPOQ* (rs16861205 and rs62292784) were negatively associated with atopy. The allele A of rs16861205 was a protective factor in the general group and in the non-overweight group. This protection was lost in overweight individuals. In a 4-year longitudinal study, this allele was positively associated with serum adiponectin concentrations (Siitonen et al., 2011), suggesting that this SNP is acting as a protective factor by increasing the levels of adiponectin.

In the *LEP*, the rs11763517 (C allele), rs11760956 (A allele) and rs2167270 (A allele) were negatively associated with asthma. *In silico* analyses on Ensembl revealed that they are located in non-coding region, with modifying impact, but with no known clinical significance. In our study, we observed that these three SNPs remain protecting for asthma in non-overweight individuals. However, overweight individuals lose such protection, demonstrating the impact of adiposity opposite to the effect of this variant. Until now, there are no publications that report its association with asthma and/or obesity.

The absence of a protective association between rs11763517, rs11760956 and rs2167270 with asthma, in overweight individuals, is relevant. According to Khan and Awan (Khan and Awan, 2016), leptin-mediated signaling in obesity suffers an interruption that can induce impairment of lung function. Recently, a paper has reported that high serum leptin levels have been positively correlated with asthma in Egyptian children; as well as higher BMI in asthmatic individuals, compared to non-asthmatic (Elngar et al., 2019). Although our work has not dosed serum leptin, it supposes a potential biological effect due to the pre-applied association in the literature of increased leptin and/ or disturbances in its action in overweight individuals, as possible factors related to the non-observation of asthma protection.

Also, in *LEP*, the rs12706832 (allele G) was associated as a risk factor for asthma for the first time in the literature. To assess the combined effect of alleles on asthma risk we performed a polygenic risk score to asthma including the risk alleles of the six SNP previously associated with asthma described here. The individuals who carry 7 or more risk

alleles were more than twice as likely to have asthma. No previous study was found in literature considering variants occurring together in such genes for asthma.

The GWAS study (Zhu et al., 2020) related 4 subtypes of asthma to obesity. Molecular mechanisms for comorbid asthma and obesity have been suggested, with inferences about inflammation, and evidence of increased asthma risk associated with increased BMI. However, the genes studied in our research have not been described, which confirms the non-generalization of the results for ethnic populations out of Europe. Moreover, the strongest genetic correlation was observed for late onset asthma (Zhu et al., 2020).

For atopy, five polymorphisms in *LEP* were significantly associated. Three of them (rs3828942, rs12706832 and rs2278815) with an increase in risk of up to 60% (all SNPs are located in the intron). Among the three variants negatively associated (rs17151919, rs2167270, rs11760956) in our analysis, we highlight the rs17151919 which is a missense variant, probably benign and with moderate impact according with SIFT (0.25) and Polyphen 2 (0.293). The allele A of rs17151919 has been associated with reduced leptin levels in African Americans (Khan, 2016). It has been described in association of this SNP with high body weight and waist circumference in young Caucasian and African American adults (Friedlander et al., 2010).

The A allele of rs2167270 showed a negative association with atopy in our study. In this context, Banihani et al. (Banihani et al., 2018) evaluated the association of rs2167270 with the incidence of atopic dermatitis. The GG genotype was more frequent among the cases, and the AG genotype had a higher prevalence among the controls. It corroborates with

our findings, evidencing that the A allele is negatively associated with atopy. The influence of this SNP, according to Hoffstedt et al. (Hoffstedt et al., 2002), occurs in the expression of leptin at the transcriptional level, affecting its secretion.

When we stratified the groups, overweight individuals lost the significant association of protection of the polymorphic allele in both variants (rs2167270 and rs17151919), demonstrating the impact of excess weight on the association observed with atopy.

One of the limitations of our study was the impossibility of evaluating the causal relationship of the associations found. Another limitation was using BMI/Age Z-Score as the only variable that measures body adiposity (Neovius et al., 2004). Attention to the possible ethnic peculiarities in this study is relevant, as there is a predominance of African ancestry, which is important for broadening the knowledge of possible differential associations in the predominant population in the country of study and for purposes of comparison with other ethnicities and/or nationalities.

In addition, the sample stratified by BMI did not reach the ideal statistical percentage, due to the design of the study for asthma. However, an attempt to demonstrate the possible impact of adiposity on genetic results related to asthma and atopy may be relevant to influence the design of new research, paired by adiposity, to investigate the impact of variants in genes encoding adipokines in the asthma phenotype and obesity.

Finally, the measurement of mRNA and adipokines in serum and tissues in individuals with or without obesity, can confirm the hypotheses of differential associations between the polymorphisms in this study and different asthma phenotypes.

Conclusion

Our findings demonstrate that SNPs on the LEP and ADIPOQ genes may have an impact on atopy and asthma. Our results also indicate that overweight may have a modifying effect on the associations leading to a loss of the protective effect attributed to the alleles on the studied genes. These markers may be useful in the future to guide patients with such genotypes to control the body weight and thus reduce the risk of developing allergic diseases. We suggest that detailed functional studies should be performed to better investigate and understand the role of these SNPs in the asthma-obese phenotype in children and adults, and the presence of association with serum adipokine levels.

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References

- Alcantara-Neves, N.M., Veiga, R.V., Dattoli, V.C.C., Fiaccone, R.L., Esquivel, R., Cruz, Á.A., Cooper, P.J., Rodrigues, L.C., Barreto, M.L., 2012. The effect of single and multiple infections on atopy and wheezing in children. *J. Allergy Clin. Immunol.* 129. <https://doi.org/10.1016/j.jaci.2011.09.015>
- Azizpour, Y., Delpisheh, A., Montazeri, Z., Sayehmiri, K., Darabi, B., 2018. Effect of childhood BMI on asthma: A systematic review and meta-analysis of case-control studies. *BMC Pediatr.*

18, 1–13. <https://doi.org/10.1186/s12887-018-1093-z>

Banihani, S.A., Elmadhoun, R.A., Khabour, O.F., Alzoubi, K.H., 2018. The rs2167270 polymorphism of leptin gene is associated with atopic dermatitis. *Dermatoendocrinol.* 10, 1–5. <https://doi.org/10.1080/19381980.2018.1454191>

Barreto, M.L., Cunha, S.S., Alcântara-Neves, N., Carvalho, L.P., Cruz, Á.A., Stein, R.T., Genser, B., Cooper, P.J., Rodrigues, L.C., 2006. Risk factors and immunological pathways for asthma and other allergic diseases in children: Background and methodology of a longitudinal study in a large urban center in Northeastern Brazil (Salvador-SCAALA study). *BMC Pulm. Med.* 6. <https://doi.org/10.1186/1471-2466-6-15>

Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J., Park, J., Hitz, B.C., Weng, S., Cherry, J.M., Snyder, M., 2012. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 22, 1790–1797. <https://doi.org/10.1101/gr.137323.112>

Costa, G.N.O., Dudbridge, F., Fiaccone, R.L., da Silva, T.M., Conceição, J.S., Strina, A., Figueiredo, C.A., Magalhães, W.C.S., Rodrigues, M.R., Gouveia, M.H., Kehdy, F.S.G., Horimoto, A.R.V.R., Horta, B., Burchard, E.G., Pino-Yanes, M., Del Rio Navarro, B., Romieu, I., Hancock, D.B., London, S., Lima-Costa, M.F., Pereira, A.C., Tarazona, E., Rodrigues, L.C., Barreto, M.L., 2015. A genome-wide association study of asthma symptoms in Latin American children. *BMC Genet.* 16, 1–11. <https://doi.org/10.1186/s12863-015-0296-7>

7

Cruz, A.A., Camargos, P.A., Urrutia-Pereira, M., Stelmach, R., 2018. Global Alliance against Chronic Respiratory Diseases (GARD) Brazil success case: Overcoming barriers. *J. Thorac. Dis.* 10, 534–538. <https://doi.org/10.21037/jtd.2018.01.40>

- 473 D’Innocenzo, S., Matos, S.M.A., Prado, M.S., Santos, C.A.S.T., Assis, A.M.O., Cruz, A.A.,
474 Marchioni, D.M.L., Rodrigues, L.C., Barreto, M.L., 2014. Padrão alimentar, asma e sibiló
475 atópico e não atópico em crianças e adolescentes: Estudo SCAALA, Salvador, Bahia, Brasil.
476 Cad. Saude Publica 30, 1849–1860. <https://doi.org/10.1590/0102-311X00165513>
- 477 de Lima Azambuja, R., da Costa Santos Azambuja, L.S.E., Costa, C., Rufino, R., 2015.
478 Adiponectin in Asthma and Obesity: Protective Agent or Risk Factor for More Severe
479 Disease? Lung 193, 749–755. <https://doi.org/10.1007/s00408-015-9793-8>
- 480 Deng, X., Ma, J., Yuan, Y., Zhang, Z., Niu, W., 2019. Association between overweight or obesity
481 and the risk for childhood asthma and wheeze: An updated meta-analysis on 18 articles and
482 73 252 children. Pediatr. Obes. 14. <https://doi.org/10.1111/ijpo.12532>
- 483 Ding, Y. et al, 2015. Plasma adiponectin concentrations and adiponectin gene polymorphisms are
484 associated with bronchial asthma in the Chinese Li Population. Iran J Allergy Asthma
485 Immunol 14, 292–297.
- 486 Elngar, E.F., Zekry, O.A., Ali, M.M., Shora, H.A., 2019. Asthma Severity and Serum Leptin Level
487 in Obese Egyptian Children 2, 1–8.
- 488 Fox, E.A., Wright, A.E., Fumagalli, M., Vieira, F.G., 2019. NgsLD: Evaluating linkage
489 disequilibrium using genotype likelihoods. Bioinformatics 35, 3855–3856.
490 <https://doi.org/10.1093/bioinformatics/btz200>
- 491 Friedlander, Y., Li, G., Fornage, M., Williams, O.D., Lewis, C.E., Schreiner, P., Pletcher, M.J.,
492 Enquobahrie, D., Williams, M., Siscovick, D.S., 2010. Candidate Molecular Pathway Genes
493 Related to Appetite Regulatory Neural Network, Adipocyte Homeostasis and Obesity: Results
494 from the CARDIA Study. Ann. Hum. Genet. 74, 387–398. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-1809.2010.00596.x)
495 [1809.2010.00596.x](https://doi.org/10.1111/j.1469-1809.2010.00596.x)

- 496 Froidure, A., Mouthuy, J., Durham, S.R., Chanez, P., Sibille, Y., Pilette, C., 2016. Asthma
497 phenotypes and IgE responses. *Eur. Respir. J.* 47, 304–319.
498 <https://doi.org/10.1183/13993003.01824-2014>
- 499 Global Initiative for Asthma., 2020. Global Strategy for Asthma Management and Prevention.
- 500 Hoffsted, J., Eriksson, P., Mottagui-Tabar, S., Arner, P., 2002. A polymorphism in the leptin
501 promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin.
502 *Horm. Metab. Res.* 34, 355–359. <https://doi.org/10.1055/s-2002-33466>
- 503 IBGE. Instituto Brasileiro de Geografia e Estatística, Coordenação de Trabalho e Rendimento,
504 2011. Pesquisa de Orçamentos Familiares: 2008-2009. Análise do Consumo Alimentar
505 Pessoal no Brasil, Biblioteca do Ministerio do Planejamento, Orçamento e Gestão.
506 <https://doi.org/ISSN 0101-4234>
- 507 Jensen, M.E., Wood, L.G., Gibson, P.G., 2012. Obesity and childhood asthma - Mechanisms and
508 manifestations. *Curr. Opin. Allergy Clin. Immunol.* 12, 186–192.
509 <https://doi.org/10.1097/ACI.0b013e3283508df5>
- 510 Kanu, J.S., Gu, Y., Zhi, S., Yu, M., Lu, Y., Cong, Y., Liu, Yunkai, Li, Y., Yu, Y., Cheng, Y., Liu,
511 Yawen, 2016. Single nucleotide polymorphism rs3774261 in the AdipoQ gene is associated
512 with the risk of coronary heart disease (CHD) in Northeast Han Chinese population: A case-
513 control study. *Lipids Health Dis.* 15. <https://doi.org/10.1186/s12944-015-0173-4>
- 514 Khan, A.R., Awan, F.R., 2016. Leptin resistance: A possible interface between obesity and
515 pulmonary-related disorders. *Int. J. Endocrinol. Metab.* 14, 1–6.
516 <https://doi.org/10.5812/ijem.32586>
- 517 Khan, R.J., 2016. Modification of the association between the polymorphisms of adipokine genes

- 518 and serum adipokine levels by vitamin D status in African Americans: The Jackson Heart
519 Study. *FASEB J.* 30.
- 520 Kim, K.W., Shin, Y.H., Lee, K.E., Kim, E.S., Sohn, M.H., Kim, K.E., 2008. Relationship between
521 adipokines and manifestations of childhood asthma. *Pediatr. Allergy Immunol.* 19, 535–540.
522 <https://doi.org/10.1111/j.1399-3038.2007.00690.x>
- 523 Kyriakou, T., Collins, L.J., Spencer-Jones, N.J., Malcolm, C., Wang, X., Snieder, H., Swaminathan,
524 R., Burling, K.A., Hart, D.J., Spector, T.D., O'Dell, S.D., 2008. Adiponectin gene ADIPOQ
525 SNP associations with serum adiponectin in two female populations and effects of SNPs on
526 promoter activity. *J. Hum. Genet.* 53, 718–727. <https://doi.org/10.1007/s10038-008-0303-1>
- 527 Lambrecht, B.N., Hammad, H., 2015. The immunology of asthma. *Nat. Immunol.* 16, 45–56.
528 <https://doi.org/10.1038/ni.3049>
- 529 Leiria, L.O.S., Martins, M.A., Saad, M.J.A., 2015. Obesity and asthma: Beyond TH2 inflammation.
530 *Metabolism.* <https://doi.org/10.1016/j.metabol.2014.10.002>
- 531 Magrone, T., Jirillo, E., 2015. Childhood obesity: Immune response and nutritional approaches.
532 *Front. Immunol.* <https://doi.org/10.3389/fimmu.2015.00076>
- 533 Maniscalco, M., Paris, D., Melck, D.J., D'Amato, M., Zedda, A., Sofia, M., Stellato, C., Motta, A.,
534 2017. Coexistence of obesity and asthma determines a distinct respiratory metabolic
535 phenotype. *J. Allergy Clin. Immunol.* 139, 1536-1547.e5.
536 <https://doi.org/10.1016/j.jaci.2016.08.038>
- 537 Matos, S.M.A., Jesus, S.R., Saldiva, S.R.D.M., Prado, M.S., D'Innocenzo, S., Assis, A.M.O.,
538 Rodrigues, L.C., Alcantara-Neves, N.M., Cruz, Á.A., Simões, S.D.M., Barreto, M.L., 2011.
539 Overweight, asthma symptoms, atopy and pulmonary function in children of 4-12 years of

- age: Findings from the SCAALA cohort in Salvador, Bahia, Brazil. *Public Health Nutr.* 14, 1270–1278. <https://doi.org/10.1017/S1368980011000462>
- Moraes, T.J., Sears, M.R., Subbarao, P., 2018. Epidemiology of Asthma and Influence of Ethnicity. *Semin. Respir. Crit. Care Med.* 39, 3–11. <https://doi.org/10.1055/s-0037-1618568>
- Muc, M., Mota-Pinto, A., Padez, C., 2016. Association between obesity and asthma - Epidemiology, pathophysiology and clinical profile. *Nutr. Res. Rev.* 29, 194–201. <https://doi.org/10.1017/S0954422416000111>
- Muiya, N., Al-Najai, M., Tahir, A.I., Elhawari, S., Gueco, D., Andres, E., Mazhar, N., Altassan, N., Meyer, B.F., Alshahid, M., Dzimiri, N., 2013. The 3'-UTR of the adiponectin Q gene harbours susceptibility loci for atherosclerosis and its metabolic risk traits. *BMC Med. Genet.* 14, 1–9. <https://doi.org/10.1186/1471-2350-14-127>
- Mukadam, S., Zacharias, J., Henao, M.P., Kraschnewski, J., Ishmael, F., 2018. Differential effects of obesity on eosinophilic vs. non-eosinophilic asthma subtypes. *J. Asthma* 55, 720–725. <https://doi.org/10.1080/02770903.2017.1365886>
- Nagel, G., Koenig, W., Rapp, K., Wabitsch, M., Zoellner, I., Weiland, S.K., 2009. Associations of adipokines with asthma, rhinoconjunctivitis, and eczema in German schoolchildren. *Pediatr. Allergy Immunol.* 20, 81–88. <https://doi.org/10.1111/j.1399-3038.2008.00740.x>
- Nailwal, M., Chauhan, J.B., 2017. Computational analysis of high risk missense variant in human UTY Gene: A candidate gene of AZFa sub-region. *J. Reprod. Infertil.* 18, 298–306.
- Neovius, M., Linné, Y., Barkeling, B., Rössner, S., 2004. Discrepancies between classification systems of childhood obesity. *Obes. Rev.* 5, 105–114. <https://doi.org/10.1111/j.1467-789X.2004.00136.x>

- 562 Nyambuya, T.M., Dlodla, P.V., Mxinwa, V., Nkambule, B.B., 2020. Obesity-related asthma in
563 children is characterized by T-helper 1 rather than T-helper 2 immune response: A meta-
564 analysis. *Ann. Allergy, Asthma Immunol.* <https://doi.org/10.1016/j.anai.2020.06.020>
- 565 Onis, M. de, Adelheid W Onyango, Elaine Borghi, Amani Siyam, C.N.& J.S., Objective, 2007.
566 Development of a WHO growth reference for school-aged children and adolescents. *Bull.*
567 *World Health Organ.* 660–667. <https://doi.org/07.043497>
- 568 Periyalil, H.A., Gibson, P.G., Wood, L.G., 2013. Immunometabolism in obese asthmatics: Are we
569 there yet? *Nutrients* 5, 3506–3530. <https://doi.org/10.3390/nu5093506>
- 570 Pitchon, R.R., Alvim, C.G., de Andrade, C.R., Lasmar, L.M. de L.B.F., Cruz, Á.A., Reis, A.P. dos,
571 2019. Asthma mortality in children and adolescents of Brazil over a 20-year period. *J. Pediatr.*
572 *(Rio. J.)* <https://doi.org/10.1016/j.jped.2019.02.006>
- 573 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar,
574 P., De Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: A tool set for whole-genome
575 association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575.
576 <https://doi.org/10.1086/519795>
- 577 Ramya, K., Ayyappa, K.A., Ghosh, S., Mohan, V., Radha, V., 2013. Genetic association of
578 ADIPOQ gene variants with type 2 diabetes, obesity and serum adiponectin levels in south
579 Indian population. *Gene* 532, 253–262. <https://doi.org/10.1016/j.gene.2013.09.012>
- 580 Rastogi, D., Holguin, F., 2017. Metabolic dysregulation, systemic inflammation, and pediatric
581 obesity-related asthma. *Ann. Am. Thorac. Soc.* 14, S363–S367.
582 <https://doi.org/10.1513/AnnalsATS.201703-231AW>
- 583 Rubio-Chavez, L.A., Rosales-Gomez, R.C., Rubio-Chavez, K.H.L., Ramos-Nuñez, J.L., Garcia-

- 584 Cobian, T.A., Camargo-Hernandez, G., Sanchez-Corona, J., Gutierrez-Rubio, S.A., 2020. The
585 rs822396 Polymorphism of the ADIPOQ Gene Is Associated with Anthropometric, Clinical,
586 and Biochemical Alterations Related to the Metabolic Syndrome in the Mexican Population.
587 *Metab. Syndr. Relat. Disord.* 18, 243–250. <https://doi.org/10.1089/met.2019.0045>
- 588 Siitonen, N., Pulkkinen, L., Lindström, J., Kolehmainen, M., Eriksson, J.G., Venojärvi, M., Ilanne-
589 Parikka, P., Keinänen-Kiukaanniemi, S., Tuomilehto, J., Uusitupa, M., 2011. Association of
590 ADIPOQ gene variants with body weight, type 2 diabetes and serum adiponectin
591 concentrations: The Finnish Diabetes Prevention Study. *BMC Med. Genet.* 12.
592 <https://doi.org/10.1186/1471-2350-12-5>
- 593 Silva, R.D.C.R., Assis, A.M.O., Goncalves, M.S., Fiaccone, R.L., Matos, S.M.A., Barreto, M.L.,
594 Pinto, E.D.J., Silva, L.A. Da, Rodrigues, L.C., Alcantara-Neves, N.M., 2013. Prevalence of
595 Wheezing and its Association with Body Mass Index and Abdominal Obesity in Children. *J.*
596 *Asthma* 50, 267–273. <https://doi.org/10.3109/02770903.2012.757774>
- 597 Solé, D., Aranda, C.S., Wandalsen, G.F., 2017. Asthma: epidemiology of disease control in Latin
598 America – short review. *Asthma Res. Pract.* 3, 4. <https://doi.org/10.1186/s40733-017-0032-3>
- 599 Stern, J., Pier, J., Litonjua, A.A., 2020. Asthma epidemiology and risk factors. *Semin.*
600 *Immunopathol.* 42, 5–15. <https://doi.org/10.1007/s00281-020-00785-1>
- 601 Szymczak-Pajor, I., Pawliczak, R., 2017. Adipose tissue as a potential source of hormones and
602 cytokines involved in asthma pathogenesis. *Alergol. Pol. - Polish J. Allergol.* 4, 132–138.
603 <https://doi.org/10.1016/j.alergo.2017.10.001>
- 604 Tenesa, A., Navarro, P., Hayes, B.J., Duffy, D.L., Clarke, G.M., Goddard, M.E., Visscher, P.M.,
605 2007. Recent human effective population size estimated from linkage disequilibrium. *Genome*
606 *Res.* 17, 520–526. <https://doi.org/10.1101/gr.6023607>

- Vijayakanthi, N., Grealley, J.M., Rastogi, D., 2016. Pediatric obesity-related asthma: The role of metabolic dysregulation. *Pediatrics* 137. <https://doi.org/10.1542/peds.2015-0812>
- Wang, Y., Zhao, L.P., Dudoit, S., 2006. A fine-scale linkage-disequilibrium measure based on length of haplotype sharing. *Am. J. Hum. Genet.* 78, 615–628. <https://doi.org/10.1086/502632>
- WORLD HEALTH ORGANIZATION, 1995. Physical status: The use of and interpretation of anthropometry, Report of a WHO Expert Committee., World Health Organization.
- Youssef, D.M., Elbehidy, R.M., Shokry, D.M., Elbehidy, E.M., 2013. The influence of leptin on Th1/Th2 balance in obese children with asthma. *J. Bras. Pneumol.* 39, 562–568. <https://doi.org/10.1590/s1806-37132013000500006>
- Yuan, Y., Jiang, H., Kuang, J., Hou, X., Feng, Y., Su, Z., 2012. Genetic Variations in ADIPOQ Gene Are Associated with Chronic Obstructive Pulmonary Disease. *PLoS One* 7, 1–8. <https://doi.org/10.1371/journal.pone.0050848>
- Yuksel, H., Sogut, A., Yilmaz, O., Onur, E., Dinc, G., 2012. Role of adipokines and hormones of obesity in childhood asthma. *Allergy, Asthma Immunol. Res.* 4, 98–103. <https://doi.org/10.4168/aair.2012.4.2.98>
- Zhu, Z., Guo, Y., Shi, H., Liu, C.L., Panganiban, R.A., Chung, W., O'Connor, L.J., Himes, B.E., Gazal, S., Hasegawa, K., Camargo, C.A., Qi, L., Moffatt, M.F., Hu, F.B., Lu, Q., Cookson, W.O.C., Liang, L., 2020. Shared genetic and experimental links between obesity-related traits and asthma subtypes in UK Biobank. *J. Allergy Clin. Immunol.* 145, 537–549. <https://doi.org/10.1016/j.jaci.2019.09.035>

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All authors approve the final version and they assume responsibility related to its accuracy and integrity.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Highlights

1. Genetic variants in *ADIPOQ* and *LEP* are associated with asthma
2. Increasing in the number of risk allele is associated with a higher risk of asthma
3. BMI changes the association of genetic variants with asthma and atopy

***ADIPOQ* and *LEP* variants on asthma and atopy: genetic association modified by overweight**

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RSC performed experiments and data analyses, explained the main results and wrote the final version. APCM reviewed the literature and contributed to data analysis and discussion. HSS performed experiments, analyzed genescore and helped with data interpretation. RCRS drafted the manuscript and contributed with the methodology nutrition design, SMAM revised the manuscript and contributed with the methodology nutrition design, MLB designed the SCAALA, collected and created the database, and reviewed the manuscript. NMAN designed the SCAALA, collected and created the database, and reviewed the manuscript. CAVF contributed to the conception and design of this study, drafted, wrote and verified the manuscript. RdSC designed this study, and drafted, wrote and verified the manuscript analyzing results according to the immunogenetic. All authors approve the final version and they assume responsibility related to its accuracy and integrity.

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Abbreviations/Acronyms.

ADIPOQ. Adiponectin

BMI. Body Mass Index

Chr. Chromosome

696 CI. Confidence Intervals

697 DNA. Deoxyribonucleic acid

698 HWE. Hardy-Weinberg Equilibrium

699 LD. Linkage Disequilibrium

700 *LEP*. Leptin

701 MAF. Minor Allele Frequency

702 OR. Odds Ratio

703 SCAALA. Social Changes Asthma and Allergy in Latin America

704 SNP. Single-Nucleotide Polymorphisms

705 SPT. Skin Prick Test

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