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Variants in proinflammatory genes IL1RL1, IL1B and IRF4 are associated with overweight in a pediatric Brazilian population

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ABSTRACT

Background: Obesity is a chronic complex disease with great prevalence for children all over the world. Characterized for low-grade inflammation associated with several comorbidities such as resistance and type 2 diabetes mellitus (T2DM).
Objectives: To investigate whether genetic variants in *IL10, IL1RL1, IL1B, IRF4, TNF, IL6*, and *IL33* genes are associated with being overweight in children.
Methods: We performed the genotyping of 1004 children using Illumina 2.5 Human Omni bead chip, and association analysis on the genetic variants and the overweight through logistic regression adjusted for sex, age and components principal.
Results: Of the seven genes analyzed, 16 SNVs significantly associated. Eleven variants in *IL1RL1*, two in *IL1B* and one in *IRF4* genes increased overweight risk and two SNVs in *IL1RL1* were associated with protection against overweight. The rs2287047-A was negatively associated (OR: 0.66, CI95%: 0.19–0.45) and had a reduced *IL1RL1* expression in whole blood (p 0.033) *in silico* eQTL. The rs12203592-T, in *IRF4,* was positively associated with being overweight, and led to an increased gene expression in whole blood ($p < 0.001$) and adipose tissue ($p < 0.001$).
Conclusion: These results suggest that genetic variants in inflammatory genes may play an important role in the development of overweight in children.

1. Introduction

Excess of body weight has been identified as the fifth leading risk factor for overall death and is associated with an increased risk for comorbidities, such as cancer and asthma (Hruby and Frank, 2015; Sharma et al., 2019). According to World Health Organization (WHO), in 2017, over 340 million children and teenagers, aged 5–19, were overweight or obese worldwide. Obesity is described as a chronic

Abbreviations: ADD, additive; Chr, chromosome; CI, confidence interval; DOM, dominant; eQTL, Expression quantitative trait locus; WHO, World Health Organization; T2DM, type 2 diabetes mellitus; VAT, visceral adipose tissue; IL, interleukin; TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; *Tregs*, *Regulatory T cells*; VAT, Treg cells in visceral adipose tissue; IRF4, transcription factor 4; IRFs, Interferon Regulation Factors; SNVs, Single nucleotide variants; SCAALA, Social Changes Asthma and Allergy in Latin America; BMI, body mass index; MAF, minor allele frequency; PCA, principal components analysis; NCBI, National Center for Biotechnology Information; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; TG, triglycerides.

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disease resulting from abnormal or excessive fat accumulation in adipose tissue (Onis et al., 2007). Its etiology involves genetic, hormonal and environmental aspects (Wang et al., 2011). Moreover, in recent years, obesity has been considered a low-grade inflammatory disease directly related to insulin resistance and T2DM (Martins et al., 2014; Zatterale et al., 2020).

Overweight or obesity related inflammation begins in visceral adipose tissue (VAT) and the major cells responsible for such inflammation are macrophages, T and B lymphocytes, natural killers, and neutrophils (Trim et al., 2018). Those cells express a large amount of proinflammatory mediators, such as interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) (Winer and Winer, 2012). In obese individuals, the increase of free fatty acids derived from disrupted adipocytes or their apoptosis results in the infiltration of classically activated macrophages (M1) (Carobbio et al., 2019; Ye, 2013). Adipose tissue macrophage produces TNF-α, IL-6 and other proinflammatory cytokines in the VAT. By its turn, these cytokines are released into the bloodstream which produces a systemic inflammation (Ye, 2013), impairing the functions of various organs, such as liver, heart, muscle, and brain (Divella et al., 2016; Hotamisligil, 2006).

However, regulatory T cells (Treg) in visceral adipose tissue (VAT-Treg cells) are specialized in the prevention of obesity-associated inflammation, insulin sensitivity and glucose tolerance by producing significant levels of IL-10 (Vasanthakumar et al., 2015). The presence of IRF4 (transcription factor 4) is necessary to activate the expression and activation of *IL1RL1/ST2* to stimulate IL-10 production (Becker et al., 2017). The *IL1RL1/ST2*, an IL-33 receptor, plays an important role in the signaling to express IL-10 and maintain VAT-Treg cells activity on the homeostasis and sustenance of the visceral adipose tissue (Vasanthakumar et al., 2015).

Both, genetics and epigenetics alterations that could affect both anti-inflammatory and proinflammatory mechanisms linked to VAT immune response may play a role in obesity. Indeed, some evidence suggests the relative importance of genetic individualities in body weight and human adiposity (Rankinen et al., 2006; Vimaleswaran et al., 2012). Therefore, SNVs in candidate genes linked to VAT homeostasis are important to identify new mechanisms or pathways that could be potential targets for therapeutic interventions (Rankinen et al., 2006). This could have a great impact on public health (Rankinen et al., 2006; Seidel, 2010). In this way, here we investigated the association between variants in *IL10, IL1RL1, IL1B, IRF4, TNF, IL6* and *IL33* genes associated with inflammation in VAT in overweight (Fig. 1).

2. Materials and methods

2.1. Population and study design

The study population included 1004 unrelated children between 5 and 11 years old from the city of Salvador, Northeastern Brazil. Anthropometric measures and blood samples used here were obtained from unrelated children that were originally recruited from the conduction of a sanitation program study on the occurrence of childhood monitoring in 2005. The population sample refers to a longitudinal study of the project called SCAALA (Social Changes Asthma and Allergy in Latin America) (Barreto et al., 2006; Figueiredo et al., 2010). This work was conducted under the approval of the Ethics Committee of the

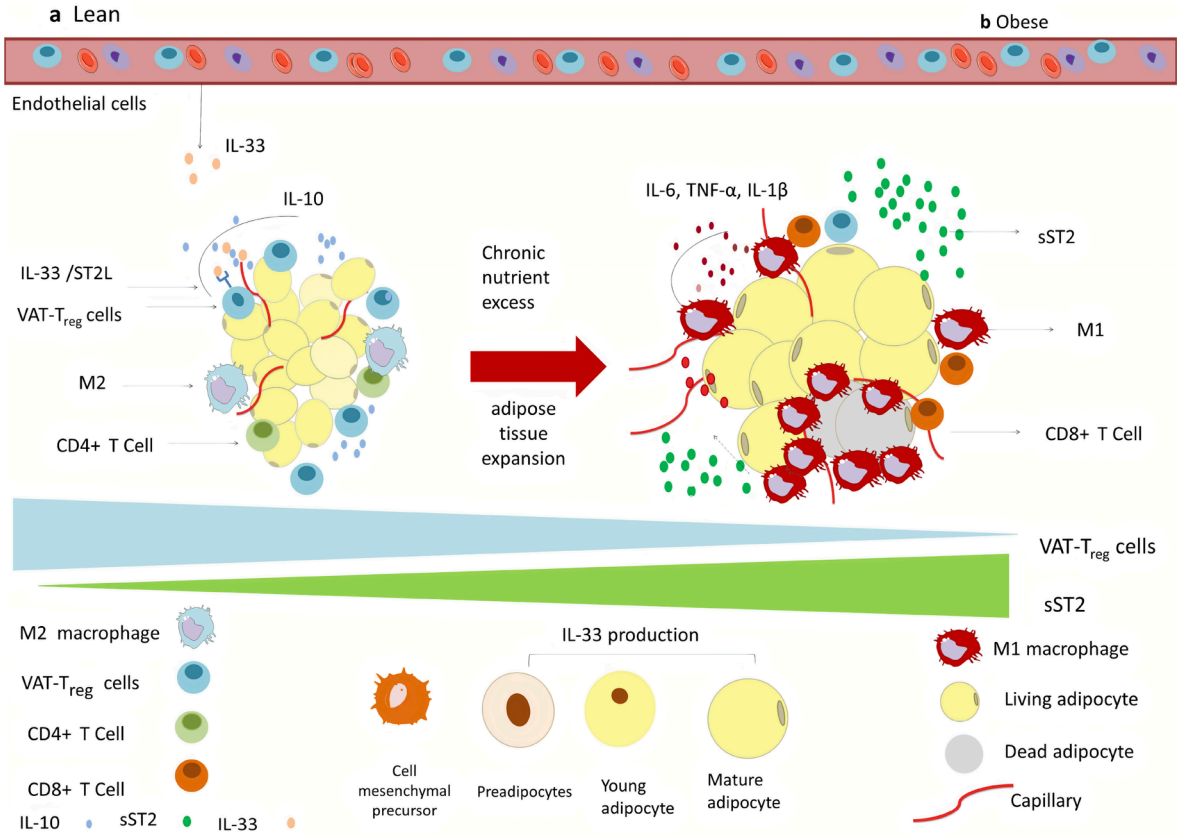

Fig. 1. Obesity-Associated Adipose Tissue Inflammation In the lean state, adipose tissue contains regulatory immune cells (blue and green). Adipocytes and endothelial cells–derived IL-33 and ST2 receptors are responsible for the maintenance of the abundance of adipose Treg and the anti-inflammatory profile due to IL-10 secretion. (b) In contrast, obese adipose tissue contain high amount of proinflammatory cytokines, such as L-6, TNF-α, IL-1β, produced by infiltrated M1 macrophages, and sST2 soluble produced by adipocytes as an obesity-induced adipokine that attenuates IL-33 signaling and disrupts VAT-Treg cells. IL: interleukin; VAT: visceral adipose tissue; M: macrophages; TNFα: tumor necrosis factor alpha; sST2: Soluble suppression of tumorigenesis-2 (also called *IL1RL1*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Children in northeastern Brazil have 2 dietary patterns named Western and Prudent (Silva et al., 2013). We have previously described the dietary patterns of our population (D'Innocenzo et al., 2011). Basically, 4 groups main groups were described (1: fruit, vegetables, pulses, cereals and sea food; 2: milk and dairy products, ketchup/mustard/mayonnaise and chicken; 3: fried food, sweets, snacks, soda/artificial fruit juice and 4: processed meat products, eggs, and red meat) (D'Innocenzo et al., 2011). Patterns of food intake in children depend on the socio-economic conditions of their families and the choice of healthier food is associated with a higher socioeconomic status. Silva et al. (2013) demonstrated that the pattern 1 has been negatively associated with another inflammatory condition, asthma, indicating a protection of inflammation with this more Mediterranean-kind of diet.

2.2. Overweight definition

Weight, height and age data were collected to assess the children's anthropometric status. A portable electronic scale (Filizola R, model E-150, São Paulo, Brazil) and a portable stadiometer (Leicester) properly calibrated were used to measure weight and height, respectively. Each measurement (weight and height) was performed in duplicate, considering the average, as the final measurement. Variations of 100 g of weight and 0–1 cm for height were allowed, as described in Matos et al. (Matos et al., 2011). Then, the body mass index (BMI) was calculated and, according to the age and sex of the children, following World Health Organization (WHO) reference tables to characterize excess weight, children with $\geq +1$ standard deviation of the median were considered overweight and those with values below 1, no overweight (Onis et al., 2007). In the present work, children who were overweight, obese and severely obese were considered overweight.

2.3. DNA extraction and genotyping

Peripheral blood leukocytes were used for DNA extraction according to the FlexiGene DNA Kit (Qiagen, Hilden, Germany) following manufacturer's recommendations. DNA samples from 1,004 subjects were genotyped using the Illumina 2.5 Human Omni Bead Chip. The variants' within *IL10, IL1RL1, IL1B, IRF4, TNF, IL6* and *IL33* genes were obtained from their initial and final position presented in the supplementary material (Table S1). The variants screening was conducted following the criterion exclusion of: Hardy–Weinberg equilibrium (HWE) with p-value lower than 0.05 in the controls and minor allele frequency (MAF) lower than 0.01.

2.4. Statistical analysis

Multiple logistic regression was performed between variants and overweight adjusted for sex, age and population stratification as previously described (Lima-Costa et al., 2015). Population was done based on principal components analysis (PC1 to African and PC2 to European). The first two PCs were the most representative of the population (Lima-Costa et al., 2015).

We tested for possible associations between co-variates and our outcome overweight, especially, in the context of asthma, since this cohort was designed for asthma risk factors. However, using Mann-Whitney test we did not find any significant associations between overweight and asthma symptoms/ markers of allergy, so they were not included in the model.

Three genetic models were used in all analyses, additive, dominant, and recessive. The permutation analysis (10,000 permutations) was performed to solve the problem of multiple comparisons. This has been managed as a potential method to control the false discovery rate (Lage-Castellanos et al., 2010). The linkage disequilibrium was performed in Haploview software, we report r^2 value to verify the SNVs that are inherited in genetics blocks corrected by frequency (Browning and Browning, 2007). All genetic data were analyzed using PLINK 1.9 software (Purcell et al., 2007). For haplotype analysis was used SNPStats platform (https://www.snpstats.net/start.htm) (Solé et al., 2006).

2.5. In silico annotation

In silico analyses were performed in the National Center for Biotechnology Information (NCBI) platform (www.ncbi.nlm.nih.gov) where the information regarding function of each SNV was obtained.

In addition, we use RegulomeDB (a database that assists interpretation of regulatory variants in the human genome) for identifies possible regulatory regions and functional variants through computational predictions and manual annotations. This database determines a score for the variant that ranges from one to six (regulomedb.org) representing an increasing scale, which demonstrates its most likely functional consequence (Boyle et al., 2012). Finally, the Haploreg platform (Ward and Kellis, 2012) was used to provide the functional impact of genetic variants in different human tissues. In such databases, information is obtained on histones of promoters and enhancers that overlap the region where the variant is found.

2.6. In silico expression analysis

In silico expression was performed, using the Genotype-Tissue Expression platform (GTEx – www.gtexportal.org), in blood, subcutaneous adipose and visceral adipose tissues, according to the genotype of each SNV. The analysis also highlights the expression quantitative trait loci (eQTLs), which infers how genetic components can alter gene expression, such as silent mutations that alter the stability of mRNAs, splicing changes, and expression of non-functional proteins (Lonsdale et al., 2013).

3. Results

3.1. Characteristics of the study population

The study included 847 controls and 157 cases (Table 1). Mean age of cases and controls were 7.41 and 7.24, respectively. Among the obese children, 59% were males. There was no difference between the group's control and case.

3.2. Description of the SNVs

The variants in the seven genes interconnected with inflammation in VAT (*IL10, IL1RL1, IL1B, IRF4, TNF, IL6, and IL33*) were analyzed. From these genes, 251 SNVs were extracted from the Illumina genotyping chip, in 1.004 subjects. From those markers, 7 were excluded due to the HWE deviation and 26 SNVs due to MAF $< 1\%$. No sample was excluded based on low genotyping error rate ($<10\%$). Thus, a total of 218 SNVs were included in the association analysis.

Table 1
Characteristics of nutritional status, age group, and gender in the studied population.

	Subject grous (n = 1004)		
Variables	**Control Non Overweight**	**Case Overweight**	P-value
	847	157	
Age mean (SD*)	7.24 (1.46)	7.41 (1.49)	0.202
Sex			
Male	453 (53%)	92 (59%)	0.237
Female	394 (47%)	65 (41%)	

Mean (standard deviation)*. Data were analyzed using the chi squared test#.

Among the seven genes analyzed, there were only three with variants associated with being overweight. SNVs significantly associated with overweight were characterized based on functional and genomic regulatory aspects (Table 2). Non-associated SNVs were presented in supplementary material (Table S2).

3.3. Association between IL1RL1, IL-1B, and IRF4 SNVs with overweight

Aiming to investigate which variants were related to overweight on the highlighted genes from the previous analysis, we observed thirteen associated variants in *IL1RL1,* two in *IL1B*, and one in *IRF4* (Table 3).

SNV: single nucleotide variant; OR: odds ratio; 99% CI: 99% confidence interval; Perm: permutational P value.

The majority of variants in the *IL1RL1* were positively associated with overweight. Using the additive model, rs3917291-A (polymorphic allele found in the analyzed population) (OR: 1.56 CI95%: 1.05–2.32), rs12477295-A (OR: 1.44 CI95%: 1.04–2.01), rs6750958-G (OR: 1.59 CI95%: 1.01–2.50), and rs13394668-G (OR: 1.58 CI95%: 1.01–2.49); in the dominant model rs3917291-A (OR: 1.69 CI95%: 1.08–2.63); and in the recessive model rs12477295-A (OR: 4.15 CI95%: 1.60–10.75), rs6750958-G (OR:7.82 CI95%: 1.28–47.74), rs13394668-G (OR: 7.93 CI95%: 1.30–48.45), rs10203841-T (OR: 1.68 CI95%:1.11–2.56), rs992153-T (OR: 2.90 CI95%: 1.37–6.17), rs2041751-G (OR: 2.12 CI95%: 1.11–4.05), rs13007967-G (OR: 1.60 CI95%: 1.01–2.53), rs2310186-G (OR: 1.58 CI95%: 1.01–2.50), rs1024794-C (OR: 3.21 CI95%: 1.14–9.03), and rs4851541-T (OR: 1.73 CI95%: 1.02–2.92).

The SNVs rs3917292-A in both additive (OR: 0.35, CI95%: 0.14–0.90), and dominant (OR: 0.36, CI95%: 0.14–0.89) models, as well as the rs2287047-A (OR: 0.66, CI95%: 0.19–0.45) in the dominant model were negatively associated with overweight.

Among *IL1B* variants, two SNVs rs1071676-C (OR: 2.75 CI95%: 1.85–6.98) and rs1143639-T (OR: 2.75 CI95%: 1.85–6.98) were associated to risk of overweight in the recessive model. Regarding *IRF4,* the rs12203592-T was positively associated with overweight in the additive (OR: 1.80 CI95%: 1.03–3.14) and dominant (OR: 1.87 CI95%: 1.06–3.30) model.

3.4. Haplotype-based association of IL1RL1 SNVs with overweight

We performed haplotype analysis of the *IL1RL1* gene using the SNPstats, three variants for time. Haplotype 1: rs10203841, rs2041751, and rs12477295 (OR: 1.71 CI95%: 1.09–2.67). Haplotype 2: rs2041751, rs12477295, rs992153 (OR: 1.52 CI95%:1.08–2.14), Haplotype 3: rs12477295, rs992153, rs6750958 (OR: 1.63 CI95%: 1.15–2.30), Haplotype 4: rs992153, rs6750958, rs13394668 (OR 1.69 CI95%: 1.07–2.67), the latter alleles doubling the risk for overweight, Haplotype 5: rs6750958, rs13394668, rs13007967 (OR: 2.03 CI95%:1,17–3.52) variants together almost doubling the chance of risk to overweight (Table 4). A polygenetic risk score was also performed; however, no significant result was found.

3.5. Linkage disequilibrium

The linkage disequilibrium (LD) analysis was performed on the *IL1RL1*, *IL1B*, and *IRF4* genes, using values equal or >80% for r^2 to be considered as strong LD (Fig. 2).

3.6. GTEx: in silico eQTL analyses

Using the GTEx platform we tested eQTL for 16 overweight-associated variants in whole blood, subcutaneous adipose and visceral adipose tissues. The variants rs12203592 and rs2287047 were differentially expressed among them.

The T allele of rs12203592 increased expression of the *IRF4* in whole blood ($p < 0.0011$) (Fig. 3A), subcutaneous adipose tissue ($p = 0.00082$) (Fig. 3B), and visceral adipose tissue ($p = 0.000032$) (Fig. 3C). On the other hand, the of rs2287047-A was associated with reduced expression of the *IL1RL1* in whole blood ($p = 0.036$) (Fig. 3D), but not in subcutaneous adipose tissue ($p < 0.76$) or visceral adipose tissue ($p < 0.83$) (Fig. 3).

4. Discussion

We found genetic variants in *IRF4*, *IL1B*, and *IL1RL1*, genes associated with being overweight in children. The rs12203592-T and

Table 2
Characterization of the significant SNVs associated with overweight in Brazilian children.

SNV	Regulation	Promoter histone marks in Adipose*	Enhancer histone marks in Adipose*	Proteins bound*	Motifs changed*	RegulomeDB score	MAF	Function
IL1RLI Gene								
rs3917291	Distal, RNA Binding	–	H3K4me1_Enh	–	–	6	0.07	Intron variant
rs3917292	RNA Binding	–	15_EnhAF, H3K4me1_Enh, H3K27ac_Enh	–	–	4	0.04	Intron variant
rs12477295	–	–	H3K27ac_Enh	–	–	4	0.14	Intron variant
rs6750958	Distal	–	–	–	–	–	0.05	Intron variant
rs13394668	Proximal	–	–	–	–	4	0.05	Intron variant
rs2287047	Distal, RNA Binding, eQTL	H3K9ac_Pro	13_EnhA1, H3K4me1_Enh, H3K27ac_Enh	–	–	6	0.49	Intron variant
rs992153	–	–	H3K4me1_Enh, H3K27ac_Enh	–	–	5	0.19	Intron variant
rs10203841	Distal	H3K9ac_Pro	H3K4me1_Enh, H3K27ac_Enh	–	–	6	0.41	Intron variant
rs2041751	eQTL	–	H3K4me1_Enh, H3K27ac_Enh	–	–	3a	0.22	Intron variant
rs13007967	eQTL	–	H3K4me1_Enh	–	–	3a	0.39	Intron variant
rs2310186	Distal	H3K9ac_Pro	–	–	–	4	0.391	Intron variant
rs1024794	Distal	H3K9ac_Pro	H3K4me1_Enh	–	–	4	0.14	Intron variant
rs4851541	eQTL	H3K9ac_Pro	H3K4me1_Enh	–	–	5	0.29	Intron variant
IL1B Gene								
rs1071676	Proximal,Distal, RNA Binding	–	–	–	–	6	0,175	Utr variant 3 prime
rs1143639	Distal, RNA Binding	–	–	–	–	5	0,172	Intron variant
IRF4 Gene								
rs12203592	RNA Binding	–	–	–	–	2b	0,039	Intron variant

CHR, chromosome; SNV, single nucleotide variant; MAF, minor allele frequency; utr,untranslated region.

Table 3
Significant associations between *IL1RL1, IL1B,* and *IRF4* SNVs and overweight, using a logistic regression adjusted for sex, age, and 1 and 2 PCs.

Gene	SNVs	Genotype	Case (153)	Control (814)	Model	OR (CI 95%)	PERM	p – value
IL1RL1	rs3917291	GG	121(79.0%)	701(86.1%)	**ADD**	**1.56(1.05–2.32)**	**0.0231**	**0.0264**
		AG	30(19.6%)	106(13.0%)	**DOM**	**1.69(1.08–2.63)**	**0.02275**	**0.019**
		AA	2(1.3%)	7(0.8%)	REC	1.38(0.28–6.79)	1	0.6894
	rs3917292	GG	148(96.7%)	746(91.5%)	**ADD**	**0.35(0.14–0.90)**	**0.0225**	**0.0294**
		AG	5(3.26%)	66(8.09%)	**DOM**	**0.36(0.14–0.89)**	**0.02978**	**0.0282**
		AA	0(0%)	3(0.3%)	REC	NA	NA	NA
	rs12477295	GG	106(69.2%)	608(74.6%)	**ADD**	**1.44(1.04–2.01)**	**0.0195**	**0.02704**
		AG	38(25.4%)	196(24%)	DOM	1.33(0.91–1.95)	0.1272	0.1375
		AA	8(5.2%)	10(1.2%)	**REC**	**4.15(1.60–10.75)**	**0.00331**	**0.0033**
	rs6750958	AA	129 (84.8%)	721 (89.5%)	**ADD**	**1.59(1.01–2.50)**	**0.0382**	**0.04324**
		GA	20 (13.1%)	83 (10.1%)	DOM	1.49(0.90–2.46)	0.1544	0.1096
		GG	3 (1.9%)	2 (0.2%)	**REC**	**7.82(1.28–47.74)**	**0.03593**	**0.02586**
	rs13394668	AA	129 (84.8%)	721 (89.4%)	**ADD**	**1.58(1.01–2.49)**	**0.0430**	**0.04528**
		GA	20 (13.1%)	82 (10.1%)	DOM	1.48(0.90–2.44)	0.1544	0.1211
		GG	3 (1.9%)	2 (0.2%)	**REC**	**7.93(1.30–48.45)**	**0.03221**	**0.0248**
	rs2287047	GG	48 (31.3%)	196 (24%)	ADD	0.82(0.64–1.06)	0.1272	0.1438
		AG	70 (45.7%)	430 (52.7%)	**DOM**	**0.66(0.19–0.45)**	**0.0342**	**0.0339**
		AA	35 (22%)	189 (23.1%)	REC	0.95(0.63–1.43)	0.6923	0.8159
	rs10203841	CC	48 (31.3%)	282 (34.7%)	ADD	1.26(0.98–1.62)	0.05701	0.0602
		TC	68 (44.4%)	402 (49.5%)	DOM	1.16(0.80–1.69)	0.4286	0.4215
		TT	37 (24%)	128 (15%)	**REC**	**1.68(1.11–2.56)**	**0.0158**	**0.0141**
	rs992153	CC	95 (62.0%)	529 (65.6%)	ADD	1.31(0.96–1.77)	0.1317	0.0791
		TC	47 (30.7%)	256 (31.7%)	DOM	1.18(0.82–1.70)	0.3617	0.3486
		TT	11 (7.1%)	21 (2.6%)	**REC**	**2.90(1.37–6.17)**	**0.0080**	**0.0054**
	rs2041751	AA	88 (57.5%)	497 (60.9%)	ADD	1.25(0.94–1.66)	0.1202	0.1151
		GA	51 (33.3%)	282 (34.6%)	DOM	1.16(0.82–1.65)	0.2603	0.3926
		GG	14 (9.1%)	36 (4.4%)	**REC**	**2.12(1.11–4.05)**	**0.0110**	**0.02241**
	rs13007967	AA	52 (34.2%)	292 (35.8%)	ADD	1.19(0.92–1.54)	0.2	0.1818
		GA	71 (46.7%)	415 (50.9%)	DOM	1.07(0.74–1.55)	0.7273	0.6863
		GG	29 (19%)	107 (13%)	**REC**	**1.60(1.01–2.53)**	**0.0479**	**0.0434**
	rs2310186	TT	53 (34.6%)	296 (36.3%)	ADD	1.18(0.92–1.53)	0.2	0.1849
		GT	71 (46.4%)	411 (50.4%)	DOM	1.08(0.75–1.55)	0.7273	0.6694
		GG	29 (18%)	107 (13%)	**REC**	**1.58(1.00–2.50)**	**0.0479**	**0.04813**
	rs1024794	TT	109 (71.2%)	613 (75.3%)	ADD	1.35(0.96–1.89)	0.1078	0.08371
		CT	38 (24.8%)	191 (23.4%)	DOM	1.27(0.86–1.87)	0.2128	0.2215
		CC	6 (3.9%)	10 (1.2%)	**REC**	**3.21(1.14–9.03)**	**0.02402**	**0.02635**
	rs4851541	GG	81 (52.9%)	401 (49.2%)	ADD	1.03(0.79–1.34)	0.6667	0.7918
		TG	51 (33.3%)	344 (42.2%)	DOM	0.85(0.60–1.21)	0.4054	0.3912
		TT	21 (13%)	70 (8.5%)	**REC**	**1.73(1.02–2.92)**	**0.03438**	**0.0403**
IL1B	rs1071676	GG	98 (64.9%)	535 (66.8%)	ADD	1.182(0.85–1.63)	0.5	0.3111
		CG	46 (30.4%)	251 (31.3%)	DOM	1.08(0.75–1.56)	0.666	0.6648
		CC	7 (4.6%)	14 (1.7%)	**REC**	**2.75(1.85–6.98)**	**0.024**	**0.03299**
	rs1143639	CC	98 (64.9%)	537 (67.1%)	ADD	1.19(0.86–1.65)	0.351	0.2809
		TC	46 (30.4%)	249 (31.1%)	DOM	1.09(0.76–1.58)	0.526	0.6132
		TT	7 (4.6%)	14 (1.7%)	**REC**	**2.75(1.85–6.98)**	**0.024**	**0.0329**
IRF4	rs12203592	CC	134 (88.2%)	761 (93.3%)	**ADD**	**1.80(1.03–3.14)**	**0.044**	**0.0385**
		TC	18 (11.7%)	53 (6.50%)	**DOM**	**1.87(1.06–3.30)**	**0.020**	**0.0304**
		TT	0 (0%)	1 (0.1%)	REC	NA	NA	NA

Table 4
Risk haplotype for SNVs on ***IL1RL1*** and overweight.

Haplotype	rs10203841	rs2041751	rs12477295	Freq	**Odds Ratio/95% Confidence interval**	P-value
Reference	C	A	G	0.45	1	
	T	G	A	0.08	**1.71 (1.09–2.67)**	**0.019**
Haplotype	rs2041751	rs12477295	rs992153	Freq	OR (95% CI)	P-value
Reference	A	G	C	0.734	1	
	G	A	T	0.1288	**1.52 (1.08–2.14)**	**0.017**
Haplotype	rs12477295	rs992153	rs6750958	Freq	OR (95% CI)	P-value
Reference	G	C	A	0.7406	1	—
	A	T	A	0.1289	**1.63 (1.15–2.30)**	**0.0057**
Haplotype	rs992153	rs6750958	rs13394668	Freq	OR (95% CI)	P-value
Reference	C	A	G	0.7504	1	—
	C	G	A	0.0584	**1.69 (1.07–2.67)**	**0.026**
Haplotype	rs6750958	rs13394668	rs13007967	Freq	OR (95% CI)	P-value
Reference	A	G	A	0.5888	1	—
	G	A	G	0.0395	**2.03 (1.17–3.52)**	**0.012**

Adjusted by gender, age and principal components 1 and 2.

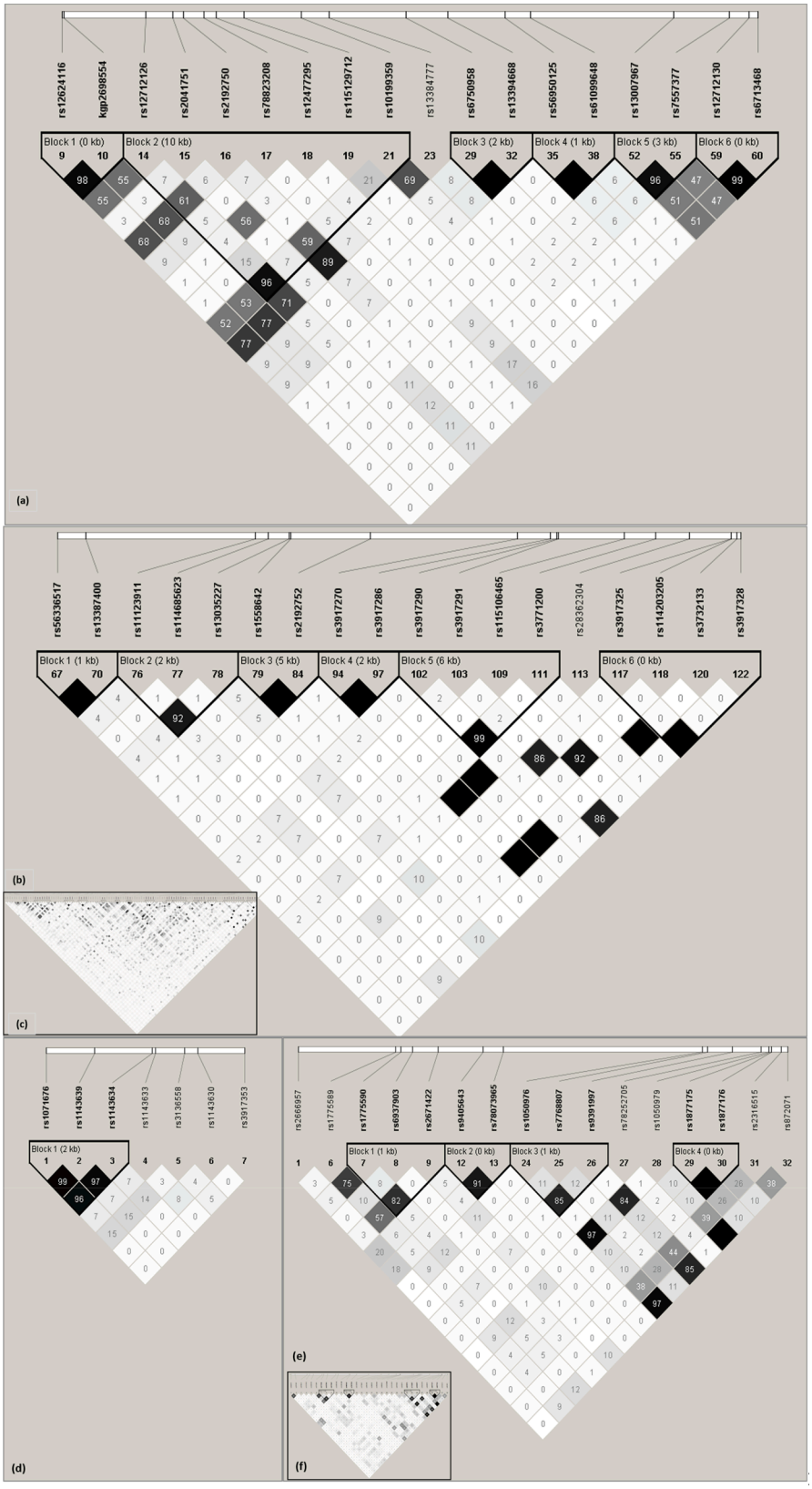

Fig. 2. Pairwise LD within Haploview using the R^2. Color intensity and value in square indicate the degree of confidence in the r2 value and how much the SNPs are in LD. *IL1RL1*, the figure was divided into two (Fig. 2A and B) the complete LD is shown in Fig. 2C. In the same Fig. 2D a LD block was found in the *IL1B* gene with three SNVs. The LD of the *IRF4* gene is shown in Fig. 2E (the complete LD is shown in Fig. 2F).

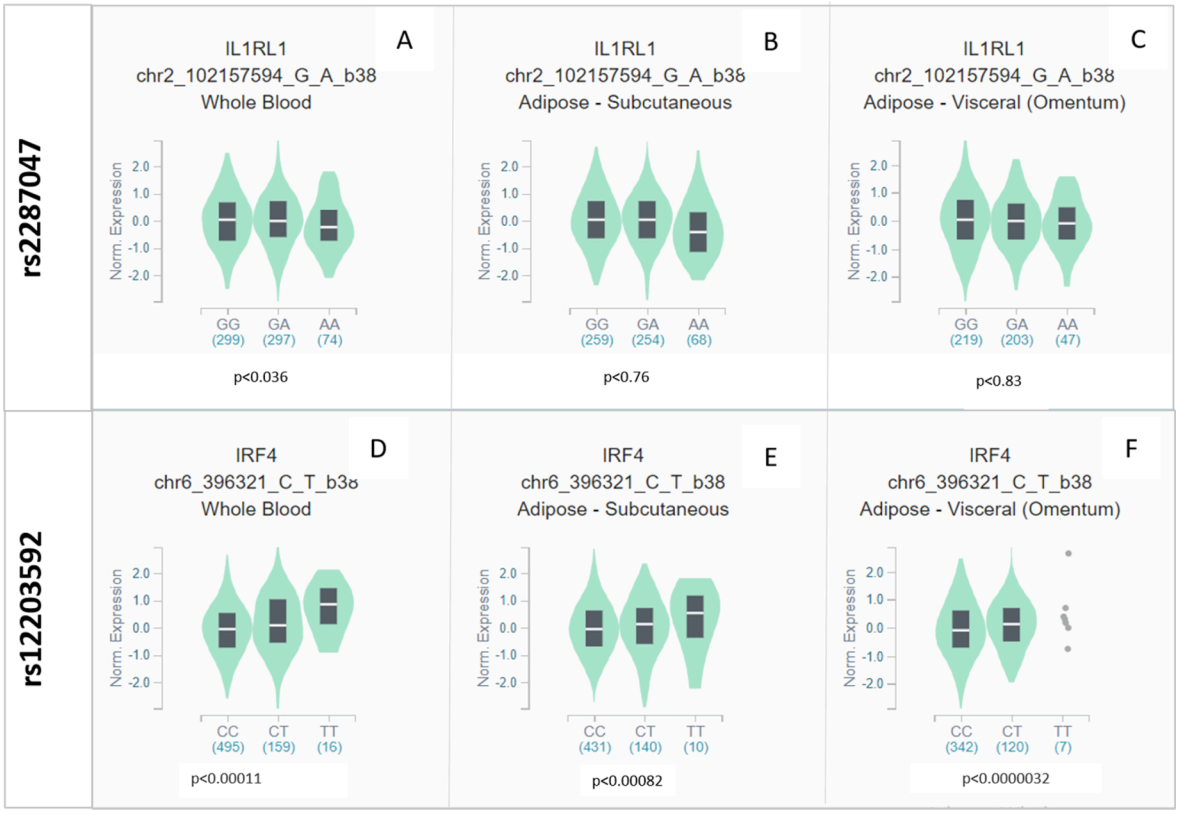

Fig. 3. GTEx-Gene expression level of *IL1RL1* rs2287047 whole blood (A), subcutaneous adipose (B) and visceral tissue (C) and *IRF4* rs12203592 in human in whole blood (D), subcutaneous adipose (E) and visceral tissue (F).

rs2287047-A variants, in the *IRF4* and *IL1RL1* genes, respectively, had a difference in expression in their respective genes.

Interferon Regulation Factors (IRFs) are a large family with >10 members which play important roles in transcriptional regulation of the immune system (Paun a Pitha, 2007). *IRF4* is the only member of this family that controls genetic expression and regulators of lymphocytes, macrophages, B cells, dendritic cells and VAT-T_{reg} cells development (Paun and Pitha, 2007; Vasanthakumar et al., 2015). In addition, *IRF4* is a regulator of adipogenesis and a critical determinant of the transcriptional response to nutrient availability through lipolysis in adipocytes (Eguchi et al., 2011). Eguchi et al. (Eguchi et al., 2011) studied *IRF4* knockout mice (Irf4−/−) in adipocytes and identified that these animals had increased adiposity and impaired lipolysis (Eguchi et al., 2011). These evidences reinforce the possible role of this gene in the homeostasis of lipid metabolism, and adipogenesis.

In our study, rs12203592-T of *IRF4* was positively associated with two-fold the risk of being overweight. Additionally, the T allele was significantly associated with increased expression of *IRF4* gene in the whole blood and in subcutaneous and visceral adipose tissue (Fig. 2 D, E and F). Although there are some other factors that could influence *IRF4* expression, our results demonstrate that its expression could be related to the overweight condition. Since *IRF4* induces the expression of cytokines such as IL-4 and IL-10 (Orekhov et al., 2019). These cytokines are important in the context of obesity. IL-4 is known to inhibit the accumulation of lipids in adipose tissue and IL-10 regulates the production of proinflammatory cytokines and chemokines in VAT (El-Wakkad et al., 2013; Mattos et al., 2016). Both cytokines act as obesity regulators by sensitizing insulin and glucose tolerance, promoting local and systemic homeostasis (El-Wakkad et al., 2013; Han et al., 2015).

The interleukin IL-1β, encoded by the *IL1B* gene, is an important cytokine produced by M1-type macrophages and perpetuates the inflammatory state in obesity. It seems that its expression is related to the exposure of lipids such as LDL- cholesterol, and triglycerides (TG) (Maintinguer Norde et al., 2018). Genetic variants in *IL1B* have already been associated with the increased atherosclerotic process and increased lipid concentration in the elderly population (Netea and Dinarello, 2011). In our study, rs1071676-C, and of rs1143639-T, increased the weight of being overweight by almost three-fold (Table 3). Although these two variants described here have never been described in any other previous obesity study, other variants of the same gene have been associated with obesity (Maculewicz et al., 2021). A variant rs1143623 in *IL1B* has been identified to affect glucose levels in overweight T2DM patients on use of metformin. This same variant (rs1143634) was in disequilibrium linkage (Fig. 2D) with rs1071676-C and rs1143639-T described in this study.

In this study, the variants rs2041751-G and rs4851541-T in the *IL1RL1* were associated with risk for overweight and scored 3a and 5, respectively, in RegulomeDB (Table 2), which represents that both of them can cause a DNA motif alteration and/or transcription factor (TF) binding (HaploReg platform). The variant rs2041751-G and rs4851541-T altered four DNA motifs. They often can indicate sequence-specific binding sites for proteins, such as nucleases and TF. In addition, the rs2041751-G occurs in a region with promoter histone marks in multiple tissues and cells, especially in immune cells, which indicates a predicted promoter region. This variant interacts with TF in B-lymphocyte-induced maturation protein 1 (Blimp-1), controls patterns of gene expression in T lymphocytesis (Martins et al., 2006) and is essential for normal development and immunity (Bikoff et al., 2009). These data suggest that both SNVs are potentially regulatory variants (Ward and Kellis, 2012).

The rs3917292-A and rs2287047-A in *IL1RL1*, were associated with protection for overweight. The rs2287047-A decreases the expression of the *IL1RL1* gene in whole blood (Fig. 3 D). This variant has already been associated with protection against an inflammatory disease in adults (Näkki et al., 2010). IL-33/ST2 is critical for the development, maintenance and expansion of the VAT Tregs (Beltrán et al., 2010). Studies

show that sST2 levels are increased in adults with diseases related to obesity (Lin et al., 2016), and other inflammation-related diseases (Beltrán et al., 2010; Bergis et al., 2016; Marc. et al., 2008; Rehman et al., 2008; Watanabe et al., 2018).

Ragusa et al. (Ragusa et al., 2017) demonstrated that sST2 expression was reduced in adipose tissue in obese animals. Yu et al. (Yu et al., 2020) identified seven variants in *IL1RL1* associated with the risk of obesity in 175 obese individuals (BMI $\geq$ 25) when compared to the control (BMI $<$ 25.0) and concluded that *IL1RL1* variants may be associated with mediators of inflammation. Zeyda et al. (2013) find increased IL-33 and ST2/*IL1RL1* expression levels in VAT and in the subcutaneous adipose tissue of severely obese humans and in diet-induced obese mice. The increase or decrease of ST2 in individuals seems to be deleterious in obesity, further studies need to be carried out.

Transcriptional factors such as *BATF* and *IRF4* are essential for the expression of *IL1RL1*. The *IRF4, IL33* and *IL1RL1* are indispensable for the development of VAT-Treg cells. However, proinflammatory cytokines produced by macrophages, such as IL-1β, may help to increase the inflammatory state mainly by inhibiting or reducing the VAT-Treg cells population (Fig. 1). We hypothesize that these variants may contribute to the perpetuation of the inflammatory state in obesity.

In addition, Caradonna et al. (2018) have described that the presence of IL-1β in activated differentiated Caco-2 cell line acts as modulator of DNA methylation in such cells which points out for a complex interaction between genomic factors and environment. Even though gene expression can be altered by both genetic variations and epigenetic modifications and that they can both affect the same gene or region, it seems that genetic polymorphisms is more important than the epigenetics changes (Naselli et al., 2014).

Thus, such evidence may reinforce the results found here where we describe the association of such variants with overweight, since this is also related to a proinflammatory state.

No association with SNVs in *TNF*, *IL10* and *IL6* (complete list of all SNVs in supplementary material (Table S2) were found in this study. Other studies that sought the relationship between variants in *IL6* and obesity have already been carried out (Hu et al., 2018; Ibrahim et al., 2017), however, not all found associations. Maculewicz et al. (2021) also did not find any relationship between obesity parameters and *IL6* variants in a homogeneous population of young people between 19 and 26 years old in Poland. Another study in Brazil, with children aged between 7 and 9 years, also did find association in *IL6* variants and the anthropometric parameters, blood pressure levels and biochemical markers assessed in the study (Todendi et al., 2015).

Other studies also found no significant association between genetic variants in the TNF-a promoter G-308A and G-238A with overweight or obesity (Hedayati et al., 2012; Walston et al., 1999). The fact that there was no association of genetic variants in the genes of *IL6, TNF* and *IL10* with overweight may be related to factors such as the analyzed population (children) and ethnicity (Maculewicz et al., 2021).

There are several factors for childhood overweight, in addition to those mentioned above. Genetic variants in body weight regulatory genes can influence behavioral responses, such as appetite control, food preference, and simple carbohydrate consumption (Heianza and Qi, 2017). Variants in genes such as visfatin, responsible for adipogenesis, adiponectin and leptin responsible for insulin sensitization and appetite control. Adiponectin and leptin have already been associated with childhood overweight, including in the population studied (Coelho et al., 2021). The literature demonstrates that alterations in the production of this adipokines are associated with a higher BMI in children, however, when treated early with a healthier lifestyle, such as the intake of antioxidant and anti-inflammatory foods characteristic of a Mediterranean diet and the regular practice of physical activities, achieves a significant decrease in the BMI of children/adolescents (Pejsova et al., 2019; Siegrist et al., 2013). This is because the adoption of a healthier diet has a protective role against inflammatory diseases (Shahbazi et al., 2021), and a lower risk of developing overweight, obesity and abdominal obesity (Notario-Barandiaran et al., 2020) therefore can maintain the efficient action of adiponectin and leptin (Pejsova et al., 2019). Physical activity and a more balanced diet tend to reduce the expression of risk variants (Heianza and Qi, 2017). Furthermore, the early identification of risk polymorphisms for obesity may intensify the control and surveillance of weight and biochemical parameters in the routine of clinical practice, and may even lead to changes in guidelines for patients at risk.

5. Conclusion

In conclusion, this is the first study to analyze several genetic variants in pro-inflammation genes associated with overweight in Brazilian children. Our results suggest that variants in the *IRF4* transcription factor, *L1RL1* receptor and *IL1B* cytokine genes may play an important role in the development of childhood overweight. In addition, with understanding and knowledge of the genotypes found here, they can help in personalized medicine and help patients who need to control their body weight. Functional level investigations should be carried out.

6. Limitations

This study was pediatric population-based study focused in being a representative sampling of our city, Salvador, Bahia Brazil. Considering the phenotype obesity/overweight, a convenience sampling was applied for the present study and were included all participants with anthropometric data available as well as genetic data. This is a limitation of our work, however, to our knowledge, no study was published so far involving several genes associated to inflammation stratified by overweight. Thus, considering the relevant and unprecedented nature of these results, we believe that their dissemination is important to guide the realization of future studies that must be designed with this sample size consideration in mind. In addition, several papers have been published investigating the role of variants in pro-inflammatory genes in obese adults, however, this topic is still scarce when it comes to children and adolescents, therefore, making comparisons between these studies and ours difficult.

APCM performed experiments, analyzed data, and wrote the manuscript. HMPT helped with experimental design and data interpretation. RSC Helped write the manuscript. GQ, TSJ and HSS Performed the haplotype analysis NMAN and MLB contributed to the study design and revised the manuscript RSC revised the manuscript LCP and CAF designed the study and revised the manuscript. SMAM, SD and RFCS contributed with the fieldwork and data collection and reviewed the manuscript.

CRediT authorship contribution statement

Raísa Santos Coelho: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Formal analysis. **Ana Paula Castro Melo:** Writing - Review & Editing, Investigation. **Hátilla dos Santos Silva:** Methodology, Formal analysis, Investigation, Writing - Review & Editing. **Rita de Cássia Ribeiro Silva:** Resources, Investigation, Methodology, Writing - Review & Editing. **Sheila Maria Alvim Matos:** Investigation, Writing - Review & Editing, Methodology **Maurício Lima Barreto:** Resources, Investigation, Methodology, Writing - Review & Editing. **Neuza Maria Alcântara-Neves:** Project administration, Funding acquisition, Resources, Investigation, Methodology, Writing - Review & Editing. **Camila Alexandrina Viana de Figueiredo:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing - Review & Editing. **Ryan dos Santos Costa:** Conceptualization, Writing - Original Draft, Writing - Review & Editing, Formal analysis, Investigation, Supervision.

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

Declaration of Competing Interest

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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Appendix A. Supplementary material

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References

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 Pulmon. Med. 6 https://doi.org/10.1186/1471-2466-6-15.

Becker, M., Levings, M.K., Daniel, C., 2017. Adipose-tissue regulatory T cells: critical players in adipose-immune crosstalk. Eur. J. Immunol. 47 (11), 1867–1874. https://doi.org/10.1002/eji.201646739.

Beltrán, C.J., Núñez, L.E., Díaz-Jiménez, D., Farfan, N., Candia, E., Heine, C., López, F., González, M.J., Quera, R., Hermoso, M.A., 2010. The ST2/IL-33 axis in immune cells during inflammatory diseases. Inflamm. Bowel Dis. 16, 1097–1107. https://academic.oup.com/ibdjournal/article/16/7/1097/4628398.

Bergis, D., Kassis, V., Radeke, H.H., 2016. High plasma sST2 levels in gastric cancer and their association with metastatic disease. Cancer Biomark. 16 (1), 117–125. https://doi.org/10.3233/CBM-150547.

Bikoff, E.K., Morgan, M.A., Robertson, E.J., 2009. An expanding job description for Blimp-1/PRDM1. Curr. Opin. Genet. Dev. 19 (4), 379–385. https://doi.org/10.1016/j.gde.2009.05.005.

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 (9), 1790–1797. https://doi.org/10.1101/gr.137323.112.

Browning, S.R., Browning, B.L., 2007. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am. J. Hum. Genet. 81 (5), 1084–1097. https://doi.org/10.1086/521987.

Caradonna, F., Cruciata, I., Schifano, I., La Rosa, C., Naselli, F., Chiarelli, R., Perrone, A., Gentile, C., 2018. Methylation of cytokines gene promoters in IL-1β-treated human intestinal epithelial cells. Inflamm. Res. 67 (4), 327–337. https://doi.org/10.1007/s00011-017-1124-5.

Carobbio, S., Guénantin, A.-C., Samuelson, I., Bahri, M., Vidal-Puig, A., 2019. Brown and beige fat: from molecules to physiology and pathophysiology. Mol. Cell Biol. 1864 (1), 37–50.

Coelho, R. Santos, Melo, A. Paula Castro, Silva, H. dos Santos, Silva, R. De Cassia Ribeiro, Matos, S. Maria Alvim, Barreto, M. Lima, Alcântara-Neves, N. Maria, Figueiredo, C. Alexandrina Viana de, Costa, R. do Santos, 2021. ADIPOQ and LEP variants on asthma and atopy: genetic association modified by overweight. Gene 781, 145540.

D'Innocenzo, S., Marchioni, D.M.L., Prado, M.S., Matos, S.A., Pereira, S.R.S., Barros, A.P., Sampaio, L.R., Assis, A.M.O., Rodrigues, L.C., Barreto, M.L., 2011. Condições socioeconômicas e padrões alimentares de crianças de 4 a 11 anos: Estudo SCAALA-salvador/bahia. Revista Brasileira de Saude Materno Infantil 11 (1), 41–49. https://doi.org/10.1590/S1519-38292011000100005.

Divella, R., De Luca, R., Abbate, I., Naglieri, E., Daniele, A., 2016. Obesity and cancer: the role of adipose tissue and adipo-cytokines-induced chronic inflammation. J. Cancer 7 (15), 2346–2359. https://doi.org/10.7150/jca.16884.

Eguchi, J., Wang, X., Yu, S., Kershaw, E.E., Chiu, P.C., Dushay, J., Estall, J.L., Klein, U., Maratos-Flier, E., Rosen, E.D., 2011. Transcriptional Control of Adipose Lipid Handling by IRF4. Cell Metab. 13 (3), 249–259. https://doi.org/10.1016/J.CMET.2011.02.005.

El-Wakkad, A., Hassan, N.E.M., Sibaii, H., El-Zayat, S.R., 2013. Proinflammatory, anti-inflammatory cytokines and adiponkines in students with central obesity. Cytokine 61 (2), 682–687. https://doi.org/10.1016/J.CYTO.2012.11.010.

Figueiredo, C.A., Barreto, M.L., Rodrigues, L.C., Cooper, P.J., Silva, N.B., Amorim, L.D., Alcantara-Neves, N.M., 2010. Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect. Immun. 78 (7), 3160–3167. https://doi.org/10.1128/IAI.01228-09.

Han, J.M., Wu, D., Denroche, H.C., Yao, Y., Verchere, C.B., Levings, M.K., 2015. IL-33 reverses an obesity-induced deficit in visceral adipose tissue ST2 + T regulatory cells and ameliorates adipose tissue inflammation and insulin resistance. J. Immunol. 194 (10), 4777–4783. https://doi.org/10.4049/jimmunol.1500020.

Hedayati, M., Sharifi, K., Rostami, F., Daneshpour, M.S., Zarif Yeganeh, M., Azizi, F., 2012. Association between TNF-α promoter G-308A and G-238A polymorphisms and obesity. Mol. Biol. Rep. 39 (2), 825–829. https://doi.org/10.1007/s11033-011-0804-4.

Heianza, Y., Qi, L., 2017. Gene-diet interaction and precision nutrition in obesity. Int. J. Mol. Sci. 18 (4) https://doi.org/10.3390/ijms18040787.

Hotamisligil, G.S., 2006. Inflammation and metabolic disorders. Nature 444 (7121), 860–867.

Hruby, A., Frank, B.H., 2015. The epidemiology of obesity: a big picture. Pharmacoeconomics 33 (7), 673–689. https://doi.org/10.1007/s40273-014-0243-x.The.

Hu, M., Yu, Z., Luo, D., Zhang, H., Li, J., Liang, F., Chen, R., 2018. Association between -174G>C polymorphism in the IL-6 promoter region and the risk of obesity A meta-analysis. Medicine (United States) 97 (33). https://doi.org/10.1097/MD.0000000000011773.

Ibrahim, O.M., Gabre, A.A., Sallam, S.F., El-Alameey, I.R., Sabry, R.N., Galal, E.M., Tawfik, S.M., Zarouk, W.A., Mosaad, R.M., Ramadan, A., 2017. Influence of Interleukin-6 (174G/C) gene polymorphism on obesity in Egyptian children. Open Access Macedonian J. Med. Sci. 5 (7), 831–835. https://doi.org/10.3889/oamjms.2017.175.

Lage-Castellanos, A., Martínez-Montes, E., Hernández-Cabrera, J.A., Galán, L., 2010. False discovery rate and permutation test: an evaluation in ERP data analysis. Stat. Med. 29 (1), 63–74. https://doi.org/10.1002/sim.3784.

Lima-Costa F, M, Rodrigues C, L, Barreto L, M, Horta L, B, et al., Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative)., 2015. Scientific Reports, 5 (1), 1–7. https://doi.org/10.1038/srep09812.

Lin, Y.H., Zhang, R.C., Hou, L.B., Wang, K.J., Ye, Z.N., Huang, T., Zhang, J., Chen, X., Kang, J.S., 2016. Distribution and clinical association of plasma soluble ST2 during the development of type 2 diabetes. Diabetes Res. Clin. Pract. 118, 140–145. https://doi.org/10.1016/j.diabres.2016.06.006.

Lonsdale, J., Thomas, J., Salvatore, M., Phillips, R., Lo, E., Shad, S., Foster, B., 2013. The GTEx Consortium. the genotype-tissue expression (GTEx) project. Nat. Genet. 45 (6), 580–585. https://doi.org/10.1038/ng.2653.The.

Maculewicz, E., Antkowiak, B., Antkowiak, O., Mastalerz, A., Białek, A., Cywińska, A., Borecka, A., Humińska-Lisowska, K., Garbacz, A., Lorenz, K., Szarska, E., Michałowska-Sawczyn, M., Dziuda, Ł., Cięszczyk, P., 2021. IL-6 polymorphisms are not related to obesity parameters in physically active young men. Gene 12 (10), 1498.

Maintinguer Norde, M., Oki, E., Ferreira Carioca, A.A., Teixeira Damasceno, N.R., Fisberg, R.M., Lobo Marchioni, D.M., Rogero, M.M., 2018. Influence of IL1B, IL6 and IL10 gene variants and plasma fatty acid interaction on metabolic syndrome risk in a cross-sectional population-based study. Clin. Nutr. 37 (2), 659–666. https://doi.org/10.1016/j.clnu.2017.02.009.

Marc, S., Morrow, D.A., Higgins, L.J., MacGillivray, C., Guo, W., Bode, C., Rifai, N., Cannon, C.P., Gerszten, R.E., Lee, R.T., 2008. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-Type natriuretic peptide in patients with ST-elevation myocardial infarction. Circulation 117, 1936–1944. https://doi.org/10.1038/jid.2014.371.

Martins, L.M., Oliveira, A.R.S., Cruz, K.J.C., Torres-Leal, F.L., Marreiro, D.D.N., 2014. Obesity, inflammation, and insulin resistance. Braz. J. Pharm. Sci. 50 (4), 677–692.

Martins, G.A., Cimmino, L., Shapiro-Shelef, M., Szabolcs, M., Herron, A., Magnusdottir, E., Calame, K., 2006. Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat. Immunol. 7 (5), 457–465. https://doi.org/10.1038/ni1320.

Matos, S.M.A., Jesus, S.R., Saldiva, S.R.D.M., Prado, M.S., D'Innocenzo, S., Assis, A.M.O., Rodrigues, L.C., Alcantara-Neves, N.M., Cruz, Á.A., Simões, S.D.M., Barreto, M.L., 2011. 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 (7), 1270–1278. https://doi.org/10.1017/S1368980011000462.

Mattos, R.T., Medeiros, N.I., Menezes, C.A., Fares, R.C.G., Franco, E.P., Dutra, W.O., Rios-Santos, F., Correa-Oliveira, R., Gomes, J.A.S., 2016. Chronic low-grade inflammation in childhood obesity is associated with decreased il-10 expression by monocyte subsets. PLoS ONE 11 (12), 1–14. https://doi.org/10.1371/journal.pone.0168610.

Näkki, A., Kouhia, S.T., Saarela, J., Harilainen, A., Tallroth, K., Videman, T., Battié, M.C., Kaprio, J., Peltonen, L., Kujala, U.M., 2010. Allelic variants of IL1R1 gene associate with severe hand osteoarthritis. BMC Med. Genet. 11 (1), 1–10. https://doi.org/10.1186/1471-2350-11-50.

Naselli, F., Catanzaro, I., Bellavia, D., Perez, A., Sposito, L., Caradonna, F., 2014. Role and importance of polymorphisms with respect to DNA methylation for the expression of CYP2E1 enzyme. Gene 536 (1), 29–39. https://doi.org/10.1016/j.gene.2013.11.097.

Netea, M.G., Dinarello, C.A., 2011. More than inflammation: interleukin-1β polymorphisms and the lipid metabolism. J. Clin. Endocrinol. Metabol. 96 (May), 1279–1281. https://doi.org/10.1210/jc.2011-0685.

Notario-Barandiaran, L., Valera-Gran, D., Gonzalez-Palacios, S., Garcia-de-la-Hera, M., Fernández-Barrés, S., Pereda-Pereda, E., Fernández-Somoano, A., Guxens, M., Iñiguez, C., Romaguera, D., Vrijheid, M., Tardón, A., Santa-Marina, L., Vioque, J., Navarrete-Muñoz, E.M., 2020. High adherence to a mediterranean diet at age 4 reduces overweight, obesity and abdominal obesity incidence in children at the age of 8. Int. J. Obes. 44 (9), 1906–1917. https://doi.org/10.1038/s41366-020-0557-z.

Onis, M., Onyango, W.A., Borghi, E., Siyam, A., Chizuru, N., Siekmanna, J., 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85 (660), 667.

Orekhov, A.N., Orekhova, V.A., Nikiforov, N.G., Myasoedova, V.A., Grechko, A.V., Romanenko, E.B., Zhang, D., Chistiakov, D.A., 2019. Monocyte differentiation and macrophage polarization. Vessel Plus 201, 9. https://doi.org/10.20517/2574-1209.2019.04.

Paun, A., Pitha, P.M., 2007. The IRF family, revisited. Biochimie 89 (6-7), 744–753.

Pejsova, H., Hubacek, J.A., Zemankova, P., Zlatohlavek, L., 2019. Baseline leptin/adiponectin ratio is a significant predictor of BMI changes in children/adolescents after intensive lifestyle Intervention. Exp. Clin. Endocrinol. Diabetes 127 (10), 691–696. https://doi.org/10.1055/a-0859-7041.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575. https://doi.org/10.1086/519795.

Ragusa, R., Cabiati, M., Guzzardi, M.A., D'Amico, A., Giannessi, D., Del Ry, S., Caselli, C., 2017. Effects of obesity on IL-33/ST2 system in heart, adipose tissue and liver: study in the experimental model of Zucker rats. Exp. Mol. Pathol. 102 (2), 354–359. https://doi.org/10.1016/j.yexmp.2017.03.002.

Rankinen, T., Zuberi, A., Chagnon, Y.C., Weisnagel, S.J., Argyropoulos, G., Walts, B., Pérusse, L., Bouchard, C., 2006. The human obesity gene map: The 2005 update. Obesity 14 (4), 529–644. https://doi.org/10.1038/oby.2006.71.

Rehman, S.U., Mueller, T., Januzzi, J.L., 2008. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. J. Am. Coll. Cardiol. 52 (18), 1458–1465. https://doi.org/10.1016/j.jacc.2008.07.042.

Seidel, G.E., 2010. Brief introduction to whole-genome selection in cattle using single nucleotide polymorphisms. Reprod. Fertil. Dev. 22 (1), 138–144. https://doi.org/10.1071/RD09220.

Shahbazi, R., Sharifzad, F., Bagheri, R., Alsadi, N., Yasavoli-Sharahi, H., Matar, C., 2021. Anti-inflammatory and immunomodulatory properties of fermented plant foods. Nutrients 13 (5), 1516.

Sharma, V., Coleman, S., Nixon, J., Sharples, L., Hamilton-Shield, J., Rutter, H., Bryant, M., 2019. A systematic review and meta-analysis estimating the population prevalence of comorbidities in children and adolescents aged 5 to 18 years. Obes. Rev. 20 (10), 1341–1349. https://doi.org/10.1111/obr.12904.

Siegrist, M., Rank, M., Wolfarth, B., Langhof, H., Haller, B., Koenig, W., Halle, M., 2013. Leptin, adiponectin, and short-term and long-term weight loss after a lifestyle intervention in obese children. Nutrition 29 (6), 851–857. https://doi.org/10.1016/J.NUT.2012.12.011.

Silva, R.D.C.R., Assis, A.M.O., Cruz, A.A., Fiaccone, R.L., Dinnocenzo, S., Barreto, M.L., Da Silva, L.A., Rodrigues, L.C., Alcantara-Neves, N.M., 2013. Dietary patterns and wheezing in the midst of nutritional transition: a study in Brazil. Pediatry., Allergy, Immunol., Pulmonol. 26 (1), 18–24. https://doi.org/10.1089/ped.2012.0182.

Solé, X., Guinó, E., Valls, J., Iniesta, R., Moreno, V., 2006. SNPStats: a web tool for the analysis of association studies. Bioinformatics 22 (15), 1928–1929. https://doi.org/10.1093/bioinformatics/btl268.

Todendi, P.F., Klinger, E.I., Ferreira, M.B., Reuter, C.P., Burgos, M.S., Possuelo, L.G., Valim, A.R.M., 2015. Association of IL-6 and CRP gene polymorphisms with obesity and metabolic disorders in children and adolescents. Anais Da Academia Brasileira de Ciencias 87 (2), 915–924. https://doi.org/10.1590/0001-3765201520140364.

Trim, W., Turner, J.E., Thompson, D., 2018. Parallels in immunometabolic adipose tissue dysfunction with ageing and obesity. Front. Immunol. 9 (FEB) https://doi.org/10.3389/fimmu.2018.00169.

Vasanthakumar, A., Moro, K., Xin, A., Liao, Y., Gloury, R., Kawamoto, S., Fagarasan, S., Mielke, L.A., Afshar-Sterle, S., Masters, S.L., Nakae, S., Saito, H., Wentworth, J.M., Li, P., Liao, W., Leonard, W.J., Smyth, G.K., Shi, W., Nutt, S.L., Koyasu, S., Kallies, A., 2015. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. Nat. Immunol. 16 (3), 276–285.

Vimaleswaran, K.S., Tachmazidou, I., Zhao, J.H., Hirschhorn, J.N., Dudbridge, F., Loos, R.J.F., 2012. Candidate genes for obesity-susceptibility show enriched association within a large genome-Wide association study for BMI. Hum. Mol. Genet. 21 (20), 4537–4542. https://doi.org/10.1093/hmg/dds283.

Walston, J., Seibert, M., Yen, C.-J., Cheskin, L.J., Andersen, R.E., 1999. Tumor necrosis factor-a-238 and -308 polymorphisms do not associate with traits related to obesity and insulin resistance. Brief Genet. Rep. Ort. 48, 2096–2098.

Wang, Y.C., McPherson, K., Marsh, T., Gortmaker, S.L., Brown, M., 2011. Health and economic burden of the projected obesity trends in the USA and the UK. The Lancet 378 (9793), 815–825. https://doi.org/10.1016/S0140-6736(11)60814-3.

Ward, L.D., Kellis, M., 2012. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucl. Acids Res. 40 (D1), 930–934. https://doi.org/10.1093/nar/gkr917.

Watanabe, M., Nakamoto, K., Inui, T., Sada, M., Honda, K., Tamura, M., Ogawa, Y., Yokoyama, T., Saraya, T., Kurai, D., Ishii, H., Takizawa, H., 2018. Serum sST2 levels predict severe exacerbation of asthma. Respir. Res. 19 (1), 1–10. https://doi.org/10.1186/s12931-018-0872-2.

Winer, S., Winer, D.A., 2012. The adaptive immune system as a fundamental regulator of adipose tissue inflammation and insulin resistance. Immunol. Cell Biol. 90 (8), 755–762. https://doi.org/10.1038/icb.2011.110.

Ye, J., 2013. Mechanisms of insulin resistance in obesity. Front. Med. 7 (1), 14–24. https://doi.org/10.1007/s11684-013-0262-6.

Yu, G.I., Song, D.K., Shin, D.H., 2020. Associations of IL1RAP and IL1RL1 gene polymorphisms with obesity and inflammation mediators. Inflamm. Res. 69 (2), 191–202. https://doi.org/10.1007/s00011-019-01307-y.

Zatterale, F., Longo, M., Naderi, J., Raciti, G.A., Desiderio, A., Miele, C., Beguinot, F., 2020. Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. Front. Physiol. 10 (January), 1–20. https://doi.org/10.3389/fphys.2019.01607.

Zeyda, M., Wernly, B., Demyanets, S., Kaun, C., Hämmerle, M., Hantusch, B., Schranz, M., Neuhofer, A., Itariu, B.K., Keck, M., Prager, G., Wojta, J., Stulnig, T.M., 2013. Severe obesity increases adipose tissue expression of interleukin-33 and its receptor ST2, both predominantly detectable in endothelial cells of human adipose tissue. Int. J. Obesity 37 (5), 658–665. https://doi.org/10.1038/ijo.2012.118.