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ORIGINAL ARTICLE

SH2B1 variants as potential causes of non-syndromic monogenic obesity in a Brazilian cohort

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Abstract

Purpose *SH2B1* gene encodes an important adaptor protein to receptor tyrosine kinases or cytokine receptors associated with *Janus* kinases. This gene has been associated with the structural and functional modulation of neurons and other cells, and impacts on energy and glucose homeostasis. Several studies suggested that alterations in this gene are strong candidates for the development of obesity. However, only a few studies have screened *SH2B1* point variants in individuals with obesity. Therefore, the aim of this study was to investigate the prevalence of *SH2B1* variants in a Brazilian cohort of patients with severe obesity and candidates to bariatric surgery.

Methods The cohort comprised 122 individuals with severe obesity, who developed this phenotype during childhood. As controls, 100 normal-weight individuals were included. The coding region of *SH2B1* gene was screened by Sanger sequencing.

Results A total of eight variants were identified in *SH2B1,* of which p.(Val345Met) and p.(Arg630Gln) variants were rare and predicted as potentially pathogenic by the in the silico algorithms used in this study. The p.(Val345Met) was not found in either the control group or in publicly available databases. This variant was identified in a female patient with severe obesity, metabolic syndrome and hyperglycemia. The p.(Arg630Gln) was also absent in our control group, but it was reported in gnomAD with an extremely low frequency. This variant was observed in a female patient with morbid obesity, metabolic syndrome, hypertension and severe binge-eating disorder.

Conclusion Our study reported for the first time two rare and potentially pathogenic variants in Brazilian patients with severe obesity. Further functional studies will be necessary to confirm and elucidate the impact of these variants on SH2B1 protein function and stability, and their impact on energetic metabolism.

Level of evidence Level V, cross-sectional descriptive study.

Keywords *SH2B1* · Non-syndromic monogenic obesity · Sanger sequencing · Bariatric surgery · Mutations · Leptin-melanocortin pathway

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Introduction

SH2B1 gene (Src-homology 2B adaptor protein 1) is located on chromosome 16p11.2. It encodes four isoforms (α, β, δ and γ), differing only in their COOH termini. This gene code a scaffold protein implicated in signaling downstream of a diversity of receptor tyrosine kinases and cytokine receptors/Janus kinase (Jaks) complexes. These comprise receptors for leptin, insulin, insulin-like growth factor 1 (IGF1), nerve growth factor (NFG), growth hormone (GH), platelet-derived growth factor (PGDF), glial cell-derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF) [1–7]. Some of these receptors have a crucial role in food intake and energy expenditure, indicating that SH2B1 is likely to be involved in the etiology of obesity [8–10].

Alterations in *SH2B1* have been suggested as strong candidates for the development of obesity based on animal models, genome-wide association studies (GWAS) and genomic structural variation observations. In vivo studies indicated an influence of SH2B1 in body weight regulation, as *Sh2b1* null mice exhibit obesity, severe hyperphagia, insulin resistance, glucose intolerance, hyperleptinemia, and often, aggressive behavior [3, 4]. Additionally, neuron-specific restoration of SH2B1 expression rescued the metabolic disorders and obesity [5].

GWAS showed that variants near *SH2B1* gene were associated with body mass index (BMI) [11–13]. Speliotes and colleagues [12] have performed a large-scale GWAS using 249,796 participants of European descent and identified genetic variants close to or in 32 loci associated to BMI, including the *SH2B1* gene. Furthermore, a common variant [rs7498665; p.(Thr484Ala)] was repeatedly associated with obesity or increased BMI in different European cohorts [14–16]. However, the association was not observed in a study performed in a Japanese cohort [17].

Copy number variation was shown to contribute to the genetic architecture of severe obesity development. Bochukova and coworkers [18] have reported a deletion of approximately 200 kilobases (Kb) in the 16p11.2 region that harbor the *SH2B1* gene segregating with severe early-onset obesity in Caucasian patients. Interestingly, two of three patients carrying the corresponding reciprocal duplication were reported as underweight. These results suggest that this region may have a key role on energy balance and could influence BMI by altering on gene dosage [19]. Moreover, a longer de novo ~ 1.7-megabase (Mb) deletion at the chromosome 16p11.2 was identified in two patients with severe obesity and mild development delay. This large deletion included the ~ 200-kb deletion and extended through a 593-kb region previously associated with obesity, autism and intellectual disability [18, 20–22].

Only a few studies investigated rare and potentially deleterious variants in *SH2B1*. Doche and coworkers [23] have reported five carriers of potentially loss-of-function mutations in the heterozygous state [p.(Pro90His), p.(Thr175Asn), p.(Pro322Ser), and p.Phe344Leufs*20] in a cohort of 300 participants with mixed European descent. Functional analyses showed that all variants impact GH/NGF-mediated signaling, but only the frameshift variant affected the leptin signaling. The patients exhibited severe early-onset obesity, hyperphagia, insulin resistance, developmental delay and behavioral abnormalities, such as tendency for social isolation and aggression. Later, the same group also described four novel variants [p.(Thr546Ala), p.(αArg663Val) p.(αVal695Met) and p.(αAla723Val)] present in patients with severe early-onset obesity and insulin resistance. Only the patient carrying p.(Thr546Ala) presented mild developmental delay [24].

Volckmar and colleagues [25] have screened 95 children and adolescents with extreme obesity and identified two rare novel genetic alterations (g.9483C/T [p.βThr656Ile/p.γPro674Ser] and g.10182C/A) and five common variants. Functional analyses suggested that the p.βThr656Ile/p.γPro674Ser did not impact leptin signaling. Recently, Foucan and colleagues [26] have screened 59 genes involved in monogenic forms of obesity or diabetes in a cohort of 25 schoolchildren with obesity from Guadeloupe Island. They identified five carriers of rare heterozygous variants in four genes involved in monogenic obesity, of which one was a likely pathogenic variant [p.(Pro90His)] in *SH2B1*.

Furthermore, Zhen and coworkers [27] sequenced the exonic and flanking region of *SH2B1* in 338 children with obesity and 221 normal-weight Chinese Han children. Their results showed two rare and non-potentially pathogenic variants were unique in children with obesity [p.(Val209Ile) and p.(Met465Thr)]. By whole exome sequencing, Stahel and coworkers [28] have analyzed 45 adults with extreme obesity (BMI > 50 kg/m^2) from Canada, which resulted in the identification of one non-potentially deleterious *SH2B1* variant (p.(αArg663Val)). No pathogenic variants were observed in children and/or adolescents with severe obesity from Turkey [29, 30]. Furthermore, also no pathogenic coding or splice site variants were observed in 500 children with severe obesity of the Genetics of Obesity Study (GOOS) [18].

Flores and coworkers [2] have identified 15 variants in children with severe obesity, in which four alterations lie in Pleckstrin homology (PH) domain [p.(Arg270Trp), p.(Arg270Gln), p.(Glu299Gly) and p.(Pro322Ser)]. In vivo experiments suggested that this region controls energy balance and glucose homeostasis. Lastly, Aerts and colleagues [31] performed a *SH2B1* gene screening in a cohort of Belgian population. They found six rare missense variants in children with obesity [p.(Pro19Leu), p.(Thr175Asn),

p.(Ser188Leu), p.(αAla667Val) and p.(αAla672Thr)]; however, no functional tests were performed.

To date, *SH2B1* point mutations associated with non-syndromic monogenic obesity remains limited to a few individuals in the world. Due to its rarity and its importance to energetic metabolism, we aimed to investigate the prevalence of *SH2B1* variants in a cohort of patients with severe obesity from Rio de Janeiro, Brazil, and to describe the clinical and inflammatory phenotypes of individuals carrying potential pathogenic *SH2B1* variants.

Materials and methods

Study population

This study was designed and performed as a cross-sectional study including 122 patients with severe obesity (97 females [79.5%] and 25 males [20.5%]), aged 18–65 years (median of 37.0 [28; 45]) in Rio de Janeiro, Southeast region of Brazil. These unrelated participants were recruited from a non-governmental organization, called Rescue Group to Self-Esteem and Citizenship of the Obese (in Portuguese, "Grupo de Resgate à Autoestima e Cidadania do Obeso"). Participants presented severe obesity (BMI $\geq$ 35 kg/m^2) and developed obesity during childhood (0–11 years). The period of onset of obesity was self-reported. All patients were candidate to undergo bariatric surgery. Exclusion criteria were pregnancy, lactation, consumption of any medicine to lose or gain weight; as well as the presence of symptoms suggestive of syndromic obesity (cognitive delay, dysmorphic characteristics, and organ-specific developmental abnormalities). To analyze the possible recurrence of observed variants in a control group from our population, a total of 100 unrelated patients with normal weight (18.5 $\leq$ BMI $\leq$ 24.9 kg/m^2) were selected (median of age 28.0 [24.0; 36.0]). The exclusion criteria were pregnancy, lactation and the use of medication to lose or gain weight. All these participants were volunteers recruited from public hospitals in Rio de Janeiro, Brazil. This study involving human participants was approved by Ethics Committee of the Oswaldo Cruz Foundation (CAAE: 09225113.0.0000/ Protocol Nº: 346.634) and Grande Rio University (CAAE: 64908114.4.0000.5283/ Protocol Nº: 5283). The patients/participants provided written informed consent to participate in this study.

Anthropometry and body composition

Anthropometric indices as well as blood pressure were determined after a 12 h fasting period, as previously described [32]. Briefly, body weight, height, neck, waist and hip circumferences were measured. Body adipose index (BAI), BMI and waist-hip ratio (WHR) were calculated for each participant. BAI was calculated using the formula: hip circumference/(height$^{1.5}$) – 18. BMI was calculated as weight (kg) divided by the square of height (m).

Biochemical and inflammatory markers analyses

Blood samples were collected after a 12 h overnight fasting period. Fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) were measured by oxidase–peroxidase method (BioSystems). Low-density lipoprotein cholesterol (LDL-c) was calculated using Friedewald formula. The measurement of hemoglobin glycated (HbA1c) and C-reactive protein (CRP) was carried out using latex agglutination method and turbidimetric inhibition immunoassay (TINIA), respectively.

Serum concentration of leptin, resistin, monocyte chemoattractant protein-1/CCL2 (MCP1), and plasminogen activator inhibitor-1 (PAI-1) were measured by Human Adipocyte Magnetic Bead (Millipore-Merck [cat# HADCYMAG-61 k]) on Bio-Plex 200 Multiplexing Analyzer System, according to the manufacturer's protocol.

SH2B1 mutation analyses

Mutational analysis was performed on genomic DNA isolated from peripheral blood extracted using QIAamp Blood Kit (Qiagen, Valencia, CA, USA), according to manufacturers. A total of eight PCR amplicons were designed with Primer3 tool to screen the coding region of *SH2B1* gene (ENST00000618521.4). The sequence of primers and PCR conditions are available upon request. After amplifications, the PCR products were visualized on 1% agarose gels and purified with ExoSAP kit (Thermo Fisher Scientific, Waltham, MA, United States).

Sequencing was carried out with Big Dye Terminator kit v.3.1 (Applied Biosystems, Austin, TX, USA) on a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing conditions were previously described [33]. The electropherograms were analyzed and aligned with their reference sequence ENST00000618521.4 provided by Ensembl database using the software BioEdit. Identified variants were confirmed by resequencing of a second PCR. All patients were previously screened for other candidate genes for non-syndromic monogenic obesity, such as *melanocortin 4 receptor* (*MC4R*), *leptin* (*LEP*), *proopiomelanocortin* (*POMC*), *melanocortin 2 receptor accessory proteins 2* (*MRAP2*) and *BDNF* [34–36]. After these analyses, we performed the screening of the *SH2B1* gene in our cohort.

Bioinformatic tools

SH2B1 genomic, transcript and protein sequences were obtained from Ensembl (http://www.ensembl.org/) and Uniprot database (https://www.uniprot.org/). All Identified variants were cross-checked against the follow publicly database: PubMed, Google scholar, Clinvar, dbSNP (https://www.ncbi.nlm.nih.gov/), Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org/), and Online Archive of Brazilian Mutations (ABraOM) (http://abraom.ib.usp.br/). Variants not identified in databases or published articles were classified as novel. All variants identified received the nomenclature recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). Furthermore, all reported/found variants received the nomenclature according to *SH2B1* isoforms. Three *SH2B1* isoforms sequences were available on public database: α (ENST00000684370.1/ Q9NRF2), β (ENST00000395532.8/ Q9NRF2-2) and γ (ENST00000359285.9/ Q9NRF2-3).

Pathogenic effects of the identified missense variant were predicted by in-silico analysis using seven different programs: Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (https://sift.bii.a-star.edu.sg/), Mutation Taster (http://www.mutationtaster.org/), Mutation assessor (http://mutationassessor.org/r3/), Mupro Tool (http://mupro.proteomics.ics.uci.edu/), I-mutant 2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.html), Revel (https://sites.google.com/site/revelgenomics/). Moreover, amino acid conservation was analyzed using aligning sequences from 8 species using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Finally, our identified variants were classified according to the published criteria of pathogenicity of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [37] available on VarSome (https://varsome.com/).

Results

Basic clinical characteristics

This study included 122 unrelated Brazilian patients with severe obesity, who developed this phenotype during childhood. The basic phenotypical characteristics in the case group are presented in Table 1. Generally, as expected, the individuals with severe obesity presented higher anthropometric measurements and biochemical levels when compared to the control group; exceptions were height and HDL-C. Regarding the obesity and inflammatory measurements, only leptin was different between the groups, which was higher in case group.

SH2B1 molecular screening

The entire coding region of *SH2B1* gene was screened in our patients. In total, eight variants were found in our sample: six rare missense variations [p.(Ala99Val), p.(Val345Met), p.(Ser410Phe), p.(Glu514Gln), p.(Arg630Gln) and p.(αAla663Val)], one common polymorphism [p.(Thr484Ala)] and one synonymous variant (p.Val729=). Overall, the frequency of *SH2B1* variants (rare and common) was 29.5% in our cohort. Only p.(Thr484Ala) was found in a homozygous state. No individual presented more than one variant. Figure 1 shows the location of the found variants in the SH2B1 protein. Except for p.(Val345Met), all variants occur in unknown functional domains of the protein.

An overview of our results together with the reported allele frequencies in the gnomAD and in the ABraOM database are shown in Table 2. Our in-silico analyses were performed using seven algorithms to predict the possible impact of *SH2B1* missense variants on protein structure and function. Among variants, four were predicted as potential deleterious by the used algorithms [p.(Val345Met), p.(Ser410Phe), p.(Glu514Gln) and p.(Arg630Gln)].

The p.(Val345Met) mutation was predicted to be pathogenic by all algorithms. The valine at the codon 345 of SH2B1 exists in a highly conserved region among multiple species (Fig. 2a). Moreover, this variant was not reported in publicly available exomes/genomes and in our 100 normal-weight individuals. All these findings suggest that this may be a potential disease-causing mutation. The potential impact was also interpreted according to current standards and guidelines, which suggested that alteration is a variant with uncertain significance (VUS).

The p.(Ser410Phe) was predicted to be nonpathogenic by five out of seven softwares used in this study (Mutation Taster and Mupro tool). This variant was also observed in gnomAD and AbraOM database. According to ACMG/AMP classification, p.(Ser410Phe) is a benign variant. In addition, p.(Glu514Gln) variant was also predicted as pathogenic by four out of seven software used in this study (Polyphen-2, SIFT, Mutation Taster and Mupro Tool). However, this variant was observed in the Brazilian public database (ABraOM) with a low frequency and also in one normal-weight control. These results indicate that this p.(Glu514Gln) may not be disease causing.

Finally, the p.(Arg630Gln) alteration was classified as deleterious by four algorithms (Polyphen-2, Mutation taster, Mupro Tool and I-mutant 2.0). It also affects an amino acid located in an evolutionary conserved position across several species from human (*Homo sapiens)* to frog (*Xenopus tropicalis*) (Fig. 2b). This variant was found in the gnomAD with a low frequency (MAF < 0.0001). All reported patients were heterozygous for this variant in this database. It is noteworthy, however, that the p.(Arg630Gln) variant was not

Table 1 Participants' demographic, anthropometric, clinical and laboratory data

Parameters	Patients		Controls		*p*
	n	Values	*n*	Values	
Age (years)	122	37.0 (28; 45)	100	28.0 (24.0; 36.0)	< 0.001
Gender					
Female	122	97 (79.5)	100	71 (71.0)	0.142
Male		25 (20.5)		29 (29.0)	
Weight (kg)	122	128.9 (114.0; 146.9)	100	62.5 (56.8; 62.7)	< 0.001
Height (m)	122	1.64 (1.58; 1.70)	100	1.66 (1.62; 1.75)	0.008
BMI (kg/m^2)	122	47.0 (42.8; 52.6)	100	22.8 (21.1; 23.8)	< 0.001
BAI (%)	122	49.2 (44.4; 55.7)	95	27.7 (25.7; 30.6)	< 0.001
Waist circumference (cm)	122	137.0 (126.0; 144.0)	100	82.5 (77.0; 87.0)	< 0.001
Hip circumference (cm)	122	141.8 (133.0; 151.2)	100	100.0 (96.6; 104.0)	< 0.001
WHR	122	0.97 (0.91; 1.00)	98	0.83 (0.78; 0.87)	< 0.001
SBP (mm Hg)	61	131.0 (121.0; 148.5)	84	118.5 (110.0; 126.0)	< 0.001
SBD (mm Hg)	61	88.0 (79.0; 97.5)	84	81.0 (74.2; 89.7)	0.012
Glucose (mg/dL)	85	97.0 (92.0; 105.5)	79	91.0 (86.0; 97.0)	< 0.001
Cholesterol total (mg/dL)	98	192.0 (162.2; 221.7)	87	181.0 (156.0; 207.0)	0.153
HDL-cholesterol (mg/dL)	98	44.5 (40.0; 52.2)	87	59.0 (49.0; 70.0)	< 0.001
LDL-cholesterol (mg/dL)	95	118.0 (97.0; 143.0)	86	102.5 (85.7; 124.5)	< 0.001
Tryglicerides (mg/dL)	98	123.5 (84.7; 166.5)	87	81.0 (65.0; 108.0)	< 0.001
Hemoglobin glycated (%)	66	5.65 (5.10; 6.50)	68	4.90 (4.60; 5.50)	< 0.001
CRP (mg/dL)	66	1.08 (0.56; 1.64)	67	0.17 (0.06; 0.31)	< 0.001
Leptin (pg/mL)	96	2644.7 (2058.5; 3380.0)	34	565.1 (204.6; 853.4)	< 0.001
MCP1 (pg/mL)	96	246.3 (134.2; 366.8)	34	246.8 (137.8; 317.6)	0.593
PAI-1 (pg/mL)	96	25,210.0 (17057.9; 31,640.4)	34	22,475.3 (15,648.3; 29,631.7)	0.199
Resistin (pg/mL)	96	8330.6 (5708.7; 10,843.6)	34	7580.53 (4671.8; 9438.5)	0.175
Metabolic syndrome					
Yes	93	73 (78.4)	81	3 (3.70)	< 0.001
No		20 (23.6)		78 (96.3)	
Hypertension					
Yes	122	83 (68.0)	100	10 (10.0)	< 0.001
No		39 (31.9)		90 (90.0)	

p value for differences between patients with obesity and normal weight. Data are presented as median (interquartile) for continuous variables, and number (percentage) for categorical variables

BAI body adiposity index, *BMI* body mass index, *CRP* C-reactive protein, *DBP* diastolic blood pressure, *HDL-cholesterol* high-density lipoprotein-cholesterol, *LDL-cholesterol* low-density lipoprotein-cholesterol, *MCP1* monocyte chemoattractant protein 1, *NA* not available, *PAI-1* plasminogen activator inhibitor-1, *SBP* systolic blood pressure, *WHR* waist–hip ratio

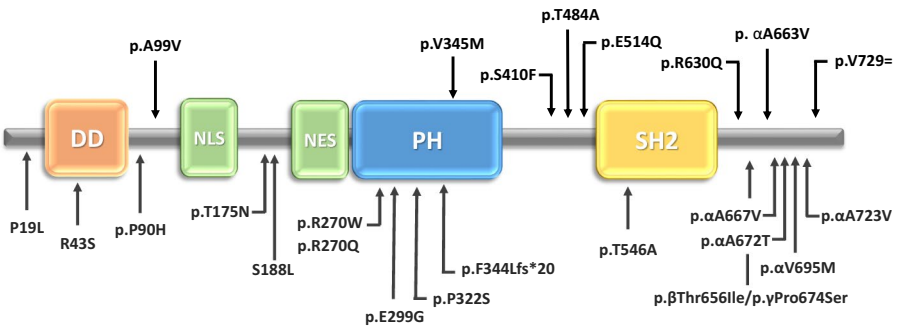

Fig. 1 Schematic representation of SH2B1 protein domains and location of the variants identified in this study (black, top of the figure), and the previous potentially pathogenic variants found in the literature (gray, bottom of the figure). These previous variants were classified as potentially pathogenic according to their own manuscript [2, 23, 24, 26, 31]

Table 2 Coding *SH2B1* variants identified in our cohort

ID	Mutation (cDNA)	Mutation (protein)	MAF gnomAD (%)		MAF ABraOM	MAF our study (%)	ClinVar	Poly-phen-2	SIFT	Mutation Taster	Mutation Assessor	Protein stability		Revel	VarSome (ACMG)
			Exomes	Genomes								Mupro Tool	I-mutant 2.0		
rs144126859	c.296C > T	p.(Ala99Val)	0.0002131	0.0005740	0.000854	0.0041	Not reported	Benign	Tolerated	Polymorphism	Neutral	Decrease	Increase	Likely benign	Likely benign
rs947055242	c.1033G > A	p.(Val345Met)	NA	NA	NA	0.0041	Not reported	Probably damaging	Deleterious	Disease causing	Medium	Decrease	Decrease	Likely disease causing	VUS
rs115698674	c.1229C > T	p.(Ser410Phe)	0.0002068	0.001020	0.002135	0.0041	Not reported	Benign	Tolerated	Disease causing	Neutral	Decrease	Increase	Likely benign	Likely benign
rs7498665	c.1450A > G	p.(Thr484Ala)	0.351047	0.34468	0.329206	0.3360	Not reported	Benign	Tolerated	Polymorphism	Neutral	Increase	Decrease	Likely benign	Benign
rs893059694	c.1540G > C	p.(Glu514Gln)	NA	NA	0.001281	0.0041	Not reported	Possibly damaged	Deleterious	Disease causing	Neutral	Decrease	Increase	Likely benign	Likely benign
rs760535051	c.1889G > A	p.(Arg630Gln)	0.000007959	0.00003187	NA	0.0041	Not reported	Probably damaging	Tolerated	Disease causing	Neutral	Decrease	Decrease	Likely benign	Likely benign
rs190981290	c.1988C > T	p.(αAla663Val)	0.007079	0.005364	0.005551	0.0082	Benign	Benign	Tolerated	Polymorphism	Neutral	Increase	Increase	Likely disease causing	Likely benign
rs28698920	c.2187G > T	p.Val729=	0.003214	0.01037	0.007259	0.0041	Benign	–	–	–	–	–	–	–	Likely benign

ACMG American College Medical Genetics, *MAF* Minor Allele Frequency, *NA* Not available, *VUS* variant of uncertain significance

Fig. 2 Alignment by Mutation Taster of a portion of the *SH2B1* amino acid sequence across species, in which the position of p.(Val345Met) (**A**) and p.(Arg630Gln) (**B**) variants is showed in the red squares. The white squares showed the non-conserved position

identified in our control sample, suggesting that there may be a potential role for this variant in the patient's phenotype. Nevertheless, ACMG/AMP classified this variant as a likely benign.

Clinical phenotype of patients carrying potential pathogenic variants

Here, we have identified two *SH2B1* variants potentially contributing to severe obesity. Table 3 shows the clinical detail of the patients carrying the possible deleterious variants.

The patient carrying the p.(Val345Met) was a 48-year-old female participant. On clinical examination, her body weight was 82.4 kg and she was 1.53 m tall with BMI of 35.2 kg/m^2. She had metabolic syndrome (reduced HDL-cholesterol, increased waist circumference and fasting plasma glucose (FPG) and was hyperglycemic (reference range < 99 mg/dL). Her triglyceride profile was within normal range. In addition, her leptin levels were higher than the other patients with obesity in our cohort (3604.38 pg/mL vs 2644.7 pg/mL, respectively). She was medicated for gastric reflux and urinary incontinence. The patient reported that her sibling is obese.

The patient harboring the p.(Arg630Gln) was a 33-year-old female with morbid obesity. In 2015 the patient was included in our study, her body weight was 116 kg for 1.67 m with BMI of 41.6 kg/m^2. Her comorbidities included metabolic syndrome and hypertension, and she was on pharmacological treatment using an ACE inhibitor. Except for HDL-cholesterol, her lipid profile was within normal range.

Table 3 Anthropometric, clinical and inflammatory profile of patients carrying potential *SH2B1* pathogenic variants

Variables	Carriers	
	p.(Val345Met)	p.(Arg630Gln)
Age (years)	58	33
Gender	Female	Female
Weight (kg)	82.4	116
Height (m)	1.53	1.67
BMI (kg/m^2)	35.2	41.6
BAI (%)	48.1	46.6
Waist circumference (cm)	105	122
Hip circumference (cm)	125	139.5
Neck circumference	38	39
WHR	0.84	0.87
Blood pressure (mm Hg)	122/78	142/97
FPG (mg/dL)	113	88
Cholesterol total (mg/dL)	216	143
HDL-cholesterol (mg/dL)	38	41
LDL-cholesterol (mg/dL)	154	82
Triglycerides (mg/dL)	118	98
HbA1c (%)	5.5	NA
CRP (mg/dL)	1.28	NA
Leptin (pg/mL)	3604.38	2803.4
MCP1 (pg/mL)	168.02	290.88
PAI-1 (pg/mL)	24,940.24	20,197.38
Resistin (pg/mL)	8331.75	8861.87
Hypertension	No	Yes
Metabolic syndrome	Yes	Yes
Type 2 diabetes	No	No

BAI body adiposity index, *BMI* body mass index, *CRP* C-reactive protein, *FPG* Fasting Plasma Glucose, *HDL-cholesterol* high-density lipoprotein-cholesterol, *LDL-cholesterol* low-density lipoprotein-cholesterol, *HbA1c* glycated hemoglobin, *WHR* waist–hip ratio, *NA* data not available

She presented severe binge-eating disorder (BED) and consumed a median of 3709 kcal per day, distributed as 51.89% carbohydrates, 11.94% proteins, and 36.17% total fats. In addition, she exhibited normal intellectual development and was diagnosed with Generalized Anxiety Disorder (GAD) after multiple panic attacks. This patient underwent bariatric surgery (sleeve) in 2019. In pre-surgery, her body weight was 120.3 kg with BMI of 43.74 kg/m^2. After 18 months, her body weight was 83.5 kg (BMI: 30.3 kg/m^2). Recently, she underwent plastic surgery.

The patient has 4 children, which 3 were biological (2 daughters and 1 son) and 1 was adopted (daughter). The patient reported that her father and youngest daughter (13 years; BMI: 32.2 kg/m^2) presented obesity; however, her son exhibits normal weight (19 years; BMI: 24.3 kg/m^2) (Supplemental Fig. 1). We have collected biological material of her normal weight son and obese youngest

daughter to perform the segregation analysis. However, the p.(Arg630Gln) variant was not found in either family member. Therefore, the segregation analysis was inconclusive.

Discussion

SH2B1 is a relatively new candidate gene associated with non-syndromic monogenic obesity. This gene encodes an intracellular adaptor protein involved in a signaling downstream of receptor tyrosine kinases (e.g., receptors for leptin, BDNF and insulin). These signaling pathways have a crucial role in food intake control, energy expenditure and/or glucose homeostasis, suggesting the importance of SH2B1 for the etiology of obesity as well as insulin resistance [1–6].

It is reasonable to speculate that the loss-of-function variants in *SH2B1* may contribute to obesity development. However, up to date, only a few studies observed rare and potentially pathogenic *SH2B1* variants in individuals with obesity [2, 23, 25, 31]. In this context, we carried out an *SH2B1* screening analysis on adults with severe obesity, who developed this phenotype during childhood. A total of eight variants were identified in our study. Our results suggested that the p.(Val345Met) and p.(Arg630Gln) were rare and potentially pathogenic.

The SH2B1 exhibits four isoforms, differing only in their COOH termini. These isoforms share 631 NH_2-terminal amino acids containing a dimerization domain, Pleckstrin homology domain (PH), src-homology 2 (SH2) domain, nuclear localization sequence (NLS), and nuclear export sequence (NES) [2, 6, 38, 39]. Several rare and potentially deleterious variants were observed into the region shared by all isoforms, and were associated with clinical phenotypes such as hyperphagia, severe obesity, insulin resistance and in some cases, developmental delay and aggressive behavior [2, 23, 24, 26]. Regarding our identified variants, p.(Val345Met) and p.(Arg630Gln) are also located in this common region, and mostly likely affect all isoforms. However, only p.(Val345Met) lies in a known domain.

The p.(Val345Met) was identified in a 48-year-old female patient with severe obesity (BMI: 35.2 kg/m^2). The patient also showed metabolic syndrome and hyperglycemia. The valine at codon 345 is located in a highly conserved position among different species from frog (*Xenopus tropicalis*) up to human (*Homo sapiens*), suggesting that this region may be functionally important. Regarding the protein structure, this variant lies on PH domain, which was shown to have a crucial role on energy and glucose homeostasis. According to this study, homozygous mice containing a two-amino acids deletion in the PH exhibited obesity, insulin resistance and glucose intolerance [2].

To the best of our knowledge, the p.(Val345Met) variant was not previously reported in the literature. However, other studies have identified potentially pathogenic variants in the PH domain. Flores and coworkers [2] have analyzed a large sample from the GOOS studies, which comprise individuals with severe early-onset obesity. A total of 15 variants was identified, of which four alterations lay within the PH domain [p.(Arg270Trp), p.(Arg270Gln), p.(Glu299Gly) and p.(Pro322Ser)]. Interestingly, they observed that patients harboring variants in or near this domain exhibited particularly high HOMA-IR scores. It was not possible to evaluate the HOMA-IR of our patient; however, we observed that this proband exhibited increased levels of leptin when compared to other individuals with severe obesity and also with controls in our sample. This phenotype is in line with the hyperleptinemia observed in *Sh2b1* null mice [3, 4].

Doche and colleagues [23] also reported two pathogenic variants [p.(Pro322Ser) and p.Phe344Leufs*20] in PH domain. Both variants were inherited from overweight or obese parents. In addition, the patients with deleterious variants had hyperphagia, obesity and maladaptive behaviors (tendency for social isolation and/or aggression). Our proband with p.(Val345Met) variant also has obesity, but no behavioral abnormalities were reported. Combining our results with the literature, we suggest that p.(Val345Met) might be a potentially disease-causing variant.

The p.(Arg630Gln) was identified in a 33-year-old female patient with morbid obesity (BMI: 41.6 kg/m^2), metabolic syndrome and hypertension. The proband also exhibited severe BED and high energy intake per day. Regarding our in-silico analysis, our results showed that the four algorithms indicate an effect on the SH2B1 protein, and also that the amino acid arginine is located in a conserved position. Interestingly, this arginine is located in the second to last amino acid shared by all SH2B1 isoforms. In addition, the p.(Arg630Gln) was also absent in our control group and found in public databases with low frequency (heterozygous state). Since clinical data are not available in these databases, we could not exclude that these patients might be obese. All these findings indicated that this variant might be potentially pathogenic; however, ACMG/AMP classified this variant as likely benign. Therefore, we suggest that further functional analyses are needed to investigate the possible impact on protein, and consequently, the pathogenicity of this variant.

Both variants, p.(Val345Met) and p.(Arg630Gln), were not reported in the literature. However, previous studies have identified potentially *SH2B1* pathogenic variants in individuals with obesity at a frequency of about 1%, in line with our results [23, 25, 31]. Despite deleterious variants of *SH2B1* being rare, their identification may reveal previously unrecognized individuals with non-syndromic monogenic obesity for further clinical management and allow their family members to receive appropriate genetic counseling.

Limitations of our study include (1) the period of obesity onset was self-reported; (2) structure and functional analyses were not carried out to elucidate the impact of the identified variants on SH2B1 protein; (3) family members of p.(Val345Met) variant were not available to further characterize its segregation.

In summary, we have reported for the first time the presence of potentially *SH2B1* pathogenic variants in Brazilian patients with severe obesity. We also have described the clinical phenotype of these probands. Our study supports the importance of screening the *SH2B1* gene to detect potential patients who might benefit from medical management and genetic counseling. Further functional analyses in vitro and/ or in vivo are necessary to elucidate the impact of these variants in the protein structure and function, and to confirm their pathogenicity.

What is already known on this subject?

Non-syndromic monogenic obesity can be caused by rare and deleterious variants in the *SH2B1* gene, resulting in severe early-onset obesity and hyperphagia. However, only a few studies have screened this gene in patients with obesity.

What this study adds?

We have found two rare variants predicted to be potentially deleterious by in-silico algorithms in Brazilian patients with severe and childhood-onset obesity.

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Author contributions ACPF, PHC and PTB: the conception and design of the study; JFNN, FCCM, ELR and JRIC: acquisition of data; ISSA, KCRS, ACPF, GMA, AC and LP: analysis and interpretation of data; ACPF: wrote the manuscript; VMZ, PTB, MCJ and CMM-M: revising it critically for important intellectual content; All authors read and approved the final version.

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Data availability statement Datasets are available on request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval The studies involving human participants were approved by Ethics Committee of the Oswaldo Cruz Foundation (CAAE: 09225113.0.0000/ Protocol Nº: 346.634) and Grande Rio University (CAAE: 64908114.4.0000.5283/ Protocol Nº: 5283). The patients/participants provided written informed consent to participate in this study.

Informed consent All participants provided informed consent prior to their participation.

Consent to participate All patients gave their written informed consent prior to enrollment in this study.

References

1. Li Z, Zhou Y, Carter-Su C et al (2007) SH2B1 enhances leptin signaling by both Janus kinase 2 Tyr813 phosphorylation-dependent and -independent mechanisms. Mol Endocrinol 21:2270–2281. https://doi.org/10.1210/me.2007-0111
2. Flores A, Argetsinger LS, Stadler LKJ et al (2019) Crucial role of the SH2B1 PH domain for the control of energy balance. Diabetes 68:2049–2062
3. Duan C, Yang H, White MF, Rui L (2004) Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. Mol Cell Biol 24:7435–7443
4. Ren D, Li M, Duan C, Rui L (2005) Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. Cell Metab 2:95–104
5. Ren D, Zhou Y, Morris D et al (2007) Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. J Clin Invest 117:397–406
6. Maures TJ, Kurzer JH, Carter-Su C (2007) SH2B1 (SH2-B) and JAK2: a multifunctional adaptor protein and kinase made for each other. Trends Endocrinol Metab 18:38–45. https://doi.org/10.1016/j.tem.2006.11.007
7. Cote JL, Vander PB, Ellis M et al (2022) The nucleolar δ isoform of adapter protein SH2B1 enhances morphological complexity and function of cultured neurons. J Cell Sci 135:jcs259179
8. Yazdi FT, Clee SM, Meyre D (2015) Obesity genetics in mouse and human: back and forth, and back again. PeerJ 3:e856. https://doi.org/10.7717/peerj.856
9. Pigeyre M, Meyre D (2018) Monogenic obesity. Pediatric obesity. Springer, Cham, pp 135–152
10. da Fonseca ACP, Mastronardi C, Johar A et al (2017) Genetics of non-syndromic childhood obesity and the use of high-throughput DNA sequencing technologies. J Diabetes Complicat 31:1549–1561
11. Willer CJ, Speliotes EK, Loos RJF et al (2009) Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41:25–34. https://doi.org/10.1038/Ng.287
12. Speliotes EK, Willer CJ, Berndt SI et al (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42:937–948. https://doi.org/10.1038/ng.686
13. Thorleifsson G, Walters GB, Gudbjartsson DF et al (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 41:18

14. Beckers S, Zegers D, Van Gaal LF, Van Hul W (2011) Replication of the SH2B1 rs7498665 association with obesity in a Belgian study population. Obes Facts 4:473–477
15. Renström F, Payne F, Nordström A et al (2009) Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. Hum Mol Genet 18:1489–1496
16. Holzapfel C, Grallert H, Huth C et al (2010) Genes and lifestyle factors in obesity: results from 12 462 subjects from MONICA/KORA. Int J Obes 34:1538–1545
17. Hotta K, Nakamura M, Nakamura T et al (2009) Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. J Hum Genet 54:727–731
18. Bochukova EG, Huang NI, Keogh J et al (2010) Large, rare chromosomal deletions associated with severe early-onset obesity. Nature 463:666–670
19. Jacquemont S, Reymond A, Zufferey F et al (2011) Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11. 2 locus. Nature 478:97–102
20. Walters RG, Jacquemont S, Valsesia A et al (2010) A new highly penetrant form of obesity due to deletions on chromosome 16p11. 2. Nature 463:671–675
21. Weiss LA, Shen Y, Korn JM et al (2008) Association between microdeletion and microduplication at 16p11. 2 and autism. N Engl J Med 358:667–675
22. Jarick I, Vogel CIG, Scherag S et al (2011) Novel common copy number variation for early onset extreme obesity on chromosome 11q11 identified by a genome-wide analysis. Hum Mol Genet 20:840–852
23. Doche ME, Henning E, Cline JM et al (2012) Human SH2B1 mutations are associated with maladaptive behaviors and obesity. J Clin Invest 122:4732–4736. https://doi.org/10.1172/JCI62696.4732
24. Pearce LR, Joe R, Doche ME et al (2014) Functional characterization of obesity-associated variants involving the α and β isoforms of human SH2B1. Endocrinology 155:3219–3226. https://doi.org/10.1210/en.2014-1264
25. Volckmar A-L, Bolze F, Jarick I et al (2012) Mutation screen in the GWAS derived obesity gene SH2B1 including functional analyses of detected variants. BMC Med Genom 5:1–10
26. Foucan L, Larifla L, Durand E et al (2018) High prevalence of rare monogenic forms of obesity in obese Guadeloupean Afro-Caribbean children. J Clin Endocrinol Metab 103:539–545
27. Zheng Z, Hong L, Huang X et al (2013) Screening for coding variants in FTO and SH2B1 genes in Chinese patients with obesity. PLoS ONE 8:e67039
28. Stahel P, Sud SK, Lee SJ et al (2019) Phenotypic and genetic analysis of an adult cohort with extreme obesity. Int J Obes 43:2057–2065
29. Türkyilmaz A, Yarali O, Kurnaz E, Cayir A (2021) Screening of MC4R, LEP, LEPR, POMC, SH2B1, and SIM1 genes in Turkish children with severe early-onset obesity. Med Sci Int Med J 10(2):328
30. Akıncı A, Türkkahraman D, Tekedereli I et al (2019) Novel mutations in obesity-related genes in Turkish children with non-syndromic early onset severe obesity: a multicentre study. J Clin Res Pediatr Endocrinol 11:341
31. Aerts E, Beckers S, Zegers D et al (2015) Genetic and structural variation in the SH2B1 gene in the Belgian population. Mol Genet Metab 115:193–198
32. da Fonseca ACP, Abreu GM, Zembrzuski VM et al (2019) The association of the fat mass and obesity-associated gene (FTO) rs9939609 polymorphism and the severe obesity in a Brazilian population. Diabetes, Metab Syndr Obes Targets Ther 12:667
33. da Fonseca ACP, da Fonseca GP, Marchesini B et al (2020) Genetic variants in the activation of the brown-like adipocyte pathway and the risk for severe obesity. Obes Facts 2:130–143
34. da Fonseca ACP, de Medeiros AG, Palhinha L et al (2021) A rare potential pathogenic variant in the BDNF gene is found in a Brazilian patient with severe childhood-onset obesity. Diabetes, Metab Syndr Obes Targets Ther 14:11
35. da Fonseca ACP, de Medeiros AG, Zembrzuski VM et al (2019) Identification of the MC4R start lost mutation in a morbidly obese Brazilian patient. Diabetes, Metab Syndr Obes Targets Ther 12:257
36. da Fonseca ACP, Abreu GM, Zembrzuski VM et al (2020) Study of LEP, MRAP2 and POMC genes as potential causes of severe obesity in Brazilian patients. Eat Weight Disord Anorexia, Bulim Obes 26(5):1399–1408
37. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med 17:405–424. https://doi.org/10.1038/gim.2015.30
38. Yousaf N, Deng Y, Kang Y, Riedel H (2001) Four PSM/SH2-B alternative splice variants and their differential roles in mitogenesis. J Biol Chem 276:40940–40948
39. Maures TJ, Chen L, Carter-Su C (2009) Nucleocytoplasmic shuttling of the adapter protein SH2B1β (SH2-Bβ) is required for nerve growth factor (NGF)-dependent neurite outgrowth and enhancement of expression of a subset of NGF-responsive genes. Mol Endocrinol 23:1077–1091