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ORIGINAL ARTICLE

Study of *LEP*, *MRAP2* and *POMC* genes as potential causes of severe obesity in Brazilian patients

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Abstract

Purpose Monogenic forms of obesity are caused by single-gene variants which affect the energy homeostasis by increasing food intake and decreasing energy expenditure. Most of these variants result from disruption of the leptin–melanocortin signaling, which can cause severe early-onset obesity and hyperphagia. These mutation have been identified in genes encoding essential proteins to this pathway, including leptin (*LEP*), melanocortin 2 receptor accessory proteins 2 (*MRAP2*) and proopiomelanocortin (*POMC*). We aimed to investigate the prevalence of *LEP*, *MRAP2* and *POMC* rare variants in severely obese adults, who developed obesity during childhood. To the best of our knowledge, this is the first study screening rare variants of these genes in patients from Brazil.

Methods A total of 122 Brazilian severely obese patients (BMI ≥ 35 kg/m^2) were screened for the coding regions of *LEP*, *MRAP2* and *POMC* by Sanger sequencing. All patients are candidates to the bariatric surgery. Clinical characteristics were described in patients with novel and/or potentially pathogenic variants.

Results Sixteen different variants were identified in these genes, of which two were novel. Among them, one previous variant with potentially deleterious effect in *MRAP2* (p.Arg125Cys) was found. In addition, two heterozygous mutations in *POMC* (p.Phe87Leu and p.Arg90Leu) were predicted to impair protein function. We also observed a *POMC* homozygous 9 bp insertion (p.Gly99_Ala100insSerSerGly) in three patients. No pathogenic variant was observed in *LEP*.

Conclusion Our study described for the first time the prevalence of rare potentially pathogenic *MRAP2* and *POMC* variants in a cohort of Brazilian severely obese adults.

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

Keywords Leptin–melanocortin pathway · *LEP* · *POMC* · *MRAP2* · Severe obesity

Introduction

Obesity is characterized as an abnormal or excessive body fat mass accumulation, which is measured using a Body Mass Index (BMI, BMI ≥ 30 kg/m^2) [1]. This disease has

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emerged as a serious health and economic problem worldwide, affecting both quality and expectancy life [1, 2]. Globally, there are at least 650 million adults and 124 million children/adolescents (5–19 years) with obesity. In Brazil, 19% of men and 26% of women were obese in 2016 [3]. Epidemiological studies showed that patients with high BMI have an increased risk for mortality caused by heart diseases, cancer and type 2 diabetes, being also associated with an estimated reduction of 6.5–13.7 years of life expectancy [4, 5].

The development of obesity occurs due to environmental changes, resulted of energy imbalance between caloric intakes and expended [1]. One of the most important neuroendocrine systems responsible for controlling appetite, food intake and energy utilization is leptin–melanocortin pathway, located in the hypothalamus [6–8]. Leptin (LEP) is mainly produced by white adipose, when the body has adiposity or energy replacement. This hormone crosses the blood–brain barrier and binds to its receptor in the neurons of hypothalamic arcuate nucleus. Leptin binding inhibits the neuropeptide Y and agouti-related protein expression as well as induces the proopiomelanocortin (POMC) production [9–11]. POMC is cleaved and processed by prohormone convertase 1/3, which produces α- and β melanocyte-stimulating hormone [12–14]. These products bind to melanocortin-3 and melanocortin-4 receptors (MC3R and MC4R), stimulating their activity. Both MC3R and MC4R signaling are regulated by melanocortin 2 receptor accessory proteins 2 (MRAP2) [15, 16]. The activation of MC3R and MC4R results in a decreased energy intake and an increased basal energy expenditure [8, 11, 14].

Over the past two decades, a large number of different rare genetic variants in the ligands or receptors which disrupt the signaling of the leptin–melanocortin pathway were identified, leading to hyperphagia, severe early-onset obesity and some cases endocrine abnormalities [15, 17–19]. These variants were found affecting nearly 2–5% of the severe obese subjects [20]. This genetic form of obesity is caused by a single-gene mutation and is transmitted as autosomal recessive or dominant Mendelian traits [11, 14].

One of the first evidence of *LEP* associated with monogenic form of obesity was described by Montague et al. [21]. They identified two severely obese cousins with low serum leptin levels and a homozygous frameshift mutation (c.398delG) in the *LEP* gene. They were born from a highly consanguineous family of Pakistani ancestry. Congenital *LEP* deficiency is extremely rare and was identified mostly in populations with high rate of inbreeding. The affected patients exhibited low/no detectable leptin levels in the circulation or biologically inactive protein [21–30].

Similar to *LEP*, loss-of-function mutations found in *MRAP2* and *POMC* were associated with monogenic obesity disorders [15, 31]. The first cases of severe obesity due to POMC deficiency was found in probands from Germany. Both children showed an early-onset obesity, adrenal insufficiency and red hair pigmentation [32]. Recently, Çetinkaya et al. [33] reported a young female patient with POMC deficiency caused by a homozygous mutation in exon 3 (c.206delC). Besides early-onset obesity, the patient exhibited rapid increase in growth and red hair. Additionally, Cirillo et al. [34] have described a young male patient from Egyptian ethnic origin with a homozygous nonsense mutation (p.Gln68Ter) causing severe early-onset obesity and hypocortisolism, but without the distinctive red hair phenotype. POMC deficiency was also found in a few probands from India, Turkey, Iraq, Hispanic origin and Caucasian ancestry [31, 35–39]. Although POMC and LEP deficiency are an extremely rare autosomal recessive disorder, several studies have reported that the defective allele in the heterozygous state contributed to the obesity risk [40–43].

Up to date, few studies have carried out a mutation analysis in the coding region of *MRAP2* [15, 44–46]. However, five rare heterozygous variants of in this gene were found in patients with severe early-onset obesity. No specific endocrine abnormalities were described in these cases [15, 45]. In mice, *Mrap2* knockout also caused severe obesity at a young age [15]. Interestingly, a heterozygous nonsynonymous mutation (p.Ala40Ser) was identified in a patient with Prader–Willi syndrome, suggesting a possible role of MRAP2 in this disease phenotype [44]. Recently, Baron et al. [46] have performed a large-scale sequencing of *MRAP2* and identified seven loss-of-function mutations which caused monogenic obesity associated with hyperphagia, hyperglycemia and hypertension.

Epidemiological studies of monogenic obesity are limited in the Brazilian population. Therefore, the aimed of our study was to assess the prevalence of *LEP*, *POMC* and *MRAP2* variants in a sample of 122 severely obese patients, which developed obesity during childhood. Additionally, clinical characteristics were also described for patients carrying potentially pathogenic mutations.

Materials and methods

Study population

This cross-sectional study comprised a cohort of 122 obese adults (97 females [79.5%] and 25 males [20.5%]) with early-onset obesity, aged 18–65 years, from Rio de Janeiro, Southeast region of Brazil. The inclusion criteria were patients with severe obesity (BMI $\geq$ 35 kg/m^2) which developed obesity during childhood (0–11 years). The period of obesity onset was obtained by self-report. The exclusion criteria were pregnancy, lactation, the use of medication to lose or gain weight; and the presence of symptoms suggestive of

syndromic obesity. All patients were candidates to undergo the bariatric surgery in Brazil. They were recruited from a non-governmental organization, called Rescue Group to Self-Esteem and Citizenship of the Obese. All probands were previously screened for *MC4R* mutations. Additionally, novel and pathogenic variants were screened in one hundred normal-weight individuals ($18.5 \leq BMI \leq 24.9$ kg/m^2) from public hospitals in the same city.

Mutation analyses of LEP, MRAP2 and POMC genes

Genomic DNA was extracted from peripheral blood samples using QIAamp Blood Kit (Qiagen, Valencia, CA, USA). All patients were screened for the entire coding region of *LEP* (ENST00000308868.4), *MRAP2* (ENST00000257776.4) and *POMC* (ENST00000405623.5) genes using Sanger sequencing. The first exon of these genes was not sequenced, because they are not translated. We designed 2 (*LEP*) or 3 (*POMC* and *MRAP2*) set of primers to amplify the coding region of studied genes using Primer3Plus software. The information about the DNA sequence of primers, PCR reagents and cycling conditions are presented in Supplementary Table S1. The products of PCR were visualized on 1.5% agarose.

Amplicons were purified by Sweep Clean up kit (Applied Biosystems, Vilnius, Lithuania) and sequenced using Big Dye Terminator kit v.3.1 (Applied Biosystems, Austin, TX, USA) on 3730xl DNA analyzer (Applied Biosystems). Reactions were performed in a total volume of 10 µl, including 10–40 ng of PCR products, 1X sequencing buffer, 1.0 µl Big Dye Terminator kit v.3.1 and 0.32 pmol of primer. The sequencing of these products was carried out by following conditions: 40 cycles of 94 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. The products were analyzed and aligned with the sequence provided for the National Centre for Biotechnology Information (NCBI) and Ensembl database using BioEdit software. Mutations identified were confirmed by bidirectional sequencing of a second PCR.

Biochemical, anthropometric and blood pressure variables

Body weight, height, waist and hip circumferences were measured as previous described [47, 48]. BMI and waist–hip ratio (WHR) were obtained from each participant. BMI was calculated as weight (kilograms) divided by the square of height (meters). Blood pressure was also measured by wrist monitor. Hypertension was diagnosed using blood pressure $\geq 140 \times 90$ mm Hg, or use of antihypertensive drugs therapy [49].

After an overnight fast, plasma glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglyceride (TG) were measured by oxidase–peroxidase method (BioSystems). Low-density lipoprotein cholesterol (LDL-c) was obtained using Friedewald formula: LDL-c = TC − HDL-c − TG/5. C-reactive protein (CRP) and hemoglobin glycated (HbA1c) were measured by latex agglutination method and turbidimetric inhibition immunoassay (TINIA), respectively. Patients using medication for these biochemical or blood pressure parameters had their levels excluded from statistical analyses.

Bioinformatic tools

LEP, *MRAP2* and *POMC* sequences (genomic and protein) were obtained through the NCBI (https://www.ncbi.nlm.nih.gov/) and Ensembl database (https://www.ensembl.org/). Pathogenicity of missense variants were estimated by Polyphen-2 (Polymorphism Phenotyping), SIFT (Sorting Intolerant From Tolerant) and Mutation Taster programs. Bases insertion and deletion were analyzed using only Mutation Taster. All variants found were checked in dbSNP (https://www.ncbi.nlm.nih.gov/), Exome Aggregation Consortium (ExAC) (https://exac.broadinstitute.org) and 1000 Genomes project database (https://www.internationalgenome.org/) in order to verify previous identification in the literature.

Results

Clinical characteristic of study population

Clinical characteristics of the severely obese patients are shown in Table 1.

Description of identified variants

The coding region of *LEP*, *MRAP2* and *POMC* was screened in a cohort of 122 patients with severe obesity and early-onset, revealing 16 variants in our cohort. Of these variants, 5 were missense, 10 were synonymous and 1 was in frame insertion. One missense mutation (*POMC*; p.Arg90Leu) was novel as well as one synonymous variant (*POMC*; p.Gly182=). An overview of the identified variants is presented in Table 2.

LEP gene

We identified four previous reported variants, including one missense (p.Val94Met) and three synonymous (p.Gln55=, p.Pro68= and p.Asn103=). All of them were identified in a heterozygous state. Val94Met is a common polymorphism located in exon 3 and it was found in 5 participants. In silico analyses using three different prediction programs (Polyphen-2, SIFT and Mutation taster) were performed in

Table 1 Characteristics of the individuals screened for *LEP*, *MRAP2* and *POMC* variants

Variables	*n*	Patients
Age (years)	122	37.0 (28; 45)
Gender		
Female	122	97 (79.5)
Male		25 (20.5)
Weight (kg)	122	128.9 (114.0; 146.9)
Height (m)	122	1.64 (1.58; 1.70)
BMI (kg/m^2)	122	47.0 (42.8; 52.6)
BAI	121	49.1 (44.3; 55.6)
Waist circumference (cm)	121	137.0 (126.0; 144.0)
Hip circumference (cm)	121	141.8 (133.0; 151.2)
WHR	121	0.97 (0.91; 1.00)
SBP (mm Hg)	61	131.0 (121.0; 148.5)
SBD (mm Hg)	61	88.0 (79.0; 97.5)
Glucose (mg/dl)	85	97.0 (92.0; 105.5)
Cholesterol total (mg/dl)	98	192.0 (162.2; 221.7)
HDL-cholesterol (mg/dl)	98	44.5 (40.0; 52.2)
LDL-cholesterol (mg/dl)	95	118.0 (97.0; 143.0)
Tryglicerides (mg/dl)	98	123.5 (84.7; 166.5)
Hemoglobin (%)	66	5.65 (5.10; 6.50)
CRP (mg/dl)	66	1.08 (0.56; 1.64)

Data are presented as median values (interquartile range 25–75%) for quantitative traits and *n* (%) for qualitative parameters

BMI Body Mass Index, *WHR* Waist–hip ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL-cholesterol* high-density lipoprotein cholesterol, *LDL-cholesterol* low-density lipoprotein cholesterol *CRP*, C-reactive protein

our study, which suggested that Val94Met is a non-disease causing.

MRAP2 gene

The sequencing of *MRAP2* led us to identification of four different variants, one missense (p.Arg125Cys) and three synonymous (p.Gln14 =, p.Asn159 = and p.His201 =). All of them were earlier reported. Patients carrying p.Arg125Cys or p.Gln14 = were all heterozygous; while, the others variants (p.Asn159 = and p.His201 =) were found in either a heterozygous or homozygous form. Asn159 = and His201 = were polymorphic in our sample and several patients were compound heterozygous for these variants. In addition, one rare missense mutation (p.Arg125Cys) was identified. This substitution was predicted to be deleterious by PolyPhen-2 software; however, divergent results were observed using SIFT and Mutation Taster. The mutated allele (T) was infrequently found in subjects of ExAC and was not observed in our normal-weight participants. Furthermore, the residue is not located in a highly conserved region across different organisms. This proband did not show a pathogenic mutation in *MC4R*, *LEP* and *POMC* genes.

POMC gene

Eight *POMC* sequence variants were found in our cohort, including four rare synonymous mutations (p.Cys6 =, p.Ser94 =, p.Gly182 = and p.Asp217 =), three missense variants (p.Asp53Gly, p.Phe87Leu and p.Arg90Leu) and a 9-bp insertion (p.Gly99_Ala100insSerSerGly). All variants were heterozygous, except for the inframe insertion which was found as homozygous state in three patients. Among the three identified missense variants, one was not previous described (p.Arg90Leu). This variant was absent in dbSNP, 1000 genomes and ExAC database.

The potential impact of Asp53Gly, Phe87Leu, Arg90Leu and 9-bp insertion was predicted by Bioinformatic analyses. Our results showed that Phe87Leu was "probably damage" and "disease causing" by Polyphen-2 and Mutation Taster, respectively. However, it was predicted as "tolerate" by SIFT. Additionally, Arg90Leu was predicted as "possibly damage" and "deleterious" by Polyphen-2 and SIFT, respectively. Nevertheless, it was predicted as non-disease causing using Mutation Taster. Furthermore, both variants were not identified in our 100 normal-weight individuals. Asp53Gly and 9-bp insertion were predicted to have benign effect and were found in our control group. Phenylalanine at codon 87 (p.Phe87Leu) and Arginine at codon 90 (p.Arg90Leu) are located in a highly conserved protein position among different species from human up to zebrafish.

Clinical characterization of probands carrying potential pathogenic mutations

MRAP2 Arg125Cys variant was identified in a 37-year-old male proband with BMI of 41.8 kg/m^2, which reported early-onset severe obesity. On clinical examination, his current weight was 146.2 kg, but the maximum self-reported weight was 150 kg. His neck circumference was 50 cm; waist circumference, 133 cm; hip circumference, 121 cm and WHR, 1.10. The patient had high blood pressure 154/110 mm Hg and presented arterial hypertension, being treated pharmacologically by ACE inhibitor. We also measured biochemical parameters, in which fasting plasma glucose was 94 mg/dl, total cholesterol, 251 mg/dl; HDL-cholesterol, 53 mg/dl; TC, 481 mg/dl; HbA1c, 5.1% and CRP 0.56 mg/dl. The patient presented metabolic syndrome and did not report family history of obesity.

In *POMC*, the Phe87Leu mutation was detected in a 26-year-old female patient, weight of 123 kg and BMI of 46.1 kg/m^2. However, her maximum self-reported weight was 150 kg and BMI of 56.4 kg/m^2. Her neck circumference was 39 cm; waist circumference, 119 cm; hip

Table 2 Identification of variants in LEP, MRAP2 and POMC genes

Gene	Location	ID	Mutation (cDNA)	Mutation (protein)	Exon	Type of mutation	MAF ExAC (%)	MAF our study (%)	Polyphen-2	Mutation Taster	SIFT	ClinVar
LEP	7q32.1	rs138908051	165G > A	Gln55 =	3	Synonymous	0.017	0.410	–	–	–	na
		rs140510728	204C > G	Pro68 =	3	Synonymous	0.010	0.410	–	–	–	na
		rs17151919	280G > A	Val94Met	3	Missense	0.792	2.05	Bening	Polymorphism	Tolerated	Benign/Likely benign
		rs28954113	309C > T	Asn103 =	3	Synonymous	0.203	0.820	–	–	–	na
MRAP2	6q14.3	rs775755022	42A > G	Gln14 =	2	Synonymous	0.002	0.410	–	–	–	na
		rs148904867	373C > T	Arg125Cys	4	Missense	0.017	0.410	Bening	Polymorphism	Deleterious	na
		rs9449776	477C > T	Asn159 =	4	Synonymous	11.39	25.0	–	–	–	na
		rs2875382	603C > T	His201 =	4	Synonymous	25.21	39.75	–	–	–	na
POMC	2p23.3	rs8192605	18C > T	Cys6 =	2	Synonymous	0.535	0.410	–	–	–	With Uncertain significance allele
		rs28932470	158A > G	Asp53Gly	3	Missense	0.20	0.410	Bening	Polymorphism	Tolerated	With Uncertain significance allele
		rs10654394	297_298insAGC AGCGGC	Gly99_ Ala100ins-SerSerGly	3	Inframe insertion	5.77	2.460	–	Polymorphism	–	With Likely benign allele
		rs199636726	261C > A	Phe87Leu	3	Missense	0.140	0.410	Probably damage	Disease causing	Tolerated	na
		Novel	269G > T	Arg90Leu	3	Missense	na	0.410	Possibly damage	Polymorphism	Deleterious	na
		rs28930368	c.282C > T	Ser94 =	3	Synonymous	7.610	0.410	–	–	–	Likely benign
		Novel	c.546C > A	Gly182 =	3	Synonymous	na	0.410	–	–	–	na
		COSM1019221	651C > T	D217 =	3	Synonymous	na	0.410	–	–	–	na

MAF minor frequency allele, na data not available

circumference, 148 cm and WHR, 0.80. The blood pressure was 121/85 mm Hg. Regarding biochemical measurements, her fasting plasma glucose was 98 mg/dl, total cholesterol, 141 mg/dl; HDL-cholesterol, 47 mg/dl; LDL-cholesterol, 79 mg/dl; TC, 73 mg/dl; HbA1c, 6.2% and CRP 1.3 mg/dl. Further, this patient had polycystic ovary syndrome and depression. She also reported a family history of obesity (mother and sibling).

Finally, *POMC* Arg90Leu was found in a 39-year-old female case. On anthropometric examination, her BMI was 49.5 kg/m^2; weight, 141.3 kg; neck circumference, 51.5 cm; waist circumference, 152 cm; hip circumference, 137 cm and WHR, 1.11. The blood pressure was 123/84 mm Hg. In addition, her fasting plasma glucose was 99 mg/dl, total cholesterol, 189 mg/dl; HDL-cholesterol, 46 mg/dl; LDL-cholesterol, 114 mg/dl and TC, 145 mg/dl. This patient had type 2 diabetes, hypertension, metabolic syndrome and depression. She takes medicine to control blood pressure (beta-adrenergic blocking agents and ACE inhibitor) and glucose. No family history of obesity was reported.

Discussion

In the present study, we have screened the entire coding region of *LEP*, *MRAP2* and *POMC* genes in patients with severe obesity from Southeast of Brazil. To the best of our knowledge, this is the first study investigating rare variants in these candidate genes associated to monogenic obesity in our population. As result, we identified sixteen different variants (synonymous, missense and inframe insertion) of which two was novel. Of these mutations, three were predicted to have functional impact on the proteins.

Among the studied genes, *LEP* encodes a hormone produced mainly by the white adipose tissue. It plays a critical role on regulating energy intake and expended through its actions on the hypothalamus [8]. Different variants have been reported and associated with monogenic obesity, caused by the absence (undetectable or very low) of leptin circulation or biologically inactive protein [21–23, 26]. Treatment of patients with recombinant human leptin leads to rapid reduction in food intake and body fat mass [50, 51]. However, monogenic obesity caused by pathogenic mutations in *LEP* is extremely rare [21, 24–26]. In our cohort, no patient with leptin deficiency or pathogenic variants was identified.

MRAP2 is mainly expressed in adrenal cells and in the brain, including hypothalamus and brainstem [52]. It has been suggested that the MRAP2 modulates MC4R/MC3R signaling, two important components in the leptin–melanocortin pathway and consequently it is involved in energy balance. For that reason, the association of MRAP2 with obesity development could be due to disruption of this signaling [15, 16]. In zebrafish, the homologous isoform of MRAP2 in mammals increases the MC4R sensitivity to its agonist [53]. Additionally, Asai et al. [15] have showed that *Mrap2* knockout mice develop severe early-onset obesity and late-onset hyperphagia, even when receiving the same amount of food as wild-type mice.

Up to date, few studies can be found regarding *MRAP2* mutation analysis and obesity in human. Asai et al. [15] have screened the coding region and intron–exon boundaries of *MRAP2* in a European large cohort of children and adolescents with or without obesity. They identified four rare missense variants present only in the case group (p.Glu34Ter, p.Asn88Tyr, p.Leu115Val and p.Arg125Cys), in which one was clearly disruptive (p.Glu34Ter). Moreover, in silico analyses also suggested that Asn88Tyr and Arg125Cys variants were potentially pathogenic, indicating that the rare heterozygous *MRAP2* mutations may contribute to severe early-onset obesity. Furthermore, one missense mutation (p.Arg125His) was found in two obese children and adolescents from Germany [44]. Similarly, Schonnop et al. [45] have reported three missense mutations (p.Arg125His, p.Ala137Thr and p.Gln174Arg) and two synonymous variants (p.Asn159 = and p.His201 =) from severely obese patients. Only Gln174Arg was predicted to have a potential functional impact by in silico programs and in vitro analyses (slightly reduced MC4R signaling). Recently, Baron et al. [46] have carried out a large-scale sequencing of *MRAP2* using 9418 subjects and identified 23 rare heterozygous variants in obese children and adults. Functional analysis showed that six loss-of-function mutations (c.-3_7del, p.Gly31Val, p.Phe62Cys, p.Asn77Ser, p.Arg102Ter and p.Pro195Leu) caused monogenic obesity, which was also associated with hyperphagia, hyperglycemia and hypertension.

In our study, we have identified one missense (p.Arg125Cys) and three synonymous variants (p.Gln14 =, p.Asn159 = and p.His201 =). Only Gln14 = was not described by previous studies. Arg125Cys was identified in a 37-year-old male subject with BMI of 41.8 kg/m^2, who developed obesity during childhood. Our patients also showed hypertension and metabolic syndrome. This heterozygous *MRAP2* variant involved a C > T base change in the exon 4. Our in silico analyses predicted that this variant is disease causing by SIFT software; however, the other programs showed a conflicting results. Moreover, this variant was not found in our normal-weight individuals. We also identified that the residue is not located in highly conservative position across the species. Recently, Baron et al. [46] have identified Arg125Cys in 13 subjects (6 obese, 4 overweight and 3 normal-weight individuals). They have classified this genetic variation as variant with uncertain significance (VUS). Interestingly, Liang et al. [16] have recently reported that Arg125 was only conserved in primates and altered the three-dimensional conformation of

MRAP2 structure. Moreover, in vitro analyses showed that the Arg125Cys mutation impaired α-MSH-induced MC4R or MC3R stimulation and reduced the surface expression of MC3R. These results demonstrated that this variant have a functional impact on regulating MC3R/MC4R signaling. Based on the literature and our findings, we suggest that the presence of Arg125Cys contribute to development of severe obesity in human.

POMC is a prohormone which encodes a several biologically active peptides after processed by tissue-specific proteolysis, including α-melanocyte-stimulating hormone (α-MSH) [54, 55]. This peptide is produced by anorexigenic neurons in the hypothalamus and acts on MC3R and MC4R, regulating the control of appetite and energy balance [54, 56]. Homozygous or heterozygous compound loss-of-function mutations has been associated with severe obesity development in humans. Similar to *LEP*, those variants are also very rare [17, 18, 31, 36]. However, POMC haploinsufficiency also appears to alter the obesity risk [36, 57]. Charllis et al. [58] found a heterozygous missense mutation (Arg236Gly) which produced an aberrant fusion protein. This variant was co-segregated with early-onset obesity over three generations and was absent in British Caucasian normal-weight controls, suggesting an increased risk of obesity development in carriers.

Several mutational-screening studies have also observed a higher prevalence of obesity or an increased weight in subjects with loss of one copy of the *POMC* gene, indicating a dosage effect of this gene product on body weight control [18, 32, 36, 41, 42]. Farooqi et al. [36] have reported a novel homozygous frameshift mutation, predicted to disrupt production of all POMC-derived peptides. They observed that heterozygous family members had greater frequency of obesity when compared to wild type. Clement et al. [17] identified a homozygous frameshift mutation (p.Pro74fs) in female patient of North African ancestry with hyperphagia and early-onset obesity, in which her heterozygous parents were obese or overweight. Despite her heterozygous sisters showed normal weight, they exhibited a high score for restraint eating, suggesting a tendency to control food intake to main the body weight. Experimental studies also reported that heterozygous mice for a null *POMC* mutation became obese on a high-fat chow due to increased food intake [58].

Among our severely obese probands, we identified four synonymous variants (p.Cys6=, p.Ser94=, p.Gly182=and p.Asp217=), a 9-bp insertion (p.Gly99_Ala100insSerSerGly) and three missense mutations (p.Asp53Gly, p.Phe87Leu and p.Arg90Leu), of which two were not previous reported (p.Gly182=and p.Arg90Leu). This 9-bp insertion may occur by slipped strand mispairing, resulting in the addition of three amino acids (Ser-Ser-Gly) in the 16-kDa fragment carboxyl terminal portion of γ-MSH [59, 60]. The functional implication of this insertion is still not elucidated; however, higher leptin and insulin levels were observed in heterozygous obese patients when compared to homozygous wild-type subjects [59, 61]. It has been suggested that this insertion might impact on mRNA stability or posttranslational processes of POMC peptide [60]. We identified three homozygous patients with severe obesity phenotype and family history of obesity. However, we also identified this variant in our control group. More functional analyses and case–control studies with large cohort are necessary to determine whether this insertion can influence the adiposity.

We also found two missense mutations, Phe87Leu and Arg90Leu, predicted to have pathogenic effect by two of the three in silico softwares used. These variants were not observed in our normal-weight individuals. Both variants were located in a highly conservative position among different species, suggesting that region may have an important role on the protein. Phe87Leu was infrequently reported in the ExAC (21/15,526 alleles), while Arg90Leu was absent in the ExAC, 1000 genomes and dbSNP databases. In the literature, Phe87Leu was also identified in only one French severely obese child. Segregate and in vitro were not performed with this variant, because the function of this region in the protein was not elucidate [17]. Therefore, we could not clarify whether Phe87Leu and Arg90Leu could affect on development of severe obesity. Further functional analyses are necessary to elucidate their possible causative effects.

This study has several limitations that might be taken into account before analyzing the results. First, we could not perform the segregate analysis because no family members were available to this study. Second, the period of obesity onset was self-reported. Third, we did not carry out functional analysis to elucidate the impact of the identified variants.

In summary, this is the first study to sequence the coding regions of *LEP*, *MRAP2* and *POMC* genes, which are involved in the leptin–melanocortin signaling. We reported sixteen variants, of which two were novel. Among these mutations, one previous mutation in *MRAP2* (p.Arg125Cys) appeared to have a functional impact on protein structure and function were identified. In addition, two missense variants in *POMC* (p.Phe87Leu and p.Arg90Leu) were found and our in silico analyses did not clarify whether these variants influence the development of severe obesity. All these variants were absent of our normal-weight group. Further functional or expression analyses are necessary to determine the pathogenicity of these POMC variants.

What is already known on this subject?

Non-syndromic monogenic obesity is caused by mutation in single genes, which disrupts the energy homeostasis. Most of these mutation result from disruption of the leptin–melanocortin pathway, resulting in severe early-onset obesity and hyperphagia. These pathogenic mutations have been identified in some genes, such as *LEP*, *MRAP2* and *POMC*.

What does this study add?

We have identified potentially deleterious *MRAP2* and *POMC* variants in Brazilian patients with severe and childhood-onset obesity.

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Availability of data and material The data and material will be available as requested.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

Ethical approval This study was approved by Ethics Committee of the Oswaldo Cruz Foundation.

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

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