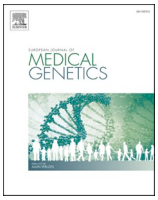
European Journal of Medical Genetics 66 (2023) 104728

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg



Next-generation sequencing of 12 obesity genes in a Portuguese cohort of patients with overweight and obesity

Licínio Manco [a,*], Janet Pereira [a,b], Teresa Fidalgo [a,b], Marina Cunha [c], José Pinto-Gouveia [d], Cristina Padez [a], Lara Palmeira [d]

[a] Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra, Portugal
[b] Department of Clinical Hematology, Centro Hospitalar e Universitário de Coimbra, Portugal
[c] Miguel Torga Superior Institute, Coimbra, Portugal
[d] Center for Research in Neuropsychology and Cognitive and Behavioral Intervention (CINEICC), Faculty of Psychology and Educational Sciences, University of Coimbra, Portugal

ARTICLE INFO

Handling Editor: A. Verloes

Keywords:
Next generation sequencing
Severe obesity
Monogenic obesity
Mutations

ABSTRACT

We examined 12 monogenic obesity genes in 72 Portuguese individuals with overweight and obesity (class 1 and class 2), some of which with suspected genetic obesity, to identify known or unknown potential obesity variants. Genomic DNA was analyzed for variants in genes *LEP*, *LEPR*, *MC4R*, *POMC*, *PCSK1*, *BDNF*, *NTRK2*, *SIM1*, *SH2B1*, *UCP3*, *GCG* and *ADCY3* through next generation sequencing (NGS). The impact of the rare variants was investigated in the ClinVar database and using *in silico* tools for prediction of pathogenicity. Four potential pathogenic missense variants were detected at the heterozygous state in five individuals: two in the *ADCY3* gene, NM_004036.5:c.1153G > A (p.Val385Ile) (rs756783003) and NM_004036.5:c.1222G > A (p.Gly408Arg) (rs201606553), one in gene *SH2B1*, NM_001145795.1:c.127C > A (p.Arg43Ser) (rs547678855), and the fourth in gene *POMC* NM_000939.4:c.706C > G (p.Arg236Gly) (rs28932472), which was found in two individuals. Moreover, six rare variants near splicing sites were also identified, as well as eight rare synonymous variants. In summary, some potential pathogenic rare missense variants were identified, two of them in *ADCY3* gene, the most recently identified gene as having a role in monogenic obesity. Further analysis should be performed to confirm the clinical relevance of these variants.

1. Introduction

Obesity is a multifactorial disorder characterized by excess body fat, involving complex interactions between environmental and genetic factors (Lin and Li, 2021). While environmental alterations have increased obesity rates worldwide during the last decades, family and twin studies estimated a body mass index (BMI) heritability between 40% and 70%, highlighting the impact of genetics on the risk of obesity (Wardle et al., 2008; Silventoinen et al., 2010). However, genetic and non-genetic factors' contribution to the actual global obesity pandemic remains to be fully understood.

Genetic variation associated with common forms of obesity has been identified through large-scale population studies in candidate genes or recently through genome-wide association studies (GWAS). On the other hand, rare monogenic forms of Mendelian obesity (non-syndromic), resulting from alteration in a single gene, have been identified in cohorts of patients with severe and early-onset (<10 years old) obesity via Sanger and massively parallel sequencing (Loos and Yeo, 2022).

Most causative proteins in monogenic forms of obesity (non-syndromic) are in the hypothalamic leptin-melanocortin pathway, a key circuit for the regulation of food intake and energy homeostasis (Loos and Yeo, 2022; Beckers et al., 2009; Yeo et al., 2021). Generally, mutations in *LEP*, *LEPR* and *MC4R* genes represent the most common cause of monogenic forms of obesity. In particular, pathogenic mutations in *MC4R*, with more than 200 mutations identified so far, were found in up to 5% of cases of severe early-onset obesity (Farooqi et al., 2003; Kühnen et al., 2019; Wade et al., 2021).

Despite all the research studies already conducted in the last two decades, a genetic basis for obesity is still far from being fully understood. Meanwhile, several loci recently discovered by GWAS include genetic variants with subtle effects in or near the same genes that were first identified for extreme and early-onset obesity, disrupting

* Corresponding author. Research Centre for Anthropology and Health (CIAS), Department of Life Sciences University of Coimbra, 3000-456, Coimbra, Portugal.
E-mail address: lmanco@antrop.uc.pt (L. Manco).

https://doi.org/10.1016/j.ejmg.2023.104728
Received 24 March 2022; Received in revised form 28 December 2022; Accepted 10 February 2023
Available online 10 February 2023

components of the leptin-melanocortin signaling pathway (Loos and Yeo, 2022). In this regard, the present study aimed to identify, in a cohort of adult individuals with overweight and obesity, some of them with suspected genetic obesity, novel or previously known pathogenic variants in genes of the melanocortin pathway, including *MC4R*, *BDNF*, *SH2B1*, *POMC*, *LEP*, *LEPR*, *NPY*, *SIM1*, *NTRK2*, *PCSK1* and *ADCY3*.

2. Materials and methods

2.1. Study population

A sample of 72 unrelated individuals, aged 28–48 years old (mean 40.76), from Portuguese ancestry, was selected with the following Body Mass Index (BMI) classifications: 20 individuals with overweight, BMI 25–29.9 kg/m^2 (mean 27.94); 20 individuals with obesity class 1, BMI 30–34.9 kg/m^2 (mean 31.75); and 32 individuals with obesity class 2, BMI 35–39.9 kg/m^2 (mean 38.04). Written informed consent was obtained from all individuals before collection of samples. The study was conducted in accordance with Declaration of Helsinki and was approved by the Ethics Committee of Centro Hospitalar e Universitário de Coimbra (CHUC).

2.2. Next generation sequencing

Targeted next-generation sequencing (NGS) of all exons and adjacent regions in 12 selected genes, including *LEP*, *LEPR*, *MC4R*, *POMC*, *PCSK1*, *BDNF*, *NTRK2*, *SIM1*, *SH2B1*, *UCP3*, *GCG* and *ADCY3* was performed. This list contains 10 genes of the leptin-melanocortin pathway, previously demonstrated to be involved in monogenic obesity, and the two additional candidate genes *UCP3* (that encodes a mitochondrial transporter protein) linked to severe obesity (Argyropoulos et al., 1998) and *GCG* (encodes glucagon) associated with diabetes and other disorders (Lindquist et al., 2021). DNA was isolated from total blood using the QIAmp® DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and massively parallel sequencing was performed using the Ion S5™ next-generation sequencing system (Thermo Fisher Scientific). Primer sequences were designed using Ion AmpliSeq Designer (Thermo Fisher Scientific, Massachusetts, USA). The library preparation, NGS and bioinformatic analysis workflow were performed as previously described (Fidalgo et al., 2017).

2.3. Variant selection and interpretation

To analyze the results obtained in the NGS, the criteria used was the inclusion of missense, synonymous, frameshift and intronic near splice site variants. The minimum depth of coverage required for each nucleotide in the ROI to be considered for further analysis was ≥100x. All variants that resulted from these filters with MAF ≤0.01 were searched in the Varsome (https://varsome.com/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases. *In silico* analysis was performed for rare missense variants to predict the possible consequences of the amino acid changes on the structure and function of the protein by using the Ensembl genome browser (https://www.ensembl.org/index.html) and the human Variant Effect Predictor (VEP) cache file that contain precalculated predictions from SIFT and PolyPhen-2 for amino acid change. Moreover, the variant deleteriousness rankings from the Combined Annotation Dependent Depletion (CADD) tool scores and the Genomic Evolutionary Rate Profiling (GERP) conservation scores displayed in Ensembl, were also addressed in all rare variants. Frequencies of the identified variants were compared with global frequencies in the Genome Aggregation Database (gnomAD) v2.1.1 (https://gnomad.broadinstitute.org/). (Databases and online tools were accessed on 5 December 2022).

2.4. Statistical analysis

Group comparisons were performed by means of the independent samples *t*-test. A Mann-Whitney *U* test was run to determine if there were differences in BMI between groups. Statistical analyses were performed using IBM® SPSS® Statistics v.27 (SPSS Inc, Chicago, IL, USA).

3. Results

The number of the identified missense, synonymous, frameshift and intronic (near splicing sites) variants found in the 72 analyzed individuals is detailed in Table 1. Twenty-two different rare variants were identified in the 12 genes in a total number of 30 individuals: eight missense variants (MAF <0.01) in 10 individuals, eight synonymous variants in 12 individuals and six intronic rare variants located near splice sites in 8 individuals.

Stratifying the study sample into three BMI-based groups, the rare variants were found in 11 individuals with severe obesity, 8 individuals with obesity and 11 individuals with overweight; this difference was not statistically significant (chi-square = 0.864; p = 0.649). Considering all the coding missense variants in addition to the rare variants, 157 coding and rare variants were found in 32 individuals with severe obesity, 98 in 20 individuals with obesity and 93 in 20 individuals with overweight; again, the difference was not statistically significant between the three groups (chi-square = 0.034; p = 0.983).

From all the 48 identified different variants, 11 (22.9%) were found at the *ADCY3* gene, followed by genes *PCSK1* and *LEPR* with 6 (12.5%) different variants each. Most of the 12 analyzed genes showed variants in more than 75% of the individuals, with three genes (*ADCY3*, *BDNF* and *LEPR*) showing variants in 100% of samples; the *POMC* gene showed variants in about 47% of the individuals, and the *MC4R* and *GCG* genes showed variants in only 3 and 2 samples respectively (Fig. 1).

3.1. Rare missense variants

The identified rare missense mutations (MAF <0.01 in gnomAD), all found in the heterozygous state, are detailed in Table 2. The most suggestive variants potentially involved as disease-causing are as follows:

- The *ADCY3* NM_004036.5:c.1153G > A (p.Val385Ile) (rs756783003) variant found in a subject with severe obesity (BMI = 38 kg/m^2) classified as "possibly damaging" in PolyPhen-2 (scores 0.804 and 0.54) and as "tolerated" in SIFT (score 0.06).
- The *ADCY3* NM_004036.5:c.1222G > A (p.Gly408Arg) (rs201606553) variant found in a subject with overweight (BMI = 26.2 kg/m^2) classified as "possibly damaging" and "probably damaging" in PolyPhen-2 (scores 0.673 and 0.994, respectively) and as "tolerated" in SIFT (score 0.12).

Table 1
Frequency of common (MAF >0.01) and rare (MAF <0.01) variants found in 72 adult individuals with overweight/obesity.

Impact	MAF	Frequency		Genes
		total	different	
Missense	>0.01	317	11	LEPR, ADCY3, SIM1, PCSK1, BDNF, SH2B1
	<0.01	10	8	MC4R, ADCY3, SH2B1, LEPR, POMC
Synonymous	>0.01	386	14	ADCY3, UCP3, LEPR, POMC, UCP3, NTRK2, BDNF, PCSK1
	<0.01	12	8	LEPR, POMC, ADCY3, SIM1, NTRK2, UCP3, LEP
Intronic_near splice site	<0.01	8	6	GCG, ADCY3, PCSK1, POMC, SH2B1
Frameshift	>0.01	1	1	POMC

Abbreviations: MAF, minor allele frequency.

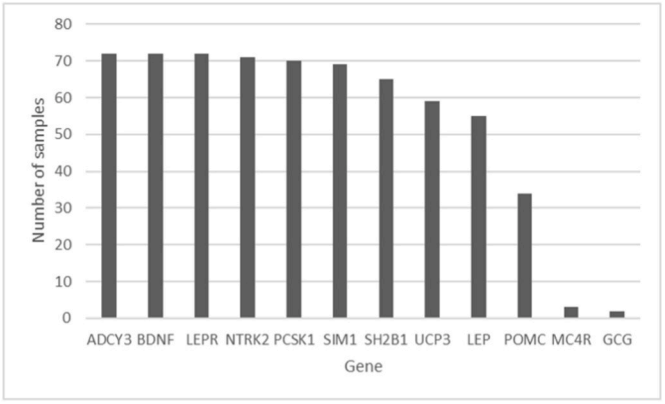


Fig. 1. Frequency of variants found in the 12 analyzed genes. Most of the genes showed variants in more than 75% of the samples, with three genes (*ADCY3*, *BDNF* and *LEPR*) showing variants in 100% of the samples. The *POMC* gene showed variants in about 47% of the samples, and the *MC4R* and *GCG* genes showed variants in only 3 and 2 samples, respectively.

- The *SH2B1* NM_001145795.1:c.127C > A (p.Arg43Ser) (rs547678855) variant found in a subject with severe obesity (BMI = 36.3 kg/m^2) classified as "possibly damaging" in PolyPhen-2 (scores 0.688; 0.859) and as "deleterious" in SIFT (score 0.03).
- The *POMC* NM_000939.4:c.706C > G (p.Arg236Gly) (rs28932472) variant found in two subjects, one with obesity (BMI = 31.7 kg/m^2) and the second with overweight (BMI = 27.8 kg/m^2). This variant was documented in ClinVar with conflicting interpretations of pathogenicity and was classified as "probably damaging" in PolyPhen-2 (score 0.987) and as "deleterious" in SIFT (score 0).

All above mentioned rare missense variants were classified with high CADD scores (>20), indicating that are predicted to be functionally relevant, and positive GERP scores, suggesting they are under strong evolutionary constraint. Two additional rare missense variants were also found, each in one individual, NM_001145795.1 (SH2B1):c.296C > T (p.Ala99Val) (rs144126859) and NM_002303.6 (LEPR):c.976C > T (p.Arg326Cys) (rs771516479), both classified as "benign" and "tolerated" in Polyphen-2 and SIFT, respectively.

The two well-known *MC4R* variants NM_005912.2:c.751A > C(p.Ile251Leu) (rs52820871) and NM_005912.3:c.307G > A (p.Val103Ile) (rs2229616) were found in two and one individuals, respectively. These two variants were classified as "benign/likely benign" in ClinVar, as well as "benign" and "tolerated" in Polyphen-2 and SIFT, respectively.

3.2. Rare synonymous variants

On regards other additional rare variants, eight different synonymous variants were found in 12 patients, all at the heterozygous state (Table 3). One individual depicts the two variants rs193922651 and rs181883754. The four variants rs72792178, rs144491317, rs13306517 and rs181883754 were found in two different individuals each, increasing the probability of having some clinical significance.

3.3. Rare variants near splicing sites

Six intronic variants near canonical splice-sites (±10), putative affecting splicing, were found in genes *GCG*, *ADCY3*, *PCSK1*, *POMC* and *SH2B1* (Table 4). The *POMC* variant NM_000939.4:c.132+6T > C has not been described previously. The *SH2B1* variant NM_001145795.1: c.1513+5C > T (rs117918991), was found in three different individuals (frequency = 0.021) with severe obesity, without other identified rare variants, which suggest for a role in obesity.

4. Discussion

In this study we focused on rare variants in 12 candidate genes that could contribute to weight gain in 72 individuals with overweight or obesity (class 1 and class 2). Twenty-two different rare variants were identified in 30 individuals (41.67%), eight of them are coding missense variants (Table 1). The *in silico* tool analysis suggest functional significance in four of these variants (Table 2).

The *POMC* NM_000939.4:c.706C > G (p.Arg236Gly) (rs28932472) variant (MAF = 0.00259), was found at heterozygous state in two subjects which display obesity (BMI = 31.7 kg/m^2) and overweight (BMI = 27.8 kg/m^2) (Table 2). This variant is documented in ClinVar with conflicting interpretations of pathogenicity (uncertain significance and benign) and is predicted to be "probably damaging" in PolyPhen-2 and "deleterious" in SIFT. The CADD method for position Arg236 generates a high predictive score of 31, taken together with a highly evolutionary conservation (GERP score 1.9). This *POMC* coding missense variant was previously identified in heterozygous state in seven patients with obesity (Kleinendorst et al., 2018; Echwald et al., 1999) and in two children with severe early-onset obesity (Challis et al., 2002). The p.Arg236Gly substitution is predicted to disrupt a dibasic processing site between beta melanocyte-stimulating hormone (beta-MSH) and beta-endorphin (Challis et al., 2002). All these data suggest to the deleteriousness of

Table 2
Rare missense variants of potential interest identified at heterozygous state in a cohort of 72 adult individuals with overweight/obesity.

Chr position (hg38)	Gene	Cases	dbSNP	Variant	Consequence	Freq total (gnomAD)	ClinVar	*In silico* tools (Ensembl; VEP)			
								CADD	GERP	PolyPhen-2	SIFT
Chr1:65598786	LEPR	1	rs771516479	NM_002303.6:c.976C > T	p.Arg326Cys	0.000012	–	15.2	−1.84	B (0.34)	T (0.15)
Chr2:24841302	ADCY3	1	rs756783003	NM_004036.5: c.1153G > A	p.Val385Ile	0.000078	–	22.8	0.43	PD (0.804; 0.54)	T (0.06)
Chr2:24840006	ADCY3	1	rs201606553	NM_004036.5: c.1222G > A	p.Gly408Arg	0.000125	–	23.2	3.53	PD (0.673); PrD (0.994)	T (0.12)
Chr2:25161179	POMC	2	rs28932472	NM_000939.4:c.706C > G	p.Arg236Gly	0.00259	US/B	31	1.9	PrD (0.987)	D (0)
Chr16:28866221	SH2B1	1	rs547678855	NM_001145795.1: c.127C > A	p.Arg43Ser	0.0000085	–	24.5	–	PD (0.688; 0.859)	D (0.03)
Chr16:28866390	SH2B1	1	rs144126859	NM_001145795.1: c.296C > T	p.Ala99Val	0.00025	–	8.071	–	B (0)	T (0.55)
Chr18:60372043	MC4R	1	rs2229616	NM_005912.3:c.307G > A	p.Val103Ile	0.016	B	18.52	3.12	B (0.021)	T (0.5)
Chr18:60371599	MC4R	2	rs52820871	NM_005912.3:c.751A > C	p.Ile251Leu	0.0069	B/LB	18.81	3.94	B (0.005)	T (1)

Abbreviations: B, benign; LB, likely benign; T, tolerated; D, deleterious; PD, possibly damaging; PrD, Probably Damaging; DC, disease causing; US, Uncertain Significance; VEP, Variant Effect Predictor; CADD, Combined Annotation Dependent Depletion; GERP, Genomic Evolutionary Rate Profiling.

Table 3
Rare synonymous variants of potential interest identified at heterozygous state in a cohort of 72 adult individuals with overweight/obesity.

Chr position (hg38)	Gene	Cases	dbSNP	Variant	Protein	Freq total (gnomAD)	ClinVar	*In silico* tools	
								CADD	GERP
Chr1: 65636940	LEPR	1	rs193922651	NM_002303.6:c.3423C > T	p.Tyr1141 =	0.000159	LB	4.756	−0.25
Chr2: 24827904	ADCY3	1	rs139420185	NM_001320613.1:c.2430C > T	p.His810 =	0.00043	–	0.071	−7.26
Chr2: 24821548	ADCY3	2	rs72792178	NM_004036.5:c.3096C > T	p.Asn1032 =	0.00591	B	7.07	−3.24
Chr2:25164755	POMC	1	rs8192605	NM_000939.4:c.18C > T	p.Cys6 =	0.00582	US/B/LB	9.439	0.01
Chr6:100448598	SIM1	1	rs753737612	NM_005068.3:c.624G > A	p.Val208 =	0.0000518	US/LB	11.15	−1.31
Chr7:128252093	LEP	2	rs13306517	NM_000230.3:c.75A > G	p.Gln25 =	0.00775	B/LB	1.999	−3.34
Chr9: 85021281	NTRK2	2	rs144491317	NM_006180.6:c.2361C > A	p.Val787 =	0.00328	B	7.578	0.05
Chr11:74006980	UCP3	2	rs181883754	NM_003356.3:c.63C > G	p.Gly21 =	0.0000039	–	11.23	1.58

Abbreviations: B, benign; LB, likely benign; US, Uncertain Significance; CADD, Combined Annotation Dependent Depletion; GERP, Genomic Evolutionary Rate Profiling.

Table 4
Rare variants of potential interest located near splicing sites identified at heterozygous state in a cohort of 72 adult individuals with overweight/obesity.

Chr position (hg38)	Gene	Cases	dbSNP	Variant	Freq total (gnomAD)	Impact	ClinVar	*In silico* tools	
								CADD	GERP
Chr2:25164635	POMC	1	–	NM_000939.4:c.132+6T > C	–	Splicing	–	–	–
Chr2: 24830835	ADCY3	1	rs80261757	NM_004036.5:c.2056–10T > C	0.000525	Splicing	–	5.275	−2.39
Chr2:162147348	GCG	1	rs5649	NM_002054.5:c.254+5G > A	0.005	Splicing	B	18.90	1.29
Chr5: 96400195	PCSK1	1	rs200973203	NM_000439.5:c.1197-9C > T	0.00209	Splicing	US/B	15.53	0.63
Chr16:28871988	SH2B1	3	rs117918991	NM_001145795.1:c.1513+5C > T	0.0109	Splicing	–	0.821	–
Chr16:28873442	SH2B1	1	rs200470848	NM_001387430.1:c.1898-5T > C	0.00437	Splicing	B	14.67	–

Abbreviations: B, benign; LB, likely benign; US, Uncertain significance; CADD, Combined Annotation Dependent Depletion; GERP, Genomic Evolutionary Rate Profiling.

this missense mutation with a putative causal role in obesity. The POMC deficiency causes severe obesity that begins at an early age due to excessive feeding (hyperphagia) that remain for life (Kleinendorst et al., 2018). In our study, two other rare *POMC* variants were found in two subjects: the synonymous variant NM_000939.4:c.18C > T (p.Cys6 =) (rs8192605) (MAF = 0.00582) (Table 3) and a new rare variant near a splicing site (NM_000939.4:c.132+6T > C) (Table 4); however, further segregation analysis or functional studies are needed to evidence the pathogenicity of these mutations.

Two *ADCY3* rare coding missense variants were found at heterozygous state in two different individuals: the NM_004036.5:c.1222G > A (p.Gly408Arg) variant (MAF = 0.000125) (rs201606553), detected in one individual with overweight (BMI = 26.2 kg/m^2), and the NM_004036.5:c.1153G > A (p.Val385Ile) variant (MAF = 0.000078) (rs756783003), detected in one individual with severe obesity (BMI = 38 kg/m^2). These two missense variants herein identified have not been previously discussed in the context of obesity. However, the two *ADCY3* variants can be found in public databases (e.g., in Varsome), and the *in silico* tool Polyphen-2 reported the two missense mutations as "possible damaging" (Table 2). In the current investigation, we have also identified at heterozygous state two additional rare synonymous *ADCY3* variants in three individuals with obesity (Table 3) and one variant near a splice site in one individual (Table 4). Several *ADCY3* variants have been implicated as a potential cause of monogenic obesity (Saeed et al., 2018; Grarup et al., 2018; AbouHashem et al., 2022). Moreover, a SNP in *ADCY3* (rs11676272) has been correlated with obesity through GWAS (Stergiakouli et al., 2014). Adenylyl cyclase 3 (ADCY3) plays essential roles in the regulation of adiposity and glucose homeostasis. Specific inhibition of ADCY3 in the primary cilia resulted in increased food intake and significant weight gain (Tian et al., 2018). Although these findings may suggest functional significance of the two identified *ADCY3* rare missense variants in the present study, a definitive conclusion cannot be drawn in the absence of segregation analyses.

Two individuals were heterozygous carriers for two coding missense variants in the *SH2B1* gene: one individual for NM_001145795.1:c.127C > A (p.Arg43Ser) (rs547678855) (MAF = 0.0000085) and the second for NM_001145795.1:c.296C > T (p.Ala99Val) (rs144126859) (MAF = 0.00025). In particular, the p.Arg43Ser variant was classified as "possibly damaging" in Polyphen-2 and "deleterious" in SIFT, and the CADD tool generates a high predictive score of 24.5. Several *SH2B1* variants have been detected among obese children and adolescents with insulin resistance (Volckmar et al., 2012; Doche et al., 2012; Pearce et al., 2014; Saeed et al., 2022) and predicted (by PolyPhen-2) to have a functional effect (probably damaging) (Giuranna et al., 2018). The Src homology 2B adaptor protein 1 (SH2B1) is a signaling molecule downstream of the leptin receptor in the leptin-melanocortin pathway. This protein is recruited for neurotrophin receptors, including the neurotrophic receptor tyrosine kinase 2 (NTRK2), that binds the brain-derived neurotrophic factor (BDNF) (Lin and Li, 2021). It was shown that human obesity-associated *SH2B1* variants, increase expression of genes in the leptin (JAK/STAT or AKT) signaling pathway which implies that the main mode of action might affect leptin signaling rather than insulin signaling (Giuranna et al., 2018).

Two individuals depict the known variant in the *MC4R* gene NM_005912.3:c.751A > C (p.Ile251Leu) (MAF = 0.0069) (rs52820871), at the heterozygous state. Also, the well-known *MC4R* missense variant NM_005912.3:c.307G > A (p.Val103Ile) (rs2229616) (MAF = 0.016) was found in a third subject in the heterozygous state. However, both variants were reported in the literature through meta-analyses as ameliorating obesity (Heid et al., 2005; Stutzmann et al., 2007; Wang et al., 2010). Both minor alleles 251Leu and 103Ile have gain-of-function properties causing a slightly increased MC4R function, which is concordant with this weight-lowering effect (Lotta et al., 2019).

In conclusion, here, we present a large group of 72 individuals with overweight and obesity (class 1 and class 2) for which a diagnostic targeted NGS gene panel analysis was performed. The analysis of 12 genes (10 of them from the leptin-melanocortin pathway, previously demonstrated to be involved in monogenic obesity) allowed to identify likely pathogenic coding missense mutations in genes *ADCY3*, *SH2B1* and *POMC* for five subjects. Further segregation analyses or functional studies are needed to evidence the pathogenicity of these mutations. Of note, the analysis of *ADCY3*, the most recent identified gene as having a role in monogenic obesity, showed two potential pathogenic variants.

CRediT authorship contribution statement

Licínio Manco: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Janet Pereira:** Investigation, Methodology, Formal analysis. **Teresa Fidalgo:** Investigation, Supervision, Data curation. **Marina Cunha:** Investigation. **José Pinto-Gouveia:** Investigation. **Cristina Padez:** Funding acquisition, Writing – review & editing. **Lara Palmeira:** Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

The data that has been used is confidential.

Acknowledgements

This work was in part supported by the Fundação para a Ciência e a Tecnologia (FCT) under the CIAS institutional grant UIDB/00283/2020.

References

AbouHashem, N., Zaied, R.E., Al-Shafai, K., Nofal, M., Syed, N., Al-Shafai, M., 2022. The spectrum of genetic variants associated with the development of monogenic obesity in Qatar. Obes. Facts. https://doi.org/10.1159/000521851, 10.1159/000521851.

Argyropoulos, G., Brown, A.M., Willi, S., et al., 1998. Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. J. Clin. Invest. 102, 1345–1351.

Beckers, S., Zegers, D., Van Gaal, L.F., Van Hul, W., 2009. The role of the leptin-melanocortin signalling pathway in the control of food intake. Crit. Rev. Eukaryot. Gene Expr. 19, 267–287. https://doi.org/10.1615/critreveukargeneexpr.v19.i4.20. PMID: 19817705.

Challis, B.G., Pritchard, L.E., Creemers, J.W., et al., 2002. A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. Hum. Mol. Genet. 11, 1997–2004.

Doche, M.E., Bochukova, E.G., Su, H.W., 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.

Echwald, S.M., Sorensen, T.I., Andersen, T., et al., 1999. Mutational analysis of the proopiomelanocortin gene in Caucasians with early onset obesity. Int. J. Obes. Relat. Metab. Disord. 23, 293–298.

Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., O'Rahilly, S., 2003. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N. Engl. J. Med. 348, 1085–1095. https://doi.org/10.1056/NEJMoa022050.

Fidalgo, T., Martinho, P., Pinto, C.S., et al., 2017. Combined study of ADAMTS13 and complement genes in the diagnosis of thrombotic microangiopathies using next-generation sequencing. Res. Pract. Thromb. Haemost. 1, 69–80. https://doi.org/10.1002/rth2.12016.

Giuranna, J., Volckmar, A.L., Heinen, A., et al., 2018. The effect of SH2B1 variants on expression of leptin- and insulin-induced pathways in murine hypothalamus. Obes. Facts 11, 93–108. https://doi.org/10.1159/000486962.

Grarup, N., Moltke, I., Andersen, M.K., et al., 2018. Loss-of-function variants in ADCY3 increase risk of obesity and type 2 diabetes. Nat. Genet. 50, 172–174. https://doi.org/10.1038/s41588-017-0022-7.

Heid, I.M., Vollmert, C., Hinney, A., et al., 2005. Association of the 103I MC4R allele with decreased body mass in 7937 participants of two population based surveys. J. Med. Genet. 42, e21. https://doi.org/10.1136/jmg.2004.027011.

Kleinendorst, L., Massink, M.P.G., Cooiman, M.I., et al., 2018. Genetic obesity: next-generation sequencing results of 1230 patients with obesity. J. Med. Genet. 55, 578–586.

Kühnen, P., Krude, H., Biebermann, H., 2019. Melanocortin-4 receptor signalling: importance for weight regulation and obesity treatment. Trends Mol. Med. 25, 136–148. https://doi.org/10.1016/j.molmed.2018.12.002.

Lin, X., Li, H., 2021. Obesity: epidemiology, pathophysiology, and therapeutics. Front. Endocrinol. 12, 706978 https://doi.org/10.3389/fendo.2021.706978.

Lindquist, P., Madsen, J.S., Bräuner-Osborne, H., Rosenkilde, M.M., Hauser, A.S., 2021. Mutational landscape of the proglucagon-derived peptides. Front. Endocrinol. 12, 698511 https://doi.org/10.3389/fendo.2021.698511.

Loos, R.J.F., Yeo, G.S.H., 2022. The genetics of obesity: from discovery to biology. Nat. Rev. Genet. 23, 120–133. https://doi.org/10.1038/s41576-021-00414-z.

Lotta, L.A., Mokrosiński, J., Mendes de Oliveira, E., et al., 2019. Human gain-of-function MC4R variants show signaling bias and protect against obesity. Cell 177, 597–607. https://doi.org/10.1016/j.cell.2019.03.044 e9.

Pearce, L.R., Joe, R., Doche, M.E., 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.

Saeed, S., Bonnefond, A., Tamanini, F., et al., 2018. Loss-of-function mutations in ADCY3 cause monogenic severe obesity. Nat. Genet. 50, 175–179. https://doi.org/10.1038/s41588-017-0023-6.

Saeed, S., Janjua, Q.M., Haseeb, A., et al., 2022. Rare variant analysis of obesity-associated genes in young adults with severe obesity from a consanguineous population of Pakistan. Diabetes, db210373. https://doi.org/10.2337/db21-0373.

Silventoinen, K., Rokholm, B., Kaprio, J., Sorensen, T.I., 2010. The genetic and environmental influences on childhood obesity: a systematic review of twin and adoption studies. Int. J. Obes. 34, 29–40. https://doi.org/10.1038/ijo.2009.177.

Stergiakouli, E., Gaillard, R., Tavaré, J.M., et al., 2014. Genome-wide association study of height-adjusted BMI in childhood identifies functional variant in ADCY3. Obesity 22, 2252–2259. https://doi.org/10.1002/oby.20840.

Stutzmann, F., Vatin, V., Cauchi, S., et al., 2007. Non-synonymous polymorphisms in melanocortin-4 receptor protect against obesity: the two facets of a Janus obesity gene. Hum. Mol. Genet. 16, 1837–1844. https://doi.org/10.1093/hmg/ddm132.

Tian, Y., Peng, B., Fu, X., 2018. New ADCY3 variants dance in obesity etiology. Trends Endocrinol. Metabol. 29, 361–363. https://doi.org/10.1016/j.tem.2018.02.004.

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, 65. https://doi.org/10.1186/1755-8794-5-65.

Wade, K.H., Lam, B.Y.H., Melvin, A., et al., 2021. Loss-of-function mutations in the melanocortin 4 receptor in a UK birth cohort. Nat. Med. 27, 1088–1096. https://doi.org/10.1038/s41591-021-01349-y.

Wang, D., Ma, J., Zhang, S., et al., 2010. Association of the MC4R V103I polymorphism with obesity: a Chinese case control study and meta-analysis in 55,195 individuals. Obesity 18, 573–579.

Wardle, J., Carnell, S., Haworth, C.M., Plomin, R., 2008. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am. J. Clin. Nutr. 87, 398–404. https://doi.org/10.1093/ajcn/87.2.398.

Yeo, G.S.H., Chao, D.H.M., Siegert, A.M., et al., 2021. The melanocortin pathway and energy homeostasis: from discovery to obesity therapy. Mol. Metabol. 48, 101206 https://doi.org/10.1016/j.molmet.2021.101206.