

## **Heterozygous pathogenic variants in *POMC* are not responsible for monogenic obesity: implication for MC4R agonist use**

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## Abstract

**Purpose.** Recessive deficiency for proopiomelanocortin (POMC) causes childhood-onset severe obesity. Cases can now benefit from the MC4R agonist setmelanotide. Furthermore, a phase 3 clinical trial is evaluating setmelanotide in heterozygotes for *POMC*. We performed a large-scale genetic analysis assessing the effect of heterozygous, pathogenic *POMC* variants on obesity.

**Methods.** A genetic analysis was performed in a family including two cousins with childhood-onset obesity. We analyzed the obesity status of heterozygotes for pathogenic *POMC* variants in the Human Gene Mutation Database (HGMD). The association between heterozygous, pathogenic *POMC* variants and obesity risk was assessed using 190,000 exome samples from UK Biobank.

**Results.** The two cousins carried a compound heterozygous, pathogenic variant in *POMC*. Six siblings were heterozygotes; only one of them had obesity. In HGMD, we identified 60 heterozygotes for pathogenic *POMC* variants, of whom 14 had obesity. In UK Biobank, heterozygous, pathogenic *POMC* variants were not associated with obesity risk, but modestly increased body mass index levels.

**Conclusions.** Heterozygous, pathogenic *POMC* variants do not contribute to monogenic obesity, but slightly increase BMI. Setmelanotide use in patients with obesity which would only be based on the presence of a heterozygous *POMC* variant can be questioned.

*Keywords:* obesity; Hypocortisolism; POMC; Heterozygous; Variant.

## Introduction

Obesity and overweight are global public health problems, and 671 million individuals (12% of the world's adult population) suffer from obesity (NCD Risk Factor Collaboration Data) <sup>1</sup>. Obesity is a multifactorial background <sup>1,2</sup>. It includes rare, monogenic forms mostly due to pathogenic variants in genes involved in the leptin-melanocortin pathway (including *MC4R*, *LEP*, *LEPR*, *POMC*, *PCSK1*, etc.) which regulates food intake <sup>2,3</sup>. These monogenic forms are characterized by severe early-onset weight gain, diversely associating eating behavior disorders, neurodevelopmental disorders, pituitary deficiencies, and polymalformative syndromes <sup>2</sup>.

*POMC* deficiency was firstly described in 1998 <sup>4</sup>. *POMC* encodes proopiomelanocortin that is the precursor of numerous biologically active peptides including  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH) and adrenocorticotrophic hormone (ACTH);  $\alpha$ -MSH is the ligand for melanocortin 4 receptor (MC4R) and melanocortin 1 receptor (MC1R), and ACTH binds to MC2R (melanocortin 2 receptor) <sup>5</sup>. The patients carrying biallelic pathogenic *POMC* variants present with severe early-onset obesity along with a neonatal corticotrophic insufficiency, sometimes associated with skin hypopigmentation with red hair <sup>4</sup>. In these cases, obesity was ascribed to a loss of inhibitory tone of appetite via the lack of ligation of  $\alpha$ -MSH to MC4R; neonatal hypocortisolism was related to the absence of ligation of ACTH to MC2R; and skin hypopigmentation with red hair was explained by the lack of ligation of  $\alpha$ -MSH to MC1R <sup>5</sup>. Other endocrine features such as central hypothyroidism and hypogonadism may also be present <sup>6</sup>.

Patients who suffered from severe, early-onset obesity can now be treated by molecules targeting the melanocortin pathway. ObeRar cohort aims to personalize individual care management using innovative therapies (ClinicalTrials.gov Identifier: NCT04604626). The MC4R agonist setmelanotide has been proven to be effective in patients with obesity who

were recessively deficient for *POMC*, *LEPR* and *PCSK1*<sup>3,7,8</sup>; and consequently, this treatment has recently been approved by the United States Food and Drug Administration and the European Medicines Agency<sup>8</sup>. Setmelanotide is currently being evaluated in patients with obesity who carry rare, heterozygous *POMC* variants (ClinicalTrials.gov Identifier: NCT05093634). However, the effect of heterozygous *POMC* variants on obesity (including severe, early-onset forms) is still elusive.

To address this question, we analyzed a large non-consanguineous family, including two cousins who suffered from severe obesity and neonatal hypocortisolism, along with progressive multiple pituitary deficiencies. We identified three heterozygous, pathogenic *POMC* variants in this family; the two cousins carrying a compound heterozygous *POMC* variant as expected. We then carefully analyzed the heterozygotes of the family; and we performed a review of the literature. We finally used UK Biobank exome data to analyze the effect of heterozygous, pathogenic *POMC* variants on obesity risk and body mass index (BMI) levels.

## **Materials and Methods**

**Study participants.** We collected clinical data of two first cousins (Patient A and Patient B) from pediatric and adult medical records related to endocrinology. We reported family data focusing on the heterozygotes for pathogenic *POMC* variants. Following French laws, we obtained the consents from patients' and their relatives'.

We performed a review of the literature and gathered clinical and biological data of the heterozygotes ( $n=60$ ) for rare, pathogenic *POMC* variants from Human Gene Mutation Database (HGMD Pro 2022 v3).

We used participants ( $n=187,727$  individuals) with available exome data from UK Biobank. This research is part of UK Biobank research application #67575.

In HGMD, obesity in adults was classified into three categories (according to Centers for Disease Control and Prevention): Class I obesity (or 'obesity'), BMI of 30-34.9 kg/m<sup>2</sup>; Class II obesity, BMI of 35-39.9 kg/m<sup>2</sup>; Class III obesity, BMI  $\geq 40$  kg/m<sup>2</sup>. Severe obesity was defined by BMI  $\geq 35$  kg/m<sup>2</sup> in adults.

Classification of childhood obesity was done according to available data. Childhood obesity was defined by  $\geq 2$  SDS-BMI (with z-score, according to World Health Organization) or a BMI  $\geq 95$ th percentile (according to Centers for Disease Control and Prevention). Severe obesity was defined by a  $\geq 3$  SDS-BMI (z-score), BMI  $\geq 120\%$  of the 95th percentile or BMI  $\geq 35$  kg/m<sup>2</sup> (according to Centers for Disease Control and Prevention).

In UK Biobank the case control for adult obesity was defined, with cases having BMI  $\geq 30$  kg/m<sup>2</sup> and controls having BMI  $< 25$  kg/m<sup>2</sup>. The case control for adult overweight was defined with cases having BMI  $\geq 25$  kg/m<sup>2</sup> and controls having BMI  $< 25$  kg/m<sup>2</sup>. BMI data outliers were excluded using Tukey methods (quartiles +/- 3 times IQR).

**Genetic analyses in the family.**

**Karyotyping.** Conventional cytogenetic analysis was performed on peripheral blood lymphocytes from Patient A using the 550-band including GTG (G-bands after trypsin and Giemsa) and RHG (R-bands by heating using Giemsa) banding for family members.

**DNA microarray.** Array comparative genomic hybridization (CGH) was performed in Patient A using Agilent 8×60k oligoarrays (SurePrint G3 Human CGH Microarray 8×60k). Random primer labelling and hybridization were carried out with sex-matched reference DNA according to the manufacturer's recommendations and results were analyzed using cytogenomics (v4.0.2.21) software (Agilent Technologies).

**Sanger sequencing.** Genomic DNA was PCR-amplified using sets of flanking intronic primers for direct sequencing of *TBX19*, *PROPI* and *LHX3* as previously described for Patient A<sup>9</sup>. Amplification was carried out for this patient, using the Hot Start Taq polymerase kit protocol (Qiagen). Sequencing was assessed with a 3130 XL Genetic analyzer (Life Technologies). Sequences were analyzed using Variant Reporter software (Life Technologies)<sup>10</sup>. A solo strategy was used for Patient B, following protocols which were previously described<sup>11</sup> to analyse *PIT1*, *PROPI*, *OTX2* and *GLI2*. POMC-targeted sequencing was performed as a second step in Patient B following standard protocols.

**Exome sequencing and analysis of siblings.** Exome sequencing was performed in Patient A. For this purpose, we used Human Core Exome Kit (Twist Bioscience) and Illumina sequencing (on the NovaSeq6000 system). Sequence reads were aligned to the Human Genome Reference Sequence (GRCh38). Based on the clinical data of Patient A, we analyzed 114 genes linked with monogenic obesity. Sanger sequencing focusing on *POMC* (NM\_001035256.3) was then performed in the paternal grandparents of Patient A, her mother, her brother, and the close family of Patient B (including seven siblings in total). Primer sequences and PCR conditions are available upon request.

**Analyses of genetic variants.** To assess the pathogenicity of the variants we used the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) <sup>12</sup>. For the supporting pathogenic criterion PP3, we used PolyPhen-2 (HumDiv), SIFT and Mutation Taster. Variants addressing the supporting pathogenic criterion PP4 were previously reported homozygous or compound heterozygous in patients with severe early-onset obesity and adrenal insufficiency. Variants addressing the supporting pathogenic criterion PP5 have already been reported pathogenic or likely pathogenic in ClinVar and/or HGMD database (Pro 2022 v3). None of the pathogenic or likely pathogenic (P/LP) variants reported in the present manuscript had a minor allele frequency (MAF) > 0.1% in the Genome Aggregation Database (gnomAD; v2.1.1).

**Genetic analyses in UK Biobank.**

***Detection of P/LP POMC variants.*** We used exome data from pVCF format (field #23156). All P/LP POMC variants were heterozygous. All P/LP POMC variants had a coverage higher than 16 reads and a quality GQ score higher than 40.

***Statistical analyses for association studies.*** We used the MiST method <sup>13</sup> to assess the effect of P/LP POMC variants on overweight or obesity and BMI, as previously described <sup>14</sup>. Briefly, heterozygous P/LP POMC variants were analyzed as a single cluster. We obtained a score statistics  $S(\pi)$  for the mean effect ( $\pi$ ) of the study population. These analyses were adjusted for age, sex, and ancestry (*i.e.* first five genetic principal components available in the field #22009). The BMI data were not normally distributed so we use the log-transformed to get a normal shape. As none analyses had a significant p-value related to the heterogeneity of the cluster ( $\tau$ ), we only showed p-values related to the effect of the cluster ( $\pi$ ) below.

## Results

### Genetic investigation of the two cousins with childhood-onset severe obesity and hypocortisolism

We describe the phenotypes of two first cousins (Patient A and Patient B) from a nonconsanguineous family, referred for neonatal hypocortisolism associated with early-onset and severe obesity (**Table 1**). The weight growth chart revealed a  $> 3$  SDS-BMI at nine months for Patient A (**Supplementary Figure 1**) and  $> 3$  SDS-BMI at 15 months for Patient B (**Supplementary Figure 2**). Additionally, Patient A had drug-resistant epilepsy, possibly due to these episodes of neonatal hypoglycemia. Patient B suffered from psychomotor sequelae possibly due to cerebral anoxia and convulsive crises. They subsequently suffered from combined pituitary hormonal deficiency (CPHD) (**Table 1**). Their skin color was pale but not associated with red hair (**Table 1**).

In Patient A, the first genetic investigations including karyotyping, CGH-array, and targeted sequencing of genes linked with hypopituitarism failed to find any P/LP genetic event. In Patient B, genetic investigations also failed to find a genetic etiology. At the age of 28 years, Patient B was found to carry a compound heterozygous pathogenic variant in *POMC* (NM\_001035256.3:c.416dup p.(Tyr139\*) and c.84C>A p.(Cys28\*)) (**Supplementary Figure 3**). These two variants were genetically null and were not present in GnomAD (**Supplementary Table 1**). As the clinical presentations were similar in the two cousins, a targeted *POMC* sequencing was then performed in Patient A. This patient only shared c.416dup p.(Tyr139\*) variant, which was inherited from her mother (**Figure 1**). As a single heterozygous *POMC* variant was unlikely to explain alone Patient A's severe early-onset obesity and hypocortisolism, we performed exome sequencing that revealed a second pathogenic *POMC* variant (c.-11C>A; **Supplementary Figure 3; Supplementary Table 1**).



This non-coding variant has already been shown to be pathogenic in the literature via *in vitro* assays<sup>4,15</sup>, and is very rare in GnomAD (MAF of  $2.8 \times 10^{-5}$ ).

Following the genetic diagnosis of *POMC* deficiency, daily setmelanotide injections were administered to both patients and led to significant decrease in weight and feeling of hunger. Notable side effects have been melanocyte hyperstimulation, responsible for melanoderma in both patients.

### **Familial description of siblings carrying a heterozygous pathogenic variant in *POMC***

In the family, we then investigated the phenotypes of the heterozygotes for P/LP *POMC* variants. Indeed, when the heterozygotes have severe, early-onset obesity, they may be eligible for setmelanotide treatment according to the current clinical trial (NCT05093634). Only one out of six heterozygotes of the family for P/LP *POMC* variants presented with obesity (**Figure 1**). However, Patient A's father only suffered from mild obesity Class I, which occurred during adulthood. Unfortunately, he passed away, and we were not able to collect further information or perform a genetic test. Patient A's mother who carried c.416dup p.(Tyr139\*) variant did not present with overweight (BMI of 20 kg/m<sup>2</sup>) at 61 years (**Figure 1**). Her hormonal profile was normal (data not shown). Patient A's brother who carried the c.-11C>A variant had a normal weight (BMI of 24 kg/m<sup>2</sup>) at 29 years old (**Figure 1**). His hormonal workup showed no pituitary deficiency (data not shown). No other heterozygotes for a P/LP *POMC* variant had overweight (BMI value between 20 and 24 kg/m<sup>2</sup>). We did not have the current BMI of one of the heterozygotes in the family, but she did not report history of obesity (Patient I.2; **Figure 1**). The median BMI value in the family was normal at  $24 \pm 4.2$  kg/m<sup>2</sup>. In conclusion, in this family with strong evidence of variants in *POMC* causing obesity, the heterozygote state was not associated with severe, childhood obesity or with pituitary dysfunction.

### **Effect of rare, heterozygous P/LP *POMC* variants on obesity**

To support further these negative findings, we then questioned the contribution of rare, heterozygous P/LP *POMC* variants to obesity in various cohorts. We first collected from the literature the data linked to obesity on heterozygotes for P/LP *POMC* variants. We noticed 16 heterozygous P/LP *POMC* variants in HGMD (**Table 2**), that were carried by 60 patients (including c.-11C>A variant) (**Supplementary Table 1**). Among these patients, only 14 had obesity (23%), 27 had overweight (45%) and 19 had normal weight (32%) (**Table 2**). Among the 11 adults with obesity, only two patients had Class III obesity, and three patients had Class II obesity. Among the three children with obesity, only one children had severe obesity (**Table 2**). Obesity was less severe and the onset was later in heterozygotes when compared to carriers of biallelic P/LP *POMC* variants (data not shown). No heterozygotes were reported with pituitary hormone deficiency or intellectual disability (data not shown). Therefore, even in the literature linked to obesity, carrying a heterozygous P/LP *POMC* variant does not lead to a dominant form of obesity.

We finally analyzed the 190K exome samples from UK Biobank. We identified 17 rare, heterozygous P/LP variants in *POMC* (**Supplementary Tables 1 and 2**). Among the heterozygotes for a rare, P/LP *POMC* variant, 39.2% had obesity while the obesity rate was 42.4% in non-heterozygotes. When adjusting for age, sex and ancestry the burden of rare, P/LP *POMC* variants did not have a significant effect on obesity risk ( $P=0.47$  with an odds ratio [OR] of 0.84, 95% confidence interval [CI]: 0.51-1.3; **Table 3**). Furthermore, we did not find a significant association between risk of overweight or obesity and the burden of rare, P/LP *POMC* variants (68.8% overweight or obesity in heterozygotes *versus* 67.0% overweight or obesity in non-heterozygotes;  $P=0.57$  with an OR of 1.1, 95% CI: 0.78-1.6; **Table 3**). In contrast, the burden of rare, heterozygous P/LP *POMC* variants was only

significantly associated with a slight increase of BMI (26.8 kg/m<sup>2</sup> in heterozygotes *versus* 26.7 kg/m<sup>2</sup> in non-heterozygotes;  $P=8.5\times 10^{-5}$  with an effect [ $\pi$ ] of  $0.070 \pm 0.018$ ; **Table 3**).

## Discussion

Our data seem to rule out that rare, heterozygous P/LP variants in *POMC* cause a dominant form of monogenic obesity, which was illustrated by the description of a large family with three different rare P/LP *POMC* variants and different heterozygotes from large databases (*i.e.* HGMD and UK Biobank).

In the literature linked to obesity, the putative association between obesity and heterozygous P/LP variants in *POMC* is highly debatable as based on case reports with heterogeneous criteria for pathogenicity evaluation. Indeed, in most cases, the patients described carried heterozygous variants of uncertain significance (VUS) or variants with a high MAF in GnomAD<sup>16-24</sup>. We have identified only a few reports suggesting a possible association between Class I obesity and heterozygous *POMC* in children<sup>16,22</sup>. Another study suggested a relationship between several heterozygous variants in *POMC* and severe obesity<sup>24</sup>. However, the classification of variants may be questionable by the fact that the authors classified the variants into three groups according to their functional effects (high / moderate / low functional impact) predicted by several software programs (PolyPhen-2, UMD-Predictor, Mutation Assessor, and Align GVGD)<sup>24</sup>. This classification can be challenged in view of the lack of reliability, due to the grouping of software programs and the establishment of a random threshold to define a damaging effect of the variants.

In our family, Patient A carries the heterozygous variant c.416dup p.(Tyr139\*). This variant had not previously been reported in the literature. Moreover, it was not found in UK Biobank. In contrast, the second cryptic c.-11C>A variant carried by Patient A has been identified in previous studies as pathogenic based on functional *in vitro* studies<sup>4,15</sup>. This variant was found

in heterozygotes suffering from obesity in one study<sup>24</sup>. However, its impact on obesity in the heterozygous state seems modest, given its presence in six individuals with normal weight in the HGMD database (**Table 2**). Of the 60 heterozygotes of the P/LP *POMC* variants reported in HGMD, only 14 had obesity (including 5 with severe obesity). In addition, these results are obviously confounded by the primary recruitment of index cases with biallelic, P/LP *POMC* variants within families with obesity. To assess the impact of these variants on BMI and obesity risk, we overcame this issue by studying exome data from UK biobank that is a large population study. The heterozygotes for a rare, P/LP *POMC* variant were not more likely to have obesity than the non-heterozygotes. However, we only found a very modest increase in BMI (0.1 unit of BMI) associated with these variants. This effect was comparable to the effect of a common single nucleotide polymorphism found by a genome-wide association study.

In conclusion, we have no evidence from both family study and the general population that carrying a single heterozygous P/LP variant in *POMC* is causing a dominant form of (severe) obesity or is associated with a higher risk of obesity. These results have implications in precision obesity medicine as the novel MC4R agonist setmelanotide is used with great efficacy in *POMC*-deficient patients (homozygotes or compound heterozygotes)<sup>8</sup>, as we also evidenced in Patients A and B. Given the absence of demonstrated association between rare heterozygous P/LP *POMC* variants and the development of obesity, the use of setmelanotide in patients with severe obesity which would only be based on the presence of a heterozygous, pathogenic *POMC* variant can be questioned. The present study is limited by the fact that our analysis was focused on P/LP variants only. Functional studies of all heterozygous *POMC* variants associated with a more in-depth phenotyping of each heterozygote would allow us to confirm the present data.

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## **Data Availability**

Identified data have been reported including figures, tables, and supplementary records. Some information is available upon request: Primer sequences, PCR conditions, and hormonal profile of two heterozygotes within the family. Data concerning heterozygotes for *POMC* compared to carriers of biallelic P/LP *POMC* variants are also available upon request.

## **Conflict of Interest**

Authors declare that they have no competing interests.

## **Ethics declaration**

All procedures followed were following the ethical standards of the committee responsible for human experimentation (institutional and national) and the 1975 Declaration of Helsinki, as revised in 2000. Informed consent for genetic testing was required from all individuals reported. This report does not qualify for submission to an IRB or REC.

### **Author Contributions and Guarantor Statement**

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**Table 1. Clinico-biological characteristics of Patients A and B from one family.**

		<b>PATIENT A</b>		<b>PATIENT B</b>	
<b>Sex</b>		Female		Male	
<b>Compound heterozygous POMC variants</b>	<b>(NC_000002.12)</b>	25164783G>T	25161469dup	25164689G>T	25161469dup
	<b>(NM_001035256.3)</b>	c.-11C>A	c.416dup	c.84C>A	c.416dup
	<b>(NP_001030333.1)</b>	p.(?)	p.(Tyr139*)	p.(Cys28*)	p.(Tyr139*)
<b>ACMG criteria</b>		Likely pathogenic (PS3, PM2, PP4, PP5)	Pathogenic (PVS1, PM2, PP4, PP5)	Pathogenic (PVS1, PM2, PP4, PP5)	Pathogenic (PVS1, PM2, PP4, PP5)
<b>Age of obesity onset</b>		>+3SD at 9 months		>+3SD at 15 months	
<b>BMI before MC4R agonist administration</b>		43 kg/m <sup>2</sup>		42 kg/m <sup>2</sup>	
<b>Hypocortisolism</b>		Yes (at first week of life)		Yes (at first week of life)	
<b>Neonatal Hypoglycemia</b>		Yes (at first week of life)		Yes (at first week of life)	
<b>Icterus</b>		Yes (at first week of life)		Unknown	
<b>Hypothyroidism</b>		Yes (at one year)		Yes (between 3 and 6 months)	
<b>Growth hormone deficiency</b>		Yes (at 8 years)		Yes (between 3 and 6 months)	
<b>Hypogonadism</b>		Yes (no puberty)		Yes (no puberty)	
<b>Red Hair</b>		No		No	
<b>Skin color</b>		Pale		Pale	
<b>Others features</b>		Drug-resistant epilepsy		Severe convulsions complicated hypoglycemia	

**Table 2. Obesity status of patients carrying a heterozygous, P/LP *POMC* variant from HGMD.**

<b><i>POMC</i> variant (NM_001035256.3)</b>	<b><i>POMC</i> variant (NP_001030333.1)</b>	<b>Location (NC_000002.12)</b>	<b>ACMG criteria</b>	<b><math>N_{Ob}</math> (<math>N_{SevereOb}</math>)</b>	<b><math>N_{Owt}</math></b>	<b><math>N_{Nwt}</math></b>	<b>Ref</b>
c.-11C>A	p.(?)	25164783G>T	PS3, PM2, PP4, PP5	0 (0)	0	6	4,25–27
c.44C>G	p.(Ala15Gly)	25164729G>C	PS3, PM2, PP1, PP5	1 (0)	5	0	28
c.83G>T	p.(Cys28Phe)	25164690C>A	PS3, PM2, PP3, PP5	1 (1)	2	0	29
c.109C>T	p.(Leu37Phe)	25164664G>A	PS3, PM2, PP3, PP5	2 (0)	2	0	29,30
c.151A>T	p.(Lys51*)	25161734T>A	PVS1, PM2, PP4, PP5	0 (0)	1	0	25
c.206del	p.(Pro69Leufs*2)	25161680del	PVS1, PM2, PP4, PP5	6 (3)	8	2	31–33
c.223dup	p.(Arg75Profs*44)	25161666dup	PVS1, PM2, PP4, PP5	1 (0)	1	5	34
c.231C>A	p.(Tyr77*)	25161654G>T	PVS1, PM2, PP4, PP5	0 (0)	1	1	35
c.256C>T	p.(Arg86*)	25161629G>A	PVS1, PM2, PP4, PP5	0 (0)	1	1	36
c.296del	p.(Gly99Alafs*59)	25161590del	PVS1, PM2, PP4, PP5	0 (0)	1	0	25
c.313G>T	p.(Glu105*)	25161572C>A	PVS1, PM2, PP4, PP5	0 (0)	1	0	4,25
c.400_401insGG	p.(Glu134Glyfs*25)	25161485_25161486insCC	PVS1, PM2, PP4, PP5	0 (0)	1	0	25
c.430T>C	p.(Phe144Leu)	25161455A>G	PS3, PM1, PP3, PP5	2 (0)	1	2	18,37
c.433C>T	p.(Arg145Cys)	25161452G>A	PS3, PM1, PM2, PP3, PP4, PP5	0 (0)	2	1	27
c.433del	p.(Arg145Alafs*13)	25161453del	PVS1, PM2, PP4, PP5	0 (0)	0	1	4,25
c.605_616delAGGCCGACCTGGinsGGGCCAGGC CGACGTGT	p.(Gln202_Glu206delinsArgAlaGlnAlaAspVal*)	25161280-25161269delins18	PVS1, PM2, PP5	1 (1)	0	0	32
<b>TOTAL</b>				<b>14 (5)</b>	<b>27</b>	<b>19</b>	

$N_{Nwt}$ , number of heterozygotes with normal weight (*i.e.* with BMI < 25 kg/m<sup>2</sup>);  $N_{Ob}$ , number of heterozygotes with obesity (*i.e.* with BMI ≥ 30 kg/m<sup>2</sup> in adults and standardised BMI [SDS] ≥ 2 or a BMI ≥ 95th percentile in children);  $N_{Owt}$ , number of heterozygotes with



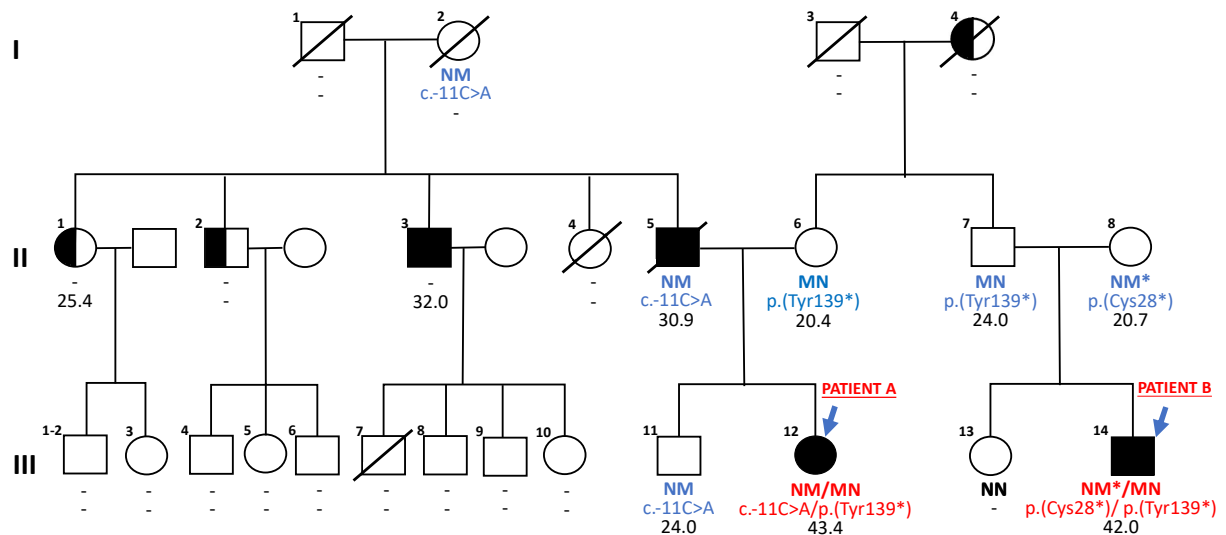
overweight (*i.e.* with BMI between 25 and 30 kg/m<sup>2</sup>);  $N_{SevereOb}$ , number of heterozygotes with severe obesity (*i.e.* with BMI  $\geq 35$  kg/m<sup>2</sup> in adults and SDS  $\geq 3$  or BMI  $\geq 120\%$  of the 95th percentile or BMI  $\geq 35$  kg/m<sup>2</sup> in children); **Ref**, references.

**Table 3. Association of rare, heterozygous, pathogenic *POMC* variants with adiposity, in UK Biobank.**

Trait	<i>N</i>	% or median [IQR] of the trait in heterozygotes	% or median [IQR] of the trait in non-heterozygotes	OR [CI]	Effect ± SE	<i>P</i> -value
BMI	187,727	26.8 [24.5-29.2]	26.7 [24.1-29.8]	-	0.070 ± 0.018	8.5×10 <sup>-5</sup>
CC OWT	187,222	68.8	67.0	1.1 [0.78-1.6]	-	0.57
CC OB	107,219	39.2	42.4	0.84 [0.51-1.3]	-	0.47

Association analyses were performed using the MiST method adjusted for age, sex, and ancestry. BMI was logarithmically before analysis. *BMI*, body mass index; *CC OB*, case-control analysis for obesity (participants with obesity *versus* participants with normal weight); *CC OWT*, case-control analysis for overweight or obesity (participants with overweight or obesity *versus* participants with normal weight); *CI*, confidence interval; *IQR*, interquartile range; *OR*, odds ratio; *SE*, standard error.

**Figure 1. Pedigree with three rare, pathogenic *POMC* variants, including Patient A and Patient B with a compound heterozygous *POMC* variant.**



Pedigree of the family shows the weight status of each member, as well as genetic status. Under each member, we have shown the genetic status when it is known, then the BMI of the individual. Circles represent female participants and squares male participants. A slash through the symbol indicates that the family member is deceased. Black symbols represent patients with obesity. The half-filled indicates individuals with overweight. Patient III.12 was Patient A and Patient III.14 was Patient B.

*NM*, heterozygous variant c.-11 C>A; *MN*, heterozygous variant c.416dup p.(Tyr139\*); *NM\**, heterozygous variant c.84C>A p.(Cys28\*); *NM/MN* or *NM\*/MN*, compound heterozygous variant; *NN*, non-carriers; -, unavailable data.

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