Rare melanocortin-3 receptor mutations with \textit{in vitro} functional consequences are associated with human obesity

Monica Mencarelli$^1$, Beatrice Dubern$^{2,3,4}$, Rohia Alili$^{2,3,4}$, Sabrina Maestrini$^1$, Lina Benajiba$^{2,3,4}$, Mariantonella Tagliaferri$^6$, Pilar Galan$^7$, Maura Rinaldi$^6$, Chantal Simon$^{8,9}$, Patrick Tounian$^{2,3,4}$, Serge Hercberg$^7$, Antonio Liuzzi$^6$, Anna Maria Di Blasio$^1$ and Karine Clement$^{2,3,4}$

$^1$Molecular Biology Laboratory, Istituto Auxologico Italiano, 28921 Verbania, Italy, $^2$INSERM, U-872, Nutriomique (team 7) 75006 Paris, France, $^3$University Pierre and Marie Curie-Paris 6, Cordeliers Research Center, 75006 Paris, France, $^4$Assistance-Publique Hôpitaux de Paris, Research Center on Human Nutrition, Pitié-Salpêtrière Hospital, 75013 Paris, France, $^5$Gastroenterology and Nutrition Department, Armand-Trousseau hospital, Assistance-Publique Hôpitaux de Paris, 75012 Paris, France, $^6$Istituto Auxologico Italiano, Department of Internal Medicine, 28921 Verbania, Italy, $^7$INSERM U557, UMR, INRA U1125, CNAM, CRNH-IdF, Bobigny, Public Health Department, Avicenne Hospital and University Paris 13, Bobigny, France, $^8$INSERM U-870, INRA U-1235, Human Nutrition Research Center, Hospices Civils de Lyon, University of Lyon, Oullins, France and $^9$Louis Pasteur University of Strasbourg, Medical Faculty, EA 1801, 67000 Strasbourg, France

Received September 22, 2010; Revised October 11, 2010; Accepted October 21, 2010

In contrast to the melanocortin 4 receptor, the possible role of the melanocortin 3 receptor (MC3R) in regulating body weight is still debated. We have previously reported three mutations in the \textit{MC3R} gene showing association with human obesity, but these results were not confirmed in a study of severe obese North American adults. In this study, we evaluated the entire coding region of \textit{MC3R} in 839 severely obese subjects and 967 lean controls of Italian and French origin. \textit{In vitro} functional analysis of the mutations detected was also performed. The total prevalence of rare \textit{MC3R} variants was not significantly different in obese subjects when compared with controls ($P = 0.18$). However, the prevalence of mutations with functional alterations was significantly higher in the obese group ($P = 0.022$). In conclusions, the results of this large study demonstrate that in the populations studied functionally significant MC3R variants are associated with obesity supporting the current hypothesis that rare variants might have a stronger impact on the individual susceptibility to gain weight. They also underline the importance of detailed \textit{in vitro} functional studies in order to prove the pathogenic effect of such variants. Further investigations in larger cohorts will be needed in order to define the specific phenotypic characteristics potentially correlated with reduced MC3R signalling.

**INTRODUCTION**

The widespread increase in the prevalence of obesity is certainly driven by environmental changing, but it is now well recognized that genetic susceptibility may influence the outcome (1,2). Family and twin studies have shown that genetic factors contribute 40–70% to the variation in BMI (3,4). In recent years, genome-wide association studies have highlighted that several common variants with small effects (5–8) contribute to the genetic predisposition to obesity and that, based on their allelic frequencies, the population attributable risk must be taken into consideration. In addition, rare variants associated, in mice and in humans, with a phenotype of severe early-onset obesity have been reported in genes coding for molecules participating in the pathways that regulate energy homeostasis (9–20). Among these genes,
Table 1. Anthropometric characteristics of the subjects studied

<table>
<thead>
<tr>
<th></th>
<th>Obese subjects</th>
<th>Normal weight subjects</th>
<th>SUVIMAX population (n = 416)</th>
<th>ICAPS population (n = 337)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>French children (n = 496)</td>
<td>Italian adults (n = 343)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.6 ± 4.5</td>
<td>11.2 ± 0.7</td>
<td>51 ± 0.31</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>319/160</td>
<td>294/94</td>
<td>290/126</td>
<td>142/72</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.35 ± 7.3</td>
<td>44.8 ± 7.0</td>
<td>22.2 ± 0.1</td>
<td>22.4 ± 2.7</td>
</tr>
</tbody>
</table>

Table 2. Summary of rare variants identified in the coding region of MC3R gene in French and Italian cases and controls

<table>
<thead>
<tr>
<th>Nucleotide variation</th>
<th>Amino acid change</th>
<th>Obese subjects</th>
<th>Normal weight subjects</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>French (n = 496)</td>
<td>Italian (n = 343)</td>
<td>French (n = 753)</td>
</tr>
<tr>
<td>T-4C</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>G50C</td>
<td>S17T</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>T260C</td>
<td>I87T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G472T</td>
<td>D158Y</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G529A</td>
<td>V177I</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C745T</td>
<td>L249F</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C769A</td>
<td>R257S</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C839G</td>
<td>T280S</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C853G</td>
<td>L285V</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G877A</td>
<td>A293T</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1003A or I335S</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1004C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1082C</td>
<td>X361S</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9/496 (1.81%)</td>
<td>4/343 (1.16%)</td>
<td>7/753 (0.92%)</td>
<td>1/214 (0.46%)</td>
</tr>
</tbody>
</table>

*Mutations already described by Calton et al. (22).
*Mutations reported by Mencarelli et al. (42).

In order to investigate whether MC3R gene rare variants are associated with human obesity, we analysed the coding sequence of this gene in 1249 French and 557 Italian unrelated subjects. Table 1 shows the anthropometric characteristics of the subjects enrolled in the study. We identified subjects carrying the already reported T6K and the V81I variants which showed a near complete linkage disequilibrium. Their allelic frequency was 10 and 14% in the Italian and French population, respectively, in line with those detected in other Caucasian cohorts studied (43–46). As demonstrated in a previous study (42), these variants do not have any effect on MC3R function. Five subjects were heterozygous for new synonymous sequence variants (F12F, S69S, L95L, I226I, Q270Q) that were not further analysed as they do not change the amino acid and, presumably, would not affect receptor signalling. In addition to the three rare variants previously described (42), we also identified eight missense mutations and a single nucleotide variant in the promoter of the gene, all found in an heterozygous state. Some of these rare variants were present only in the obese populations (T-4C, S17T, D158Y, V177I, A293T, T280S, I335S), others were identified only in normal weight subjects (I87T, R257S, L285V) and the variants L249F and X361S were present in both groups (Table 2). The frequency of MC3R rare variants carriers was not statistically different between the Italian and French subjects both in the obese and control groups (Table 2). Moreover, no statistically significant differences were observed when the frequency of rare variants carriers in total obese subjects (1.55%) was compared with that in total lean ones (0.83%) (\( P = 0.18 \)).

RESULTS

Prevalence and nature of MC3R rare variants

mutations in the melanocortin receptor 4 (MC4R) have a prevalence of 1–6% in obese subjects, both children and adults, and this prevalence is always significantly higher than that reported in controls (18–19,21–36). Another melanocortin receptor, the melanocortin receptor 3 (MC3R), is expressed in the arcuate nucleus of the hypothalamus and, in rodents, has been implicated in the long-term energy homeostasis (12,37). The knock-out mouse for MC3R (MC3R-KO) is hypophagic and shows a peculiar phenotype of decreased linear growth, increased fat mass and decreased lean mass. Thus, high feed efficiency, and not hyperphagia, seems to contribute to the increased fat mass (12). Despite the characterization of the obese phenotype in MC3R-KO, the role of the MC3R in human obesity is still discussed. To date, two common variants have been described, but they are not associated with any obesity-related phenotype and thus should be considered as benign polymorphisms (38). The first reported mutation (39) was in vitro functionally active but did not segregate with obesity in the family (39–41). We subsequently studied 290 severely obese adults and found three mutations which were not present in 215 normal weight controls. Two of these mutations segregated with obesity in the family members and one of them demonstrated in vitro functional consequences (42). However, in that study, the control population was not screened for the entire coding region of the gene and, thus, the prevalence of rare variants in controls could not be determined. Recently, Calton et al. (22) investigated the prevalence and function of MC4R and MC3R mutations in two cohorts of North American severely obese and non-obese subjects. They confirmed that MC4R mutations are significantly associated with severe obesity in North American adults, but, in contrast to the previously published data, they did not support a similar role for MC3R mutations (22). To evaluate the prevalence of MC3R mutations and their possible association with obesity in other populations, in this study we screened the entire coding region of the MC3R gene in a total of 1806 subjects of French and Italian origin. In addition, we also evaluated in vitro functional consequences of each detected mutations.
In vitro functional analysis of MC3R variants

In order to investigate the possible association between an impairment of MC3R signalling and obesity, we evaluated the functional consequences of all mutations identified. The T-4C variant lies in the promoter region of the gene, thus we postulated that it could affect protein production. The WT and mutated receptor containing the 5′ flanking region presenting the mutation were cloned and transiently transfected in HEK293 cells. Western-blot analysis demonstrated that very low levels of MC3R protein were present in the lysates of cells transfected with the mutated receptor (Fig. 1). This nucleotide variant was present in two non-related French children of 15 and 13 years of age with BMI of 32 (BMI Z-score 3.3 SD) and 28.3 kg/m² (BMI Z-score 3.5 SD), respectively.

MC4R missense mutations associated with obesity lead to impaired receptor response to agonist stimulation, thus we used an in vitro assay to systematically compare the agonist activation of the MC3R variants identified in both obese and normal weight subjects to that of the WT receptor. A missense variant was considered to have functional implications when the mutated receptor showed an EC₅₀ significantly different from that of the WT receptor (P < 0.05) and/or an E₅₀max < 50% of WT receptor maximum activity. Based on these parameters, two mutations led to a complete inactivation of the receptor (D158Y and I335S) and two (S17T and T280S) led to a drastic decrease in the E₅₀max (30 and 15% of the WT receptor maximal response, respectively) (Fig. 2A). These four mutations were found in seven obese subjects who were not relatives. In contrast, the variants identified only in normal weight subjects or in both obese and normal weight subjects showed a receptor response curve similar to WT receptor (Fig. 2B). Moreover, for two variants identified only in lean subjects (I87T and L285V), the maximal response was 154 and 142% of the maximal response of the WT receptor (Fig. 2C). Based on these functional studies, the prevalence of carriers of mutations associated with a reduced MC3R signalling was higher in obese subjects than in correspondent controls both in French North Africans and in French and Italian Caucasians (Table 3). The carriers’ frequencies were not statistically different among the three groups (P > 0.05) indicating absence of heterogeneity. Furthermore, a meta-analysis of the results obtained demonstrated that functionally significant MC3R mutations were associated with obesity (P = 0.022) (Table 3). Table 4 shows some of the phenotypic characteristics of the obese carriers. It is worth note that seven of the nine carriers are adolescents with early-onset obesity.

In silico analysis of MC3R gene variants

To determine whether in silico approaches could also be used to evaluate the functional significance of MC3R gene variants, we used the computer mutation prediction programs PolyPhen (47) and Screening for Non-Acceptable Polymorphism (SNAP) (48–50). The results of the in vitro assay and the in silico predictions are shown in Table 5. It must be noted that there are a number of discrepancies between the in vitro functional evaluation and the in silico prediction. Both PolyPhen and SNAP programs correctly predicted the functional implications of the three mutations that almost abolished the response to the agonist (D158Y, I335S and T280S), but both programs failed to detect the functional importance of the S17T
alteration. Moreover, for two mutations, the in silico prediction was not supported by the results of an altered in vitro signalling. These findings suggest that the in silico approaches cannot replace in vitro functional characterization.

**DISCUSSION**

This study confirms that MC3R rare variants are present in both obese and lean subjects with a non-significantly different prevalence in the two groups. However, further analysis of the functional consequences induced by the identified variants highlights that the prevalence of heterozygotes for functionally altered variants is significantly higher in the obese subjects when compared with controls. Indeed, five of the seven variants found only in obese subjects have in vitro functional alterations that reduce or completely abolish the agonist response. In contrast, none of the rare variants found in lean controls or in both groups shows a decreased in vitro functional activity.

The MC3R is a melanocortin receptor closely related to the MC4R and expressed in the arcuate nucleus of the hypothalamus that is a locus of energy homeostasis control (12,37). For these reasons, it has been also considered key gene predisposing to obesity but in marked contrast with MC4R, its pathophysiological role is still debated.

In the mouse model, the MC3R-KO is associated with a milder phenotype than in MC4R-KO and is not clearly characterized. In a previous study, we reported three mutations in obese subjects that were not found in 215 lean controls (42). Two of them segregated with obesity in the family members studied and one was associated with impairment of the in vitro agonist response (42). Subsequently, Calton et al. (22) reported that the prevalence of rare MC3R variants with and without functional alterations was not significantly different in two large North American cohorts of obese and lean subjects. The results of the present study are partially in accordance with those reported in the North American cohorts. Indeed, we confirm that rare MC3R variants are also present in the control populations with a frequency similar to that of the obese group. Compared with the frequencies reported in the North American cohorts, the prevalence in our cohorts of European subjects is higher in both groups suggesting the potential role of ethnicity. However, the analysis of the in vitro functional effects of these variants showed that those causing partial or total impairment of MC3R signalling were present only in the obese population. Considering as benign polymorphisms the rare variants without functional implications and as mutations only the variants with functional abnormalities, the prevalence of MC3R mutations carriers was significantly different in the two groups (P = 0.022). Taken together these findings, it is tempting to speculate that, as for MC4R, impairment of MC3R signalling is associated

**Table 3.** MC3R rare variants with functional implications on the receptor activity in French and Italian cases and controls

<table>
<thead>
<tr>
<th>Nucleotide variation</th>
<th>Amino acid change</th>
<th>French subjects</th>
<th>Italian subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>G50C</td>
<td>S17T</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G472T</td>
<td>D158Y</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C839G</td>
<td>T280S</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>T1003A or T1004C</td>
<td>I335S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4/144</td>
<td>0/226</td>
</tr>
<tr>
<td>P-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Cases versus correspondent controls.

<sup>b</sup>Overall P-value derived from the meta-analysis of the three studies.

**Table 4.** Anthropometrics and biochemical parameters of the MC3R mutations carriers

<table>
<thead>
<tr>
<th></th>
<th>T-4C/+</th>
<th>S17T/+</th>
<th>D158Y/+</th>
<th>T280S/+</th>
<th>I335S/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>15</td>
<td>13</td>
<td>20</td>
<td>15</td>
<td>58</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>32</td>
<td>28</td>
<td>40</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td>BMI Z-score (SD)</td>
<td>3.3</td>
<td>3.5</td>
<td>4.0</td>
<td>3.0</td>
<td>&gt;18</td>
</tr>
<tr>
<td>Age of obesity onset</td>
<td>6</td>
<td>6</td>
<td>4.5</td>
<td>NA</td>
<td>1.5</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.7</td>
<td>4.6</td>
<td>4.6</td>
<td>4.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>36.3</td>
<td>35.8</td>
<td>44</td>
<td>NA</td>
<td>42.36</td>
</tr>
<tr>
<td>Chol (mg/dl)</td>
<td>181.7</td>
<td>189.5</td>
<td>135</td>
<td>177.9</td>
<td>143.1</td>
</tr>
<tr>
<td>HDL–C (mg/dl)</td>
<td>58</td>
<td>58</td>
<td>38.7</td>
<td>41.0</td>
<td>81.2</td>
</tr>
<tr>
<td>TGL (mg/dl)</td>
<td>79.7</td>
<td>53.1</td>
<td>44.3</td>
<td>124.0</td>
<td>44.3</td>
</tr>
</tbody>
</table>

*Human Molecular Genetics, 2011, Vol. 20, No. 2*
with human obesity. This hypothesis is in contrast with the
conclusions of the study in the North American cohorts (22).
It is worth noting that Calton et al. (22) based their conclusions
on the results obtained with a single mutation (F82S) inducing
impaired in vitro MC3R signaling and found in two normal
weight individuals. The lean phenotype described in these
MC3R mutation carriers could be the result of an incomplete
penetrance. Similar findings were previously described for
MC4R mutations. Indeed, Farooqi et al. (51) in a large study
of children with early-onset obesity reported that, for some
MC4R mutations, although all homozygotes were obese,
only 68% of heterozygotes had an obese phenotype and this
could not be explained by their in vitro function. They postu-
lated that the penetrance of MC4R mutations may vary as
function of ethnicity or genetic and environmental modifiers
may have major effects in some pedigrees.

The genetic background of the two European populations is
different from that of the North American cohorts and this could
be another reason for the differences among the two studies. In
addition, we also evaluated obese children/adolescents with an
earlier impact of the mutations on weight and this could
explain the higher frequency of functionally significant
MC3R mutations found in the present study. The
genotype–phenotype correlation in the carriers of
MC3R inactivating mutations did not allow to associate a
specific phenotype to these mutations. In the MC3R-KO
mouse, hyperphagia is not present and the increased fat
mass seems to derive from high feed efficiency. In obese sub-
jects, it is difficult to achieve a real estimate of daily caloric
intake as it is generally based on self-reported questionnaire
and it is also difficult to assess the real energy expenditure.
Thus, in evaluation, the energy balance cannot be
useful as in animals and cannot indicate the possible presence
of high feed efficiency. Looking at the anthropometric
characteristics of the nine MC3R mutation carriers, it is
worth note that seven of them are adolescents with early-onset
obesity. This finding leads to speculate that, as for MC4R, the
role of the MC3R in the development of obesity might be par-
ticularly important in childhood.

In conclusion, the results of this large study demonstrate
that functionally significant MC3R variants are associated
with obesity supporting the current hypothesis that rare var-
iants might have a stronger impact on the individual suscepti-
bility to gain weight. They also underline the importance of
detailed in vitro functional studies in order to prove the patho-
ogenic effect of such variants. In addition, our study demon-
strates that these functional studies cannot be replaced by
in silico approaches. However, evaluation in larger cohorts
will be needed in order to precise the specific phenotypic
characteristic potentially correlated with reduced MC3R sig-
alling. It could be used to identify subgroups of patients for
whom dedicated therapeutic strategies could be developed.

**MATERIALS AND METHODS**

**Subjects**

**Italian population.** The present study was conducted on 343
obese patients referred to the Division of General Medicine of
the San Giuseppe Hospital, Istituto Auxologico Italiano (Verbania, Italy),
for diagnostic or therapeutic problems related to obesity or its morbidity.
The patient group (249 females and 94 males, mean age 40 ± 12 years and
mean BMI 44.8 ± 7.0 kg/m²) included the 290 subjects previously
studied (42). A control group of 214 Italian normal weight
individuals (142 females, 72 males, mean age 36 ± 12 years,
and mean BMI 22.4 ± 2.7 kg/m²) was also screened. Obese
patients and controls were all Caucasian from Northern Italy.

**French populations.** French obese and population-based
control children were also recruited in France. A total of
479 obese children/adolescent living in the Paris area (319
females and 160 males, mean age 13.6 ± 4.5 years, mean
BMI Z-score 4.3 ± 1.1 SD) were recruited at Trouseau

---

### Table 5. Summary of in vitro and in silico functional implications of the MC3R variants identified

<table>
<thead>
<tr>
<th>Nucleotide variation</th>
<th>Amino acid change</th>
<th>Population screening No. obese carriers (n = 839)</th>
<th>No. control carriers (n = 967)</th>
<th>In vitro functional study</th>
<th>In silico prediction PolyPhen SNAP Classification Assayed difference compared with WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-4C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G50C</td>
<td>S17T</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T260C</td>
<td>I87T</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G472T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G529A</td>
<td>V1771</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C745T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T280S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C839G</td>
<td>S17T</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1082C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1003A or T1004C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1002C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For PolyPhen predictions: benign is denoted as ‘−−’, possibly damaging is denoted as ‘+-’ and probably damaging is denoted as ‘++’. For SNAP predictions: neutral is denoted as ‘-’ and non-neutral as ‘+’, both followed by (RI: % expected accuracy). For in vitro classification, no functional alterations is denoted as ‘−’. and functional alterations as ‘+’., followed by the difference observed from WT.

*Mutations reported for the first time by Calton et al. (22).*
In silico prediction of missense mutations effects

PolyPhen prediction: missense variants identified by sequencing were classified based on their potential impact on protein function or structure (benign, possibly damaging, or probably damaging) using a new version of the PolyPhen method (47). These predictions are based on the analysis of multiple sequence alignments of homologous proteins, functional annotation and structural information if available (47). The new version of PolyPhen constructs multiple sequence alignment using a pipeline of several existing programs for aligning sequences, alignment quality control and clustering of sequences.

SNAP prediction: SNAP (Screening for Non-Acceptable Polymorphisms) is a neural-network-based method (49) that uses information about sequence conservation, per residue predictions of secondary structure, solvent accessibility and flexibility, and, if available, experimental functional annotations. The server output is a binary classification of the mutation’s functional effect (neutral/non-neutral) in combination with the reliability index (RI) of prediction (integer score ranging from 0 to 100).
Statistics analysis

The prevalence of rare MC3R mutation carriers and of functionally significant rare mutations carriers in severely obese subjects and lean subjects were analysed by exact Fisher’s test. Fisher’s weighted Z-score test was used for the meta-analysis of the data obtained in the different cohorts. Best-fit estimates of the ligand concentration needed to achieve 50% of maximum effect (EC50s) were obtained by non-linear regression fitting of the dose–response curves using GraphPad Statistics Software. Differences in EC50 of mutant receptors and wild-type receptors were analysed by ANOVA and Dunnett’s post-test. A P-value < 0.05 was assumed as statistical significant.

Acknowledgements

We thank the registered dialecticians, nurses of the Center of Research on Human Nutrition and “region Ile de France”, Paris as well as the patient and family for their collaboration.

Conflict of Interest statement. None declared.

Funding

We thank region Ile de France (CPER contact) for supporting this work, as well as Institut Benjamin Delessert and Guigoz, Paris (to K.C. and B.D.).

References


