Effect of the melanocortin-3 receptor C17A and G241A variants on weight loss in childhood obesity\textsuperscript{1–3}

Nicola Santoro, Laura Perrone, Grazia Cirillo, Paolo Raimondo, Alessandra Amato, Carmine Brienza, and Emanuele Miraglia del Giudice

ABSTRACT

Background: The central melanocortin system is critical for the long-term regulation of energy homeostasis. Melanocortin-3 receptor (MC3R) knock-out mice, despite being hypophagic, have increased fat mass and higher feed efficiency than do their wild-type littermates.

Objective: The aim was to evaluate whether, in childhood obesity, MC3R variants are associated with changes in fatness reduction as a consequence of a weight-reduction program.

Design: Molecular screening of the MC3R coding region in 184 obese children, 77 girls and 107 boys [\(z\) (±SEM) body mass index (BMI); in kg/m\(^2\); age 9.2 ± 2 y], was performed. BMI was evaluated at baseline and after 6 and 12 mo of the weight loss program.

Results: No new mutations were found. Two previously described polymorphisms, C17A (Thr6Lys) and G241A (Val81Ile), were observed in 20 patients in almost complete linkage disequilibrium. No new mutations were found. Two previously described polymorphisms, C17A (Thr6Lys) and G241A (Val81Ile), were observed in 20 patients in almost complete linkage disequilibrium. No significant differences in BMI \(z\) scores were observed at baseline of the weight-loss program between the genotypes; however, at follow-up, heterozygotes showed a significantly higher BMI \(z\) score (\(P = 0.03\)). When the patients were divided according to the amount of weight lost, a higher prevalence of heterozygotes was observed among subjects who lowered their BMI \(z\) score \(< 1.5\) (\(P = 0.03\)).

Conclusion: These results suggest a gene-diet interaction between the MC3R C17A and G241A variants and a weight loss program for the ability to lose weight in childhood obesity. Am J Clin Nutr 2007;85:950–3.

KEY WORDS MC3R gene, polymorphism, childhood obesity, weight loss, BMI \(z\) score

INTRODUCTION

Obesity is a major pediatric problem in Western countries (1). In an effort to find new therapeutic strategies, research on genetic factors predisposing to obesity has been encouraged. Starting from leptin gene cloning, several mechanisms that underlie the central control of feeding behavior and energy expenditure have been highlighted (2).

Five receptors belonging to the melanocortin receptor (MCR) family have been cloned. Two of them, melanocortin-3 receptor (MC3R) and MC4R, are involved in body-weight regulation (2). Both are receptors for \(\alpha\)-melanocyte stimulating hormone, a bioactive peptide derived from the prohormone proopiomelanocortin (3). Several MC4R mutations have been found and functionally characterized in obese humans (4, 5). The clinical characteristics of subjects who carry these kind of mutations are early onset of obesity, accelerated height velocity, advanced bone age, and hyperinsulinemia (5). Heterozygous patients show an intermediate phenotype between the wild-type homozygotes and mutated allele homozygotes, suggesting a gene dose effect for MC4R mutations (5). MC3R seems to have an important role in the regulation of energy storage and differs from MC4R, which is primarily involved in food intake regulation. Remarkably, despite the increased adiposity seen in MC3R knock-out mice (mc3r\(-/-\)), these animals do not exhibit increased food intake, which suggests increased feed efficiency and defective energy partitioning as causes of fat mass accumulation (6–8).

Linkages between markers on chromosome 20q13, where MC3R maps, and type 2 diabetes, plasma insulin concentrations, and increased fat mass have been reported (9–12). Molecular screenings performed on obese or diabetic patients showed the presence of two MC3R common variants, which were in almost complete linkage disequilibrium (C17A and G241A) (13–16). Recently, it has been shown that obese children homozygous for the MC3R allele, ie, carrying both variants, are more prone to become obese than are subjects who carry the wild-type allele or those who are heterozygotes (17). However, longitudinal studies to assess the influence of these MC3R variants on the individual capability in losing weight after a weight loss program have not been performed. Therefore, we studied the potential gene-diet interaction between MC3R gene variants and a weight loss program on the ability to lose weight during a 1-y follow-up intervention in a group of obese children.

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SUBJECTS AND METHODS

One hundred eighty-four unrelated Italian obese children and adolescents, 77 females and 107 males \( [x (\pm \text{SEM}) \text{ body mass index (BMI) } z \text{ score: } 3.3 \pm 2.3; \text{ age: } 9.2 \pm 2 \text{ y}] \) of 630 obese children referred to the Department of Pediatrics of the Second University of Naples between January 1998 and December 2002 for a weight loss program, were enrolled in the study. They represented the subgroup of patients who completed the 12-mo follow-up for the weight loss program, whereas the other 446 children dropped out during the follow-up and, for this reason, were not included in the study. The procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Informed consent was obtained from the parents. The ethical committee of the Second University of Study of Naples approved the study.

The subjects were evaluated at 0800 after an overnight fast. Height was measured by a Harpenden stadiometer, and weight was assessed by a balance beam scale. These measurements were recorded by the same operator and repeated in duplicate. Obesity was defined when BMI exceeded the 97th percentile for sex and age according to reference values; zeta scores for BMI (BMI \( z \) scores) were calculated \((18, 19)\). Pubertal stage was assessed by using the Tanner criteria \((20)\).

The subjects were submitted to a weight loss program. They consumed a nutritionally balanced \((50\% \text{ of energy as carbohydrates, } 30\% \text{ of energy as fat, and } 20\% \text{ of energy as protein})\) self-selected diet of common foods \((60\% \text{ of the recommended dietary allowances for age and sex})\). All subjects underwent lifestyle modifications. They followed a program based on physical exercise and behavioral therapy, including individual psychological care of the child and his or her family. The habitual level of physical activity during the program was assessed by a standardized questionnaire \((21)\). Information was obtained on the number of hours of exercise and other leisure time activities. The number of hours of physical activity per week were summed and expressed as a score \((21)\). Measurements were repeated after 6 and 12 mo.

Genomic DNA was collected from nucleated white blood cells. The \( MC3R \) coding region was PCR-amplified by using 2 couples of primers to generate overlapping fragments: \( MC3R\alpha F \) \((5'-\text{CTACACAGCA-TATGACCG-3')}\) and \( MC3R\alpha R \) \((5'-\text{CTCTACGTTGGAT-GGAAAGT-3')}\) to amplify a 582-base pair \((\text{bp})\) fragment and \( MC3R\beta F \) \((5'-\text{CTACACAGCA-TATGACCG-3')}\) and \( MC3R\beta R \) \((5'-\text{CTCTACGTTGGAT-GGAAAGT-3')}\) to amplify a 582-base pair \((\text{bp})\) fragment. Reactions were carried out by using the following conditions: denaturation at 95 °C for 5 min followed by 35 cycles of 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C for both fragments. Amplification products were analyzed by bidirectional sequencing with the use of Big Dye terminator (Applera, Foster City, CA) and electrophoresed on an automatic sequencer \((\text{ABI PRISM } 310; \text{ Perkin Elmer, Foster City, CA})\). One hundred sex- and age-matched nonobese control subjects were screened. This group of controls was recruited as previously reported \((22)\).

A chi-square test was performed to assess whether the observed genotype frequencies were in Hardy-Weinberg equilibrium and to test the differences in allele frequency between the obese subjects and the lean controls. Linkage disequilibrium between markers was assessed as previously described \((23)\). Data are expressed as means \( \pm \text{SDs}\). Mean BMI \( z \) score of the subjects who were homozygous for the wild allele and heterozygous for the mutated allele at baseline, 6 mo, and 12 mo were compared by using analysis of variance \((\text{ANOVA})\). ANOVA for repeated measures was used where appropriate. Patients were divided into 2 groups according to the degree of BMI \( z \) score reductions; one group was composed of subjects who lowered their BMI \( z \) score \( >1.5 \) (which represents the mean of the reduction in BMI \( z \) scores of the overall population investigated) and another group was made up of children who lowered their BMI \( z \) score \( <1.5 \). The prevalence of the genotypes among the 2 groups of patients was evaluated by a chi-square test. All statistical analyses were performed with the SAS Statistical Software Package version 8.2 \((\text{SAS Institute, Cary, NC})\).

RESULTS

One hundred fifty-one \((82\%)\) of the patients were prepubertal \((83 \text{ males})\). The clinical features of the study population before the weight-loss program are shown in Table 1.

Molecular screening did not show any new mutation in the \( MC3R \) coding region in either the obese children or the lean controls. Two previously described polymorphisms were detected: C17A \((\text{Thr6Lys})\) and G241A \((\text{Val81Ile})\). Because these polymorphisms were in almost complete linkage disequilibrium

<p>| TABLE 1 |
| Clinical features of the subjects at baseline according to sex and ( MC3R ) polymorphism|
| Wild type ((n = 164)) | Heterozygous ((n = 20)) |</p>
<table>
<thead>
<tr>
<th></th>
<th>Males ((n = 98))</th>
<th>Females ((n = 66))</th>
<th>Males ((n = 9))</th>
<th>Females ((n = 11))</th>
<th>( P ) for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>9.0 ( \pm 2.0 )</td>
<td>9.2 ( \pm 2.1 )</td>
<td>9.5 ( \pm 1.8 )</td>
<td>9.4 ( \pm 2.0 )</td>
<td>0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.3 ( \pm 15.1 )</td>
<td>53.8 ( \pm 19.0 )</td>
<td>54.0 ( \pm 16.1 )</td>
<td>56.1 ( \pm 15.0 )</td>
<td>0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>136.2 ( \pm 15.0 )</td>
<td>136.9 ( \pm 17.7 )</td>
<td>138.0 ( \pm 14.0 )</td>
<td>137.5 ( \pm 17.4 )</td>
<td>0.9</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>29.9 ( \pm 4.2 )</td>
<td>29.3 ( \pm 3.9 )</td>
<td>28.4 ( \pm 4.0 )</td>
<td>29.5 ( \pm 4.1 )</td>
<td>0.6</td>
</tr>
<tr>
<td>BMI ( z ) score</td>
<td>3.4 ( \pm 2.2 )</td>
<td>3.3 ( \pm 2.1 )</td>
<td>3.1 ( \pm 2.3 )</td>
<td>3.2 ( \pm 2.5 )</td>
<td>0.9</td>
</tr>
</tbody>
</table>

\(^{1}\) Anthropometric features of the subjects homozygous for the \( MC3R \) wild-type allele and heterozygotes \((\text{G241A})\) were compared by using 2-factor ANOVA with interaction. When the main effect of genotype or sex was evaluated, no statistically significant differences in age, weight, height, BMI, and BMI \( z \) score were observed either between wild-type homozygotes and heterozygotes or between the males and females.

\(^{2}\) Two-factor ANOVA.

\(^{3}\) \( \pm \) SD \((\text{all such values})\).
TABLE 2
Change in BMI z score according to MC3R polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Wild type (n = 164)</th>
<th>Heterozygous (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>z ± SD</td>
<td>Change</td>
</tr>
<tr>
<td><strong>BMI z score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.4 ± 2.2</td>
<td>0</td>
</tr>
<tr>
<td>6 mo</td>
<td>2.1 ± 1.4</td>
<td>−38</td>
</tr>
<tr>
<td>12 mo</td>
<td>1.7 ± 1.1</td>
<td>−50</td>
</tr>
</tbody>
</table>

1 A 3-factor repeated-measures ANOVA with sex and genotype as between-subjects factors and time (baseline, 6 mo, and 12 mo) as a within-subject factor was used to analyze the linear trend of BMI z score depending on MC3R genotype. This analysis showed a significant effect of sex (P < 0.001) and genotype (P = 0.03) on BMI z score, whereas no significant effect of sex (P = 0.2) was observed. A significant genotype × time interaction was observed.

2 Calculated as baseline BMI z score minus BMI z score at each time period (× 100/baseline BMI z score).

3 Statistically significantly different from the wild-type MC3R genotype after 12 mo, P = 0.03 (one-factor ANOVA).

(D' = 1.0, R² = 0.93) and therefore all heterozygous children had both mutations, only the C17A variant was used to test for association studies. Twenty obese children (10.8%; 11 girls) and 12 controls (12.4%; 4 girls) were heterozygous for the rare allele. Rare allele homozygotes were not found. The allelic frequencies were not significantly different between the obese and control subjects (chi-square: 0.5; P > 0.1). At baseline, there was no statistically significant difference (P > 0.1) in mean age, as well as in weight, height, and BMI, between the subjects who were homozygous for the wild allele and those who were heterozygous for the rare allele (Table 1). During the weight-loss program, the level of habitual physical activity, expressed as a score, was not significantly different (P > 0.1) between the groups. After 12 mo, the obese children who carried the rare allele lowered their BMI z score less than did the wild-type allele homozygotes, independent of sex (Table 2). After a 12-mo follow-up, 88 (5 heterozygotes) subjects reduced their BMI z score >1.5 and 96 (15 heterozygotes) reduced their BMI z score <1.5. Eighty-eight (5 heterozygotes) subjects reduced their BMI z score >1.5, and 96 (15 heterozygotes) reduced their BMI z score <1.5. **MC3R** heterozygotes (C17A and G241A); □, MC3R wild-type homozygotes. A chi-square test was used to test the prevalence of heterozygotes in the 2 groups (chi-square: 4.3; P = 0.03).

Interestingly, we found that the copresence of both polymorphisms on the same allele was associated with changes in the reduction of BMI that occurred during a weight-loss program. In particular, heterozygotes showed a major difficulty in losing weight compared with wild type homozygotes.

Recently, in vitro studies conducted by Feng et al (17) showed that double homozygosity for MC3R sequence variants C17A and G241A affected melanocortin receptor function. The double mutant, in fact, showed a reduced total binding capacity despite a preserved binding affinity (17). Homozygosity for these sequence variants appeared more frequently in African Americans than in whites, among whom the frequency of the sequence variants was rare, and was associated with increased adiposity and insulin concentrations (17). Because our sample was composed exclusively of white children, we did not find, as expected, rare allele homozygotes. Moreover, consistent with the previous report, we did not observe significant differences in fatness between the wild type homozygotes and the heterozygotes at baseline of the weight-loss program. This suggests, therefore, that the co-occurrence of the 2 variants in heterozygotes may influence the ability of an individual to lose weight more so than does the predisposition of an individual to become obese. Consistent with the MC4R mutation, a dose-dependent effect (24) may also be hypothesized for MC3R gene variants, but this effect is evident only when a greater feed efficiency is required, such as during a weight-loss program. The mechanism through which the co-occurrence of these 2 variants can affect the ability of an individual to lose weight is suggested by results obtained in animal studies. In fact, it has been speculated that the increased fatness of mc3r−/− mice may be a consequence of a defect in energy partitioning (8, 25). It has been shown that mc3r−/−−/− mice have a lower respiratory quotient when fed a low-fat feed and a higher respiratory quotient when fed a high-fat feed than do their wild-type littermates, which suggests a transient reduction in the ratio of fatty acid to carbohydrate oxidation despite increased fat intake (8). Consistent with these data, a recent report showed that...
if these mice were submitted to a low-fat diet, fatty acid oxidation in red oxidative muscle isolated from gastrocnemius was significantly reduced compared with wild-type mice (26).

Studies conducted in humans showed that an energy-restrictive diet caused a shift in substrate metabolism associated with an enhanced fatty acid utilization by skeletal muscle, probably, via the sympathetic system (26–29). During a weight-loss program, the patients who carry the C17A and G241A MC3R variants, being unable to adequately increase their feed efficiency experience a greater difficulty in losing weight, likely because of their inability to adequately increase fatty acid oxidation. Examples of gene polymorphisms that influence the phenotype only under particular condition are known. For example the long allele of INS VNTR has been shown to cause hyperinsulinaemia only in obese subjects, in whom higher insulin secretion is required (30). In conclusion, these data showed that MC3RC17A and G241A variants may play a crucial role in the ability of an obese child to lose weight during a weight-loss program.

None of the authors has conflict of interest, and all authors take full responsibility for the article.

NS and EMG had primary responsibility for the protocol development, analytic framework for the study, and writing of the manuscript. LP supervised the design and execution of the study and contributed to the writing of the manuscript. GC and PR participated in the development of the protocol, in the preliminary data analysis, and laboratory work. AA and CB were responsible for the patient screening and outcome assessment.

REFERENCES