

## CLINICAL STUDY

## Ghrelin gene polymorphisms and ghrelin, insulin, IGF-I, leptin and anthropometric data in children and adolescents

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### Abstract

**Background:** Previous investigations on the ghrelin gene reported three common polymorphisms (Arg51Gln, Leu72Met, and Gln90Leu), but their role in overweight and obese individuals remains to be clarified.

**Objective:** To ascertain whether these genetic variants could influence ghrelin secretion and play a part in predisposing to earlier onset of obesity or in modulating the overweight phenotype in childhood.

**Design and methods:** Mutational analysis of the entire ghrelin gene and total and acylated plasma determinations were performed in 81 obese or overweight children and adolescents (46 were obese and 35 overweight: Ob/Ow). We also recruited 168 normal-weight healthy controls (72 young adults and 96 children) for mutational or plasma ghrelin analysis.

**Results:** Median total and acylated plasma ghrelin concentrations were significantly lower in Ob/Ow individuals than in controls (175 pg/ml compared with 345 pg/ml,  $P < 0.0001$ , and 95 pg/ml compared with 114 pg/ml,  $P < 0.0001$ , respectively). The ghrelin gene variants showed similar allele frequencies in the Ob/Ow individuals and in controls; in the former, they were not associated with any change in total and acylated circulating ghrelin concentrations or anthropometric data. The Leu72Met status was associated with a positive family history for obesity (75% for Leu72Met compared with 39% for Leu72Leu,  $P = 0.03$ ) and with a greater percentage of newborns born 'large for gestational age' (33% for Leu72Met compared with 5% for Leu72Leu,  $P = 0.03$ ), but in the control group it was related to a lower mean body mass index z-score ( $-0.03$  for Leu72Met and  $-0.47$  for Leu72Leu,  $P = 0.04$ ).

**Conclusion:** Our present findings do not support the hypothesis that the ghrelin gene polymorphisms have a relevant impact in the secretion of total and acylated ghrelin.

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### Introduction

Ghrelin, a recently described endogenous ligand for the growth hormone secretagogue receptor type 1a (GHS-R1a) (1) is a strong stimulator of growth hormone release (2), but may act also as potent appetite-stimulating hormone (3). The human ghrelin gene is located on chromosome 3, at locus 3p25–26, and consists of 4 exons and 3 introns (4). The major product is a 28 amino acid peptide acylated with an *n*-octanoyl at Ser at position 3. Post-transductional mechanisms yield different isoforms of ghrelin, which either are acylated at Ser with a decanoyl or decenoyl group, or have a deletion of the C-terminal Arg at position 28 (5). The *n*-acyl addition is essential for its binding to the receptor and for its biological activity (6).

Ghrelin is mostly secreted by the X/A-like cells in the gastric fundus (7), although it is also expressed in

bowel, pancreas, kidney, gonads, placenta, hypothalamus and pituitary gland (1, 8–13). Plasma ghrelin is mostly des-acylated and inactive at the hypothalamus (14), whereas at this site acylated ghrelin influences neuropeptide Y (NPY) and agouti-related protein (AGRP) neurones in the arcuate nucleus (7, 15). In bone marrow, des-acylated ghrelin has a direct adipogenic action (16). Chronic administration of ghrelin increases food intake and body weight, and decreases energy expenditure, whereas acute treatment stimulates the secretion of growth hormone both in rodents and in humans (3, 17). Stimulation of appetite and food intake after acute administration of ghrelin has been shown in humans (17). Normal fluctuations of circulating ghrelin may have a major impact on food intake, as ghrelin peaks precede meals (18). This suggests that derangement in the ghrelin system could have a role in obesity. However, ghrelin secretion

is negatively associated with body weight and insulin secretion (19, 20) and obese individuals generally have significantly lower circulating ghrelin concentrations than lean controls (21). A notable exception to this negative association between ghrelin concentrations and body weight is represented by Prader–Willi syndrome which, although generally characterised by obesity, has the feature of absolutely or relatively increased ghrelin concentrations (22). Thus it has been suggested that, at least in some cohorts of dysgenetic patients, genetic alterations in the ghrelin system would play a part in the pathogenesis of obesity and eating disorders (23).

Previous studies on ghrelin genetic variants provided contradictory findings as to their role in obesity (24). Three polymorphisms have been described (25, 26): a single base substitution G152A, with Gln replacing Arg at codon 28 of mature ghrelin, C214A with Met replacing Leu at codon 72, or A269T with Leu replacing Gln at codon 90 in the prepro-ghrelin. The 72Met variant seems to be associated with an earlier onset of obesity (25), but it has been proposed that, later, 72Met could be protective against the accumulation of fat (24). The aim of our study was to test whether genetic variants in the ghrelin gene could play a part in predisposing to early onset of obesity or be associated with anthropometric data and secretion of total and acylated ghrelin, leptin, insulin or insulin-like growth factor (IGF)-I in overweight and obese children, and to compare them in control children with normal weight.

## Materials and methods

### Study participants

A total of 249 unrelated Caucasian individuals were recruited for this study and were divided in two groups. The first group consisted of 81 obese or overweight (46 were obese and 35 overweight: Ob/Ow) children and adolescents (M/F 40/41; median age 10.1 years, range 2.4–15.9 years; mean body mass index (BMI)  $28.42 \pm 5.47$  kg/m<sup>2</sup>). They were tested before the beginning of treatment by diet, undergoing a complete investigation to exclude endocrine and central nervous system disorders, hypothalamic tumours and

genetic syndromes. According to the Italian reference charts for BMI, obesity was defined when BMI exceeded the 99th centile for sex and age, and overweight was defined when BMI was between the 85th and the 99th centiles. Among the Ob/Ow children and adolescents, 86% were still obese or overweight after 1 year of dieting treatment. The second group consisted of 168 healthy normal-weight controls (72 young adults and 96 children) recruited among our Medical School students, staff and children or adolescents referred for regular health check-ups, with no history of obesity in childhood (Table 1). All 96 normal-weight children were enrolled as controls for plasma ghrelin determination, and 18 of them were also available for analysis of the ghrelin gene. Seventy-two young adults were enrolled as controls only for mutational analysis of the ghrelin gene.

All weight and height data were measured by two trained examiners. BMI was calculated as body weight divided by height squared (kg/m<sup>2</sup>). The BMI of each individual was converted by the LMS method (summarises the data in terms of three smooth age specific curves called L (lamda), M (mu) and S (sigma)) to a standard BMI z-score for the child's age, using Italian reference tables (27). The Ob/Ow and normal-weight groups were matched for age and pubertal development, the latter evaluated according to Tanner (28). Data on gestational age, weight at birth and at 1 year of age, age at onset of obesity, family history for obesity, and eating disorders were collected for obese children by means of a semi-structured interview. Babies with a birth weight for gestational age and sex greater than the 97th centile of the Neonatal Italian Standards (29) were defined as 'large for gestational age'. A positive family history for obesity identified families in which at least one close relative (parents, brothers and sisters) expressed obesity.

The study was approved by the Ethics Committee of our hospital, and written informed consent was obtained from all participants and parents of children.

Blood samples were drawn from the 81 Ob/Ow and the 96 normal-weight children and adolescents after an overnight fast and were immediately mixed with aprotinin (0.6 TIU/ml) to inhibit protease activity, centrifuged at 1600 *g* for 15 min at 4 °C and stored at –80 °C until tested for total ghrelin, leptin, insulin

**Table 1** Baseline characteristics of study participants. Age is expressed as median (range), BMI z-score as mean  $\pm$  s.d.

	Obese children (n = 46)	Overweight children (n = 35)	Normal-weight children (n = 96)	Normal-weight young adults (n = 72)
Males/females	27/19	13/22	33/63	15/57
Age (years)	10.3 (2.4–15.9)	10 (5.9–15.6)	9.4 (1.5–16.9)	27.5 (22–36)
BMI z-score	2.61 $\pm$ 0.51	1.77 $\pm$ 0.28	–0.27 $\pm$ 0.96	–0.11 $\pm$ 0.76
LGA/AGA	6/40	2/33	ND	ND
Positive/negative family history for obesity	32/14	16/19	ND	ND

LGA, large for gestational age; AGA, adequate for gestational age; ND, not done.

and IGF-I concentrations. Blood samples for the measurement of acylated ghrelin concentrations were drawn in 57 Ob/Ow and in 44 normal-weight children and adolescents as described above.

Blood samples were collected for DNA extraction from all 81 Ob/Ow individuals and 90 normal-weight individuals (72 adults and 18 children).

### Mutational analysis of the ghrelin gene

DNA was extracted from peripheral white cells by the salting-out procedure (30). For the Ob/Ow individuals, the entire prepro-ghrelin coding region, part of the 5'-UTR and part of the 3'-UTR were analysed by direct sequencing using the ABI Prism 3100 DNA Analyzer (Applied Biosystem, Foster City, CA, USA), whereas for all the healthy normal-weight individuals the mutational screening was performed by a wave-denaturing high-performance liquid chromatography system (Wave, Transgenomic, Santa Clara, CA, USA), followed by subsequent direct sequencing of the positive heteroduplex samples. The product was amplified by the polymerase chain reaction (PCR) technique. Two primers for each of the four exons that encode for the prepro-hormone were designed to include non-coding regions. Their sequences were as follows:

Exon 1: forward primer, 5'-AGGCACATGAGAAGGGAG-3'; reverse primer, 5'-TGAGGTCAGACCCAGAGTCC-3' = 322 bp GHR1-amplicon.

Exon 2: forward primer, 5'-TCTGGGCTTCAGTCTTCTCC-3'; reverse primer, 5'-CTGTTCAGTCCACCTCTCC-3' = 315 bp GHR2-amplicon.

Exon 3: forward primer, 5'-CCTGAAGAGAGGCAGAGAGC-3'; reverse primer, 5'-CAGAGGTTGGGAAAACTCA-3' = 292 bp GHR3-amplicon.

Exon 4: forward primer, 5'-TCACCACCGTGAAGA-AAAG-3'; reverse primer, 5'-ATTCGCCTCTGAGCTTGTA-3' = 200 bp GHR4-amplicon.

The PCR amplification was carried out in a volume of 30  $\mu$ l, containing 200 ng DNA, 20 pmol each primer, 0.1 mmol/l each of the dNTPs (Amersham Pharmacia Biotech Inc., New Jersey, NJ, USA) and 0.75 unit AmpliTaq Gold DNA Polymerase (Applied Biosystems). Each PCR was performed at 95 °C for 6 min, followed by 36 cycles at 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 40 s, and one cycle at 72 °C for 5 min, using a thermal cycler DNA (Techne, Cambridge, UK). Both the size of the primers and their position in the ghrelin genomic sequence (GenBank Accession Number: AF296558) were designed to include the splicing sites and for optimal detection by Wave DHPLC.

### Ghrelin and hormone measurements

Total plasma ghrelin (acylated and des-acylated) was measured by RIA (Phoenix Pharmaceuticals, Inc., Belmont, USA) after plasma extraction in reverse-phase C18 columns. One millilitre plasma was loaded onto

pre-equilibrated C18 columns (Strata, Phenomenex, Torrance, CA, USA). The columns were washed with 6 ml 1% trifluoroacetic acid (Sigma-Aldrich, Milano, Italy) and eluted with 3 ml 60% acetonitrile (Sigma-Aldrich) in 1% trifluoroacetic acid. The eluant was dried and dissolved in 1 ml RIA buffer. Reconstituted samples were then measured in duplicate in the competitive RIA. Based on our data, sensitivity was 3 pg/tube and the intra-assay coefficient of variation (CV) was 3.9% at 14 pg/tube and 4.9% at 95 pg/tube.

The acylated form of ghrelin was measured by a competitive radioimmunoassay (Linco Research, Inc., St Charles, MO, USA) using a specific antibody for the biologically active form of ghrelin with the acyl group on Ser 3. When 100  $\mu$ l plasma was used, sensitivity was 10 pg/ml and intra-assay CV was 9.4% at 62 pg/ml and 5.9% at 232 pg/ml. The specificity of the test was 100% for human ghrelin and <0.1% for des-acylated ghrelin.

Insulin was measured by chemiluminescent enzyme-labelled immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA). Sensitivity was 2  $\mu$ IU/ml. The intra-assay and interassay CV were 2.5–8.3% and 4.4–8.6% respectively. IGF-I was measured in duplicate by a two-site radioimmunoassay (Diagnostic System Laboratories Inc., Webster, TX, USA) after an acid-ethanol extraction. Sensitivity was 0.8 ng/ml. The intra-assay and interassay CV were 1.5–3.4% and 1.5–8.2%. Leptin was measured in duplicate by RIA using a commercially available kit (Linco Research Inc.). The sensitivity was 0.5 ng/ml. The intra-assay and interassay CV were 3.4–8.3% and 3.0–6.2%.

### Statistical analysis

Two-sided  $\chi^2$ -test was used to assess whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. Insulin, IGF-I and BMI data are expressed as mean  $\pm$  s.d. Because leptin and ghrelin concentrations were skewed towards low values, data for leptin and total and acylated ghrelin were expressed as median and interquartile range (IQR). The influence of genetic variants of the ghrelin gene on total and acylated plasma ghrelin concentrations and on the age at onset of obesity was assessed by the Mann-Whitney *U* and the Kruskal-Wallis analysis of variance tests, and the influence on BMI *z*-score at diagnosis was assessed by Student's *t*-test. The influence of the three polymorphisms of the prepro-ghrelin gene on birth weight corrected for gestational age and family history for obesity was tested by  $\chi^2$  and Fisher's exact test. To study the influence of the Leu72Met polymorphism on hormone concentrations controlled for age at onset of obesity, sex and BMI *z*-score, we used general linear models. We report multiple *R* and  $\beta$  coefficient with 95% confidence interval (CI) for significantly independent variables according to sigma-restricted

parameterisation. All the analyses were performed with Statistica 6.0 software (StatSoft, Inc., Tulsa, OK, USA) and a *P* value less than 0.05 was accepted as statistically significant.

## Results

### Genetic analysis

Heterozygous genotypes for the three polymorphisms (Arg51Gln, Leu72Met and Gln90Leu) were found in 81 Ob/Ow children and adolescents and 90 controls. Frequencies for the 51 Gln allele in Ob/Ow individuals and in normal-weight controls were 0.0062 and 0.0055 respectively ( $P = 1.0$ ), those for the 72Met allele were 0.074 and 0.078 ( $P = 0.89$ ), and those for the 90Leu allele were 0.037 and 0.033 ( $P = 0.85$ ). The allele frequencies were in Hardy–Weinberg equilibrium. Genotype distributions in Ob/Ow and normal-weight children are shown in Table 2.

Similarly, when data for the 46 obese and the 35 overweight children were analysed separately, no difference in allele frequencies was found between them. Frequencies for the 51 Gln allele in obese and in overweight children were 0.011 and 0 respectively ( $P = 1.0$ ), those for the 72Met allele were 0.076 and 0.071 ( $P = 0.9$ ), and those for the 90Leu allele were 0.043 and 0.029 ( $P = 0.7$ ).

New single nucleotide substitutions were also identified: a C/T transition was found at position –36 bp in the 5'-UTR in one Ob/Ow and in one normal-weight individual; a G/A transition was found at position 412 in the 3'-UTR in one Ob/Ow child and a C/T silent mutation was found at position 315 in the third coding exon (codon 105 (ATC → ATT)) of the prepro-ghrelin gene in one normal-weight individual.

### Ghrelin and hormone concentrations

Median (IQR) total plasma ghrelin concentration in 81 Ob/Ow children was 175 (138–276) pg/ml, significantly less than that in 96 control children and adolescents, in whom it was 345 (202–531) pg/ml ( $P < 0.0001$ , Mann–Whitney *U* test). Similarly, concentrations of acylated ghrelin were significantly lower in 57 Ob/Ow children than in 44 normal-weight children (95 (77–114) pg/ml compared with 114 (98–138) pg/ml;  $P < 0.0001$ ), and the ratio of

acylated to total ghrelin was significantly greater in Ob/Ow individuals than in normal-weight children (43 (33–74)% compared with 28 (20–47)%;  $P = 0.0003$ ).

Total ghrelin was negatively correlated with age and pubertal development both in Ob/Ow individuals and in normal weight children (data not shown).

Total and acylated ghrelin concentrations and the acylated/total ghrelin ratio were similar when Leu72Met was compared with Leu72Leu, and when Gln90Leu was compared with Gln90Gln in Ob/Ow children and adolescents (Table 3). The lowest values of total and acylated plasma ghrelin (45 and 39 pg/ml respectively) were found in an obese girl with the Arg51Gln substitution.

No statistically significant differences in leptin, insulin and IGF-I concentrations were found when Leu72Met was compared with Leu72Leu and when Gln90Leu was compared with Gln90Gln Ob/Ow children and adolescents (Table 4). However, a multiple design showed that, in the Ob/Ow group, mean IGF-I adjusted for age, sex and BMI z-score was  $393 \pm 42$  ng/ml in Leu72Met carriers and  $266 \pm 17$  ng/ml in Leu72Leu carriers (multiple  $R = 0.72$ ,  $P < 0.0001$ ;  $\beta$  coefficient for Leu72Met status = 0.28, 95% CI 0.08 to 0.49,  $P = 0.008$ ;  $\beta$  coefficient for age = 0.69, 95% CI 0.47 to 0.91,  $P < 0.0001$ ).

### Polymorphisms and phenotypes

To verify whether polymorphisms could influence the obese phenotype, we compared anamnestic and anthropometric data of the Ob/Ow children and adolescents who were heterozygotes for Leu72 or Gln90 alleles with those of counterparts who were homozygotes for Leu72 or Gln90 alleles. Weight centile at 1 year of age and BMI z-score at diagnosis were similar in Leu72Met compared with Leu72Leu and in Gln90Leu compared with Gln90Gln Ob/Ow children. Mean  $\pm$  s.d. BMI z-score was  $2.26 \pm 0.62$  in Leu72Leu carriers,  $2.16 \pm 0.44$  in Leu72Met carriers,  $2.25 \pm 0.60$  in Gln90Gln carriers and  $2.21 \pm 0.55$  in Gln90Leu carriers.

Median (IQR) age at onset of obesity tended to be lower, although not significantly so, in the 12 Leu72Met carriers than in 69 Leu72Leu carriers (2.5 (1–4.5) years compared with 5 (3–6.5) years;  $P = 0.08$ ). The genotype Leu72Met was associated with a positive family history for obesity (75% for Leu72Met compared with 39% for Leu72Leu;

**Table 2** Genotype distributions (%) in Ob/Ow and normal-weight individuals.

	Arg51Arg	Arg51Gln	Leu72Leu	Leu72Met	Gln90Gln	Gln90Leu
Ob/Ow children ( <i>n</i> = 81)	80 (98.8%)	1 (1.2%)	69 (85.2%)	12 (14.8%)	75 (92.6%)	6 (7.4%)
Normal-weight individuals ( <i>n</i> = 90)	89 (98.9%)	1 (1.1%)	76 (84.4%)	14 (15.6%)	84 (93.3%)	6 (6.7%)

**Table 3** Total and acylated ghrelin concentrations and acylated/total ghrelin ratio (median, IQR) in the Ob/Ow children according to ghrelin gene polymorphisms.

	Total ghrelin (pg/ml)	Acylated ghrelin (pg/ml)	Acylated/total ghrelin (%)
Leu72Leu	181 (138–278) (n = 69)	92 (77–105) (n = 49)	43 (34–74) (n = 49)
Leu72Met	169 (140–388) (n = 12)	112 (80–131) (n = 8)	50 (31–84) (n = 8)
Gln90Gln	173 (138–278) (n = 75)	95 (76–115) (n = 52)	44 (33–75) (n = 52)
Gln90Leu	253 (181–322) (n = 6)	90 (78–105) (n = 5)	43 (33–53) (n = 5)

No significant differences (Mann–Whitney *U* test) between Leu72Leu and Leu72Met or between Gln90Gln and Gln90Leu.

**Table 4** Hormone concentrations in the Ob/Ow children according to ghrelin gene polymorphisms. Insulin and IGF-I values are expressed as mean  $\pm$  s.d. leptin values as median (IQR).

	Insulin ( $\mu$ U/ml)	IGF-I (ng/ml)	Leptin (ng/ml)
Leu72Leu (n = 69)	12.1 $\pm$ 6.5	284 $\pm$ 150	20 (13–26)
Leu72Met (n = 12)	12.9 $\pm$ 8.6	352 $\pm$ 151	21 (16–27)
Gln90Gln (n = 75)	12.7 $\pm$ 7.2	299 $\pm$ 159	21 (13–26)
Gln90Leu (n = 6)	10.2 $\pm$ 3.9	288 $\pm$ 27	17 (16–18)

No significant differences between Leu72Leu and Leu72Met or between Gln90Gln and Gln90Leu.

$P = 0.03$ , Fisher's exact test) and with an greater percentage of babies born large for gestational age (33% for Leu72Met compared with 5% for Leu72Leu;  $P = 0.03$ , Fisher's exact test).

In normal-weight individuals, BMI z-score was significantly lower in Leu72Met than in Leu72Leu carriers ( $-0.03 \pm 0.7$  compared with  $-0.47 \pm 0.99$ ;  $P = 0.04$ , Student's *t*-test).

Neither the Arg51Gln nor the Gln90Leu genotypes seemed to be associated with an obesity phenotype.

## Discussion

The present study has demonstrated the existence of polymorphisms in the ghrelin gene, some variants of which are described here for the first time. These ghrelin gene polymorphisms, however, were not associated with any changes in circulating ghrelin concentrations or with overweight or obesity. The three polymorphisms in the ghrelin gene reported in other studies (24, 26, 31) were also found in our study population, although without any association with obesity or overweight. In

our groups, the frequencies for the 72Met allele were similar to that described in the White participants in the Heritage Family Study, the Swedish Obese Subjects Study and the Quebec Family Study (24), whereas the frequency for the 90Gln allele in our Ob/Ow children and adolescents was about 50% of that reported for German children (0.037 compared with 0.063) (26), probably reflecting genetic/ethnic heterogeneity between the two populations.

Several studies to date have demonstrated that ghrelin is involved in the control of appetite and energy balance (32). In agreement with previous reports in the literature, we found decreased circulating ghrelin concentrations in our obese children, but we first showed that secretion of both total and acylated ghrelin was significantly lower. Although des-acylated ghrelin seems to be the major form of circulating ghrelin (33), only the acylated ghrelin exhibits neuroendocrine activity (14, 34). However, Thompson *et al.* (16) have shown that both forms of ghrelin may promote bone marrow adipogenesis, whereas only acylated ghrelin probably acts centrally to suppress growth hormone lipolytic activity during prolonged starvation. Thus the proportion of total ghrelin to des-acylated ghrelin could be relevant for regulating the balance between adipogenesis and lipolysis in response to nutritional status (35). In our study, both total and acylated ghrelin concentrations were lower in Ob/Ow than in normal-weight children, whereas the proportion of acylated ghrelin was greater in Ob/Ow than in normal-weight children (43% compared with 28%), showing that obesity and overweight are associated with a relatively lower decrease in acylated ghrelin as compared with total ghrelin. The acylation of the Ser 3 represents a fatty acid moiety that might facilitate passage through the blood–brain barrier (36), acting as a ligand for the hypothalamic and pituitary GHS-R1a and exerting an orexigenic activity through the NPY/AGRP system and the orexin pathway (32). The proportion of acylated ghrelin, as expected, was not influenced by the ghrelin gene variants, being 50% in the case of Leu72Met, 43% for Leu72Leu and Gln90Leu, and 44% for Gln90Gln.

Previous studies (25) of the genetic variants of ghrelin forms gave contradictory results for or against the hypothesis that genetic variants are the cause of obesity (24). Specifically, in two studies (19, 25) the genotype Leu72Met appeared to be associated with an earlier age at self-reported onset of obesity, whereas the findings of another study suggested the Leu72Met status could be protective against the accumulation of fat and metabolic co-morbidities (24). Moreover, a Leu72Met status does not seem to influence basal insulin secretion (19) or ghrelin plasma concentrations, and was associated with greater concentrations of IGF-I in the Black individuals in the Heritage Family Study (24). Similarly, in our Ob/Ow children, the Leu72Met status was associated with greater neonatal

weight-for-age, earlier age at onset of obesity, unmodified plasma ghrelin and insulin secretion and greater IGF-I concentrations. In contrast, in normal-weight children and adults, the BMI z-score was significantly lower in 72Met carriers. These conflicting results could suggest that the Leu72Met genotype may be involved in energy metabolism and fat accumulation by modulating phenotypic weight during different periods of life (37), but its role requires clarification in studies involving large numbers of obese, overweight and constitutionally lean individuals. In addition, as suggested by Ukkola *et al.* (38), the substitution at codon 72 might have effects on the products of prepro-ghrelin, which could have some as yet unknown functional significance, leading to some physiological effects through binding to specific ghrelin receptors. It is unlikely that GHS-R1a is the only receptor involved in the regulation of the adipogenesis mediated by the ghrelin system; the action of des-acylated ghrelin in bone marrow adipogenesis is independent of GHS-R1a (16).

In our series, Arg51Gln and the Gln90Leu genotypes did not seem to influence weight regulation.

In conclusion, our present findings do not support the hypothesis that ghrelin gene polymorphisms have a relevant impact on the secretion of total and acylated ghrelin, or on the regulation of food intake, energy balance and endocrine activities. As far as we know, this is the first demonstration that neither total nor acylated ghrelin is not related to ghrelin gene variants.

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