Obesity and variants of the GHRL (ghrelin) and BCHE (butyrylcholinesterase) genes

Vitor G.L. Dantas, Lupe Furtado-Alle, Ricardo L.R. Souza and Eleidi A. Chautard-Freire-Maia

Departamento de Genética, Universidade Federal do Paraná, Curitiba, PR, Brazil.

Abstract

Ghrelin coded by the GHRL gene is related to weight-gain, its deactivation possibly depending on its hydrolyzation by butyrylcholinesterase (BChE) encoded by the BCHE gene, an enzyme already associated with the body mass index (BMI). The aim was to search for relationships between SNPs of the GHRL and BCHE genes with BChE activity, BMI and obesity in 144 obese and 153 nonobese Euro-Brazilian male blood donors. In the obese individuals, a significant association with higher BChE activity, in the 72LM+72MM; -116GG genotype class (GHRL and BCHE genes, respectively) was noted. No significant differences were found otherwise, through comparisons between obese and control individuals, of genotype and allele frequencies in SNPs of the GHRL gene (Arg51Gln and Leu72Met), or mean BMI between 72LL and 72LM+72MM genotypes. Although there appears to be no direct relationship between the examined GHRL SNPs and BMI, the association of the 72M SNP with higher BChE activity in obese subjects probably points to a regulatory mechanism, thereby implying the influence of the GHRL gene on BChE expression, and a consequential metabolic role in the complex process of fat utilization.

Key words: BCHE gene, body-mass index, butyrylcholinesterase, ghrelin, GHRL gene, obesity.

Received: July 8, 2010; Accepted: December 14, 2010.

Obesity is a risk factor in many diseases, such as hypertension, coronary artery disease, type II diabetes, breast and colon cancer, constituting a current pandemic disorder.

Ghrelin (Kojima et al., 1999), a peptide related to food intake, is coded by the GHRL gene (3p25-p26). The GHS (growth hormone secretagogue) receptor is activated by acylated ghrelin, although not so with des-acyl ghrelin (Hosoda et al., 2000). In rodents, it was shown that the administration of ghrelin leads to a gain in weight by increasing food intake and reducing fat utilization (Tschöp et al., 2000). It was further proposed that the decrease in plasma ghrelin concentration found in obese individuals could represent a physiological adaptation (Tschöp et al., 2001).

Butyrylcholinesterase (BChE; EC 1.1.1.8) plasma activity is positively correlated with weight and BMI (body mass index), in a phenotype (CHE2 C5-) with approximately 90% population frequency (Chautard-Freire-Maia et al., 1991; Alcântara et al., 2003), whereas in another (CHE2 C5+; 10% population frequency), with 20% higher BChE activity than the former, it is associated with lower mean weight (Chautard-Freire-Maia et al., 1991) and lower mean BMI (Alcântara et al., 2001). This shows that individuals with innate high BChE activity tend to be thinner and that BChE synthesis is increased in individuals that gain weight, suggesting that BChE activity is important in energy balance. Data from the BChE knockout mouse that became obese and significantly heavier than wild-type littermates after an 11% fat diet indicate a role for BChE in fat utilization (Li et al., 2008). Furthermore, SNPs of the human BCHE gene (3q26.1-q26.2) have been associated to BMI (Souza et al., 2005; Furtado-Alle et al., 2008).

Considering that ghrelin desacylation may depend on BChE activity (De Vriese et al., 2004), the search was for relationships between SNPs of the BCHE and GHRL genes and the variables BChE activity, BMI and obesity.

The study involved 144 obese (BMI ≥ 30 kg/m²; mean age 36.6 years) and 153 control (20 kg/m² ≤ BMI < 25 kg/m²; mean age 36.3 years) male blood donors from Curitiba, south Brazil, ethnically characterized as Euro-Brazilians on the basis of skin, hair and facial traits. The research was approved by the National's Committee for Ethics in Research (CONEP; registration number 2063).

DNA was extracted by a salting-out method (Lahiri and Nurnberger Jr, 1991). SNPs were examined for the GHRL gene (G/A, rs34911341, p.R51Q, 346 nt and C/A, rs696217, p.L72M, 408 nt) by PCR and Single Strand Conformation Analysis. The respective primers designed for this study were: GHRL15 (TCTCCCAGAGCACAAAGGAC); GHRL13 (TTCTGCTTGACCTCCATCTTCC); GHRL25 (GGAGTCGAAGAAGCCACCA); and GHRL23 (CAGAAGCATAAAACTGCAGAGG). Data...
on genotypes for exons 1 (G/A, rs1126680, -116 nt) and 4 (G/A; rs1803274, p.A539T; 1615 nt) of the BCHE gene, and BCHE plasma activity (Dietz et al., 1972) were obtained from a previous study (Furtado-Alle et al., 2008).

Statistica for Windows (StatSoft, Inc., 1996) was used for data analysis of: means, frequencies, standard errors, standard deviations, Fisher-exact test, t-test, χ² test, linear correlations, and step-wise multiple regression analysis.

Comparisons by χ² tests showed that genotype and allele frequencies for Arg51Gln and Leu72Met SNPs of the GHRL gene did not statistically differ in obese (51RR = 99.3%, 51RQ = 0.7%, 51QQ = 0.35%, and 72LL = 88.1%, 72LM = 11.2%, 72MM = 0.3%; 72M = 6.3% in 141 and 143 subjects, respectively) or control (51RR = 98.7%, 51RQ = 1.3%; 51QQ = 0.65%, and 72LL = 87.6%, 72LM = 11.8%, 72MM = 0.6%; 72M = 6.5% in 153 subjects) individuals. Ukkola et al. (2002) also did not find any significant difference in 51Q allele frequency when comparing obese with normal individuals, but did show that there was a variation in samples of different ethnic composition. In a previous study, no significant difference was found in 72M frequency between obese and non-obese individuals (Hinney et al., 2002). Although total 72M frequency (6.4%; N = 296) in the present study was no different from that obtained for individuals from Utah (8.3%; p > 0.4), it differs significantly from those found for Han Chinese (15.6%, p < 0.01), Japanese (18.2%; p < 0.001), and African individuals (0.8%; p < 0.05), all of which from the International HapMap Project, thereby showing an ethnic difference involved in the frequency of this variant.

Multiple regression step-wise analysis (Table 1) indicated two values for beta, both significantly different from 0, when compared by t-tests: the -116A variant leads to lower BCHE activity whereas the 72M to higher. Although BCHE activity tends to be higher in obese than in non-obese individuals (Furtado-Alle et al., 2008), the 72M variant appears to contribute to elevating this even more. This significant association is a novelty, and may be considered an inference, as significance comes close to the 0.05 error limit. This may be due to a regulatory mechanism by which the presence of the 72M variant of the GHRL gene induces BCHE synthesis. The -116GG genotype is characterized by normal BCHE activity. However, in the presence of the 72M variant, mean BCHE activity is higher (t = 2.18, p < 0.05) (Table 2). High BCHE activity (> 8 KU/L) was shown in 33% of obese subjects with the 72M variant, but in only 21% of those homozygous for the 72L SNP. Although the L72M SNP is not located in the coding region for the mature ghrelin peptide, the 72M allele leads to an earlier onset of obesity (Ukkola et al., 2001; Miraglia del Giudice et al., 2004). According to Ukkola et al. (2002), variation in preproghrelin peptide could theoretically change the structure of one or more of the derived products, this leading to functional consequences.

The association between the -116A variant and lower BCHE activity (Table 1) is already known, and has been reported in obese and nonobese individuals (Furtado-Alle et al., 2008).

Table 1 - Results from step-wise multiple regression analysis that considered butyrylcholinesterase activity as dependent variable in obese individuals (N = 130).

| Independent variablesa |
|------------------------|
| Betab ± S.E. | t |
| BCHE gene | -0.21 ± 0.09 | 2.51 (p < 0.02) |
| GHRL gene | 0.18 ± 0.09 | 2.09 (p < 0.05) |

F = 5.52 (p < 0.01); r² = 0.08

aNonsignificant independent variables: age, body-mass index, A539T of the BCHE gene. bRegression coefficients for the standardized variables to a mean 0 and SD 1, allowing for comparison of the relative contribution of each independent variable in predicting the dependent variable, also comparable across variables. c(-116GG = 1, -116GA = 2). d(72LL = 1, 72LM+72MM = 2).

Table 2 - Butyrylcholinesterase mean activity in 130 obese individuals, grouped by genotypes of GHRL (Leu72Met) and BCHE (G-116A) genes.

| Genotypes | n | Mean BCHE activity (KU/L) ± S.D. |
|-----------|---|-----------------------------|
| 72LM+72MM, -116GG | 13 | 8.42 ± 4.08 |
| 72LL, -116GG | 95 | 6.55 ± 2.80 |
| 72LM+72MM, -116GA | 2 | 5.22 ± 1.89 |
| 72LL, -116GA | 20 | 5.04 ± 2.11 |

ntest = 2.18; p < 0.05 when comparing 72LM+72MM, -116GG with 72LL, -116GG.

inference, as significance comes close to the 0.05 error limit. This may be due to a regulatory mechanism by which the presence of the 72M variant of the GHRL gene induces BCHE synthesis. The -116GG genotype is characterized by normal BCHE activity. However, in the presence of the 72M variant, mean BCHE activity is higher (t = 2.18, p < 0.05) (Table 2). High BCHE activity (> 8 KU/L) was shown in 33% of obese subjects with the 72M variant, but in only 21% of those homozygous for the 72L SNP. Although the L72M SNP is not located in the coding region for the mature ghrelin peptide, the 72M allele leads to an earlier onset of obesity (Ukkola et al., 2001; Miraglia del Giudice et al., 2004). According to Ukkola et al. (2002), variation in preproghrelin peptide could theoretically change the structure of one or more of the derived products, this leading to functional consequences.

The association between the -116A variant and lower BCHE activity (Table 1) is already known, and has been reported in obese and nonobese individuals (Furtado-Alle et al., 2008).

Genotypes 72LL and 72LM+72MM did not differ significantly (t-test) in mean BMI in either control (23.05 ± 1.29 kg/m² and 23.43 ± 0.96 kg/m²; p > 0.20) or obese (32.95 ± 2.19 kg/m² and 32.79 ± 1.29 kg/m²; p < 0.05) individuals. No difference in mean BMI was found in obese (32.95 ± 2.19 kg/m² and 32.90 ± 2.7 kg/m²; p > 0.95) individuals. No difference in mean BMI was found in obese individuals, when genotypes 72LL and 72LM+72MM, identical for genotypes -116GG;539AA, -116GG;539AT or -116GA;539AT of exons 1 and 4 of the BCHE gene, respectively, were compared. Obese individuals with and without the 72M variant have already been compared (Ukkola et al., 2001), with no difference found in mean BMI.

Although the examined GHRL SNPs do not appear to be directly related to BMI, the association of the 72M SNP with higher BCHE activity in obese subjects although requiring further study, points to a regulatory mechanism, thus indicating the influence of the GHRL gene on BCHE expression and, consequently, its possible role in the complex process of fat utilization.

Acknowledgments

Financial support from CNPq, Fundação Araucária and CAPES is acknowledged.
References

Alcântara VM, Rodrigues LC, Oliveira LC and Chautard-Freire-Maia EA (2001) Association of the CHE2 locus with body mass index and butyrylcholinesterase activity. Hum Biol 73:587-595.

Alcântara VM, Oliveira LC, Réa RR, Suplicy HL and Chautard-Freire-Maia EA (2003) Butyrylcholinesterase and obesity in individuals with the CHE2 C5+ and CHE2 C5- phenotypes. Int J Obes 7:1557-1564.

Chautard-Freire-Maia EA, Primo-Parmo SL, Picheth G, Lourenço MA and Vieira MM (1991) The C5 isozyme of serum cholinesterase and adult weight. Hum Hered 41:330-339.

De Friese C, Gregoire F, Lema-Kisoka R, Waebroeck M, Robberecht P and Delporte C (2004) Ghrelin degradation by serum and tissue homogenates: Identification of the cleavage sites. Endocrinology 145:4997-5005.

Dietz AA, Rubinstein HM, Lubrano T and Hodges LK (1972) Improved method for the differentiation of cholinesterase variants. Am J Hum Genet 24:58-64.

Furtado-Alle L, Andrade FA, Nunes K, Mikami LR, Souza RLR and Chautard-Freire-Maia EA (2008) Association of variants of the -116 site of the butyrylcholinesterase BCHE gene to enzyme activity and body mass index. Chem Biol Interact 175:115-118.

Hinney A, Hoch A, Geller F, Schäfer H, Siegfried W, Goldschmidt H, Remschmidt H and Hebebrand J (2002). Ghrelin gene: Identification of missense variants and a frameshift mutation in extremely obese children and adolescents and healthy normal weight students. J Clin Endocrinol Metab 87:2716-2719.

Hosoda H, Kojima M, Matsuo H and Kangawa K (2000) Purification and characterization of rat des-Q14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. J Biol Chem 275:21995-22000.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H and Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656-660.

Lahiri DK and Nurnberger Jr JL (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444.

Li B, Duysen EG and Lockridge O (2008) The butyrylcholinesterase knockout mouse is obese on a high-fat diet. Chem Biol Interact 175:88-91.

Miraglia del Giudice E, Santoro N, Cirillo G, Raimondo P, Grandone A, D’Aniello A, Di Nardo M and Perrone L (2004) Molecular screening of the ghrelin gene in Italian obese children: The Leu72Met variant is associated with an earlier onset of obesity. Int J Obes Relat Metab Disord 28:447-450.

Souza RLR, Fadel-Picheth C, Allebrandt KV, Furtado L and Chautard-Freire-Maia EA (2005) Possible influence of BCHE locus of butyrylcholinesterase on stature and body mass index. Am J Phys Anthropol 326:329-334.

Tschöp M, David L, Smiley DL and Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407:908-913.

Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E and Heiman ML (2001) Circulating ghrelin levels are decreased in human obesity. Diabetes 50: 707-709.

Ukkola O, Ravussin E, Jacobson P, Pérusse L, Rankinen T, Tschöp M, Heiman ML, Leon AS, Rao DC, Skinner JS, et al. (2002) Role of ghrelin polymorphisms in obesity based on three different studies. Obes Res 10:782-791.

Ukkola O, Ravussin E, Jacobson P, Snyder EE, Chagnon M, Sjöström L and Bouchard C (2001) Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. J Clin Endocrinol Metab 86:3996-3999.

Internet Resources

HapMap Project, http://www.hapmap.org/.

Associate Editor: Francisco Mauro Salzano

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.