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Short Communication

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

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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.

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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 $\geq$ 30 kg/m^2; mean age 36.6 years) and 153 control (20 kg/m^2 $\leq$ BMI $<$ 25 kg/m^2; 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, rs 696217, *p.L72M,* 408 nt) by PCR and Single Strand Conformation Analysis. The respective primers designed for this study were: GHRL15 (TCTCCCAGAGCACAAA GGAC); GHRL13 (TTCTGCTTGACCTCCATCTTCC); GHRL25 (GGAGTCGAAGAAGCCACCA); and GHRL23 (CAGAAGCATAAAACTGCAGAGG). Data

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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, χ^2 test, linear correlations, and step-wise multiple regression analysis.

Comparisons by χ^2 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%; *51Q* = 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%; *51Q* = 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.

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

Independent variables[a]	Beta[b] ± S.E.	t
BCHE gene[c]	-0.21 ± 0.09	2.51 ($p < 0.02$)
GHRL gene[d]	0.18 ± 0.09	2.09 ($p < 0.05$)
	$F = 5.52$ ($p < 0.01$); $r^2 = 0.08$	

[a]Nonsignificant independent variables: age, body-mass index, *A539T* of the *BCHE* gene. [b]Regression 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*[a]	13	8.42 ± 4.08
72LL; *-116GG*[a]	95	6.55 ± 2.80
72LM+72MM; *-116GA*	2	5.22 ± 1.89
72LL; *-116GA*	20	5.04 ± 2.11

[a]t-test = 2.18; $p < 0.05$ when comparing *72LM+72MM*; *-116GG* with *72LL*; *-116GG*.

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/m^2 and 23.43 ± 0.96 kg/m^2; $p > 0.20$) or obese (32.95 ± 3.29 kg/m^2 and 32.90 ± 2.7 kg/m^2; $p > 0.95$) 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.

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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 C_5 isozyme of serum cholinesterase and adult weight. Hum Hered 41:330-339.

De Vriese C, Gregoire F, Lema-Kisoka R, Waelbroeck 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/.

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