Association between leptin receptor (LEPR) and brain-derived neurotrophic factor (BDNF) gene variants and obesity: a case-control study

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Introduction: Human and animal studies provide evidence for a relevant role of the leptin receptor (LEPR) and the brain-derived neurotrophic factor (BDNF) genes in energy homeostasis.

Aim: To assess the association between human LEPR and BDNF genetic variants with adult obesity.

Design and methods: Case-control study in Pamplona (Navarra, Spain) with adult obese subjects (n = 159) and normal weight controls (n = 154). Four common polymorphisms of the LEPR gene (Lys109Arg, Gln223Arg, Ser343Ser, Lys656Asn) and 17 variants of the BDNF gene, including the Val66Met variant, were genotyped.

Results: No significant case-control differences were found in allele/genotype frequencies after adjusting for relevant co-variates. Haplotype analysis did not detect any significant association between LEPR or BDNF variants and obesity. No associations were found between LEPR variants and serum leptin levels.

Conclusions: Our results do not support a major role of LEPR or BDNF common polymorphisms in multifactorial adult obesity.

Keywords: obesity, leptin receptor, brain-derived neurotrophic factor, case-control study
The aim of this study was to assess the association between BDNF and LEPR genetic variants and obesity in adult subjects in a case-control study conducted in Pamplona (Spain).

Subjects and methods

Subjects
Participants were selected according to BMI (cases, BMI = 30 kg/m²; controls, BMI 18 – 25 kg/m²) from the Endocrinology and Occupational Health Departments at the Navarra Hospital between January 1999 and June 2000 in the city of Pamplona (Navarra, Spain). In this case-control study, the group of cases (n = 159 obese subjects; age, 42.4 ± 10.5 years; 20 men and 139 women) was compared to normal weight controls (n = 154; age, 38.6 ± 9.0 years; 41 men and 113 women). Blood samples were drawn from each participant for the extraction of genomic DNA. Exclusion criteria were exposure to hormonal treatment or development of secondary obesity due to endocrine diseases or serious intercurrent illness. Subjects with type 2 diabetes, not receiving glucose-lowering agents, were eligible as cases (9%). Controls were apparently healthy subjects with no hormonal treatment and blood pressure below 120/90. The study was approved by the Ethics Committee of the University of Navarra and all subjects provided written informed consent for participation.

Anthropometric and metabolic measurements

Anthropometric measurements and body composition were measured as described elsewhere in detail. Following a period of 12-h fast, venous blood samples were obtained and serum glucose was measured by enzymatic methods. Serum insulin was measured by radioimmunoassay and plasma leptin levels by enzyme immunoassay as previously described. As expected, Table 1 shows significant differences in anthropometric and metabolic measurements in cases versus controls.

<table>
<thead>
<tr>
<th>Table 1 Anthropometric and metabolic characteristics of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n = 159)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Weight</td>
</tr>
<tr>
<td>Body fat (%)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
</tr>
<tr>
<td>Plasma leptin (ng/ml)</td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)</td>
</tr>
</tbody>
</table>

*P-value < 0.01.

Brain-derived neurotrophic factor (BDNF) has an essential role in the development, survival and functions of neurons. Additionally, both human and animal studies provide evidence for a relevant role of the BDNF gene in energy homeostasis, probably through its effect downstream of the MC4R signaling pathway. Unger et al. showed that the selective deletion of the Bdnf gene in the ventromedial and dorsomedial hypothalamus of mice produces hyperphagic behavior and obesity. In man, BDNF haplo-insufficiency as well as de novo mutations in its receptor (encoded by NTRK2 gene) have also been related to monogenic forms of obesity. Additionally, it has been recently described that a subgroup of patients affected with the WAGR syndrome (caused by deletions in 1p13), that are hyperphagie and show larger deletions, have been associated with higher BMI concurrently with the absence of the BDNF gene.
SNP selection and laboratory methods

Human LEPR gene (gene ID = 3953) is located in chromosome 1p31 (NC_000001.9; NT_032977.8). The isoform 1 of this receptor (NM_002303.3; NP_002294.2) is composed by 20 exons and has the complete capacity of signal transduction. We have selected for this study all common SNPs in the coding sequence of the LEPR gene: Lys109Arg (rs1137100), Gln223Arg (rs1137101), Lys656Asn (rs8179183) and Ser343Ser (rs1805134). All these variants are located in the extracellular domain of the leptin receptor.

Human BDNF gene (gene ID = 627) is located in chromosome 11p13 and is comprised by two exons encoding a preprotein of 247 amino acids that is cleaved to generate a 120 amino acid mature protein, which is completely conserved between mice, rats, pigs and humans.18,19 A total of 17 SNPs located in a 57.4-kb region in human BDNF gene were genotyped (NM_170731.3), including the missense variant Val66Met (rs6265; Table 2). Nine additional SNPs in BDNF gene were also genotyped and later discarded in the analysis due their lack of variation (rs2353512, rs3750934, rs11819808, hCV 26878171, rs11030110, rs1967554, rs4258332, rs11030118 and rs7937405).

Genotypes for LEPR and BDNF were determined using the SNPlex Genotyping System (Applied Biosystems, Foster City, CA, USA). For the genotyping process, all reactions were set up in a 384-well format using various robotic systems following the manufacturer’s protocol for the SNPlex Multiplex Genotyping Systems (Applied Biosystems). The high-throughput genotyping assays were performed at the genotyping facilities of CeGen (Centro Nacional de Genotipado, Genoma Espana) in the Barcelona Node. As quality control, two HapMap samples (NA11992 and NA11993) were included in the assays to check for consistency of genotypes with the HapMap published data. All genotyping process was carried out blind with respect to phenotype.

Statistical analysis

Genotype and allele frequencies were estimated after assessing the concordance with Hardy–Weinberg equilibrium through the statistical test implemented in the HAPLOVIEW program.20 Lewontin’s D’ and the correlation coefficient between pairs of loci (r²) were calculated as measures of linkage disequilibrium (LD) for pairs of adjacent SNPs. Gene-obesity association analyses at allele, genotype and haplotype level were carried out with the UNPHASED21 and STATA v.9.0 (StataCorp 2005) packages. Adjustments by gender and age were performed in all gene-obesity statistical analyses.

Table 2 SNPs of LEPR and BDNF genes determined in this study

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position chromosome 11</th>
<th>Transcript position</th>
<th>MAF</th>
<th>Genotyping rate</th>
<th>P-value HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BDNF</strong> gene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs7124442</td>
<td>27633617</td>
<td>Exon 2 (3'UTR)</td>
<td>0.24 (T/C)</td>
<td>96.5</td>
<td>0.29</td>
</tr>
<tr>
<td>rs6265</td>
<td>27636492</td>
<td>Exon 2 (Met66Val)</td>
<td>0.23 (G/A)</td>
<td>96.8</td>
<td>0.21</td>
</tr>
<tr>
<td>rs11030102</td>
<td>27638172</td>
<td>Intron 1-2</td>
<td>0.16 (C/G)</td>
<td>94.9</td>
<td>1.0</td>
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<tr>
<td>rs11030104</td>
<td>27641093</td>
<td>Intron 1-2</td>
<td>0.24 (A/G)</td>
<td>97.4</td>
<td>0.29</td>
</tr>
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<td>rs2049045</td>
<td>27650817</td>
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<td>97.4</td>
<td>0.27</td>
</tr>
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<td>27652486</td>
<td>Intron 1-2</td>
<td>0.44 (C/A)</td>
<td>97.4</td>
<td>0.86</td>
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<tr>
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<td>27653527</td>
<td>Intron 1-2</td>
<td>0.03 (G/A)</td>
<td>99.0</td>
<td>1.0</td>
</tr>
<tr>
<td>rs7103411</td>
<td>27656701</td>
<td>Intron 1-2</td>
<td>0.25 (C/T)</td>
<td>97.4</td>
<td>0.23</td>
</tr>
<tr>
<td>rs7103873</td>
<td>27656893</td>
<td>Intron 1-2</td>
<td>0.50 (C/G)</td>
<td>96.2</td>
<td>0.31</td>
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<tr>
<td>rs5038967</td>
<td>27660056</td>
<td>Intron 1-2</td>
<td>0.24 (A/T)</td>
<td>95.8</td>
<td>0.35</td>
</tr>
<tr>
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<td>27671460</td>
<td>Intron 1-2</td>
<td>0.24 (T/C)</td>
<td>96.2</td>
<td>0.33</td>
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<tr>
<td>rs988748</td>
<td>27681321</td>
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<td>0.17</td>
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<tr>
<td>rs2030324</td>
<td>27683491</td>
<td>Intron 1-2</td>
<td>0.50 (C/T)</td>
<td>97.1</td>
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<tr>
<td>rs11030119</td>
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<td>96.5</td>
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<td>rs7934165</td>
<td>27688559</td>
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<td>0.50 (A/G)</td>
<td>95.8</td>
<td>0.23</td>
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<tr>
<td>rs10767665</td>
<td>27690434</td>
<td>Intron 1-2</td>
<td>0.49 (A/G)</td>
<td>92.9</td>
<td>0.20</td>
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<tr>
<td>rs962369</td>
<td>27690996</td>
<td>Intron 1-2</td>
<td>0.23 (C/T)</td>
<td>98.1</td>
<td>0.32</td>
</tr>
</tbody>
</table>

| **LEPR** gene                                                                 |
| rs1137100| 65809099               | Exon 4 (Lys109Arg)  | 0.22 (A/G) | 93.6            | 0.74        |
| rs1137101| 65831101               | Exon 6 (Gln223Arg)  | 0.36 (A/G) | 82.4            | 0.43        |
| rs1805134| 65839697               | Exon 9 (Ser343Ser)  | 0.17 (C/T) | 88.8            | 1.0         |
| rs8179183| 65848540               | Exon 14 (Lys656Asn) | 0.17 (G/C) | 93.5            | 0.84        |

MAF, minor allele frequency; after the allele frequency, the name of the more frequent allele is indicated first followed by the sign ’/’ and the name of the minor allele.

P-value HWE, P-value for Hardy–Weinberg equilibrium assessed in control subjects using the method implemented in the HAPLOVIEW software.
The assessment of gene–gene interactions in obesity was carried out by using the carrier status of *LEPR* genotypes as possible effect modifiers of the association between *BDNF* haplotypes and obesity, with the inclusion of relevant co-variates. The assessment of the association between serum leptin levels and obesity was carried out separately in obese and non-obese subjects, by the inclusion of the logarithm of leptin levels (dependent variable) in a linear regression model that includes *LEPR* genotypes (dummy variables for each genotype), age and gender.

### Results

Genotype frequencies did not significantly deviate from Hardy–Weinberg expectations (Table 2). Statistical analysis conducted with the HAPLOVIEW software indicated that SNPs in the *BDNF* gene showed high levels of LD both in cases and controls, especially as denoted by average values of D’ near 1 across most pair of SNPs, showing a very similar haplобlock structure in obese subjects compared to controls. High levels of LD were also found in the *LEPR* gene with D’ values ranging from 0.78 to 1 in both cases and controls. The haploblock analysis showed identical structure in *LEPR* gene variants for cases and controls. The estimated most frequent haplotype in the *LEPR* gene was AAC G, with a frequency of 0.39 in cases and 0.47 in controls. Assuming all the necessary cautions inherent in the methods for haplotype frequency estimation in unrelated subjects with high number of SNPs, the estimated most common haplotype in the *BDNF* gene appears to be TGCAGAACGATCTAAAC (43%) according to the HAPLOVIEW software, with a frequency higher than two-fold the frequency of other *BDNF* haplotypes.

Table 3 shows allele and genotype frequencies for the missense variants evaluated in this study, showing no significant case-control differences. Such lack of association persisted after the adjustment by age and gender. Likewise, no significant case-control differences were found in allele/genotype frequencies for non-coding SNPs, both in the crude analysis and after adjusting by age and gender. Additionally, crude and age–gender adjusted haplotype analysis based on adjacent SNPs using the sliding window approach did not detect any significant association between *LEPR* or *BDNF* variants and obesity. The range of *P*-values for haplotype-obesity associations for pairs of SNPs were 0.13–0.71 for *BDNF* and 0.35–0.67 for *LEPR*. The assessment of gene–gene interactions did not reveal any evidence of relevant effect modification of *LEPR* variants on the association between *BDNF* haplotypes and obesity. It is worth mentioning that our study is clearly underpowered for detecting gene–gene interactions, especially taking into account the absence of significant marginal gene–disease association effects.

As shown in Table 1, there were striking differences in serum leptin levels in obese versus non-obese subjects. The separate evaluation of men and women within cases and controls also show important differences in the logarithm of serum leptin levels by gender both in controls (5.2 ± 1.1 versus 8.9 ± 0.7; *P*-value = 0.008) and obese subjects (19.2 ± 2.9 versus 36.6 ± 2.7; *P*-value = 0.019). Multiple linear regression analysis did not detect any significant difference in serum leptin levels by *LEPR* genotypes, after adjusting by age and gender both in cases and controls.
Discussion

Since the identification of the leptin gene in 1994, important strides have been made in our understanding of the regulation of energy homeostasis. Key molecules along the leptin signaling pathway have been elucidated in studies involving patients with rare monogenic causes of obesity such as mutations in the genes encoding leptin, leptin receptor, pro-opiomelanocortin, and prohormone convertase 1, as well as those with relatively more common function-altering mutations in the gene encoding the melanocortin 4 receptor. In the present study, we investigated BDNF, one of the major signaling systems believed to function downstream of the melanocortin 4 receptor. BDNF may play an important role in energy homeostasis in humans. Hyperleptinemia is a hallmark of almost all forms of obesity; thus, understanding the role of downstream mediators of leptin action such as BDNF holds promise for laying the groundwork for the development of new pharmaceutical approaches to the global obesity epidemic.

Rare gene alterations involved in severe forms of human obesity have been described both for leptin, BDNF and their receptors (encoded by LEPR and NTRK2, respectively). These studies, in combination with animal models, have clearly established a pivotal role of leptin and leptin receptor in energy homeostasis and obesity. On the other hand, the role of common variants of LEPR gene in obesity is less clear, with a wide range of discordant effects published in the literature for the missense variants Lys109Arg, Gln223Arg and Lys656Asn, being the conclusions from meta-analysis against the existence of association between such variants and obesity. Likewise, there are inconclusive data regarding the association between common BDNF variants and common obesity, in spite that the Met66Val genotypes have been strongly suggested as a relevant variable for eating behavior in anorexia nervosa.

Both in the crude analysis and after controlling for age/gender, the single-SNP analysis conducted in our study does not indicate significant associations between BDNF common polymorphisms in the development of multifactorial obesity in adulthood. The multifactorial causation of obesity makes it difficult to detect small effects at the level of case-control studies based on cut-off points of BMI. The moderate sample size of this study may have also limited the statistical power to identify such possible small effects. As an example of this limitation, a significant effect of the SNP rs6265 of BDNF gene with obesity in a genome-wide association study has recently been reported. On the other hand, it has been suggested that methods based on a single-marker may not capture all of the available LD information contained in multi-locus haplotypes. Consequently, the haplotype analysis used in our study may constitute a more powerful approach than single-locus analyses.

As expected, we found striking differences in serum leptin levels in obese versus non-obese subjects on one hand, and in men versus women on the other hand, without significant results for LEPR variants and obesity. In relation to the possible effects of LEPR variants on circulating leptin levels, we have not detected any significant difference by genotype. Again, discordant results have been published, with some studies showing no statistically significant association while others have found that baseline leptin levels were higher for carriers of the Arg109 or Arg223 alleles in LEPR with a clearer association found among men than among women. It is likely that we did not observe the effect due to the small number of males in our population. On the other hand, both leptin and BDNF serum levels are impaired in the rare cases of leptin deficiency and in WAGR cases with deletions of BDNF. While leptin replacement has been proved to be useful for subjects with mutations in this gene, there is still a lack of evidence to assess the potential therapeutic role of leptin and/or BDNF replacement in multifactorial obesity for persons in whom insufficient hormone levels are detected.

In the present study, we have tested multiple polymorphisms and different statistical models to assess gene–obesity associations. Given the lack of a consensus in choosing the most adequate strategy for correcting for multiple tests, we have decided to show uncorrected \( P \)-values that would need adjustment to compensate multiple testing. Clearly, any kind of adjustment for multiple comparisons would yield a non-significant result in our study. Although we have not found any evidence for gene–obesity association either using a single SNP approach or in the haplotype-based analysis, both in crude or adjusted models, it is still possible that BDNF and LEPR variants may have an association with intermediate phenotypes affecting obesity such as eating behavior, as it has been suggested previously.

Conclusions

This study does not support a major role of BDNF and LEPR missense common polymorphisms in the development of multifactorial obesity in adulthood. Further studies are required to assess their effect in
intermediate phenotypes, such as those affecting energy intake and eating behavior.

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References


