Frequency of the GPR7 Tyr135Phe allelic variant in lean and obese subjects

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ABSTRACT. Background: GPR7, the endogenous coupled receptor for neuropeptide B and neuropeptide W, is expressed in several regions of the central nervous system, which are involved in the regulation of feeding behavior. GPR7 affects the regulation of energy balance through a mechanism independent of leptin and melanocortin pathways. Aim: Aim of this study was to investigate whether GPR7 gene mutations can be detected in human subjects and, in that event, if they are differently distributed among lean and obese subjects. Subjects and methods: The coding region of GPR7 were sequenced in 150 obese patients and 100 normal-weight unrelated controls. Functional studies of the allelic variants were performed. Results: One genetic GPR7 variant was found (Tyr135Phe - rs33977775) in obese subjects (13.3%) and lean control (25%). Functional studies did not reveal significant differences between the wild type and the Tyr135Phe allelic variants in their NPW-mediated capacity to inhibit forskolin-induced cAMP production. Conclusions: Screening of GPR7 gene mutations among lean and obese subjects revealed a Tyr135Phe allelic variant that was fairly common in the study population. As indicated by in vitro and in silico studies, this variant is unlikely to cause a functional derangement of the receptor.

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INTRODUCTION

Despite the general trend towards a rapid increase in the prevalence of obesity, observed especially in countries with established market economies (1-3), individual susceptibility to become obese may be extremely different within a population and genetic factors appear to be as important as environmental factors in its pathogenesis (4-7). In an effort to identify new genes that are involved in the pathogenesis of obesity, Ishii et al. found that the expression of GPR7, the endogenous G protein-coupled receptor for neuropeptide B (NPB) and W (NPW), was reduced in the hypothalamus of gold thioglucose (GTG)-induced obese mice (8). The binding of NPB or NPW to GPR7 receptor induces the activation of the inhibitory subunit of the G protein (Gxi), which ultimately results in the inhibition of the adenylate cyclase and the subsequent decrease of cAMP intracellular levels (9-12). The human 7-transmembrane receptor GPR7 has sequence similarities to opioid and somatostatin receptors, and it is encoded by a single exon gene. The initial mapping of GPR7 to human chromosome 10q11.2-q21.1 (10) has recently been changed based on an alignment of the NPB-WR1 sequence (GenBank BC069117) with the genomic sequence (GRCh37) [OMIM 600730], thus localizing the gene on chromosome 8q11.23.

GPR7 is expressed in several regions of the central nervous system, which are involved in the regulation of feeding behavior, including the dorsomedial, the paraventricular, and the arcuate nuclei of the hypothalamus (11). Male GPR7 null mice showed an adult-onset obese phenotype and increased susceptibility to become obese and develop hyperglycemia upon high fat diet feeding (8). Based on their data Ishii et al. suggested that GPR7 receptor affects the regulation of energy balance through a novel mechanism independent of leptin and the melanocortin pathways. Interestingly, this receptor that has highly conserved sequences between humans and rodents, might also play a critical role in limbic system function and stress responses (10-13). Aim of this study was to investigate whether GPR7 gene mutations can be detected in human subjects and, in that event, if they are differently distributed among lean and obese subjects.

SUBJECTS AND METHODS

Subjects

One hundred and fifty unrelated adult subjects (41 males, 109 females) with body mass index (BMI) of 35 or greater, were enrolled after informed consent and Institutional Reviewing Board approval was obtained. Body weight was measured to the nearest kilogram, and body height and circumferences were determined to the nearest centimeter. Subjects characteristics and co-morbidities are shown in Table 1. One hundred healthy non-obese adult subjects (27 males, 73 females) with BMI<25 were included as controls. The mean±SD age of the enrolled controls was 39.5±12.9 yr and their BMI 21.8±3.1 (kg/m²).

Methods

Direct nucleotide sequencing of the GPR7 gene

GPR7 coding exon was amplified from genomic DNA, isolated from whole blood using the following primers:

GPR7 Fw: 5′GTGCAGAACCGGGCTTCAGGA3′

GPR7 Rev: 5′CGCAGTTGCGGAGCGG3′

Key-words: GPR7, NPB, NPW, NPBWR1, obesity.

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PCR was performed using PCR Master Mix (Promega Corporation, WI 53711-5399) with an annealing temperature of 68 C. After purification on GENOMED columns (GmbH Poststr. 22 D-32584 Löhne, Germany), the PCR products were directly sequenced using the following primers:
GPR7Fw2: 5’GACTTCTCGTCTGGCAGAT3’
GPR7Rev2: 5’AGGACACAGATGGTGGAC3’

Sequencing reactions were then run on a CEQ 2000 XL sequencer (Beckman Coulter, Fullerton CA).

Cloning and transfections of the GPR7 wild type and mutant receptors
Wild-type and mutant GPR7 genes were first amplified using PCR primers with the following sequences:
GPR7FwClon: 5’CCGCTCGAGATGGCAGGCCTGTTCC3’
GPR7RevClon: 5’CGGATCTCTCATGGGTCGGGAC3’

These two constructs were then digested using Xhol and BamH1 and the resulting GPR7 fragments were inserted into mammalian expression vector pcDNA3 (-) (Invitrogen) to generate pcR2.1 GPR7 wild type and pcR2.1 GPR7 mutant. After sequencing the two constructs to verify the absence of cloning artifacts, the wild-type and mutant GPR7 genes were transiently transfected into COS-7 cells using Lipofectamine 2000 (Invitrogen) method. COS-7 cells were chosen based on preliminary experiments showing lack of endogenous mRNA GPR7 expression (data not shown). Cells were first grown on 10-cm Petri dishes in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 2% penicillin/streptomycin (Invitrogen), 1% fungizone (Invitrogen), and incubated at 37 C in KRH buffer. This was followed by 10 min incubation at 37 C with forskolin (1 μM) alone or in combination with three different concentrations of human Neuropeptide W-30 (NPW-30; Phoenix Pharmaceutical, Inc., CA - USA); 0.5 mmol/l isobutyryl-methyl-yanthan was added as a cAMP phosphodiesterase inhibitor. At the end of the incubation period, the medium was removed and replaced by 0.1 mol/l HCl (15). After 20 min at room temperature, the HCl solution was collected. The intracellular cAMP concentration was measured by radioimmunoassay (RIA) using a commercially available antibody (Sigma-Aldrich Corp., St. Louis, MO) and in-house radioiodinated cAMP succinyl-methyl-ester (PerkinElmer, Inc. California, US). Twenty-five microliters of HCl lysate solution were incubated in the presence of 0.1, 0.05, or 0.01 mol/l citrate buffer, 0.1 ml 1:350 diluted cAMP antibody, and approximately 12,000 cpm [125I] cAMP in 0.1 ml citrate buffer. After incubation at 4 C for 16-18 h, 1 ml 0.1% dextran T70 charcoal-activated BSA was added, and the mixture was centrifuged at 3,000 x g for 30 min to separate bound from free radioactivity. cAMP was expressed as picomoles/well, using a nine-point standard curve. All the measurements were performed in triplicate.

Statistical analysis
Statistical analysis was performed by using Statistical Package Stat-View for Windows, version 5.0 (SAS Institute, Cary, NC, USA).

The statistical methods used for frequency distribution analysis included χ2-test of significance and Z-test with the Yates correction. cAMP production in WT and mutant transfected cells was analyzed using 2 way analysis of variance. p-values <0.05 were considered statistically significant.

In silico analysis of GPR7 allelic variant
We have performed in silico analysis of the GPR7 Tyr135Phe variant using the Align-Grantham Variation-Grantham Deviation (Align-GVGD) web-based software (http://agvgd.iarc.fr). Multiple sequence alignments (MSA) from five different species were used for the GPR7 protein and the Grantham Variation (GV) and Grantham Deviation (GD) values were calculated for Tyr135Phe allelic variant. The software calculates the degree of structural difference between the mutated and the wild-type proteins, thus providing a numerical score that may range from Class C0 (no difference) to Class C65 (maximum difference), where a higher score indicates a more powerful transforming activity (16).

RESULTS
Screening for mutations of the GPR7 receptor
GPR7 coding exom was amplified from the genomic DNA of obese and normal-weight subjects and directly sequenced. Both populations were in Hardy-Weinberg equilibrium. Overall, 45 subjects exhibited a previously