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Short Communication

The reduction of Calpain-10 expression is associated with risk polymorphisms in obese children

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ABSTRACT

Excessive weight gain and obesity are major public health concerns. Childhood obesity is growing at an alarming rate. Polymorphisms in the Calpain-10 gene and the reduced expression of this gene in muscle cells and adipocytes have been associated with an increased risk of type 2 diabetes mellitus in several populations. In the present study, we explored the contribution of Calpain-10 in the development of metabolic impairment in childhood. We evaluated the presence of risk polymorphisms in the CAPN10 gene (SNP-44, SNP-43, InDel-19 and SNP-63) and the associated changes in the Calpain-10 mRNA levels in a pediatric population. A total of 161 Mexican children between 4 and 18 years old were included in this study. This population was classified into three groups according to international growth references: healthy weight (HW), overweight (OW) and obese (OB). Association studies of the anthropometric data, clinical values, genotyping and expression assays showed a decrease in the Calpain-10 mRNA and protein expression in the OW and OB groups with respect to the HW group. This decrease in the Calpain-10 mRNA expression was more evident in individuals homozygous for SNP-44 (T/T) and InDel-19 (3/3), alone ($p<0.001$ and $p=0.015$, respectively) or in combination ($p=0.017$). These polymorphisms were also associated with elevated BMI, weight percentiles, z-scores, waist circumferences, fasting glucose levels and beta cell functions in the OW and OB groups ($p<0.05$). Moreover, our results indicate a statistically significant decrease in the expression of the 75-kDa Calpain-10 isoform in the OW+OB group. The presence of polymorphisms and alterations in the expression of the CAPN10 gene at early ages might result in metabolic impairment in adulthood and should be further investigated.

1. Introduction

Obesity is becoming a worldwide epidemic. The prevalence of obesity and excessive weight gain in the Mexican population over 20 years has been reported as 34% and 43%, respectively. In the pediatric population, the prevalence of excessive weight gain and obesity ranges from 26 to 32% (Olaiz et al., 2006). Childhood obesity has been associated with adulthood obesity, premature death, disability, respiratory and psychological problems, hypertension, cardiovascular disease and insulin resistance. Moreover, weight gain, expressed as body mass index (BMI), is considered an important risk factor for metabolic syndrome (MS), diabetes and cardiovascular disease (World Health Organization, 2011).

Obesity results from the interaction between environmental and genetic factors. Different agents, such as lifestyle, physical activity and pollutants, might contribute significantly to the development of obesity (Hanis et al., 1996; Horikawa et al., 2000; Yang et al., 2007).

Abbreviations: BMI, body mass index; CAPN10, Calpain-10 gene; cDNA, complementary DNA; CT, threshold cycle; ΔC_T, CT (target gene) − CT (endogenous reference gene); $\Delta\Delta C_T$, average CT (sample of interest) − average CT (reference sample); CVs, coefficients of variation; CDC, Centers for Disease Control and Prevention; EDTA, ethylenediaminetetraacetic acid; HOMA, homeostasis model assessment; HOMA2, homeostatic model assessment (updated HOMA model); HOMA2 %B, homeostatic model assessment (beta cell function); HOMA-IR, homeostatic model of assessment—insulin resistance; HW, healthy weight children; mRNA, messenger RNA; MS, metabolic syndrome; OB, obese children; OW, overweight children; OW+OB, overweight and obese children; SEM, standard error of the mean; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; USDA/ARS, Children's Nutrition Research Center; WHO, World Health Organization.

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Polymorphisms in several genes have been associated with a predisposition to obesity (Cheng, 2005; Dedoussis et al., 2007; Yang et al., 2007). Because obesity is a major risk for type 2 diabetes mellitus (T2DM), the genes previously identified in insulin signaling and glucose uptake are currently being studied in overweight and obesity phenotypes (Cheng, 2005; Dedoussis et al., 2007; Yang et al., 2007).

Calpain-10 is associated with metabolic regulation and is one of the confirmed susceptibility genes for the development of T2DM. The CAPN10 gene encodes a calcium-dependent protease, which is present in eight different isoforms through alternative splicing. Calpain-10 isoforms have been involved in a variety of cellular processes, including insulin secretion (Marshall et al., 2005), GLUT4 translocation in response to insulin in skeletal muscle cells and adipocytes (Brown et al., 2007; Paul et al., 2003), β-cell apoptosis (Johnson et al., 2004) and mitochondrial function (Arrington et al., 2006). Although the CAPN10 gene was identified as a susceptibility gene for T2DM through genetic linkage studies and positional cloning, CAPN10 has recently been associated with insulin resistance phenotypes (Hanis et al., 1996; Horikawa et al., 2000). Variations in this gene (SNP-43 G/G, InDel-19 3/2 and SNP-63 C/T), known as haplotype 121/112, have been associated with a three-fold risk of T2DM in the Mexican-American population (Horikawa et al., 2000). Although these polymorphisms occur in non-coding regions, these mutations affect transcriptional gene regulation (Baier et al., 2000; Horikawa et al., 2000; Yang et al., 2001).

Because Calpain-10 is required for a wide variety of processes associated with the regulation of energy balance, this gene could represent a susceptibility gene for obese phenotypes. In this study, we evaluated the association of four polymorphisms in the CAPN10 gene (SNP-44, SNP-43, InDel-19 and SNP-63) in a pediatric population with overweight and obesity phenotypes and the effects of these mutations on mRNA and protein expression and other metabolic alterations.

2. Materials and methods

2.1. Subjects

A total of 161 children living in the northern area of Mexico City participated in the study. The population included individuals between 4 and 18 years of age without any severe infection or chronic disease. Anthropometric characteristics (age, sex, weight, height, waist circumference) and clinical data (glucose, insulin, cholesterol and triglycerides) were obtained from each participant. Whole blood samples were obtained under fasting conditions and stored in tubes containing EDTA until further processing. Informed consent from the legal guardian was required for each participant.

2.2. Classification of the participants into pediatric groups

The participants were classified into three groups: healthy weight (HW), overweight (OW) and obese (OB). The determinations of HW (BMI<25 kg/m^2, percentile<85 and z-score<+1), OW (BMI≥25 kg/m^2, percentile≥85 and z-score>+1 and<+2) and OB (BMI≥30 kg/m^2, percentile≥95 and z-score>+2) were performed according to international growth standards for infants WHO (World Health Organization) http://www.who.int/growthref/tools/en/, CDC (Centers for Disease Control and Prevention) www.cdc.gov and *USDA/ARS Children's Nutrition Research Center* www.bcm.edu. To compare the child growth we used the WHO AntroPlus software. To assess whether the changes were age-dependent, we re-grouped the pediatric population according to age.

2.3. Biochemical parameters

Glucose, cholesterol and triglycerides were determined from 200 μL of plasma taken under fasting conditions through enzymatic assays using a Roche Cobas Mira analyzer with commercial kits (Stanbio Laboratory, Boerne, TX, USA). Insulin levels were determined through radioimmunoassay (Siemens Healthcare Diagnostics, Los Angeles CA, USA) with a sensitivity of 8.3 pmol/L and intra/inter-assay coefficients of variation (CVs) of 5.2 and 7.3%, respectively. The homeostasis model assessment was calculated in two indices, HOMA and HOMA2, based on measurements of fasting glucose and fasting insulin. The HOMA index was calculated according to the following formula: HOMA IR = insulin (mU/L) × glucose (mmol/L)/22.5 (Matthews et al., 1985). The HOMA2 index was calculated using the HOMA2 Calculator v.2.2 software (© Diabetes Trials Unit, University of Oxford http://www.dtu.ox.ac.uk/homa).

2.4. Lymphocyte isolation

The lymphocytes for DNA, RNA and protein extractions were isolated from peripheral blood samples under fasting conditions using a 1:1 density gradient of Ficoll-Histopaque (Histopaque®-1077 H8889, Sigma-Aldrich Company, Ltd., Irvine, Ayrshire, UK).

2.5. DNA extraction and quantification

The DNA extraction was performed according to standard protocols using Nonidet P-40 (Igepal CA-630, 13 021 Sigma-Aldrich). The genomic DNA was re-suspended in TE buffer, quantified in a Nanodrop-1000 spectrophotometer (Thermo Fisher Scientific, Inc.) and stored at −20 °C until further processing.

2.6. Genotyping assays

The SNP-43 G→A (*rs3792267*) and SNP-44 T→C (*rs2975760*) polymorphisms were genotyped using allelic discrimination assays in a Real-Time PCR System (Applied Biosystems). The following primers and TaqMan-MGB probes were used for SNP-43: F 5′→3′ gcg ctc acg ctt gct; R 5′→3′ cct cac caa gtc aag gct tag c; allele 1 (G) probe 5′→3′ VIC-ctt caa acg cct tac tt and allele 2 (A) probe 5′→3′ FAM-ctt caa atg cct tac tt. For SNP-44: F 5′→3′ gca ggg cgc tca cg; R 5′→3′ gct tag cct cac ctt caa acg; allele 1 (T) probe 5′→3′ FAM-cct tac ttc aca gca ag and allele 2 (C) probe 5′→3′ VIC-cct tac ttc gca gca ag.

The InDel-19 2→3 (*rs3842570*) polymorphism was identified using end-point PCR with the following primers: F5′-gtt tgg ttc tct tca gcg tgg ag-3′ and R 5′-cat gaa ccc tgg cag ggt cta ag-3′. The PCR was performed with 40 ng of genomic DNA. The cycling conditions were 2 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C and 80 s at 72 °C and a final extension at 72 °C for 5 min. Allele 1 (two repeats of 32 bp sequences) and allele 2 (three repeats of 32 bp repeats) were identified from 155 to 187 bp fragments, respectively.

The SNP-63 C→T (*rs5030952*) polymorphism was genotyped using end-point PCR and automated DNA sequencing. A 418 bp fragment of the CAPN10 gene was amplified through end-point PCR with 200 ng of genomic DNA and F 5′-tcg gga cac tgc tgt tag gt-3′ and R 5′-ctg gct gga gtt tgg aga ag-3′ primers. The cycling conditions were 2 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 62 °C and 80 s at 72 °C and a final extension at 72 °C for 5 min. For the sequencing assays, 80 ng of PCR products were labeled with ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The labeling conditions were 2 min at 92 °C, followed by 20 cycles of 20 s at 96 °C, 10 s at 55 °C and 4 min at 60 °C and a final extension at 72 °C for 1 min. The labeled PCR products were purified using a series of alcohols and denatured in Hi-Di™ Formamide (Applied Biosystems) at 95 °C for 5 min. The PCR fragments were analyzed in a 3100-Avant Genetic Analyzer (Applied Biosystems). The presence of C identifies allele 1, and the presence of T identifies allele 2.