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Original article

Complete sequence of the ANKK1 gene in Mexican-Mestizo individuals with obesity, with or without binge eating disorder

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ABSTRACT

Background: The aim of this study was to investigate if Mexican-Mestizo individuals with obesity, with or without binge eating disorder (BED), exhibited mutations or other type of genetic variants in the sequence of *ANKK1*.
Subjects and methods: Fifty unrelated individuals (21–53 years of age) with obesity, of Mexican-Mestizo ethnic origin were included; 25 of them had BED and 25 presented obesity without BED. The diagnosis of BED was based on criteria proposed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Besides, we also analyzed 100 individuals with normal body mass index. DNA from blood leukocytes was amplified by the polymerase chain reaction and all exons of *ANKK1* were sequenced.
Results: After *ANKK1* sequencing we did not find any mutations; however, we observed various polymorphisms. One polymorphism, rs4938013 in exon 2 showed an association with obesity, whilst rs1800497 (also known as Taq1A) in exon 8, showed an association with BED ($P = 0.020$). Remarkable, for this study, the number of individuals for both polymorphisms for and additive model was sufficient to derive strong statistical power (80%, with a $P < 0.05$).
Conclusions: To our knowledge, this constitutes the first report where the complete sequences of *ANKK1* has been analyzed in individuals with obesity, with or without BED. No mutations were found; however, one polymorphism was associated with obesity, with or without BED, and another one was associated with BED.

1. Introduction

Obesity is a multifactorial disease that has reached epidemic proportions worldwide. In Mexico, the last National Survey demonstrated that the prevalence of obesity has augmented from 21.5% in 1993, to 33.3% in 2016 [1]. It has been established that obesity is associated with several psychiatric disorders [2,3], being binge eating disorder (BED) one of them [4]. One of these entities is BED which is characterized by recurrent-persistent episodes of uncontrolled binge eating with distress, in the absence of regular compensatory behaviors [5]. According to the WHO World Mental Health (WMH) Survey Initiative [6] performed in several countries, including Mexico, lifetime prevalence has been estimated at an average of 1.9%, across surveys. Individuals presenting BED, have a significantly higher BMI than those without a history of this eating disorder. Furthermore, individuals with BED are more susceptible to have other concomitant psychiatric disorders, like depression or anxiety [7].

At present, the precise etiology of BED has not been determined. This pathological entity has been considered a multifactorial disease where diverse risk factors, including genetics, play a role on its onset. It has been estimated that BED presents a heritability between 41% and 57% [8,9], which suggests that genetic variations could play an important role in its pathogenesis. Several candidate genes have been analyzed; however, the results of these studies have not been conclusive [10].

Dopamine receptor type 2 (DRD2), a G-protein coupled receptor, located on the postsynaptic dopaminergic neurons, is centrally involved in the reward-mediating mesocorticolimbic pathways. This receptor has been the focus of many studies investigating genetic variations that could be associated with

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addictive behavior and with reward mechanisms [11,12]. One of the most frequently studied functional polymorphisms of the D2 receptor was Taq1A (dbSNP_rs1800497: c.2137 G > A, p.Glu713Lys). For many years, Taq1A was thought to be located on the 3′-untranslated region of *DRD2*; however, subsequent studies demonstrated that this single-nucleotide polymorphism (SNP) was not a part of *DRD2*, but of an adjacent gene named as ankyrin repeat and kinase domain containing 1 (*ANKK1*) [13].

It has been demonstrated that *ANKK1* is activated by apomorphine, a dopaminergic agonist, indicating a potential connection between *ANKK1* and the dopaminergic system. Besides, due to its proximity with *DRD2*, it has been suggested that *ANKK1* may play an important role in modifying dopaminergic signaling, following drug exposure [14].

Although obesity has been associated with BED, there are obese individuals that never present BED. Since previous studies have only analyzed polymorphisms in *ANKK1* [11,15–18] and have not searched for the presence of mutations in the open reading-frame of this gene, as a possible cause of obesity with or without BED, the aim of this study was to investigate if individuals with obesity with or without BED, presented mutations in the sequence of *ANKK1*.

2. Subjects and methods

2.1. Subjects

The Human Research Committees of the participating institutions approved the study. Informed consent was obtained from all individuals before been included. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Twenty-five unrelated individuals presenting obesity with BED (9 males and 16 females) and twenty-five unrelated individuals presenting obesity without BED (12 males and 13 females) were recruited from the Clinic of Obesity and Eating Disorders of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. All patients were of Mexican-Mestizo ethnic origin. According to the National Institute of Anthropology, a Mexican-Mestizo is defined as a person who was born in Mexico, has a Spanish-derived last name, and has a family of Mexican ancestors back to the third generation [19]. The diagnosis of BED was determined by a psychiatrist of the clinic, who administered a semi-structured questionnaire to all individuals on a face-to-face mode. The diagnosis of this entity was based on those criteria proposed in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [5].

Body height and weight were measured at baseline examination, with the subject in a standing position, wearing a hospital gown and without shoes. Height and weight were used to calculate body mass index (BMI) (kg/m^2). Individuals were categorized as having a BMI of obesity if it was $\geq$30.0 kg/m^2. Besides, we measured body fat percentage by bio impedance using a Tanita scale (TBF 300a; Tanita Corporation, Itabashi-Ku, Tokyo, Japan).

Individuals with a previous diagnosis of bulimia nervosa, any psychotic disorder, substance abuse, alcoholism, or a serious medical/physical illness such as cancer, or heart disease, were excluded from the study.

In addition, 100 individuals were recruited as controls (38 males and 62 females). All of them **were** of Mexican-Mestizo ethnic origin and were donors at the Blood Bank of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. All of them presented a normal BMI (BMI = 22.0 ± 1.6 kg/m^2 for females, BMI = 23.3 ± 1.3 kg/m^2 for males), with ages ranging between 21–38 years and without any history of eating disorders; besides, these individuals did not present any history of major physical disorders, bulimia nervosa or a history of obesity, substance abuse, or alcoholism.

2.2. Methods

Genomic DNA was isolated from blood leukocytes using the salting out procedure described by Miller et al. [20]. DNA was amplified by PCR in 25 μl of mixture reaction, containing 100 ng of genomic DNA, 0.1 mM dNTPs, 2.0 U of KAPA Taq PCR Kit (KapaBiosystems, Massachusetts, USA), and 250 nM of each specific set of *ANKK1* primers. Primers were designed by us using Primer3web version 4.0.0 (http://primer3.ut.ee/). Due to its size, exon 8 of *ANKK1* (1306 base pairs), was divided into five fragments. The sequences of the oligonucleotides, the size of the amplified products and the annealing temperatures are under request.

After amplification, PCR products were subjected to electrophoresis on 1.2% agarose gels and stained with Midori Green DNA Stain (Nippon Genetics Europe GmbH, Düren, Germany) to verify the correct size of the expected fragments. PCR products were purified by QIAEX II (QIAGEN GmbH, Hilden, Germany). DNA sequences (100 ng DNA template/reaction) were determined by cycle sequencing on an automated DNA sequencer 3500XL (Applied Biosystems Division, Foster City, CA, USA) using the DNA Sequencing Kit BigDye™ Terminator Cycle Sequencing Ready Reaction (PE Biosystems, Foster City, CA, USA). Sequencing was performed following the protocol supplied by the manufacturer.

2.2.1. Statistical analysis

We performed a cross-sectional study. Data from the overall patient population were summarized as mean and standard deviation. To evaluate differences between groups (control and case), an unpaired Student *t*-test was used.

Deviations from Hardy-Weinberg equilibrium and the differences of the alleles and genotypes frequencies between groups were assessed by X^2 tests. Multinomial logistic regression analysis was used to determine if a specific genotype is associated with obesity, with and without BED. Odds ratio (OR) with a 95% confidence interval (CI) and an associated p-value, was calculated as a measure of association. For multiple comparisons of the

Table 1
Clinical characteristics of individuals with obesity, with or without BED.

Variables	Individuals with obesity without BED N = 25	Individuals with obesity and BED N = 25
Sex, m/f (N)	12/13	9/16
Age (years)	30 - 52	27 - 53
BMI (kg/m^2)	38.6 ± 5*	42.3 ± 6.6*
Fat (%)	39.2 ± 2.6**	42.2 ± 3.7**

BED = binge eating disorder; N = number of individuals; m = male; f = female; BMI = body mass index. The values of the age are expressed in range, the rest of the data are mean ± SD. BMI difference between individuals with obesity without BED and individuals with obesity with BED (*P < 0.02). Fat % was different between individuals with obesity without BED and individuals with obesity with BED (**P < 0.0001).