Association between the feeding behavior factors and the expression of DRD2 gene: a study of Mexican monozygotic twins

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Abstract

Introduction: The feeding behavior in humans is complex, with psychological, social, and cultural factors. This pathology consists of the desire to eat (reinforcement/reward) and the control of the act of eating (inhibitory control). It is well known that blocking post-synaptic D2 receptors in the nucleus accumbens induces a decrease of craving behavior. Due to this, to test hypothesis we measured the expression of the DRD2 gene and the expression of the type of feeding factor in a group of Mexican twins. Methods: In the study were included 18 pairs of twins (n = 36), reared together (at least up to 18 years old); native to Mexico City; to whom we applied the three factors of food instrument. Results: We observed a significant correlation between the “uncontrolled” factor and expression of the DRD2 gene (p < 0.03). Conclusion: Therefore, we propose that the expression of the DRD2 gene in subjects is a linkage to the type of feeding factor. (Gac Med Mex. 2016;152:295-8)

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Introduction

Eating behavior in the human being has been transformed from being a mainly physiological activity (satisfaction of nutritional requirements) into a complex behavior with psychological, social and cultural nuances and factors1.

It is suggested that the progressive industrialization-commercialization of food, together with a lack of control of the eating behavior, entail both inadequate nutrition and overweight2. Similarly, in the eating behavior, an adequate neurocognitive balance between the desire to eat (reinforcement/reward) and the control of the act of eating (inhibitory control) is necessary in order to prevent overweight, since the weakening of the inhibitory control in favor of the reinforcement/reward system would generate compulsive behaviors with regard to nutrition3.

The literature suggests an implication of the dopaminergic system in the regulation of eating behavior4,5. The proposed mechanism is based on the hypothesis that people with dopamine and/or dopaminergic receptors reduced levels might be predisposed to the consumption of any substance that activates dopamine.
release. Most frequently used stimulants include food, alcohol, use of opiates and nicotine. Acute use of these substances produces a sensation of wellbeing in the individual6.

Some studies have evaluated the association of the dopamine receptor DRD2 gene and suggest that this gene might be associated with obesity and eating behavior disorders6-9. In this sense, the blockage of the nucleus accumbens D2 post-synaptic receptors is known to induce a decrease in the craving behavior3,5,10.

On the other hand, the literature classifies eating behavior in 3 factors: cognitive control, uncontrolled eating and emotional eating11,12. In this idea, it is suggested that subjects with the cognitive control characteristic prefer “healthy” food; however, they can be associated with excessive food restrictions11,13,14. Individuals with the “uncontrolled” characteristic prefer greasy and salty foods; these subjects often display compulsivity to eat. Finally, individuals of the “emotional” type tend to eat more in response to stress and emotions15,16 and prefer rather elaborated foods such as cakes and sandwiches14.

Therefore, we hypothesize that the DRD2 gene expression in individuals is correlated to the type of alimentary factor. To test this hypothesis, we measured the expression of the DRD2 gene and correlated it with the alimentary factors in a group of Mexican monozygotic twins.

**Method**

**Sample**

Eighteen pairs of twins (n = 36), who were raised together (at least until 18 years of age), originating from Mexico City, were included. To test for the monozygosity of the twins, each pair underwent DNA tests with the AmpF1STR identifier® kit (Applied Biosystems) following the manufacturer’s instructions. The subjects were selected from July 2009 through August 2015 at the Carracci medical studies clinic, in Mexico City.

**Declaration of ethics**

This study was approved by the committee of ethics in research of Grupo Médico Carracci. All subjects signed an informed consent form to participate in the study.

**Data collection**

The study subjects were interviewed in order to diagnose past or present mental pathology according to the DSM-IV17. Only individuals with no psychiatric pathology were included in the study. Socio-demographic and anthropomorphic information of the subjects was obtained. The socio-demographic information was obtained based on other studies reported in the literature18. It included: Date of birth, gender, marital status, occupation, level of education and tobacco consumption. Anthropometric measurements: Anthropometric parameters included weight, height and blood pressure, according to the Mexican official standard. The measurement was collected just as reported in other studies19.

**Measurement of eating behaviors**

The subjects were applied the Three-Factor Eating Questionnaire20, version in Spanish language21. This instrument allows for three factors to be determined: cognitive restraint (capacity to inhibit the eating tendency), uncontrolled eating (eating compulsivity) and emotional type (dominance of the experience of hunger to start eating).

**Expression analysis**

The gene expression analysis was conducted in the laboratory of psychiatric and neurodegenerative diseases genomics of the National Institute of Medicine. Total RNA was extracted from lymphocytes using the TRizol reagent (Invitrogen, Toluca, Mexico) according to the manufacturer’s instructions and quantified with fluorometry using the Quant-iT Ribogreen Assay kit (Invitrogen). The RNA reading was quantified in the fluorometer (Ex = 485 nm, Em = 538 nm). In all cases, the RNA was assessed by agarose gel electrophoresis, stained with ethidium bromide in order to evaluate its integrity (Nicolini et al., 2012). cDNA synthesis was carried out using the SuperScriptTM III First-Strand Synthesis Super-Mix kit (Invitrogen) following the manufacturer’s instructions. The DRD2 gene expression was determined in a 7500 Real-Time PCR System equipment (Applied Biosystems) using TaqMan® Human DRD2 FAM (Hs002411436-m1) probes in multiplex assays (in triplicate), using ribosomal RNA as endogenous gene.

For the gene expression analysis, the 2^(-ΔΔCT) method was used (Schmittgen et al., 2007). 2^(-ΔΔCT) has been used to calculate the assessed genes relative expression. The data of this study are presented as the target genes expression change in peripheral blood lymphocytes, normalized with the endogenous gene (18s).
The real-time PCR data were expressed as CT values, where CT is defined as the cycle threshold during the PCR amplification where the product was first detected. CT average was calculated for each one of the twin pairs’ samples, the endogenous gene and the target gene. ΔCT was determined as (mean CTs of the triplicates’ target gene) minus (C-values mean). ΔΔCT represents the difference between the pairs of twins, according to the calculation with the formula ΔΔCT = (ΔCT of twins with higher body mass index - ΔCT of the other twin).

The number of times one twin is expressed with regard to the other is obtained by means of the 2-ΔΔCT formula, where, if the value obtained is expressed more or less with regard to the other twin, it is defined as an increase in RNA expression if the N-value is ≥ 1.0-fold and a decrease in RNA expression if N is ≤ 1.0-fold (Nicolini et al., 2012). The delta value between twins was obtained taking into account the body mass index (BMI), so that a twin with higher BMI was overexpressed or underexpressed with regard to the one with lower BMI.

**Statistical analysis**

Descriptive statistics was used to characterize the study population. The coefficient of variation (standard deviation divided by the mean, multiplied by 100) was used to homogenize the study groups. Pearson’s correlation coefficient was calculated to observe the correlation between gene expression and the type of eating behavior displayed by the individuals. A p-value ≤ 0.05 was considered to be significant.

**Results**

Of the 36 participants, 46.92% had overweight and obesity. The lowest difference between twins in BMI was 0.43 and the highest 7.83. Age average of the sample was 28.36 years ± 7.45. Age and BMI characteristics, as well as BMI differences by gender of the twins included in the study are shown in table 1.

The most homogeneous coefficient of variation in the three eating factors was observed in the uncontrollable type (38.18), whereas in the cognitive restraining type (42.74) and the emotional type (48.27) it was greater.

When the sample was independently analyzed, a significant correlation was observed between the “uncontrolled” factor and the DRD2 gene expression (p < 0.03) (Table 2). When the contribution of the twins was analyzed, the DRD2 gene expression was not significantly correlated with the eating types.

**Discussion**

The purpose of the study was to correlate the DRD2 gene expression and eating factors in Mexican monozygotic twins. The individuals who displayed the “uncontrolled” factor showed higher DRD2 gene expression. DRD2 gene expression has been widely studied in humans, and has been consistently associated with different behaviors, including the addictive ones3,22. Only recently, excessive eating started to be considered as an addictive behavior3. To our knowledge, this is the first study to assess the relationship between

### Table 1. Age, body mass index and body mass index variation of the Mexican twins sample

<table>
<thead>
<tr>
<th></th>
<th>Total n = 18 twins</th>
<th>Males n = 9 twins</th>
<th>Females n = 9 twins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>Age</td>
<td>28.6</td>
<td>7.45</td>
<td>28.73</td>
</tr>
<tr>
<td>BMI</td>
<td>24.95</td>
<td>4.93</td>
<td>24.76</td>
</tr>
<tr>
<td>ΔBMI</td>
<td>2.50</td>
<td>2.20</td>
<td>2.28</td>
</tr>
</tbody>
</table>

BMI: body mass index; ΔBMI: body mass index variation; SD: standard deviation.

### Table 2. Correlation between eating factors and DRD2 gene expression in a Mexican population

<table>
<thead>
<tr>
<th>Eating factors</th>
<th>Pearson’s correlation</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive restriction</td>
<td>0.005</td>
<td>0.97</td>
</tr>
<tr>
<td>Uncontrolled (impulsivity)</td>
<td>-0.36</td>
<td>0.03</td>
</tr>
<tr>
<td>Emotional</td>
<td>-0.03</td>
<td>0.84</td>
</tr>
</tbody>
</table>
The results presented in this work are in agreement with those reported in the literature, in the sense that DRD2 gene expression is directly related to compulsivity, which is the main characteristic of the “uncontrolled” factor. In other words, the higher the expression of the DRD2 gene, the higher the eating compulsivity behavior, and, therefore, a higher predisposition to have overweight or obesity. The literature suggests that behavior can participate in gene expression. However, in the analysis by twins, we observed no statistically significant correlation between the DRD2 expression and eating factors. We consider that the sample size (n = 18 pairs of twins) may have influenced on the result. Therefore, studies are required to assess this correlation using larger samples.

One strength of the study is the type of sample that was used: monozygotic twins. This type of studies provides data on the homogeneity of the genetic-parental-environmental load that can be obtained in a sample of twins raised together and eating behaviors. In addition, this approach to overweight and obesity and their correlation with gene expression opens the possibility to pharmacologic or gene therapy alternatives. On the other hand, some limitations of the study are: 1) the sample size; 2) Perinatal data of the twins such as being born with obesity and its correlation with DRD2 expression were not included in the study.

In conclusion, the results of this study provide evidence of the dopaminergic pathway participation and satisfaction in food intake. This evidence should be considered in the efforts to control body weight in the general population. Future studies with larger sample size are needed to replicate these results.

Acknowledgements

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