Contents available at **PubMed**
www.anmm.org.mx

PERMANYER

Gac Med Mex. 2016;152:295-8

GACETA MÉDICA DE MÉXICO

ORIGINAL ARTICLE

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

Alma Genis-Mendoza[1,2], Humberto Nicolini[2], Carlos Alfonso Tovilla-Zárate[3], María Lilia López-Narváez[4] and Thelma Beatriz González-Castro[5]*

[1]Hospital Infantil Juan N. Navarro, Secretaría de Salud; [2]Instituto Nacional de Medicina Genómica (INMEGEN). Mexico City; [3]Universidad Juárez Autónoma de Tabasco, Multidisciplinary Academic Division of Comalcalco, Comalcalco, Villahermosa, Tab.; [4]Hospital General de Yajalón, Secretaría de Salud, Yajalón, Tuxtla Gutiérrez, Chis.; [5]Universidad Juárez Autónoma de Tabasco, Multidisciplinary Academic Division of Jalpa de Méndez, Cunduacán, Villahermosa, Tab., Mexico

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)

Corresponding author: *Carlos Alfonso Tovilla-Zárate, alfonso_tovillaz@yahoo.com.mx*

KEY WORDS: *Eating behavior. Expression.* DRD2 *gene. Twins.*

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 factors[1].

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 overweight[2]. 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 nutrition[3].

The literature suggests an implication of the dopaminergic system in the regulation of eating behavior[4,5]. 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

Correspondence:
*Carlos Alfonso Tovilla-Zárate
División Académica Multidisciplinaria de Comalcalco
Ranchería Sur, Cuarta Sección
C.P. 86650, Comalcalco, Villahermosa, Tab., México
E-mail: alfonso_tovillaz@yahoo.com.mx

Date of reception: 06-02-2015
Date of acceptance: 20-04-2015

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 individual[3].

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 disorders[6-9]. In this sense, the blockage of the nucleus accumbens D2 post-synaptic receptors is known to induce a decrease in the craving behavior[3,5,10].

On the other hand, the literature classifies eating behavior in 3 factors: cognitive control, uncontrolled eating and emotional eating[11,12]. In this idea, it is suggested that subjects with the cognitive control characteristic prefer "healthy" food; however, they can be associated with excessive food restrictions[11,13,14]. 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 emotions[15,16] and prefer rather elaborated foods such as cakes and sandwiches[14].

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 Biosystem) 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-IV[17]. 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 literature[18]. 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 studies[19].

Measurement of eating behaviors

The subjects were applied the Three-Factor Eating Questionnaire[20], version in Spanish language[21]. 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 SuperScript™ 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 $2^{-\Delta\Delta CT}$ method was used (Schmittgen et al., 2007). $2^{-\Delta\Delta CT}$ 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*).

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

	Total n = 18 twins		Males n = 9 twins		Females n = 9 twins	
	Average	SD	Average	SD	Average	SD
Age	28.6	7.45	28.73	7.61	28.83	7.44
BMI	24.95	4.93	24.76	5.12	24.57	4.89
Δ BMI	2.50	2.20	2.28	1.95	2.61	2.26

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

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 $2^{-\Delta\Delta CT}$ 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 as 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.

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

Eating factors	Pearson´s correlation	Significance (2-tailed)
Cognitive restriction	0.005	0.97
Uncontrolled (impulsivity)	–0.36	0.03
Emotional	–0.03	0.84

The most homogeneous coefficient of variation in the three eating factors was observed in the uncontrolled 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 ones[3,22]. Only recently, excessive eating started to be considered as an addictive behavior[3]. To our knowledge, this is the first study to assess the relationship between

DRD2 gene expression profiles and eating behaviors in a Mexican population. The results indicate that this relationship is specific to the *DRD2* gene, since other studies have failed to find this relationship with other receptor subtypes such as *DRD3*[23].

The results presented in this work are in agreement with those reported in the literature[3], 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[24]. 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[20]. In addition, this approach to overweight and obesity and their correlation with gene expression opens the possibility to pharmacologic or gene therapy alternatives[5]. 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

To the Instituto de Ciencia y Tecnología del Distrito Federal (ICYTDF) for the funding granted to this project (ICyTDG/282/2009).

References

1. Sánchez-Carracedo D, Raich R, Figueras M, Torras J, Mora M. Adaptación preliminar del cuestionario de alimentación de Stunkard y Messick (Three Factor Eating Questionnaire, TFEQ) con una muestra española universitaria. Psicología Conductual. 1999;7:393-413.
2. Tudor-Locke C, Bell RC, Myers AM, et al. Controlled outcome evaluation of the First Step Program: a daily physical activity intervention for individuals with type II diabetes. Int J Obes Relat Metab Disord. 2004;28:113-9.
3. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F. Addiction: beyond dopamine reward circuitry. Proc Natl Acad Sci U S A. 2011;108:15037-42.
4. Carpenter CL, Wong AM, Li Z, Noble EP, Heber D. Association of dopamine D2 receptor and leptin receptor genes with clinically severe obesity. Obesity (Silver Spring). 2013;21:E467-73.
5. Blum K, Chen AL, Chen TJ, et al. Activation instead of blocking mesolimbic dopaminergic reward circuitry is a preferred modality in the long term treatment of reward deficiency syndrome (RDS): a commentary. Theor Biol Med Model. 2008;5:24.
6. Genis-Mendoza AD, Tovilla-Zárate CA, Nicolini H. Avances en genómica de los trastornos de la conducta alimentaria. Revista Colombiana de Psiquiatría. 2013;42:350-5.
7. Groleau P, Steiger H, Joober R, et al. Dopamine-system genes, childhood abuse, and clinical manifestations in women with Bulimia-Spectrum Disorders. J Psychiatr Res. 2012;46:1139-45.
8. Noble EP, Noble RE, Ritchie T, et al. D2 dopamine receptor gene and obesity. Int J Eat Disord. 1994;15:205-17.
9. Wang TY, Lee SY, Chen SL, et al. Association between DRD2, 5-HTTLPR, and ALDH2 genes and specific personality traits in alcohol- and opiate-dependent patients. Behav Brain Res. 2013;250:285-92.
10. Finlayson G, King N, Blundell J. The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control. Appetite. 2008;50:120-7.
11. Chacko SA, Chiodi SN, Wee CC. Recognizing disordered eating in primary care patients with obesity. Prev Med. 2015;72:89-94.
12. Karlsson J, Persson LO, Sjostrom L, Sullivan M. Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. Int J Obes Relat Metab Disord. 2000;24:1715-25.
13. Beiseigel JM, Nickols-Richardson SM. Cognitive eating restraint scores are associated with body fatness but not with other measures of dieting in women. Appetite. 2004;43:47-53.
14. de Lauzon-Guillain B, Basdevant A, Romon M, Karlsson J, Borys JM, Charles MA. Is restrained eating a risk factor for weight gain in a general population? Am J Clin Nutr. 2006;83:132-8.
15. Cappelleri JC, Bushmakin AG, Gerber RA, et al. Psychometric analysis of the Three-Factor Eating Questionnaire-R21: results from a large diverse sample of obese and non-obese participants. Int J Obes (Lond). 2009;33:611-20.
16. Ozier AD, Kendrick OW, Leeper JD, Knol LL, Perko M, Burnham J. Overweight and obesity are associated with emotion- and stress-related eating as measured by the eating and appraisal due to emotions and stress questionnaire. J Am Diet Assoc. 2008;108:49-56.
17. APA. Manual diagnóstico y estadístico de los trastornos mentales (DSM-IV). American Psychiatric Association. Editorial Masson, España; 1995.
18. Narain R, Sardana S, Gupta S, Sehgal A. Risk factors associated with tobacco habits among adolescents: a cross-sectional school-based study. Natl Med J India. 2013;26:197-202.
19. Tovilla-Zárate C, Juárez-Rojop I, Peralta Jimenez Y, et al. Prevalence of Anxiety and Depression among Outpatients with Type 2 Diabetes in the Mexican Population. PloS One. 2012;7:e36887.
20. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985;29:71-83.
21. Jauregui-Lobera I, Garcia-Cruz P, Carbonero-Carreno R, Magallares A, Ruiz-Prieto I. Psychometric properties of Spanish version of the Three-Factor Eating Questionnaire-R18 (Tfeq-Sp) and its relationship with some eating- and body image-related variables. Nutrients. 2014;6:5619-35.
22. Gardner EL. Addiction and brain reward and antireward pathways. Adv Psychosom Med. 2011;30:22-60.
23. Bruins-Slot L, Gorwood P, Bouvard M, et al. Lack of association between anorexia nervosa and D3 dopamine receptor gene. Biol Psychiatry. 1998;43:76-8.
24. Myhre R, Kratz M, Goldberg J, et al. A twin study of differences in the response of plasma ghrelin to a milkshake preload in restrained eaters. Physiol Behav. 2014;129:50-6.