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Genetic variability study of DRD4 and DAT1 in the urban population of Mexico City

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Original article

SUMMARY

There is sufficient evidence of genetic influence in psychiatric disorders, thus it has been proposed that the dopaminergic brain system could be affected in several disorders such as schizophrenia, substance abuse and attention deficit-hyperactivity disorder. In this sense the most studied genetic systems are two VNTRs: one located in exon 3 of the dopamine D4 receptor (DRD4) gene, and the other in the 3`-untranslated region of the dopamine transporter (DAT1 or SCLA6A3) gene. It has been reported that allele frequencies of these polymorphisms varied significantly among populations and this could affect the results in the association studies. Due to the previous findings, the objective of the present study was to determine the allele frequencies of DRD4 and DAT1 in an epidemiological sample of the adolescent population of Mexico City. We found that the frequencies presented in our study were in between those reported for Caucasians and those reported for the American Indigenous population, which is consistent with Euro-Indigenous crossbreeding that has occurred in Mexico. Such differences could explain the lack of consistency in several association analyses and make necessary to develop them within the Mexican population.

Key Words: DAT1, DRD4, association, HWE.

RESUMEN

Existe evidencia fehaciente de la influencia genética en los trastornos psiquiátricos y se ha propuesto que el sistema dopáminergico cerebral puede ser uno de los afectados en diversos trastornos como la esquizofrenia, el abuso de sustancias y el trastorno por déficit de atención e hiperactividad. En este sentido, los sistemas genéticos más estudiados son 2 VNTRs; uno localizado en el exón 3 del gen del Receptor a dopamina D4 (DRD4) y el otro en la región 3` no traducida del transportador a dopamina (DAT1 o SCL6A3). Se ha reportado que las frecuencias alélicas de estos polimorfismos difieren significativamente entre poblaciones y que esto puede afectar los resultados en los estudios de asociación. Debido a lo anterior, el objetivo del presente trabajo fue determinar las frecuencias alélicas del DRD4 y del DAT1 a partir de una muestra epidemiológica de la población adolescente de la Ciudad de México. Las frecuencias alélicas reportadas en el presente estudio son intermedias a las reportadas en caucásicos y poblaciones indígenas de América, lo que concuerda con la historia de mestizaje ocurrida en México. Estás diferencias pueden ayudar a explicar la falta de consistencia en diferentes estudios de asociación y hacen necesario realizarlos en población mexicana.

Palabras clave: DAT1, DRD4, asociación, HWE.

BACKGROUND

Multiple studies with families, twins and subjects given up for adoption have provided sufficient evidence of genetic influence in psychiatric disorders.[1,2] They present a complex non-Mendelian inheritance pattern with possible incomplete penetrance and variable expressivity, which suggests joint action of multiple genes of moderate or discreet effect towards environmental factors.[3,4]

Today, we know the structure and localization of multiple genes which expression products are essential for proper functioning of the Central Nervous System.[5,6] Variability on these genes may affect the expression and/or structure of the transcribed molecule and, therefore, have influence over emotional, cognitive and behavioral elements that are cardinal on the expression of symptoms or traits associated with a particular type of psychiatric disorder.

In particular, genes and their protein expression products, which take part in the dopaminergic brain system, have been considered as interesting candidates related to the manifestation of mental diseases, such as schizophrenia, substance abuse and attention deficit-hyperactivity disorder (ADHD). Among the most studied genes, in connection with such neurotransmission system, are the dopamine D4 receptor (DRD4) and the dopamine transporter (DAT1).[2,5,6]

The DRD4 gene codes for a transmembrane protein from the type-2 dopamine receptors subgroup, a member of the family of inhibitory G-proteins joined to receptors. High

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messenger RNA levels of this protein have been detected in the marrow, the prefrontal cortex, the mesencephalon, the amygdala and in the basal ganglia.[7]

Such gene is located in the subtelomeric region of the short arm of chromosome 11 being constituted by four exons. In its sequence, several polymorphisms have been described, among which a polymorphic system located in the third exon particularly stands out, which is made up by variable number tandem repeats (VNTR) of 48 base pairs (bp).[8] The number of repeats in this region varies from two to eleven, four repeats being the most common variety.[6,9]

In particular, the 7R allele has been associated with several mental diseases, such as schizophrenia, the ADHD and substance abuse, as well as with specific personality traits as the "quest for novelty".[6,7]

On the other hand, the DAT1 gene codes for a protein with 12 transmembrane domains, regulates the dopamine recapture of the synaptic space and is the main proposed mechanism for finishing the dopaminergic neurotransmission.[10] It has been described that the levels of this transporter are reduced with age and that the amount of this is affected by several mental disorders, such as Parkinson's disease, Tourette's Syndrome, major depression and the ADHD.[11] Likewise, it is one of the main molecular targets of some drugs, like cocaine and amphetamines.[10]

The gene that codes for the DAT1 is located in chromosome 5, region 15.3 of the short arm; it is about 65kb and is divided in 15 exons. Several polymorphisms have been described in its sequence; one of the most studied is a VNTR of 40 bp located in the 3`-untranslated region of the gene.[10-12] The number of repeats varies from 3 to 13. It has been reported that the 10-repeat allele is the most common.[10,11] Such common allele has been associated with several psychiatric disorders, as substance abuse and the ADHD.[13]

It bears mention that the allele frequencies of both polymorphisms vary significantly among populations[12-15] and that the risk allele reported for any mental disorder may vary in accordance with the studied population.[16]

Thus, it is very important to know the allele frequencies of these polymorphisms within Mexican population in order to conduct future association studies with different psychiatric disorders, specifically the ADHD, which has associated consistently with the 7R allele of DRD4 gene and with the 10R of DAT1 gene.[6,17-19]

METHODS AND MATERIALS

The present study was conducted according to the ethical principles described in the Declaration of Helsinki and was approved by the Scientific and Ethics Committee of the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz (INPRFM), located in Mexico City.

Subjects

The samples were obtained from adolescents who lived in the metropolitan area of Mexico City, who took part in an epidemiological study recently conducted by the INPRFM, which objective was to estimate the prevalence of mental illnesses that occur most often in this stage of the development.[20]

3,005 subjects aged between 12 and 18 years old were face-to-face assessed by an interviewer trained in the use of the CIDI-CAPI (Composite International Diagnostic Interview–Computer Assisted Personal Interview) in its adolescent-adapted version. This interview generated a psychiatric diagnosis, based on the DSM-IV-R and CIE-10 criteria. Individuals who did not comply with the criteria related to any mental disorder were appointed as non-cases. From this group 116 individuals were randomly selected.

Molecular Analysis

A mouthwash sample was obtained from the participating individuals and the DNA was extracted with the help of the Gentra Puregene Kit for DNA purification.

The amplification of the VNTR located in exon 3 of DRD4 gene was made using the *primers* reported by Lichter[21] and the conditions previously described by Aguirre.[22] In the case of the VNTR located in the 3`-untranslated region of the DAT1 the *primers* and the protocol previously reported by Kang et al. were used. (1999).[12]

The determination of the type of allele was obtained from the electrophoresis in agarose gels. The size of the alleles was established comparing the existing gel bands with molecular weight markers and/or with some previously sequenced samples. Two different researchers carried out the reading of the agarose gels blindly and most of the samples were amplified at least twice in order to ensure its correct genotyping. Dubious results were not included in the analysis.

Statistical Analysis

The Hardy-Weinberg Equilibrium (HWE) was analyzed with the subroutine of the HW program included in the LINKAGE statistical package.[23]

RESULTS

From the 116 individuals who made up the sample, only genotypes of 84 samples were obtained for DRD4 and 113 for DAT1.

The genotypic frequencies (data not shown) of both polymorphisms were in Hardy-Weinberg equilibrium (DRD4: χ^2=4.18 g.l.=15 p=0.997; DAT1: χ^2=0.095 g.l.=1 p=0.757).

The allele frequencies observed in this study are shown in tables 1 (DRD4) and 2 (DAT1), as well as their comparison with the frequencies given at other populations.[12-15]

Table 1. Allele frequencies of the type-VNTR polymorphism located in exon 3 of the DRD4 gene for several human populations

	2	3	4	5	6	7	8	9	
Caucasians	0.09	0.04	0.67	0.01	0.02	0.16	0.01	0.01	Chen et al., 1999, Vyera et al., 2003
Asians	0.15	0.01	0.79	0.03	0.02	0.01	0.00	0.00	Chen et al., 1999
Africans	0.03	0.00	0.83	0.00	0.02	0.11	0.00	0.01	Chen et al., 1999
Indigenous peoples of the Americas	0.03	0.00	0.29	0.01	0.03	0.60	0.02	0.00	Vyera et al., 2003
Chileans	0.06	0.01	0.59	0.02	0.05	0.27	0.01	0.01	Vyera et al., 2003
This study	0.03	0.01	0.58	0.02	0.01	0.35	0.00	0.00	

Regarding the DRD4 gene, it is observed that as in most of the populations, except for Asians, the alleles of 4 and 7 repeats stand for 80-90% of allele diversity.

In particular, the 7R allele frequency of the DRD4 gene, which has been associated with several psychiatric disorders, is greater in our population (35.1%), than the one reported by Caucasians (18%), but mucho smaller than the one reported for several Latin-American Indigenous populations (60%) (Table 1).

As for the DAT1 gene, we found that compared to Caucasian populations there is an increase of the 10R allele within our population (85.4% *vs*. 70%). This contrasts with the reports from South-American Indigenous populations, where this allele is practically unique (95-100%) (Table 2).

DISCUSSION

The purpose of this study was to describe allele frequencies of two polymorphic systems often used in the association and genetic linkage with psychiatric disorders studies.

To the best of our knowledge, this is the first allele frequency report of the type-VNTR polymorphism of the DAT1 3`-untranslated region within Mexican population. On the other hand, results obtained for the VNTR located in exon 3 of the DRD4 gene were similar to those previously published by our laboratory, in an independent group of healthy adult individuals who participated as controls in an association study with schizophrenia.[22]

It is important to stand out that, although the size of the sample is relatively small, it is sufficient to estimate the real frequencies of a population. In addition, we must emphasize that the samples come from an epidemiological study expressly designed for obtaining a representative sample, considering the different age groups and socio-economic level of adolescent population of Mexico City.

The genotypic frequencies from both polymorphisms were in Hardy-Weinberg equilibrium, which indicates that it is unlikely that the differences found are due to problems of typification[24,25] and strengthens the idea that the studied population is only one, thereby minimizing the possibility of population stratification problems.[25]

The fact that the frequencies for both polymorphisms are in between those reported for Caucasians and American Indigenous matches with the history of crossbreeding that has occurred in Mexico since the colonialism, showing how the urban population of Mexico City is genetically constituted. This allele behavior is similar, for example, to the reports for these same genes in the urban population of Santiago de Chile.[13]

As mentioned above, in Caucasian population the risk allele reported for psychiatric disorders, such as the ADHD, are the 7R of DRD4 gene and 10R of DAT1 gene.[6,8,17,19] It is necessary to emphasize that the frequency of such allele was higher in our population. This feature hinders, if anything, trying to replicate the results obtained in the Caucasian population, since the size of samples required to observe differences among groups of cases and controls increases significantly. Nevertheless, it is important to recall that there are reports indicating that, depending on the studied population, the risk allele may be different. It was found that in an Asian population the 2R allele of DRD4 gene is associated with the ADHD,[16] while in Caucasian populations the risk allele is 7R.[6,17-19]

In conclusion, determining the representative allele frequencies of a population of interest, for these and other polymorphisms, is an indispensable requirement for understanding the role of genes in psychiatric disorders.

Table 2. Allele frequencies of the type-VNTR polymorphism located in the DAT1 3`-untranslated region for several human populations

	10R	9R	Other	
Caucasians	0.70	0.29	0.01	Kang et al., 1999 and Vyera et al., 2003
Asians	0.91	0.06	0.03	Kang et al., 1999
Africans	0.37	0.28	0.35	Kang et al., 1999
Indigenous peoples of South America	0.10	0.00	0.00	Kang et al., 1999 and Vyera et al., 2003
Chileans	0.74	0.23	0.03	Vyera et al., 2003
This study	0.85	0.15	0.00	

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Finally, the results of this study provide very relevant information to generate an appropriate experimental design of the future studies of genetic association.

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