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Association of the *SLC6A4* gene 5HTTLPR polymorphism and ADHD with epilepsy, gestational diabetes, and parental substance abuse in Mexican mestizo children

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ABSTRACT

Introduction. Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric conditions in childhood and a multifactorial condition attributable to genetic and/or environmental influence. Allelic variants in the serotonin transporter gene (*SLC6A4*) have been associated to lower transcriptional efficiency, changes in serotonin concentration in several brain regions, and ADHD development. **Objective.** To identify the association between the *SLC6A4* alleles and ADHD diagnosis and risk factor phenotypes in children from a Mexican mestizo population. **Method.** In this study, 134 unrelated children were included and evaluated for ADHD, genotypification for the 5HTTLPR polymorphism, and identification of multiple phenotypes from their clinical records and family background for association analysis. **Results.** The following distribution of genotypes was observed: 23% SS, 49% SL, and 28% LL. From the phenotypes evaluated in the present study, gestational diabetes mellitus (p = .045), history of epilepsy (p = .047), and parental substance abuse (p = .033) showed an association with ADHD development in regression analysis along with the S variant. **Discussion and conclusion.** Results suggest that interaction of the S allele and some of the phenotypes analyzed may play a relevant role in the development of ADHD in the studied population.

Keywords: Serotonin transporter, attention deficit hyperactivity disorder, *SLC6A4* gene, allele.

RESUMEN

Introducción. El trastorno por déficit de atención e hiperactividad (TDAH) es uno de los padecimientos neuropsiquiátricos más comunes en la infancia. Como su naturaleza es multifactorial, es atribuible a influencias genéticas y/o ambientales. Las variantes alélicas del gen transportador de serotonina (*SLC6A4*) se han asociado previamente con cambios en los niveles de serotonina en algunas regiones cerebrales, así como con el desarrollo de TDAH. **Objetivo.** Identificar la posible asociación entre los alelos del gen *SLC6A4* y el diagnóstico de TDAH, así como factores de riesgo en niños mestizos mexicanos. **Método.** En el presente estudio se incluyeron 134 niños, los cuales fueron evaluados para TDAH, genotipificación del polimorfismo 5HTTLPR e identificación de múltiples fenotipos en su historia clínica y antecedentes familiares para su análisis de asociación estadística. **Resultados.** Se mostró la siguiente distribución de genotipos: 23% SS, 49% SL y 28% LL. En un modelo de regresión, los fenotipos de diabetes mellitus gestacional (p = .045), historia de epilepsia (p = .047) y el abuso de sustancias de los padres (p = .033) mostraron asociación con la variante S y el desarrollo de TDAH. **Discusión y conclusión.** El presente estudio sugiere que el alelo S en conjunto con algunos fenotipos puede cumplir un papel importante en el desarrollo de TDAH en nuestra población.

Palabras clave: Transportador de serotonina, trastorno por déficit de atención e hiperactividad, gen *SLC6A4*, alelos.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric conditions in childhood and it is characterized by an impaired neurodevelopment with inattention and hyperactivity symptoms. The worldwide-pooled prevalence for this condition is 5.29% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). However, reports of ADHD prevalence in USA show a higher and increasing numbers through time, from 7.8% in 2003 to 9.5% in 2007 (Cohen et al., 2013). In Mexico, a prevalence of 9.1% was proposed in a recent epidemiological analysis, stating the relevance of this condition (Cornejo-Escatell, Fajardo-Fregoso, López-Velázquez, Soto-Vargas, & Ceja-Moreno, 2015).

ADHD is a multifactorial condition, attributable to genetic and/or environmental influence, where the phenotype may result from the overlap of risk factors (Moreau & Waldie, 2016). Some studies have been focused in the analysis of dysregulation in the serotonergic processes and ADHD, particularly those that involve the polymorphism 5HTTLPR of the serotonin transporter gene (*SLC6A4*), where the allelic versions of the gene has been associated to changes in serotonin concentration in several brain regions. The serotonin transporter (5-HTT) is an integral membrane protein responsible for the reuptake of 5-HT from the synaptic cleft, modulating the serotoninergic neurotransmission. The human 5-HTT gene (*SLC6A4*) has been mapped to chromosome 17q11.1—q12 (Lesch et al., 1994). A 44-bp insertion/deletion polymorphism in the promoter region of this gene (5HTTLPR) has been described, which originates two alleles, L and S (long and short, respectively). The S allele has been associated with lower transcriptional efficiency, resulting in lower serotonin uptake activity when compared with the L allele (Lesch et al., 1996; Heils et al., 1996; Schenkel et al., 2011).

Using brain MRI analysis of both healthy individuals and those with internalizing problems, van der Meer et al. (2015) reported the interaction between the 5HTTLPR genotype and stress exposure on limbic and frontal brain regions involved in the regulation of social and emotional behavior, where carriers of the allele S showed less connectivity between these regions resulting in higher levels of anxiety. Hypofunctioning of frontal regions and connected subcortical structures is also a hallmark of both stress exposure and ADHD, indicating an overlap of neurobiological correlation between 5HTTLPR, stress, and ADHD (van der Meer et al., 2015).

Due to the multifactorial nature of the condition, recent reports state that for a better understanding of the disorder, the analysis of the interaction of the *SLC6A4* variants and adverse environments will be relevant. In particular, epidemiological studies by the Centers for Disease Control and Prevention's and the National Center for Health Statistics (NCHS) have shown racial/ethnic disparities, where Hispanic population exhibit a lower prevalence of ADHD when compared with other ethnic groups (Pastor, Duran, & Reuben, 2015). Considering all the information, in the present study we analyze the polymorphism 5HTTLPR for association to ADHD and adverse/risk environmental factors in a group of Mexican mestizo children.

METHOD

Participants

In the present study, 134 unrelated children were recruited among students from first to fifth grade of elementary school at the regional school district of Tepatitlan, Jalisco, Mexico, and evaluated for ADHD by a psychiatrist according to DSM-IV criteria and divided in 78 cases and 56 non-cases.

Measures

After the classification of participants (as cases or controls), additional information was retrieved from them to identify risk factors for further analysis, including diagnosis of ADHD in their mothers, fathers, and siblings and presence or absence of the following phenotypes: dysfunctional familiar environment; parents legal issues or substance abuse; gestational development of the children, including maternal age and consumption of alcohol, tobacco, or coffee; positive history of infections; gestational diabetes mellitus (GDM); preeclampsia; eclampsia; X-ray exposure; premature or post-mature delivery; newborn hypoxemia; requirement of incubator or intensive care; breastfeed; head injuries or head trauma; epilepsy; social problems; and familiar abuse.

Genotyping

Genomic DNA was isolated from saliva using the PureLink Genomic DNA Mini Kit. Polymerase Chain Reaction (PCR) for 5HTTLPR polymorphism genotyping was performed as previously described (Cook et al., 1997), with minor modifications. The forward primer used was HTTp2A, 5'-TGAATGCCAGCACCTAACCC-3', and the reverse primer HTTp2B, 5' -TTCTGGTGCCACCTAGACGC- 3'. The final amplicon product consisted of 406/450 bp fragments (S and L alleles, respectively). PCR reaction run through 40 cycles consisting of 30s at 95°C, 30s at 61°C, and 60s at 71°C, followed by 10min at 72°C. Amplification products were separated by electrophoresis on a 6% polyacrylamide gel and bands were visualized using a silver nitrate staining protocol. Subjects were classified into three genotypes: individuals homozygous for the short allele SS, those heterozygous for the short and long allele LS, and those homozygous for the long allele LL.

Statistical analysis

Genotype distribution and deviation from Hardy-Weinberg equilibrium was analyzed by χ^2 test. Logistic regression was used to examine the association between 5HTTLPR, ADHD, and the multiple phenotypes. The effect of the genetic variant on outcome was adjusted by gender and age. Results were expressed as odds ratio (*OR*), 95% CI and nominal significant differences ($p \leq .05$). Permutation test was performed for multiple testing corrections. A p value threshold of .05 after correction was used to determine significance. Statistical analysis was performed using PLINK software version 1.07 (Purcell et al., 2007) using the second most common allele for association purposes.

Ethical considerations

This study was approved by the local Institutional Review Board. Parents granted informed consent for the children to participate and provided any additional information.

RESULTS

The studied population showed an average age of 7.5 (*SD* 1.37) and gender distribution of 68% males. A higher distribution of male was observed among cases (72%) when compared to controls (60%). Distribution of genotypes can be observed in Table 1. These results did not deviate from Hardy–Weinberg equilibrium ($p > .05$).

Although in the present cohort the S variant showed a minor allele frequency (MAF) and a borderline association was found between this allele and ADHD ($p = .05$), after a permutation analysis this association became non-significant. Nevertheless, GDM ($p = .045$, $OR = 1.5$, 95% CI [1.2, 4.8]), epilepsy ($p = .047$, $OR = .59$, 95% CI [.35, .99]) and parental substance abuse ($p = .033$, $OR = 3.8$, 95% CI [1.6, 8.7]) revealed an association with ADHD using regression analysis along the S variant and was maintained after permutation test.

Table 1
Demographic information and 5HTTLPR olymorphism distribution over ADHD patients and control group

	ADHD	Control
Age		
Mean (SD)	7.6 (1.35)	7.4 (1.41)
Range	6 - 10	5 - 11
Gender		
Male (%)	56 (72)	34 (60)
Female (%)	22 (28)	22 (40)
Genotype frecuencies		
LL	34	19
LS	47	53
SS	19	28
Allele frecuencies		
L	57	45
S	43	55

DISCUSSION AND CONCLUSION

Even when this study did not find a direct association between ADHD and the 5HTTLPR polymorphisms, three relevant phenotypes showed significant association in our population: parental substance abuse, GDM, and epilepsy. Regarding substance abuse in parents of children with ADHD and the 5HTTLPR polymorphism, it was observed that it was more frequent among male than female parents. Likewise, between male parents, the most commonly abused substance was alcohol followed closely by tobacco; this tendency was inverse between female parents. Similar studies have shown an association of substance abuse in parents of child diagnosed with ADHD where the family dynamics become a cyclical pattern fed up on distress produced by child behavior (inattention and hyperactivity), increasing the risk of substance abuse in susceptible parents (maladaptive parenting), leading to reciprocal negative effects on one another (Chronis et al., 2003). In a similar context, it is notable that people with mental health disorders are more likely to experience an alcohol or substance use disorder than people without them (Watkins et al., 2014), where ADHD and substance abuse has been consistently reported, analyzed, and associated in the literature showing a development of substance abuse later in life in individuals diagnosed with ADHD in childhood (Groenman, Janssen, & Oosterlaan, 2017). Because of the multifactorial nature of these conditions and through the literature review, it can be suggested that a genetic link between this conditions may play an important role in the development of both phenotypes. In that sense, substance abuse has been analyzed previously by our research group along with the 5HTTLPR polymorphisms in Mexican population and found a positive association, attesting the relevance of *SLC6A4* variants in behavior at the current analyzed population (Peralta-Leal et al., 2015). All this information lead us to hypothesize that substance abuse and ADHD can cover the full spectrum of a more complex psychiatric disorder and not just two co-occurrence problems.

Concerning the second associated phenotype, GDM is a prenatal complication defined as glucose intolerance developed during pregnancy, and shows an expected frequency of 7% among all pregnancies. The occurrence of GDM *per se* is a risk factor for other conditions, were the onset of this condition occurs within a period of fetal brain development, leading to deficits in fine and gross motor function, lower verbal IQ language impairment, greater inattention and hyperactivity, and poorer general cognitive function (Nomura et al., 2012). Recently, it has been reported that serotonin

and related metabolites have notable functions regulating growth and development of the fetus and are involved in a great number of physiological pathways and adaptation processes during pregnancy, where serotonin metabolism is impaired in GDM (Leitner et al., 2017). Even more, a previous epigenetic study showed significant decreased DNA methylation and regulation of *SLC6A4* expression in the placenta of pregnant mothers with GDM (Blazevic et al., 2017). Both reports coincide in highlighting the relevance of BMI in the development of GDM, were *SLC6A4* polymorphism has been associated with BMI in Mexican mestizo population (Peralta-Leal et al., 2012).

Co-occurrence of epilepsy was also found in this study. Association between ADHD and epilepsy is not new and has been observed and confirmed in several studies were a bidirectional relationship has been proposed; however, the mechanisms underlying this connection are still unknown. The prevalence of ADHD in children with epilepsy is 30%-40%, which is higher than in the general pediatric population (Parisi, Moavero, Verrotti, & Curatolo, 2010). One of the proposed pathways in epilepsy development is the serotoninergic system, since 5-HT contributes to the neurodevelopment, functionality, and plasticity of the brain as it was stated before. Some studies support the hypothesis that *SLC6A4* may play an important role in the development of epilepsy by less transcriptional efficient achieved by the 5HTTLPR variants in temporal lobe epilepsy patients (Schenkel et al., 2011). Our study found an association between ADHD, epilepsy, and the S allele, offering an interesting link to better understand the association between the comorbidities observed in these patients.

This study suggests that the S allele may influence behavioral plasticity shaped by adverse environments in our population. Based on the literature review and the current results, it is suggested that families as a whole must be evaluated when a member is diagnosed with ADHD (regardless the age) for a better approach, integral treatment, and adequate follow-up. The occurrence of comorbidities such a GDM should be pursued, even after the birth of the product, to identify clinical signs that can help to integrate an early diagnosis of ADHD and start promptly with an integral family treatment in order to break up the deleterious cycle previously described.

Due to the relatively small number of subjects in this study, Type II error, through the lack of observed association between the analyzed polymorphism and ADHD, cannot be ruled out. Further analysis with a larger sample is warranted. Nevertheless, the information reported in this preliminary investigation is relevant, because the increased prevalence of ADHD among children over the years is a global health problem. In addition, the allele frequencies of the *SLC6A4* polymorphism determined in this population sample constitute a useful reference for genetic studies in ADHD and Hispanic population.

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Conflict of interests

The authors declare they have no conflict of interests.

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