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Candidate gene study reveals *DRD1* and *DRD2* as putative interacting risk factors for youth depression

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ABSTRACT

Alterations in the monoaminergic neurotransmission systems are suspected to be involved in the etiology of neuropsychiatric disorders, including depression. The role of these pathways in the risk of developing depressive symptoms during childhood or adolescence is still not completely clear. This study sought to identify putative genetic factors in genes of serotonergic and dopaminergic systems modulating the level of manifestation of depressive symptoms in children and adolescents. We analyzed 170 single nucleotide polymorphisms (SNPs) in 21 candidate dopaminergic and serotonergic genes in a non-clinical sample of 410 Costa Rican participants of ages between 7 and 18 years, assessing the severity of depressive symptoms through the Child Depression Inventory (CDI). Genotypic and haplotypic associations, as well as epistatic effects, were examined. A significant interaction effect was detected between rs1039089 in conjunction with rs877138 located upstream of the *dopamine D1 receptor* (*DRD1*) and the *dopamine D2 receptor* (*DRD2*) genes respectively, although no evidence was found for any single variant or haplotype related to a differential liability. This newly described genetic interaction among putative regulatory regions of dopamine receptors could affect the level of manifestation of depressive symptoms through an imbalance of D1-D2 heteromers and modulation of cognitive processes.

1. Introduction

Depression is the most frequent psychiatric disorder, with a lifetime risk close to 20% (Kessler et al., 2005). More than 350 million people of all ages worldwide are estimated to suffer from depression, and the global burden of this and other mental disorders are projected to increase (WHO, 2012). It is not only adults that are affected by the disease. According to the US National Comorbidity Survey-Adolescent Supplement (NCS-A), approximately 11% of adolescents have a depressive disorder by the age of 18 (Avenevoli et al., 2015). Therefore, achieving a better understanding of its neuropsychobiology at different ages and in different ethnic groups becomes a priority.

It is recognized that depression is the result of complex interactions between social, psychological and biological factors (WHO, 2012). Studies with large cohorts of twin pairs of children of ages between 3 and 12 years have revealed the influence of genetic variability over the trait. The estimated heritability, ranging from 48% up to 76%, has suggested that strictly genetic factors could play a more prominent role than the environment in the development of childhood depression (Boomsma et al., 2005). These factors, however, are still being identified.

The relation of genes implicated in the monoamine neurotransmitters systems and the susceptibility to depressive and other affective disorders has been of great interest for a long time. It has been observed that many antidepressant medications act by increasing the levels of monoamines (Ban, 2001; Yamada and Yasuhara, 2004; Berton and Nestler, 2006; Tsapakis et al., 2008). This led to the formulation of the monoamine hypothesis of depression in 1967, which proposed that the disease is caused by a decreased function of monoamines in the brain (Schildkraut and Kety, 1967).

Now it is recognized that the disorder is highly complex and cannot be solely attributed to monoamine deficiencies (Krishnan and Nestler, 2008). However, genes involved in the monoaminergic pathways are still important candidates in research efforts aimed at improving the understanding of the etiology of the depression. It has been proposed that there might be a relationship between specific symptoms of disease and the three major monoamine neurotransmitters, *i.e.*, dopamine (DA), serotonin (5-HT) and norepinephrine (NE) (Nutt, 2007).

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Even though the relevance of these systems as potential genetic risk factors for the major depressive disorder (MDD) is widely recognized, recent candidate gene and genome-wide association studies have failed to consistently identify genetic variants that could be implicated in individual susceptibility (Levinson, 2006; López-León et al., 2007; Shi et al., 2008; Wray et al., 2010). Therefore, debate still remains regarding the precise role that alterations in these neurotransmitters systems could play by themselves. The lack of reproducibility might be partially attributed to complex interactions between two or more loci, which are likely to contribute broadly to multifactorial traits (Tyler et al., 2009). Here, we have sought to analyze single nucleotide variants (SNP) in candidate genes of the dopaminergic and serotonergic pathways in a non-clinical sample of Costa Rican children and teenagers, with the aim of identifying putative genetic factors contributing to the risk of developing depressive symptoms during childhood or adolescence.

2. Methods

2.1. Participants

Our study population consisted of non-clinical (with no previous clinical diagnosis of depression) unrelated Costa Rican children and adolescents of ages between 7 and 18 years. A total of 51 public and private educational centers were contacted and information about the study was sent to 4000 parents, resulting in 650 positive responses. From August 2010 to August 2011, 575 participants were interviewed in their respective educational centers in the provinces of San José, Alajuela, Heredia and Cartago. Buccal samples were taken from children and adolescents, excluding those who were taking medication with potential effects on the central nervous system or with a previous diagnosis of an affective or posttraumatic stress disorder.

After psychometric and genotypic evaluation, a total of 410 qualifying (169 males and 241 females) with a mean age of 13 ($SD=2.3$) years were selected. All samples and psychometrical data were collected after obtaining informed consent of the parents and the children, in accordance with guidelines approved by the Ethics Committee of the University of Costa Rica.

2.2. Determination of depression scores

A general screening measure for the risk for depression was obtained using the Child Depression Inventory (CDI) Kovacs test (Lutz, 1992). This test contains 27 items describing different symptoms of childhood depression. Each item comprises three different statements, graded from 0 to 2 according to severity. The theoretical range of the scores spans from 0 to 54 and a cutoff score of 19 is suggested for clinical screening purposes in non-clinical populations. For every item, subjects were requested to choose the statements that best described themselves. Reliability of the instrument was assessed through Cronbach's alpha.

2.3. SNP selection

Variants selected for genotyping included 170 SNPs suggested by Gardner, Bertranpetit and Comas (2008) for 21 genes involved in the DA and 5-HT pathways. These correspond to many of the genes most commonly associated with neuropsychiatric disorders/behavioral traits, including genes that code for receptors, metabolizing enzymes (synthesis and degradation of neurotransmitters), transporters and signal transduction molecules.

Because of the strong European ethnic component of the Costa Rican population (Morera et al., 2003), we selected the panel suggested for European populations, of which three polymorphisms (rs10507544, rs12111696 and rs6561333) were excluded due to possible low genotyping rate. The selected panel includes variants with an average density of one SNP every 19 kb, located both within genes as well as in regions up to 30 kb away from the 3′ and 5′ ends (Table 1).

Table 1
Number and spacing of selected SNPs for tagging 21 candidate genes involved in the dopaminergic and serotonergic pathways.

Pathway	*Gene*	*Position*	*Gene length (bp)*	*N tag-SNPs*	*SNP spacing (kb)*[a]	*% Capture ($r^2 \geq 0.8$)*[b]
DA	COMT	22q11.21	28,368	11	7.34	80
	DBH	9q34	22,984	12	6.12	91
	DRD1	5q35.1	4 169	5	12.56	69
	DRD2	11q23	66,095	11	9.92	93
	DRD3	3q13.3	70,755	12	8.02	93
	DRD4	11p15.5	3 413	5	10.45	93
	DRD5	4p16.1	2 374	3	20.63	88
	MAOB	Xp11.23	115,835	7	25.88	91
	PPP1R1B	17q12	9 886	5	13.06	88
	SLC6A3	5p15.3	52,636	11	9.21	79
	TH	11p15.5	7 948	9	7.86	100
5-HT	HTR1A	5q11.2-q13	2 151	1	–	100
	HTR1B	6q13	1 542	6	11.45	80
	HTR2A	13q14-q21	65,484	14	9.06	91
	HTR2C	Xq24	326,073	4	145.75	84
	HTR4	5q31-q33	226,203	13	17.83	88
	SLC6A4	17q11.1-q12	41,683	7	10.84	90
	TPH1	11p15.3-p14	24,862	6	12.86	100
	TPH2	12q21.1	247,772	10	13.63	93
DA/5-HT	DDC	7p11	107,020	15	9.69	94
	MAOA	Xp11.3	90,601	3	28.77	87

[a] Mean spacing of the genotyped tag-SNPs for each selected gene.
[b] Percentage of non-genotyped variation captured in European populations by the selected tag-SNPs, according to Gardner et al. (2008).

2.4. Buccal sample collection and genotyping

Buccal epithelium samples were obtained by a two-minute mouth rinse using 20 ml of non-alcohol commercial mouthrinse for kids. Genomic DNA was isolated at the laboratories of the Institute of Health Investigation in Costa Rica (INISA, www.inisa.ucr.ac), using a standard alkaline lysis/proteinase K method followed by phenol-chloroform extraction. Samples were quantified by fluorimetric means using Quant-it PicoGreen (Invitrogen, Waltham, MA) at 502/523 nm.

SNPs were genotyped using the Bead Xpress Veracode platform (Illumina Inc, SanDiego, CA) in the National Genotyping Centre in Spain (CeGen, http://www.cegen.org). A total of 3 HapMap trios were genotyped and used to help in the clustering and as a control of the genotyping process.

2.5. Data quality checks

We excluded from all subsequent analysis samples with $>15\%$ missing data, SNPs with $>15\%$ missing genotypes or minor allele frequency (m.a.f.) $<5\%$ and deviations from Hardy-Weinberg equilibrium (HWE, $\chi 2$ test, $p<0.001$) were checked.

2.6. Statistical analysis

Association of single genetic variants with depression scores were evaluated through linear regressions. Since we had no reason for assuming a specific recessive/dominant, additive or heterozygote advantage/disadvantage inheritance model, we performed