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Short communication

A functional variant in MIR137, a candidate gene for schizophrenia, affects Stroop test performance in young adults

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ABSTRACT

MIR137, a brain expressed miRNA, has been identified as a top novel susceptibility gene for schizophrenia (SZ). 230 healthy participants completed the Stroop test and were genotyped for a functional Variable Number Tandem Repeat (VNTR) in MIR137 gene. MIR137 VNTR genotypes were associated with differences in Stroop facilitation and accuracies in congruent trials and for the total number of errors. This is the first study of the functional VNTR in MIR137 gene and Stroop test performance in healthy subjects. Our results could have important implications for the identification of genetic candidates for endophenotypes for SZ.

1. Introduction

Schizophrenia (SZ) is a complex and devastating psychiatric disorder, with a worldwide lifetime prevalence of around 5 per 1000 persons and an estimated heritability of around 0.64 (van Os and Kapur, 2009). Study of endophenotypes for SZ has received a particular interest in recent years, due to its potential to facilitate a better and deeper understanding of the pathophysiology of the disorder (Braff, 2015).

Stroop test is a cognitive task that allows the assessment of selective attention and interference (Henik and Salo, 2004). Alterations in both Stroop interference and facilitation have been observed in SZ and may be relevant for the understanding of the pathogenesis of this disorder (Westerhausen et al., 2011). Heritability for Stroop interference has been estimated around 50% (Swan and Carmelli, 2002). Several candidate genes, such as *BDNF* and *DRD4,* have been studied as possible correlates of Stroop interference performance in healthy subjects (Cirulli et al., 2010; Gajewski et al., 2012; Loo et al., 2008).

Recent large genome-wide association studies (GWAS) found three SNPs (rs1625579, rs1198588 and rs2660304) located in 1p21.3 with significant associations for SZ (Ripke et al., 2013; Schizophrenia Psychiatric Genome-Wide Association Study, 2011), these SNPs are located 8.7 kb downstream, 41.1 kb upstream and 400 bp upstream respectively, from the pre-miR-137 region. miR-137 is a brain expressed miRNA (Forero et al., 2010; Liang et al., 2007) that regulates several protein coding genes that are important for brain physiology (Wright et al., 2015). A recent study found that a 15 bp Variable Number Tandem Repeat (VNTR) in MIR137 gene, six bases upstream of the pre-miR region (NC_000001.10:g.98,046,178_98,046,192[3_12]; human genome assembly GRCh38.p2), can change its secondary structure, affecting the levels of the mature miRNA and downstream genes involved in synaptic processes and being associated with SZ (Strazisar et al., 2015).

Considering the role of MIR137 gene in SZ and executive function, the objective of this study was to assess the effect of MIR137 VNTR on Stroop test performance in a sample of Colombian young adults.

2. Methods

2.1. Participants

Two hundred and thirty healthy subjects were included in this study, with a mean age of 21.2 years (SD: 3.3 years), the percentage of females was 57.3%. Participants had their four grandparents born in Colombia, were unrelated and recruited from a private University in Bogotá, Colombia. The population living in Bogotá is the result of the admixture of Spaniards and Amerindians (Gonzalez-Giraldo et al., 2015) and an analysis of ancestry using highly informative markers did not find evidence of significant stratification in this population (Ojeda et al., 2013). Additional details of recruitment and general evaluation of subjects is described in

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Supplementary file. Participants provided a written informed consent and the study was approved by the institutional ethics committee.

2.2. Cognitive testing

The Stroop test was used to evaluate executive function performance and it was applied using the computerized battery of the *Psychology Experiment Building Language* (PEBL, version 0.13) (Gonzalez-Giraldo et al., 2015; Mueller and Piper, 2014) and instructions to complete the test were provided in the Spanish language. In this test, series of different words were presented into the laptop screen, one at a time. The following conditions of the Stroop test were presented to the participants of the study: neutral, congruent and incongruent (Esposito et al., 2013). Participants were asked to identify the color in which the word appeared (independently of the meaning of the word) in all conditions (Fig. S1). Testing was carried out in a distraction-free room (Clinical Simulation Laboratory, Universidad Antonio Nariño) and the test was administered to all the subjects in the same conditions. Accuracies and response times for the different Stroop trials were taken as variables of test performance for the respective analyses. Stroop facilitation scores were calculated by subtracting accuracy indexes of congruent from neutral trials and Stroop interference scores were obtained by subtracting the accuracy indexes of incongruent from neutral trials.

2.3. Genotyping and statistical analysis

Genotyping of MIR137 VNTR (NC_000001.10: g.98,046,178_98,046,192[3_12]) was performed by PCR and agarose gel electrophoresis. SNPStats program (Sole et al., 2006) was used to carry out a linear regression model (corrected for age, gender and hours of sleep) to determine the association between VNTR genotypes of MIR137 and variables of Stroop test performance (accuracies, response times, facilitation and interference). Complete details of genotyping methods and statistical analyses are described in Supplementary file.

3. Results

Descriptive data for the subjects included in this study is provided in Table S1. The most frequent alleles and genotypes for MIR137 VNTR in the current sample were 4 and 4/4 (78.0% and 58.3%, respectively). Table S2 shows the complete list of allele and genotype frequencies that were found in this study of Colombian subjects. Distribution of genotype frequencies for MIR137 VNTR was in Hardy–Weinberg equilibrium ($p=0.99$).

Mean accuracies for Stroop test were: 92% (SD: 8%) for neutral trials, 94% (SD: 6%) for congruent trials and 83% (SD: 17%) for incongruent trials. Mean response times were 848, 803 and 970 ms for neutral, congruent and incongruent trials. These variables showed a normal distribution.

A linear regression model, corrected for age, gender and hours of sleep, showed that MIR137 VNTR genotypes were associated with differences in Stroop facilitation (p value: 0.021), with subjects carrying one copy of the 4-repeat allele showing the highest Stroop facilitation scores (Table 1a; Table S3). A posthoc analysis found that there were significant differences between 4-repeat homozygous and carriers of one copy of the 4-repeat allele (p value: 0.035). These results were complemented by differences between MIR137 VNTR genotypes for mean accuracies in congruent trials (p value: 0.003) and for the total number of errors (p value: 0.016) (Table 1b, Supplementary Fig. S2). These associations were significant after correction for multiple testing (q values < 0.05). No significant differences between MIR137 VNTR genotypes for Stroop interference or for response times were found ($p > 0.05$).

Table 1a
Association between MIR137 VNTR genotypes and Stroop facilitation.

Genotype groups	Mean Stroop facilitation scores (S.E)	*p* Value
4-repeat homozygous	0.9 (0.4)	0.021
Carriers of one copy of 4-repeat	2.4 (0.5)	
4-repeat non-carriers	−3.4 (1.7)	

Table 1b
Association between MIR137 VNTR genotypes and accuracy in congruent trials and total number of errors for the Stroop test.

Genotype groups	Mean accuracy (s.e)[a]	*p* Value	Mean total number of errors (s.e)	*p* Value
4-repeat homozygous	94.63 (0.48)	0.012	9.43 (0.73)	0.042
Carriers of one copy of 4-repeat	94.54 (0.67)		10.44 (0.98)	
4-repeat non-carriers	86.48 (6.04)		20 (8.88)	

[a] Congruent trials.

4. Discussion

MIR137 VNTR (NC_000001.10:g.98,046,178_98,046,192[3_12]), a variant with direct functional effects that has been associated with SZ, modulates the promoter function (Warburton et al., 2015) and regulates the expression levels of this miRNA (Strazisar et al., 2015). In this work, we found a novel significant association between MIR137 VNTR genotypes and differences in Stroop facilitation in healthy subjects. These results are complemented with differences between MIR137 VNTR genotypes for mean accuracies in congruent trials and for the total number of errors. To our knowledge, this is the first study of the functional VNTR in MIR137 gene and Stroop test performance in healthy subjects and one of the first endophenotype studies for MIR137 gene in Latin America (Forero et al., 2014). The allele frequencies in the current study (with the 4-repeat being the most frequent allele) are similar to those described in a European sample (Warburton et al., 2015). Further studies are needed to characterize the ethnic differences in frequencies for the MIR137 VNTR (Egawa et al., 2013; Strazisar et al., 2015). The study of cognitive endophenotypes for SZ in healthy subjects has the advantages of allowing the analysis of larger sample sizes and to rule out the possible confounding effects of antipsychotic medication and other non-specific factors (Braff, 2015).

miR-137-dependent regulation of genes involved in synaptic plasticity mechanisms (Olde Loohuis et al., 2015; Siegert et al., 2015; Strazisar et al., 2015; Wright et al., 2015), could explain the functional changes in key neuronal circuits underlying the specific cognitive changes found in SZ. miR-137 is known to modulate the expression levels of several protein coding genes that are crucial for brain function and for the pathogenesis of neuropsychiatric disorders (Fig. S3), including SZ (Forero et al., 2010; Strazisar et al., 2015; Wright et al., 2013). This could help to explain the worse performance of 4 repeat non-carriers in the Stroop test. Interestingly, we observed that subjects with only one copy of the 4-repeat allele had the highest Stroop facilitation scores; future functional studies are needed to understand this finding. In addition, this association could also be evidenced in patients with

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