A functional SNP in MIR124-1, a brain expressed miRNA gene, is associated with aggressiveness in a Colombian sample

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Background: Interpersonal violence and suicide are among the main causes of mortality and morbidity around the world. In several developing countries, such as Colombia, they are among the first five entities of public health concern. Aggressiveness is an important endophenotype for aggression and suicidal behavior, having a heritability of around 50%. Exploration of classical candidate genes, involved in serotonergic and dopaminergic neurotransmission, has identified few consistent risk factors for aggressiveness. miRNAs are a novel class of molecules with a growing role in normal neural function and neuropsychiatric disorders; of special interest, miR-124 is a brain-specific miRNA that is key for neuronal plasticity. We evaluated the hypothesis that a functional polymorphism in MIR124-1 gene might be associated with aggressiveness in a Colombian sample.

Methods: The Spanish adaptation of the refined version of the Aggression Questionnaire and the abbreviated Barratt Impulsiveness Scale were applied to 170 young subjects. The functional SNP in MIR124-1 (rs531564) was genotyped by a TaqMan assay.

Results: We found a significant association between the MIR124-1 and aggressiveness in our sample, with G/G carriers having lower scores (P = 0.01). This association seemed to be specific for aggressiveness, as it was not significant for impulsiveness.

Conclusion: We showed for the first time the association of a functional polymorphism in MIR124-1 and aggressiveness. Known targets of miR-124 (such as BDNF and DRD4 genes) could explain the effect of this miRNA on behavior. A future analysis of additional novel functional polymorphisms in other brain expressed miRNAs could be useful for a deeper understanding of aggression in humans.

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1. Introduction

Aggressiveness is an important endophenotype for interpersonal violence and suicidal behavior [28,40]. According to recent data from the Global Burden of Disease Study, interpersonal violence and suicide are among the leading causes of death and disability-adjusted life years in adults around the world [19,26], especially in Low and Medium Income Countries (LMIC), such as several Latin American and African regions [32,37]. It has led to inclusion of interpersonal violence as a research priority for mental health in LMICs [39].

Heritability of human aggression has been estimated to be around 50% [29] and animal models have confirmed the important role of genetic factors in aggressive traits [2]. In a field synopsis, Vassos et al. carried out meta-analyses for polymorphisms in nine candidate genes and aggressiveness and few were significant in the pooled Odd Ratios (ORs): catechol-O-methyltransferase (COMT) in Caucasians (Cohen's d: 0.14, CI: 0.02–0.27), serotonin transporter (SLC6A4) in adults (Cohen's d: 0.11, CI: 0.01–0.19) and monoamine
oxidase A (MAOA) in females (Cohen’s d: 0.24, CI: 0.01–0.47) [45]. These results have highlighted the importance of studying novel candidate genes for aggressiveness. microRNAs (miRNAs) are a novel class of genes that have a growing role in brain physiology and neuropsychiatric disorders and endophenotypes [12,44]. Among them, miR-124 is a brain-specific miRNA, which is highly conserved in evolution and highly expressed in neurons throughout the brain [12,16,25]. It regulates a number of protein coding genes that are important for brain function and is a key miRNA for neural differentiation and brain plasticity mechanisms [12,16], due to the modulation of its direct targets and by the indirect activation of a brain-specific alternative pre-miRNA splicing [12,27]. In the human genome, there are three miRNA genes encoding miR-124, located in different genomic regions: MIR124-1 (8p23.1), MIR124-2 (8q12.3) and MIR124-3 (20q13.33). Qi et al. found that a SNP (g.9,903,189C>G, rs531564) located in the pri-miRNA region of MIR124-1 gene (ENSG00000208010) (Fig. 1B) led to differences in the expression levels of the respective mature miRNA [38]. miR-124 regulates key genes for behavior such as BDNF or GRIA3 [9,17] (Fig. 1A). These genes could be involved in the top down and bottom up pathophysiological mechanisms of aggression, involving circuits in orbital frontal cortex, anterior cingulate gyrus, amygdala and insula [40]. In an animal model of frontotemporal dementia, overexpression of MIR124-1 led to rescue of behavioral deficits in social interaction [17] and injection of silencers of MIR124-1 in animal models led to antidepressant effects and a decrease in voluntary alcohol consumption [3,4]. The aim of the current study was to test the hypothesis that a functional SNP in the brain expressed MIR124-1 gene could be associated with aggressiveness scores in a sample of young subjects.

2. Materials and methods

2.1. Participants

One hundred seventy healthy young subjects were included in this study. 124 female (72.9%) and 46 male (27.1%), with a mean age of 22.1 ± 5.6 years. Included subjects had all four grandparents born in Colombia, were unrelated and recruited from a university in Bogotá, Colombia (students of Medicine, Nursing and Clinical Laboratory undergraduate programs). The population living in Bogotá has a main Southern European genetic background, with historical admixture with Amerindians [5,33]. Participants with self-reported history of neuropsychiatric diseases, such as depression or anxiety disorders, were excluded. All participants provided written informed consent [6] and this study was approved by the respective Institutional Ethics Committee.

2.2. Phenotypic measurements

Participants completed a self-administered questionnaire, which was used to collect socio-demographic variables (age, sex and personal and familial history of neuropsychiatric disorders)
A linear regression model correcting for sex and age showed a significant association between MIR124-1 genotypes and total AQ-R-S scores \((P = 0.01,\text{ using square root transformed data})\) (Table 1 and Supplementary Fig. 1). C/G carriers showed lower aggressiveness scores. These results for total AQ-R-S scores were significant after a correction for multiple testing (using a Bonferroni correction) and there were no significant associations for MIR124-1 genotypes and AQ-R-S subscales \((P\text{ values of 0.58, 0.33, 0.88 and 0.82, for anger, hostility, physical aggression and verbal aggression factors, respectively})\).

A moderate positive correlation was found between AQ-R-S and BIS15-S scores \((r = 0.327,\text{ Pearson correlation, } P < 0.01)\). No association was found between MIR124-1 and BIS15-S scores \((P = 0.82,\text{ linear regression model correcting for sex and age})\): mean (SD) BIS15-S scores for G/G and C/C carriers were 30.9 (5.9) and 30.5 (6.4), respectively. A correction for levels of impulsiveness (BIS15-S) and depression (HADS-D) and anxiety (HADS-A) showed that the association between MIR124-1 and AQ-R-S remained significant \((P\text{ values of 0.01, 0.02 and 0.02, respectively})\).

Known interactions of mir-124 with experimentally validated targets expressed in brain are shown in Fig. 1A. Minor allele frequencies for the MIR124-1 SNP varied between different world populations (Fig. 1C).

### 4. Discussion

Aggressiveness is an important endophenotype for interpersonal violence and suicide [28, 40], two leading causes of mortality and morbidity around the world, of particular impact in developing countries and young adults [32, 37]: rates of violent death in developing and developed countries were 10.4 and 2.7 per 100,000, respectively [19, 26]. In Colombia, as in several other developing countries, interpersonal violence is one of the top five causes of disease burden [31].

Exploration of genetic factors for aggressiveness have been partially successful, highlighting the need for the analysis of novel candidates and other populations [45], which have been unexplored and could have a higher health burden [13]. In the present study, we present for the first time evidence of an association of a functional SNP in the brain expressed MIR124-1 gene and aggressiveness scores (AQ-R-S) in a Colombian sample of young subjects \((P < 0.01)\). These results seem to be specific for AQ-R-S, as we did not find a significant association of MIR124-1 with impulsiveness (BIS15-S scores, \(P = 0.82\)) and the results remained significant after correction for levels of impulsiveness, anxiety and depression and for age and gender.

A functional variant in MIR124-1 gene, known to affect expression levels of the mature miRNA, could lead to allele-specific changes in the regulation of the expression of several protein coding genes (such as BDNF, DRD4 and GRIAS3) involved in neuroplasticity and neurosignaling mechanisms [12] and related to the pathophysiology of abnormal aggression levels [24, 29].

Variants in binding sites for miR-124, located in the 3’ untranslated regions (UTRs) of different candidate genes, have been associated with schizophrenia, cognitive performance and

### Table 1

Association between genotypes of the functional SNP in MIR124-1 gene and scores of the Spanish adaptation of the refined version of the Aggression Questionnaire (AQ-R-S).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>(n)</th>
<th>Mean (SD)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>144</td>
<td>25 (0.42)</td>
<td>0.016</td>
</tr>
<tr>
<td>G/C</td>
<td>26</td>
<td>21.8 (0.67)</td>
<td></td>
</tr>
</tbody>
</table>

* Results from SNPStats program, carried out square root transformed data, were back-transformed to facilitate a better understanding of results.
clastophobia [10,18,47]. Our current results with a SNP in a brain expressed miRNA gene, extends previous reports on SNPs in miRNA binding sites on 3’UTRs of candidate genes associated with aggressiveness and related endophenotypes (in serotonin receptor 1B – HTR1B – and wolfram – WFS1 – genes) [22,24]. Limitations of the current study are the use of a single scale for the analysis of aggressiveness, a relatively small sample size, and the analysis of a single functional SNP in the MIR124-1 gene. As for many other genetic studies of behavior in healthy individuals, a large proportion of the subjects that participated in our study were women. These results need to be confirmed in future studies in other populations, using larger sample sizes and including correlations with additional behavioral and environmental variables and risk factors.

Model animals of hyperaggressive behavior, in mice and flies, have identified additional candidate genes (glutamate receptor, ionotropic, N-methyl D-aspartate 1 – GRIN1 –, for example) that could be interesting to test in future studies in humans [2]. A future genetic and epigenetic study of additional variants in miRNA genes involved in brain function, in samples of healthy subjects and patients with neuropsychiatric disorders, could identify novel candidates for aggressiveness [12,14,20,44,45].

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.eurpsy.2015.03.002.

References


Smarr KL, Keefer AL. Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). Arthritis Care Res 2011;63(Suppl. 11):S454–66.


