Association study of ~~Monoamine O~~monoamine oxidase-A gene promoter polymorphism (MAOA-uVNTR) with self-reported anxiety and other psychopathological symptoms in a community sample of early adolescents

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Abstract

The polymorphism upstream of the gene for monoamine oxidase A (MAOA-uVNTR) is reported to be an important enzyme involved in human physiology and behavior. With a sample of 228 early-adolescents from a community sample (143 girls) and adjusting for environmental variables, we examined the influence of MAOA-uVNTR alleles on the scores obtained in the *Screen for Childhood Anxiety and Related Emotional Disorders* and in the *Child Symptom Inventory-4*. Our results showed that girls with the high-activity MAOA allele had higher scores for generalized and total anxiety than their low-activity peers, whereas boys with the low-activity allele had higher social phobia scores than boys with the high-activity allele. Results for conduct disorder symptoms did not show a significant relationship between the MAOA alleles and the presence of these symptoms. Our findings support a possible association, depending on gender, between the MAOA-uVNTR polymorphism and psychopathological disorders such as anxiety, which affects high rates of children and adolescents.

Keywords: MAOA-uVNTR; Gender; Anxiety; Psychopathology; Adolescents

1 Introduction

The monoamine oxidase A (MAOA) gene possesses a variable number of tandem repeats polymorphism (MAOA-uVNTR). This polymorphism gives rise to five different alleles depending on whether there are 2, 3, 3.5, 4, or 5 copies of a sequence of 30 base pairs. The alleles have been divided into two groups according to their transcriptional activity, resulting in genotypes with low-activity (MAOA-L) and high-activity (MAOA-H) alleles (Guo, Ou, Roettger, & Shih, 2008; Sabol, Hu, & Hamer, 1998). It has long been documented that the MAOA-uVNTR polymorphism affects the MAOA gene at the transcriptional level and it has been suggested that the polymorphism is involved with diverse mental health conditions in children and adults, including major depressive disorder (Lung, Tzeng, Huang, & Lee, 2011; Rivera~~Gutiérrez, Molina, Torres-González, & Bellón~~ et al., 2009), autism spectrum disorders (Tassone et al., 2011; Verma et al., 2014), aggressive behaviors (Byrd & Manuck, 2014), panic disorder (Reif et al., 2012) and attention deficit hyperactivity disorder (ADHD) (Guan et al., 2009; Nymberg et al., 2013). This relationship between allelic variants of the MAOA-uVNTR polymorphism and a particular pattern of psychopathological symptoms was supported by previous studies both in humans and in mice (Cases et al., 1995; McDermott, Tingley, Cowden, Frazzetto, & Johnson, 2009). In this vein, it is known that MAOA is an enzyme that metabolizes neurotransmitters such as dopamine, serotonin, and norepinephrine (Jacob et al., 2005; Shih, Chen, & Ridd, 1999) which are linked to some of the abovementioned psychopathological problems. Specifically, a large body of research has confirmed the crucial role of the serotonergic and dopaminergic neurotransmission systems in the pathophysiology of emotional and behavioral disorders (Gutiérrez et al., 2004; Marceau & Neiderhiser, 2013; Maron, Nutt, & Shlik, 2012). MAOA is also considered a likely depression and anxiety candidate gene because it is also known that MAO inhibitors have been found to be effective in treating these disorders (Libert et al., 2011; Murphy, Mitchell, & Potter,

1995).

However, studies examining the main effects of MAOA variants for the psychopathological disorders of children and adolescents are relatively few (Lavigne et al., 2013) and, in addition, results are mixed. Although some studies found that MAOA-L was associated with increased conduct disorder (Foley et al., 2004; Prom-Wormley et al., 2009), others found no effects of MAOA variants on conduct problems (Huizinga et al., 2006; Widom & Brzustowicz, 2006). With regard to depression, research on the possible association with MAOA-uVNTR has also been inconclusive: whereas numerous findings implicate low-expression alleles (Cicchetti, Rogosch, & Sturge-Apple, 2007; Lavigne et al., 2013; Priess-Groben & Hyde, 2013) others have failed to find any association (Eley et al., 2004) or have found many differences between genders.

In addition, some studies have suggested that the relationship between MAOA variants and certain behaviors can usually be modulated by environmental factors (Caspi et al., 2002; Lavigne et al., 2011; Winham & Biernacka, 2013). Specifically, candidate gene ×× environmental interaction (G ×× E) studies tested the hypothesis that the effect of some environmental variable on some outcome measure (e.g. psychopathological disorders the subjects may present) depends on a particular genetic polymorphism (Keller, 2014). On the other hand, Keller (2014) warns that this type of study (G ×× E) does not properly control for confounding variables and skepticism has increased about the validity of many of these findings (Duncan & Keller, 2011; Munafo & Flint, 2009). Moreover, it is known that the vast majority of phenotypes are polygenic, which means that they are influenced by multiple genes (Fowler et al., 2009; Plomin, 2008). In addition to genetic and environment interaction, the influence of certain polymorphism may vary depending on gender. In fact, in the case of the MAOA-uVNTR polymorphism there is a certain imbalance between girls and boys because boys have only one X chromosome and MAOA is linked to it.

One of the most accepted hypotheses is the “worrier- versus warrior-gene” hypothesis. This dichotomy refers to the results of previous studies and is related to the fact that aggressive behaviors and impulsivity are associated with male subjects with MAOA-L, whereas anxious behavior is associated with MAOA-H female subjects (Huang et al., 2004; McDermott et al., 2009; Reif et al., 2012; Rivera et al., 2009). In line with this, it is known that emotional and behavioral disorders are highly prevalent, cause severe disturbances, and present a long-term course in children and adolescents from the community (Coughlan et al., 2014; Magiati et al., 2013; Merikangas et al., 2010a,b, He, Brody, et al., 2010; Merikangas, He, Burstein, et al., 2010). For this reason, study of the biological basis of these disorders could be useful for determining whether psychopathological disorders during early adolescence depend not only on environmental factors but also on biological ones.

Assuming the “worrier- versus warrior-gene” hypothesis, and in light of the uncertainty emerging from the published research, in this study we analyse, by gender, the possible association of MAOA-uVNTR polymorphism alleles with different self-reported anxiety subtypes and other psychopathological symptoms in a sample of early adolescents from the community. We hypothesized that MAOA-uVNTR polymorphism influence the presence of psychopathological symptoms in the age period studied and that this influence probably depends on gender.

2 Methods

2.1 Participants

A three3-year longitudinal study was conducted of 245 subjects (147 girls and 98 boys; mean age = 13.5; SDSD = .9). The participants were recruited from a three-phase epidemiological study of anxiety and depression disorders in the town of Reus (a Spanish town of 100,000 inhabitants). The first phase took place during the 2006/2007 academic year, the second phase during the 2007/2008 academic year and the third phase during the 2009/2010 academic year.

The baseline sample in the study was a group of 1514 subjects (794 girls and 720 boys; mean age = 10.2; SDSD = 1.2) from 13 schools randomly chosen from the town's state schools and state subsidized private schools. Screening questionnaires for emotional disorder symptoms were used to select a sample at risk of emotional disorders and risk-free control sample. Therefore, in the second phase, the participants were 562 subjects (308 girls and 254 boys), of which 405 were at risk of emotional disorders and 157 were controls. For the control group, one child without risk of emotional psychopathology was selected for every three children at risk of emotional psychopathology, matching for age, gender and type of school. During the follow-up three years after the baseline, all the participants of the second phase were contacted. 245 subjects (147 girls and 98 boys; mean age = 13.5; SDSD = .9) agreed to participate and their parents provided written informed consent. Of the 245 subjects that agreed to participate in the third phase, thirteen13 were eliminated for presenting incomplete data and four were eliminated as outliers. The final sample therefore comprised 228 subjects (143 girls and 85 boys). The socio-demographic characteristics of the sample are shown in Table 1.

Table 1 Socio-demographic and psychopathological characteristics of the sample.

	Sample of the third phase			
	Total (*n = 228)Boys (n = 85)Girls (n*n = 228)	Boys (*n* = 85)	Girls (*n* = 143)	*p*
Age (years)	13.5 (.9)	13.5 (.9)	13.6 (.9)	.319
Gender (%)		37.3	62.7	

~~Socioeconomic level~~Socioeconomic level				
Low (%)	34.6	32.9	34.6	.699
Medium (%)	44.7	43.5	44.7	
High (%)	20.6	23.5	20.6	
~~Family type~~Family type				
Nuclear (%)	83.8	87.1	81.8	.299
Single parent (%)	16.2	12.9	18.2	
~~Birthplace~~Birthplace				
Native (%)	90.4	89.4	90.9	.711
Foreign (%)	9.6	10.6	9.1	
Psychopathological variables				
~~SCARED Total (n = 228) Mean (SD) Boys (n = 85) Mean (SD) Girls (n = 143) Mean (SD) p~~SCARED	Total (*n* = 228) Mean (SD)	Boys (*n* = 85) Mean (SD)	Girls (*n* = 143) Mean (SD)	*p*
Total SCARED	19.9 (9.5)	17.9 (8.7)	21.1 (9.8)	.012
Somatic/panic	3.1 (3.1)	2.2 (2.2)	3.7 (3.4)	.001
Social phobia	5.3 (3.2)	5.3 (3.3)	5.3 (3.2)	.999
Generalized anxiety	6.3 (3.4)	5.8 (3.4)	6.6 (3.3)	.085
Separation anxiety	5.2 (3.3)	4.5 (2.7)	5.6 (3.6)	.015
~~CSI-4 Total (n = 169) Mean (SD) Boys (n = 58) Mean (SD) Girls (n = 111) Mean (SD) p~~CSI-4	Total (*n* = 169) Mean (SD)	Boys (*n* = 58) Mean (SD)	Girls (*n* = 111) Mean (SD)	*p*
Attencion deficit symptoms	7.0 (5.5)	8.2 (5.6)	6.5 (5.4)	.057
Hyperactivity/impulsivity symptoms	4.3 (5.0)	5.2 (7.7)	3.8 (5.0)	.076
Combined ADHD symptoms	11.3 (9.6)	13.3 (9.3)	10.2 (9.7)	.045
Oppositional defiant disorder symptoms	4.9 (4.2)	4.9 (4.3)	4.9 (4.2)	.931
Conduct disorder symptoms	.7 (1.5)	1.0 (2.1)	.5 (.9)	.081
Generalized anxiety symptoms	4.3 (3.4)	4.4 (3.0)	4.2 (3.5)	.734
Specific phobia symptoms	.4 (.7)	.5 (.8)	.4 (.6)	.410
Obsessions and compulsions	.4 (.8)	.4 (.9)	.3 (.6)	.316
Posttraumatic stress disorder symptoms	.2 (.6)	.2 (.6)	.3 (.6)	.697
Separation anxiety symptoms	1.1 (2.2)	.9 (1.5)	1.3 (2.4)	.289
Social phobia symptoms	3.1 (1.9)	3.3 (2.2)	2.3 (1.7)	.273
Major depressive disorder symptoms	1.6 (2.0)	1.7 (2.3)	1.5 (1.8)	.586

Dysthymic disorder symptoms	2.2 (2.3)	2.3 (2.6)	2.1 (2.1)	.573
Vocal and motor tics	.3 (.8)	.4 (1.1)	.2 (.5)	.029
Schizophrenia	.1 (.4)	.2 (.5)	.1 (.4)	.220
Autistic symptoms	1.8 (2.4)	2.4 (2.8)	1.6 (2.1)	.060
Asperger symptoms	1.2 (1.8)	1.4 (2.0)	1.1 (1.7)	.237

*p value between boys and girls.

2.2 Measures

2.2.1The The *Child Symptom Inventory-4* (Gadow & Sprafkin, 1998) is a screening instrument based on DSM-IV criteria. The parent version used in this study contains 97 items classified into 10 categories with a 4-point Likert response format. The CSI-4 has been demonstrated to be valid (Sprafkin, Gadow, Salisbury, Schneider, & Loney, 2002) and its Spanish version has shown excellent internal consistency (Cronbach's α = .99) (Angulo-Rincón et al., 2010). For this study we used all the categories except enuresis and encopresis, motor tics and vocal tics, schizophrenia, and autistic and Asperger symptoms. We created quantitative variables resulting from the sum of the items in each category, thus we created the following two variables: *any disruptive disorder*, which comprised all the ADHD categories, oppositional defiant disorder symptoms, and conduct disorder symptoms; and *any emotional disorder*, which comprised the categories related to anxiety, obsessive-compulsive disorder, and depressive disorder symptoms.

2.2.2The The *Screen for Childhood Anxiety and Related Emotional Disorders* (Birmaher et al., 1997) is a 41-item self-report questionnaire that assesses anxiety disorder symptoms in children and adolescents from 8 to 18 years old. Subjects are asked about the frequency of each symptom using a 3-point Likert response format: 0 (almost never), 1 (sometimes), and 2 (often). The reliability of the Spanish version is αα = .86. It consists of four factors called somatic/panic (12 items; α = .78), social phobia (7 items; αα = .69), generalized anxiety (9 items; αα = .69), and separation anxiety (13 items, αα = .70) (Vigil-Colet et al., 2009). A score of 25 has been considered the cut-off point for risk of anxiety (Birmaher et al., 1997; Canals, Hernández-Martínez, Cosi, & Domènech, 2012). Canals et al. (2012) also proposed cut-off scores for the SCARED factors with sensitivities between 74% and 78%. The SCARED was administered in all phases of the study.

2.2.3Stressful life events (SLE) Stressful life events (SLE) were assessed in the third phase using 10 of the 31 items of the *Adolescent Life Change Event Scale* (Yeaworth, York, Hussey, Ingle, & Goodwin, 1980). First, using a 5-point Likert response format (1 non-affected–5 highly affected), the participants indicated how they would be affected if they were in each of the proposed situations. Then they indicated which of the situations had happened to them in the previous year. We created a quantitative variable by multiplying each situation experienced by the subject in the previous year by the degree of impact they had marked on the Likert scale for the same situation. The final quantitative variable was the sum of all the situations experienced taking into account the degree of impact attributed by the subject. Cronbach's alpha was .79.

2.2.4Academic achievement Academic achievement was assessed by asking parents about the academic achievement of their children in language, social sciences, mathematics, and natural sciences. There were four items with four possible responses: 1 (fail), 2 (below average), 3 (average), and 4 (above average). We defined a quantitative variable to represent overall academic achievement using the sum of the scores in language, social sciences, mathematics, and natural sciences.

2.2.5*Socio-demographic characteristics* of the sample were collected at baseline using a questionnaire designed for this study by the authors. The children answered questions about age, gender, place of birth, date of birth, type of family, and parents' occupations. This information was corroborated by the parents. The socioeconomic status (SES) was established by the Hollingshead index (2011). This index allows the social status of each individual to be determined by categorizing his or her occupation into one of nine categories (from unskilled work to highly skilled work) and his or her level of education into one of seven categories (from non-completed primary education to completed higher education). The status score is estimated by multiplying the occupation scale value by a weight of five and the education scale value by a weight of three and then combining the two scores. We thus determined family SES on a scale from 0 to 66. This gave us three categories (low, medium and high). We considered scores under 22 to be low, scores of between 23 and 44 to be medium, and scores over 44 to be high.

2.3 Genotyping

Genomic DNA was extracted from buccal cells derived from Oragene-DNA self-collection kits (DNA, Genotek). The polymorphism 30 bp VNTR in the promoter of the MAOA gene was genotyped using a previously published protocol (Haberstick et al., 2005). Briefly, polymerase chain reaction (PCR) was performed in a total volume of 20 μl containing 20 ng of DNA, using the primers forward, 5′-D2-ACAGGCTGACCGTGGAGAAG-D2-ACAGCCTGACCGTGGAGAAG-3′ and reverse, 5′-GAACGGACGCTCCATTCGGA. PCR products included five possible fragment sizes—291, 321, 336, 351, and 381 bp (2, 3, 3.5, 4 and 5 repeats, respectively)—and were classified into two groups. The first group combined those with the 2-repeat and 3-repeat alleles and is subsequently referred to as the low-activity group of the MAOA (MAOA-L). The second group combined those with the 3.5-repeat, the 4-repeat, and the 5-repeat and is subsequently referred to as the high-activity group of the MAOA (MAOA-H). The MAOA gene is located on the X chromosome; therefore, a heterogeneous genotype does not exist in men. We classified the heterogeneous genotype (i.e. 2/3.5, 2/4, 2/5, 3/4, 3/5, 3.5/4, 3.5/5, 4/5) of girls into the high-activity group, as in other studies (Reif et al., 2012). All trials were repeated twice. If the results were negative or discordant, the trials were repeated 3, 4, or 5 times. Nine subjects were unsuccessfully genotyped for the MAOA gene and were dropped from all genetic analyses.

2.4 Procedure

This study is part of a longitudinal study. For the analyses in this paper we have used data from the third phase.

Before the beginning of the study, the project was approved by the Rovira i Virgili University ethics committee for research on individuals and by the Department of Education of the Government of Catalonia. A representative sample of subjects was then selected, and cluster sampling was conducted by randomly selecting a set of thirteen primary schools (7 state schools and 6 state-subsidized private schools) from a total of 26 schools from all five representative areas of Reus (Catalonia, Spain). We then contacted the school boards and all agreed to participate. After that, a letter was sent to all parents to inform them about the study and to ask for their written informed consent. We then conducted the study in three phases. In the third phase, the children answered the SCARED questionnaire as well as items about the presence of SLE. A saliva sample was also collected from them from which the DNA was extracted for the subsequent analyses of the MAOA-uVNTR polymorphism. The parents answered the CSI-4 questionnaire and the questions about their children's academic achievement. The participants completed the questionnaires in groups of three or four. Researchers were present to instruct the children on how to answer the surveys and use the saliva kits, and to answer any queries.

2.5 Statistical analyses

For statistical analysis we used SPSS software, version 20.0 for Windows.

First the data were tested for normality using the Kolmogorov–Smirnov and Shapiro–Smirnov and Shapiro–Wilk tests.

Descriptive data of the socio-demographic and psychopathological characteristics of the sample were presented. Values were expressed as the mean and standard deviation for the quantitative variables, and as percentages for the qualitative variables. Student's *t*-test and chi-square analyses were used to find any significant differences between boys and girls (see Table 1).

Student's *t*-test analyses were also conducted to examine possible differences between the two genotype subgroups (MAOA-L and MAOA-H) in the SCARED and CSI-4 scores. These analyses were also done separately for each gender.

On the basis of the *t*-test analysis findings, we then fitted multiple linear regression models using the enter method to explore the effect of MAOA-uVNTR polymorphism and possible confounding variables (gender, age, SLE, SES, and academic performance). Multiple linear regression models were calculated for boys and girls separately because we observed differences between girls and boys in previous exploratory analyses.

The Hardy–Weinberg equilibrium (HWE) of the genotype distributions of the girls was approximated for all samples using chi-squared tests. Because of hemizygosity in the male subjects, HWE could not be calculated for them.

Criterion for statistical significance was set at .05 such as other studies (Ducci et al., 2006; Roohi, DeVincent, Hatchwell, & Gadow, 2009).

3 Results

As the Kolmogorov–Smirnov and Shapiro–Smirnov and Shapiro–Wilk tests were not significant in any case, the sample distribution was assumed to be normal.

Table 1 shows the socio-demographic and psychopathological characteristics of the sample. Girls showed significantly higher scores of somatic/panic, separation and total anxiety than boys in the SCARED. Table 2 shows the MAOA-uVNTR alleles and genotype distributions. In girls, allele frequencies were within the Hardy–Weinberg equilibrium ($\chi^{2 = 2.127; p}$ = 2.127; p = .345). Due to hemizygosity in male subjects, the Hardy–Weinberg equilibrium could not be calculated for boys.

Table 2 Allele and genotype distribution of the MAOA-uVNTR polymorphism of the study sample.

	Alleles, *n (%)Genotypes, n (%)233.5452/22/32/3.52/42/53/33/43/3.53/53.5/3.53.5/43.5/54/44/55/5Boysn* (%)					Genotypes, *n* (%)														
	2	3	3.5	4	5	2/2	2/3	2/3.5	2/4	2/5	3/3	3/4	3/3.5	3/5	3.5/3.5	3.5/4	3.5/5	4/4	4/5	5/5
Boys	1 (1.2)	30 (35.3)	3 (3.5)	50 (58.8)	1 (1.2)	1 (0.1)	30 (4.2) 3 (.4) 50 (7.0) 1 (.1)Girls	–	–	–	30 (4.2)	–	–	–	3 (.4)	–	–	50 (7.0)	–	1 (.1)
Girls	1 (.7)	87 (60.8)	4 (2.8)	118 (82.5)	4 (2.8)	0	1 (.1)	0	0	0	19 (2.4)	62 (7.8)	2 (.3)	3 (.4)	0	2 (.3)	0	53 (6.7)	1 (.1)	0
TotalTotal	2 (.9)	117 (51.3)	7 (3.1)	168 (73.7)	5 (2.2)	1 (.1)	1 (.1)	0	0	0	49 (3.3)	62 (4.1)	2 (.1)	3 (.2)	3 (.2)	2 (.1)	0	103 (6.8)	1 (.1)	1 (.1)

Student-t's t-test analyses (see Table 3) showed that MAOA-L boys had higher social phobia factor scores than MAOA-H boys (MAOA-H group: *M* = 4.7, SD = 4.7, SD = 3.2; MAOA-L group: *M* = 6.4, SD = 3.3; t = 2.271, p = .026, d = 0 = 6.4, SD = 3.3; *t* = 2.271, *p* = .026, *d* = .52). SCARED factor scores for both genders were higher in somatic/panic, generalized anxiety and separation anxiety for MAOA-H subjects than for MAOA-L subjects but these results were not statistically significant. Taking this trend into account and to prevent the scores for the social phobia items from attenuating the SCARED total scores, we calculated SCARED total using somatic/panic, generalized anxiety, and separation anxiety factor scores. MAOA-H girls obtained higher SCARED total scores than MAOA-L girls and these results were statistically significant (MAOA-H group: *M* = 16.2, SD = 16.2, SD = 8.8; MAOA-L group: *M* = 13.5, SD = 4.7; t = -2.033, p = .048, d = 0 = 13.5, SD = 4.7; *t* = –2.033, *p* = .048, *d* = .38). In this regard, the results also showed a marked trend for generalized anxiety in girls (MAOA-H group: *M* = 6.8, SD = 6.8, SD = 3.4; MAOA-L group: *M* = 5.3, SD = 2.7; t = -1.873, p = .063, d = 0 = 5.3, SD = 2.7; *t* = –1.873, *p* = .063, *d* = .16). The analyses of the CSI-4 scores did not show statistically significant results.

Table 3 T-tt-Test analyses to examine possible differences between the two genotype subgroups (MAOA-L and MAOA-H) for the SCARED and CSI-4 scores, for both genders.

	Boys					GirlsMAOA-LMAOA-HMAOA-LMAOA-H(SCARED, n = 31)(SCARED, n = 54)(SCARED, n = 20)(SCARED, n = 123)(CSI-4, n = 23)(CSI-4, n = 35)(CSI-4, n = 16)(CSI-4, n = 95)Mean (SD)Mean (SD)tpdMean (SD)Mean (SD)tpdGirls				
	MAOA-L (SCARED, *n* = 31) (CSI-4, *n* = 23)	MAOA-H (SCARED, *n* = 54) (CSI-4, *n* = 35)	*t*	*p*	*d*	MAOA-L(SCARED, *n* = 20) (CSI-4, *n* = 16)	MAOA-H (SCARED, *n* = 123) (CSI-4, *n* = 95)	*t*	*p*	*d*
	Mean (SD)	Mean (SD)				Mean (SD)	Mean (SD)			
SCARED total score (without social phobia factor)	11.9 (5.0)	12.9 (7.4)	-–.719	.474	0.16	13.5 (4.7)	16.2 (8.8)	-2.033.0480.38SCARED Factor 1: Somatic/P–2.033	**.048**	0.38
SCARED factor 1: somatic/panic	2.1 (1.6)	2.4 (2.5)	-–.653	.515	0.14	3.0 (2.4)	3.8 (3.5)	-–1.255	.218	0.27
SCARED Factor 2: Sfactor 2: social phobia	6.4 (3.3)	4.7 (3.2)	2.271	**-026.026**	0.52	6.3 (2.8)	5.2 (3.2)	1.414	.160	0.37
SCARED Factor 3: Gfactor 3: generalized anxiety	5.8 (3.0)	5.8 (3.6)	.037	.970	0.01	5.3 (2.7)	6.8 (3.4)	-–1.873	.063	0.16
SCARED Factor 4: Sfactor 4: separation anxiety	4.0 (2.6)	4.8 (2.8)	-–1.336	.185	0.30	5.2 (2.4)	5.6 (3.8)	-–.653	.518	0.13
Any disruptive disorder (CSI-4)	33.5 (26.9)	32.3 (20.5)	.191	.849	0.05	24.3 (16.3)	26.2 (23.7)	-–.316	.753	0.09
Any emotional disorder (CSI-4)	14.7 (10.3)	13.3 (8.5)	.539	.592	0.15	11.1 (10.1)	13.5 (9.3)	-–.938	.350	0.25

*Values in bold represents *p* -<.05< .05.

On the basis of the t-test analysis results, regression models were performed to observe the effect of MAOA-uVNTR polymorphism on social phobia for boys and on total and generalized anxiety for girls. Models were adjusted for possible confounding variables. In this regard, the results showed that MAOA-uVNTR polymorphism only presented a significant association with the generalized anxiety factor scores (see Table 4). SLE was a significant factor related to all anxiety scores. Furthermore, we were aware that our results could be affected by the presence of co-occurrence between anxiety and disruptive behavior symptoms. Therefore, to determine whether this was the case, we performed the same regression analysis after removing the 10 subjects that presented comorbidity and found again that MAOA-H in girls was associated with the self-reported generalized anxiety symptoms (the model explained 12.6% of the generalized anxiety factor; $F(5, 95) =$ 2.596, p = 2.596, *p* = .031).

Table 4 Multiple linear regression of the genetic, socio-demographic and environmental variables effect on anxiety, in girls.

	SCARED Factor 3: Gfactor 3: generalized anxiety			
	B	*t*	*p*	Model
MAOA	2.366	2.427	.017	R^2ec*100 = 6.8
Stressful life events	.164	2.064	.042	$F_{5.101}$ = 2.484

Age	.181	.500	.618	$p = .037$
SES	.025	.139	.890	
Overall academic achievement	.284	2.313	.023	

Variables entered into the model: MAOA (0: low-activity; 1: high-activity); ~~S~~stressful life events (total score); ~~A~~age (years); SES (total score); ~~O~~overall academic achievement (total score).

4 Discussion

Overall, in this sample of early adolescents, our results suggest a possible association between the variants of the MAOA-uVNTR and the presence of anxiety symptoms. The results also showed different trends between girls and boys.

Our data support one part of the "worrier- versus warrior-gene" hypothesis insofar as they showed that high-activity variants of the MAOA-uVNTR polymorphism were associated with anxiety symptoms in girls, as was found in adult samples (Rivera et al., 2009; Yu et al., 2005) especially for the generalized anxiety factor and for the SCARED total scores. In this regard, at the biological level it is known that high MAOA activity degrades the serotonin, rendering it inactive in the synapses of the brain. It is also known that a dysfunction of the serotonergic system is involved in the development and pathophysiology of affective disorders such as anxiety (Lowry et al., 2008; Owens & Nemeroff, 1994). Moreover, previous studies found that MAO inhibitors were effective in treating emotional disorders (Libert et al., 2011; Reif et al., 2014), and that these disorders are more prevalent in girls (Abbo et al., 2013; Coughlan et al., 2014; Merikangas ~~et al., 2010a,b~~, He, Brody, et al., 2010; Merikangas, He, Burstein, et al., 2010). Also, in agreement with our results, a recent longitudinal study conducted with a large cohort found that low expression of MAOA was significantly related to greater happiness in women (Chen et al., 2013).

For girls, the effect of the interaction between environmental factors (SLE) and the MAOA-uVNTR polymorphism was found in generalized anxiety. For the other anxiety subtypes, only the SLE was a significant variable but we know that other genetic predispositions may exist (Arias et al., 2012; Baumann et al., 2013). On the other hand, it is important to note that unlike genetic effects, environmental influences are more time-specific, possible because experiences such as SLE are transient (Trzaskowski, Zavos, Haworth, Plomin, & Eley, 2012).

With regard to social phobia, our data presented a different pattern of results compared with findings related to anxiety and MAOA in other studies (Rivera et al., 2009; Yu et al., 2005). MAOA-L boys obtained higher social phobia scores than MAOA-H boys. This leads us to think that social phobia may present a different biological basis from other anxiety subtypes. In line with our results, Samochowiec et al. (2004) found that MAOA-uVNTR does not have a role in social phobia in female patients and suggest that another molecular mechanism may underlie their pathogenesis. Another possible interpretation of our finding is that boys with social phobia may have a hypersensitivity trait for social situations and be more concerned about questions on what opinion their peers have of them. This is related to results obtained by ~~Eisenberg et al. (2007)~~Eisenberger, Way, Taylor, Welch, and Lieberman (2007), who found that MAOA-L individuals reported higher trait interpersonal hypersensitivity and showed greater dorsal anterior cingulate cortex activity to social exclusion compared with MAOA-H individuals. Also, Baumann et al. (2013) found that male carriers of the MAOA-L allele who reported more aversive experiences in childhood exhibited a trend for enhanced anxious apprehension. In addition, previous studies have revealed a causal relationship between MAOA-L with behavioral, cognitive, neuroanatomical and neuropharmacological impairments in autism spectrum disorders (ASD) (Cohen et al., 2003; Davis et al., 2008; Yirmiya et al., 2002). Some of these studies suggested a potential role of the MAOA alleles in boys (Cohen et al., 2003; Tassone et al., 2011). These findings indicate that MAOA-L variants may be associated with social relationship problems. In fact, the pathogenesis of social phobia may be related to the pathogenesis of other disorders. There is a possibility, therefore, that social phobia is a premorbid manifestation of other psychopathological problems (such as schizoid spectrum) but more studies are needed to replicate these results.

With regard to conduct disorder symptoms, despite the "worrier- versus warrior-gene" hypothesis our findings did not show statistically significant results. Despite this, it is also known that males are three times more likely than females to have the MAOA-L genotype, which, in interaction with some form of psychosocial adversity, increases the risk of developing conduct problems such as antisocial behaviors (Byrd & Manuck, 2014; Eme, 2013; Kim-Cohen et al., 2006; Weder et al., 2009).

The present study was subject to certain limitations, such as the lack of a large sample and the lack of data on these subjects up to adulthood. We should also bear in mind that other functional polymorphisms may also be responsible for the development of the emotional and behavioral disorders studied. Nevertheless, the study examined the possible influence of environmental and genetic variables and represents a contribution to the growing body of studies on the relationship between the variant of the MAOA-uVNTR polymorphism and the most frequent child and adolescent psychopathological symptoms. Another strength of this study is that the psychopathological information was provided by the children and their parents. In short, as we hypothesized, our results suggest that MAOA-uVNTR polymorphism alleles were associated with self-reported anxiety and that this relationship depends on gender. In this regard, although our results are weak, they do indicate that the MAOA-uVNTR polymorphism may have a role in anxiety. On the other hand, our findings did not find a significant association between this polymorphism and conduct disorder symptoms and therefore the "worrier- versus warrior gene" hypothesis was not completely confirmed by our study. However, the failure to find this association does not necessarily rule out the possibility that MAOA-uVNTR polymorphism plays a role in the etiology of these symptoms. Further replication studies with large samples are needed to confirm these findings.

~~Uncited referencesEisenberger et al., 2007 and Hollingshead, 2011.~~Acknowledgements

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Highlights

- We have analysed, by gender, the association of MAOA-uVNTR polymorphism alleles with anxiety subtypes in a sample of adolescents.
- High-activity variants of the MAOA-uVNTR polymorphism were associated with anxiety symptoms in girls.
- Low-activity variants of the MAOA-uVNTR polymorphism were associated with social phobia symptoms in boys.

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