The European Journal of Psychiatry 35 (2021) 225–233

THE EUROPEAN JOURNAL OF PSYCHIATRY

www.elsevier.es/ejpsy

ORIGINAL ARTICLE

Monoamine oxidase A (*MAOA*) interaction with parenting practices on callous-unemotional traits in preschoolers

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Received 7 November 2020; accepted 1 February 2021
Available online 11 August 2021

KEYWORDS
Callous unemotional traits;
Development;
Gene by environment;
MAOA;
Parenting practices;
Preschool

Abstract
Background and Objectives: From a gene-by-environment perspective, parenting in interaction with the polymorphism in the Monoamine oxidase A (MAOA) gene (*MAOA*-uVNTR) might also be associated with increased callous-unemotional traits (CU) in preschoolers. *MAOA*-uVNTR results in differential enzyme activity, so that high-activity alleles (*MAOA*-H) are linked to reduced dopamine, serotonin, and norepinephrine availability in comparison to low-activity allele (*MAOA*-L). As *MAOA*-uVNTR has been previously described to moderate the relationship between childhood parental maltreatment and aggressive and antisocial behavior, it may also play a role in CU traits etiology.

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https://doi.org/10.1016/j.ejpsy.2021.02.003

Methods: Data was collected through questionnaires answered by parents and teachers. *MAOA*-uVNTR was genotyped in 368 Caucasian children from a community sample (51.9% male). Multiple linear regression analyses were conducted to analyze the interaction effect of *MAOA* genotypes and both positive parenting and punitive parenting practices on CU traits at two different periods (3 and 5 years old) and separately by sex.
Results: No significant interactions were found for boys. Among girls, a significant interaction effect was found for *MAOA*-LL carriers, who showed higher CU traits at age 5 when exposed to higher punitive or positive parenting at age 3.
Conclusions: Our study provides the first evidence for significant *MAOA* × early parenting effects on CU traits in preschoolers, specifically among female *MAOA*-LL carriers. This suggests that the *MAOA*-LL genotype for girls is associated with higher sensitivity to both positive and punitive parenting in girls, so that *MAOA*-LL emerges as a genotype that confers higher vulnerability to parental influences.

Introduction

Callous Unemotional (CU) traits are seen as precursors of adult psychopathy and have been added to the DSM-5 as a specifier to diagnose conduct disorder under the term 'limited prosocial emotions' (LPE) in order to identify a subgroup of children and adolescents who show a distinct prosocial and emotional functioning such as lack of empathy, lack of guilt and deficits in emotional expression.[1]

CU traits are highly heritable, and a meta-analysis estimated that genetic factors account for 42% and 68% of the variation of CU traits.[2] Takahashi et al.[3] found that the genetic effect on CU traits varies depending on the developmental path of CU traits, so that childhood-onset CU traits (around age 7) seem to be under a higher genetic influence than CU traits that develop later across adolescence. Moreover, they indicate that the course of CU traits seems to be dynamic, with environmental influences accounting for 23.5% of the variance of initial CU traits, but for 56.4% in the stability of these traits. Among the environmental factors that influence the development of childhood CU traits, parenting practices have been the focus of most studies.[4] Harsh, inconsistent parenting and corporal punishment have also been identified as risk factors for increases in CU traits in pre-schoolers.[5] At the same time, positive parenting can be considered a protective factor and strategies such as positive reinforcement, parental sensitivity and warmth have shown to predict lower CU levels among children.[6]

Certainly, not all children are equally vulnerable to certain parenting practices in the development of CU traits. Some research suggests that sensitivity to parenting practices might be explained through individual genetic variability.[7] In this sense, Gene by Environment (G × E) interaction studies have focused on the diathesis-stress model and have found that certain genotypes confer vulnerability to adverse environments. From another perspective, the differential susceptibility model[8] suggests individuals might be more susceptible to adverse parenting styles, but at the same time, might also benefit more from positive parenting practices. Both models show that genetic influences shape an individual's sensitivity towards social environments, such as parenting. While one study found a salient G × E interaction in CU trait development on BDNF and harsh parenting,[9] the question arises whether other candidate genes for CU traits might also moderate the effect of parenting practices on CU traits development.

To provide more insight into the complex relationship between genes, parenting practices and CU trait development, the current study focuses on Monoamine Oxidase A gene (*MAOA)*. Moore et al.[10] identified, among other candidate genes for CU traits, *MAOA*. This gene encodes for the Monoamine Oxidase A enzyme (MAO-A) that catalyzes the degradation of brain neurotransmitters such as serotonin, dopamine, and norepinephrine.[11] Deficits in the serotonin system have been associated with CU traits,[12] but the specific role of *MAOA* in the etiology of CU traits has remained unexamined.

The *MAOA* gene has a variable number of tandem repeats (uVNTR) polymorphism in its promoter sequence. The different allelic variants of this polymorphism are associated with changes in the transcriptional efficiency of the gene, which results in low and high enzymatic activity alleles (*MAOA*-L and *MAOA*-H, respectively).[13] As the *MAOA* gene is located on the X chromosome (Xp11.23), males inherit a single allele and are therefore hemizygous for either *MAOA*-L or *MAOA-H*, whereas females can be homozygous (*MAOA*-LL/*MAOA*-HH) or heterozygous (*MAOA*-HL). The lower MAO-A activity results in different neurochemical, neural and behavioral alterations[14] and has been identified as the ''risk allele'' for antisocial behavior[15] and aggressive behavior,[16] especially among males. *MAOA*-L has also been associated with CU traits in male adolescents with comorbid attention deficit/hyperactivity disorder.[17] Thus, neuroimaging studies have shown that the *MAOA*-L allele has an impact on altering neural circuits such as the amygdala or the prefrontal cortex, which are implicated in aggressive behavior and emotional processing.[18]

Research also indicates the existence of a robust sex-dependent G × E interaction on *MAOA*-L and childhood maltreatment, showing that males who carry the *MAOA*-

L allele and are exposed to abuse or maltreatment also develop more antisocial behavior[19] and conduct disorder.[20] Studies on females are less frequent and present less robust findings, but suggest that *MAOA*-HH confers vulnerability towards adversity, resulting in the ''risk allele'' for antisocial behavior.[15]

Based on previous G × E studies, we hypothesized the presence of sex dependent G × E interactions on CU trait development on preschoolers, so that boys who carried the *MAOA*-L allele and girls who carried the *MAOA*-HH allele and who experienced punitive parenting styles would exhibit higher levels of CU traits. At the same time, these children would show lower levels of CU traits when exposed to positive parenting practices. Moreover, we examined these G × E interactions on CU traits at two different periods (ages 3 and 5), which represent initial and ending points of preschool age. Preschool age is a developmental period in which empathy, emotional expression and conscience emerge, making it an important time in the pathway to early CU traits.[21] During these years, children are very sensitive to parenting practices and research has shown that positive parenting practices protect children from socioemotional difficulties, while harsh practices increase the risk of developing externalizing problems and CU traits.[22] Because genetic influences seem to be more important at earlier ages than later in development,[23] we hypothesized that G × E interactions would be different across age.[3]

Material and methods

Participants

In the context of a longitudinal study of psychological risk factors during development, a random sample of 2,283 children from the census of preschoolers in grade P3 (3-year-olds) in Barcelona (Catalonia, Spain) were screened for behavioral problems.[24] This began with an initial screening using the parent-administered Strengths and Difficulties Questionnaire (SDQ)[25] enriched with four additional oppositional defiant disorder items to complete the DSM-IV description. A total of 1,341 families (58.7%) agreed to participate. In a second stage of the sampling, all the children who screened positively for behavioral problems and an additional 30% of the children with negative screening scores continued and were assessed annually. Of those included, 622 families (89.4 %) agreed to participate further. No statistically significant differences in sex ($p = .82$) or type of school ($p = .85$) between participants and drop-outs were found. For the present study, which corresponds to a prospective design with independent variables assessed at age 3 and dependent variables assessed at ages 3 and 5, only Caucasian children were included, to control possible ethnic or racial variations in *MAOA* allele frequencies.[13] *MAOA* genotype was available for 368 children (59.2%). Table 1 presents the demographic information at age 3.

Table 1 Characteristics of the Sample (N = 368).

Sex; *n (%)*	Male	191 (51.9)
Socioeconomic status; *n (%)*	High	134 (36.4)
	Middle	163 (44.3)
	Low	71 (19.3)
One-parent family; *n (%)*		17 (4.6)
Age of the parents; *mean (SD)*	Mother	36.7 (4.1)
	Father	39.2 (5.4)

Materials

Individual variables

CU traits outcome. The Inventory of Callous-Unemotional Traits (ICU)[26] was answered by teachers when the children were 3 and 5 years old. The ICU is a 24-item and 4-point Likert scale questionnaire that assesses CU traits, and the total score was used. In our sample, Cronbach's alpha for the total score at both 3 years old and 5 years old was .90.

Genotype. Genomic DNA was extracted from children's buccal mucosa on a cotton swab using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain). The Polymerase Chain Reaction (PCR) was carried out using 1 μl of DNA and 14 μl of mix. The cycling parameters of the PCR were as follows: an initialization step at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C, annealing at 66 °C for 1 min, extension at 72 °C for 1 min and a final elongation at 72 °C for 15 min. The primers used were *MAOA*-Forward: 5′-ACA GCC TGA CCG TGG AGA AG-3′ (marked with fluorochrome HEX) and *MAOA*-Reverse: 5′-GAA CGG ACG CTC CAT TCG GA-3′. 1 μl of the resulting amplified DNA was mixed with 10 μl of HI-DI formamide and 0.4 μl of ROX and kept at 95 °C for 5 min before being put in the freezer for 1 min. The uVNTR polymorphism of the *MAOA* gene was genotyped using GeneMapper® Software v4.1. The genotyping success rate was 92.5% ($N = 368$), leaving 33 individuals with an undetermined uVNTR polymorphism. Ten per cent of the individuals were randomly selected for re-genotyping to confirm the validity and accuracy of the method. This re-testing showed 100% reproducibility. Regarding the Hardy-Weinberg equilibrium, the genotype of the MAOA activity for women in the sample (n = 177) was in equilibrium ($\chi^2 = 0.097$, $p = .95$). There was no need to test the equilibrium for males since their genotype distribution is the same as their allelic distribution (they only have one copy of the *MAOA* gene).

In line with previous studies, the *MAOA* genotypes were grouped according to their functionality.[19,27] The low-activity *MAOA* genotype includes individuals with the 3-repeat allele, whereas the high-activity *MAOA* genotype includes participants with 3.5, 4 or 5 repeats.

Environmental variables

The environmental variables were measured when the children were 3 years old.

Parenting Practices. The Alabama Parenting Questionnaire Preschool Revision (APQ-Pr)[28,29] consists of 24-items on a 5-point Likert scale which measures three dimensions of parenting: positive parenting, inconsistent parenting and punitive parenting. Positive parenting (12 items) and punitive parenting (5 items) scores were answered by parents when the children were 3 years old (5.8% father, 48.5% mother, 44.7% both), were taken into consideration. The positive parenting subscale measures how frequently the parent interacts in games and shared time and how often they use positive reinforcement to foster appropriate behavior. The punitive parenting subscale measures how often the

Table 2 Zero-order correlations between ICU scores at ages 3 and 5, parental styles, SES and SDQ-conduct problems at age 3, separately by sex.

	1.	2.	3.	4.	5.	6.
1. ICU at age 3		.35*	.12	.18*	.07	.54*
2. ICU at age 5	.34*		.07	.03	.08	.13
3. APQ-Pr Punitive parenting	.09	.04		−.14	−.05	.13
4. APQ-Pr Positive parenting	−.15*	.06	−.21*		−.13	.13
5. Socioeconomic status	.04	−.03	.08	.04		.01
6. SDQ-conduct problems	.57*	.13	.21*	−.17*	.03	

Above diagonal correlations for boys. Below diagonal correlations for girls. *p < .05.

parent spanks, slaps or yells at their children to punish inappropriate behavior.[30] The internal consistency in our sample showed an acceptable value for positive parenting (Cronbach's alpha = .75) but a low value for punitive parenting (alpha = .42). As both scales had few items (6 for positive parenting and 3 for corporal punishment) and most of them showed skewed distributions, inter-item mean correlation was also calculated, resulting in acceptable values of $r = .31$ for positive parenting and $r = .25$ for punitive parenting.

The Strengths and Difficulties Questionnaire (*SDQ*)[25] is a 25-item screening questionnaire for child behavior and emotional problems. Teachers answered the questionnaire when the children were 3 years old and the conduct problems subscale (Ordinal alpha = .85) was introduced as an adjusting term in linear regression models.

Procedure

The study was approved by the ethics review committee of the author's institution. Schools were informed and the participating parents had to provide written consent. The families who met the inclusion criteria and were willing to participate were contacted by telephone and interviewed at the school. The questionnaires were administered at the end of the course to guarantee that teachers knew the children they were evaluating well.

Statistical analysis

The data was analyzed using STATA 16.0 for Windows. The Type I error was fixed at .05. To compare means of APQ-Pr between genotypes, Student's t-tests were calculated for boys, while analysis of variance with post-hoc comparisons and Bonferroni correction for multiple comparisons was estimated for girls.

The G × E analyses were conducted using separate multiple linear regressions for each sex with the dependent variable being ICU scores at 3 and 5 years old (4 regression models in total). The terms entered in each model as independent variables were *MAOA* alleles, APQ-Pr Positive and APQ-Pr Punitive measured at age 3, and the first-order interactions terms between *MAOA* genotypes and the two environmental characteristics. Non-significant interactions were removed from the model and in that case main effects coefficients were reported. Conversely, in the presence of significant interaction, simple effects of each environmental variable were calculated separately for each genotype, while differences between genotypes were calculated for the mean of the two quantitative environmental variables. The SDQ conduct problems scale and socioeconomic status (SES) were included in all models as adjusting terms at baseline (age 3). The two measures of parenting style were retained in the model although its interactions had been deleted. Additionally, the ICU score at age 3 was included as a covariable in models predicting ICU score at age 5.

Normality of the dependent variable (ICU total score) was verified separately for boys and girls at age 3 and 5 using two graphical inspection techniques, boxplot and standardized normal probability plot. Inspection of the boxplot also confirmed normality of residuals for each regression model estimated.

Results

Table 2 shows the zero-order Pearson correlations between CU traits at ages 3 and 5, parenting, SES and conduct problems, separately for boys and girls. The highest positive associations were between CU traits and conduct problems at age 3, and between the two CU traits measures. There were some relevant differences among sexes. The relationship between CU traits at age 3 and positive parenting was direct for boys, but inverse for girls. Also, the association between punitive parenting and conduct problems was stronger for girls than for boys.

Allelic and genotypic frequencies and distribution of environmental factors

The H allele was present in 69.1% ($n = 132$) of the boys and the L in 30.9% ($n = 59$). The HH genotype was present in 48.6% ($n = 86$) of the girls, HL in 42.9% ($n = 76$) and LL in 8.5% ($n = 15$). Table 3 shows that there were no statistically significant differences in the two environmental scores considered in relation to the genotypes for either boys or girls.

G × E interactions on CU traits

Table 4 shows the results of the linear regressions modelling CU traits at ages 3 and 5 from *MAOA*, APQ-Pr Positive and APQ-Pr Punitive (measured when the children were 3 years old) and its interaction separately for boys and girls. No statistically significant effect was found on CU traits at age 3.

Table 3 Distribution of environmental factors at age 3 by sex and genotype.

	Boys (*n*=191)			Girls (*n* = 177)					
	H (*n* = 132)	L (*n* = 59)	*p* (H vs L)	HH (*n* = 86)	HL (*n* = 76)	LL (*n* = 15)	*p* (HH vs HL)	*p* (HH vs LL)	*p* (HL vs LL)
APQ-Pr Parenting practices									
Punitive; *mean (SD)*	3.73 (1.94)	3.63 (1.87)	.740	3.89 (1.96)	3.46 (1.64)	3.07 (1.53)	.384	.305	1
Positive; *mean (SD)*	41.06 (3.91)	40.46 (4.11)	.338	41.69 (3.80)	40.17 (3.80)	39.87 (6.08)	.054	.323	1

Table 4 MAOAx Parenting (at age 3) results on ICU scores for boys and girls at ages 3 and 5.

	Boys				*Girls*		
Response: ICU at age 3	*B*	*p*	95% *CI (B)*		*B*	*p*	95% *CI (B)*
APQ-Pr Punitive × *MAOA*		.816				.576	
APQ-Pr Positive × *MAOA*		.250				.092	
APQ-Pr Punitive parenting	0.35	.349	−0.39; 1.10		0.02	.948	−0.67; 0.72
APQ-Pr Positive parenting	0.26	.123	−0.07; 0.60		−0.07	.576	−0.33; 0.19
MAOA: L/LL vs. H/HH	−1.31	.373	−4.21; 1.59		3.16	.059	−0.12; 6.45
HL vs. HH	-	-	-		−0.01	.996	−2.61; 2.60
Response: ICU at age 5	***B***	***p***	95% ***CI (B)***		***B***	***p***	95% ***CI (B)***
APQ-Pr Punitive × *MAOA*		.906				**<.001**	
APQ-Pr Positive × *MAOA*		.126				**.002**	
APQ-Pr Punitive parenting	0.14	.697	−0.56; 0.84	for HH	0.65	.283	−0.54; 1.84
				for HL	−0.49	.453	−1.77; 0.79
				for LL	**4.17**	**<.001**	**2.26; 6.08**
APQ-Pr Positive parenting	−0.03	.872	−0.44; 0.37	for HH	0.02	.954	−0.55; 0.58
				for HL	0.06	.846	−0.51; 0.63
				for LL	**1.20**	**<.001**	**0.71; 1.68**
MAOA: L/LL vs. H/HH	−0.07	.969	−3.35; 3.21		8.05	<.001	3.82; 12.28
HL vs. HH	-	-	-		0.67	.652	−2.27; 3.61

Boys are hemizygous H or L; All regression coefficients are adjusted by SDQ conduct problems scale and SES, additionally models at age 5 are adjusted by ICU score at age 3; the B column shows main effect in absence of significant interaction, and simple effects when interaction is significant.
Bold values signifies the values are significant.

The prediction of CU traits at age 5 shows non-significant parenting effects (measured at age 3) for boys, but significant differences for girls. In the analysis of CU for girls at age 5, there was evidence of an interaction between punitive parenting (APQ-Pr Punitive) and *MAOA* gene ($p < .001$). Higher levels of punitive parenting at age 3 were associated with higher levels of CU traits only in the LL genotype subgroup ($p < .001$) (Fig. 1A). An interaction with positive parenting (APQ-Pr Positive) at age 3 was also detected when predicting CU at age 5 in the group of girls ($p = .002$) (Fig. 1B). Increased scores in APQ-Pr Positive parenting (age 3) lead to significantly higher CU scores at age 5 only for the LL genotype ($p < .001$). The effect of increasing punitive parenting at age 3 on CU traits at age 5 for LL genotype ($B = 4.17$) was larger than the effect of increasing positive parenting ($B = 1.20$).

Discussion

We analyzed the G × E interaction hypothesis that *MAOA* polymorphism moderates the impact of parenting practices at age 3 on the risk of CU traits in male and female preschoolers at two different stages (ages 3 and 5). Contrary to our hypothesis, we found that the effect of early parenting practices on CU traits is not moderated by *MAOA* alleles for boys, but it is for girls at age 5. In line with our hypothesis on age specific G × E interaction in later stages, we found a salient G × E interaction only among girls at age 5. This is in line with previous research that found that age and sex function as moderating factors of CU traits on parenting practices.[6] In our study, we moreover include genetic vulnerability in the etiology model, suggesting that girls who carry the *MAOA*-LL genotype show higher CU traits at age 5

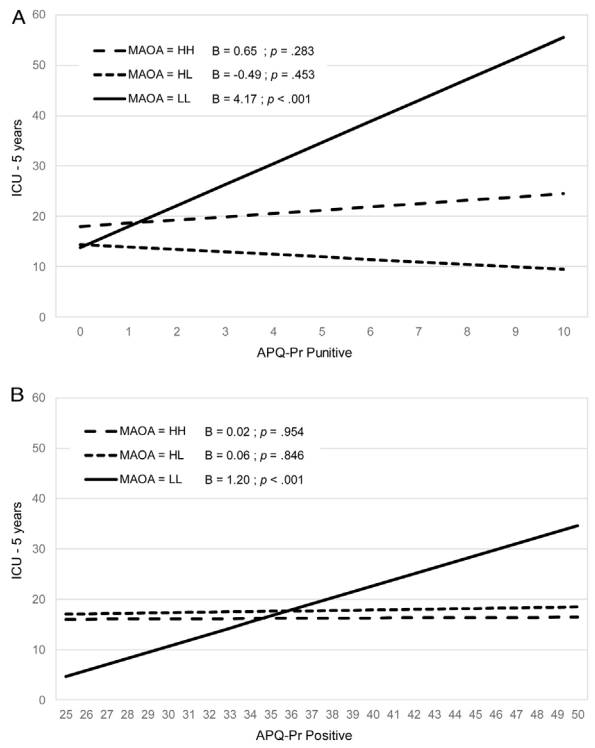

Figure 1 Regression lines for effect of APQ-Pr Punitive (A) and APQ-Pr Positive (B) at age 3 on ICU-5 years for girls depending on MAOA.
A. APQ-Pr Punitive.
B. APQ-Pr Positive.

when exposed to punitive and positive parenting at age 3. This suggests that the *MAOA*-LL genotype for girls is associated with higher sensitivity to both positive and punitive parenting in girls, so that *MAOA*-LL and not the hypothesized *MAOA*-HH allele, emerges as a genotype that confers higher vulnerability to environmental influences. Interestingly, the effect of punitive parenting on CU trait development among female *MAOA*-LL carriers was three times higher than the effect of positive parenting.

All in all, we might fail to replicate the expected G × E interactions because the studies on which we have built our hypothesis were conducted mostly in children who experienced severe early childhood experiences.[20] As the cited *MAOA* x Early adversity interactions might be of a specific nature, it is possible that *MAOA* acts as a moderating factor only when children experience extreme forms of maltreatment or trauma,[31] but not when they face less punitive environments such as parenting practices. Moreover, most *MAOA* x Early adversity interaction studies focused on antisocial behavior as an outcome, and only Fowler et al.[17] specifically addressed CU traits in their MAOA x Early adversity interaction study. Therefore, our novel and counterintuitive findings could be explained by the fact that antisocial behavior and CU traits are different constructs that may have distinct underlying G × E interactions.[32]

The sex specific G × E interaction in our study could be explained by sex differences in heritability of CU traits. Boys seem to be under greater genetic influences than girls, whereas for girls the influence of environmental factors is higher.[33] At the same time, the individual differences on vulnerability towards certain environments might be moderated by gender.[34] Thus, we studied *MAOA*, which is an X-linked gene and operates differently in males and females.[35] Females can be heterozygous and might undergo an X-inactivation of one of the alleles and show allelic expression of only one of the two alleles.[36] Therefore, understanding the effects of *MAOA* is complicated as it is unclear if one allele is inactivated or not, which leads to sex differences in *MAOA* product.[36] Moreover, G × E interactions might be under the differential effect on gender through the impact of different hormones in males and females, such as testosterone.[32] Finally, the *MAOA* promoter region also revealed to be affected by an epigenetic mechanism that involves a chemical modification to the DNA which is called DNA methylation.[37] This mechanism can modify gene expression and is considered a risk for mental disorders.[38] As studies on specific *MAOA* promoter methylation have identified higher methylation in females, especially among those with the low activity genotype,[39,40] we cannot exclude the possibility that DNA methylation influenced our findings.

Although findings are inconsistent, most studies support *MAOA*-HH as the risk allele for females on antisocial behavior.[15] Nonetheless, we report a *MAOA*-LL genotype-specific role on the increase in CU traits in 5-years old girls in interaction with early parenting practices. Our results would be in line with the alternative stream of studies that have identified *MAOA*-LL as the risk allele for antisocial behavior among females.[41,42] As this is the first study to include females in a *MAOA* x Environment interaction study on CU traits, our findings should be interpreted with caution and should be replicated to clarify which alleles might confer vulnerability towards environment in the development of CU traits among girls.

In our study, the *MAOA*-LL x Punitive parenting interaction on girls increased CU traits at age 5, which would be consistent with previous research that associates harsh parenting with CU traits.[2] When parents engage in physical and verbal abuse, communicate poorly and distance themselves from their children, this directly influences the child's ability to understand and interpret emotions and social situations.[22] Thus, when children experience harsh parenting or low parental warmth, they might react with negativity or aggression towards their parents. In turn, children with CU traits also seem to elicit more punitive parenting practices from their parents, resulting in a bidirectional influence between harsh parenting practices and child CU traits.[43] At the same time, not all children are equally sensitive towards parenting practices, so that identifying a subgroup of children (in our case, girls at age 5 who carry *MAOA*-LL alleles) can help explain the biological vulnerability towards environmental factors and its effect on CU traits.[10] Thus, our study has showed that girls who experience punitive parenting and carry the *MAOA*-LL allele increment almost three times more their CU traits than those who are exposed to positive parenting. This suggests that early harsh parenting behaviors have a deeper impact on girl's CU traits than positive parentings. In line with developmental child psychopathology theories, early harsh parenting has severe long-term effects on the biological and psychophysiological reactivity of children and impacts their stress response systems.[44] Children that have experienced punitive or coercive parenting practices might show higher levels of arousal and anxiety,

as well as altered reward-processing and fear-processing systems.[44,45] As such, punitive parenting also predicts CU traits from early childhood on, but positive parenting, in contrast, seems to have a more deteriorating effect on CU traits.[22] Interestingly, the *MAOA*-LL x Positive parenting interaction predicts higher CU traits among girls at age 5. Even though positive parenting is generally considered to prevent and reduce the risk of CU trait development among preschoolers and children,[45] studies have also shown that among preschoolers, positive parenting strategies can predict CU traits.[6] It might be that parents of children with CU traits engage in more positive parenting practices such as parental warmth or giving rewards to respond to their challenging children's CU behavior.

Moreover, girls might be under a greater parental influence, as they are generally more closely monitored by their parents.[46] Daughters are also treated with more reasoning and dialogue, whereas sons experience more authoritarian parenting practices[47] and parents might be more prone to respond at daughter's behavior with positive parenting strategies. Thus, early CU trait behavior of girls might elicit both punitive and positive parenting practices at age 3, which, at the same time, are moderated by *MAOA*-LL on CU traits at age 5.

Also, parents that have to take care of children with CU traits show higher parental inconsistencies and change their parental strategies over time.[6] Applied to our study, this would suggest that parents might initially have started with more positive reinforcement and parental warmth at age 3 to counter their daughter's emerging CU behavior,[43] but, at the same time, they also adopted more punitive practices. This would explain why girls at age 5 carrying *MAOA*-LL alleles showed higher CU traits when exposed to both positive and negative parenting at age 3. In that sense, our findings could then be an indicator of the dynamic nature of parenting practices and its reciprocal effect on CU traits, moderated by genetic vulnerabilities.

Finally, the lack of interaction at age 3 across sex might be explained by the fact that early childhood onset CU traits are under a higher genetic influence than later developed CU traits.[3] This builds on current research that analyzes genetic effect changes over time and according to different CU developing paths, in which early emerging CU traits show higher heritability. This is in line with our findings, because CU traits at age 3 (early onset CU traits) showed no G × E interaction, while CU traits at age 5 among girls (later-on CU traits) can be predicted by a G × E interaction. In our sample, it seems that it is later in development (at age 5) when the interplay of gender, genetic vulnerability and environment on CU traits becomes salient.

This study has several key strengths. It includes a prospective G × E design, with repeated assessment of CU traits. As early childhood is a period when children are very sensitive to emotion regulation, our study permitted testing whether early life environments (parenting practices) at different times were associated with the development of CU traits. Moreover, while most of the G × E research on CU has focused on high-risk children who have experienced maltreatment, our study used data from a community sample of preschoolers to study how *MAOA* interacts in less aversive environments on CU trait development. Another strength of this study is the use of the SDQ conduct problems scale as a control covariate in our regression models. CU traits are often comorbid with antisocial behavior or CD, so further isolation of the possible effect of CD provides more accurate *MAOA* x E interaction models of CU traits. Also, the socioeconomic status of the families was used as a control covariate. Finally, this study included positive and negative environmental factors in the G × E interaction models because most of the *MAOA* x E interaction studies on CU traits to date have studied genetic effects on exposure to strongly aversive environmental factors such as negative life events or harsh parenting styles.[17,48] Hence, studying positive and less aversive contexts helps to clarify how these G × E interactions work in both environments.

Nevertheless, the present study also has some notable limitations that should be considered. First, while our results are in line with previous studies focused on the *MAOA* gene,[20,49] we are aware of the methodological and statistical concerns that gene by environment (G × E) interaction studies have raised, such as small effect sizes and limited statistical power.[50] In this regard, although our total sample size (n = 368) is larger than other studies on G × E interaction on CU traits,[9] it might not have enough power to estimate small effects. Second, we cannot rule out the possibility that G-E correlations (rGE) might explain our findings,[20] so that our G × E interaction on girls might be mediated by passive rGE (parents transmit to their daughters a genetic susceptibility towards CU traits) or evocative rGE (girls with a certain genotype may show CU traits that traits elicit punitive parenting). Future research should test for the presence of rGE in the MAOAx Parenting interactions on CU traits. Third, our G × E design has focused only on one specific environmental factor (parenting), while there are other environmental factors that might be influencing CU traits.[4] Therefore, Gard et al. suggest[51] that further studies should address more complex relationships between multiple environment (G × E × E) or multiple genes (G × G × E). Thus, the authors highlight novel and more sophisticated molecular genetic approaches such as neurogenetics which provide promising results to find polygenic risk scores, instead of focusing on a single candidate gene as the present study does. Also Imaging G × E interaction studies are of interest in gaining a deeper understanding of how neural alterations mediate the effects of G × E interactions to psychopathology.[51] Fourth, there is a small number of girls in the LL genotype due to the usual genotypic distribution. Fifth, parenting practices were measured using self-reports which may be under the effect of distortions such as social desirability or individual interpretation of the items. Sixth, the study focuses on a short period of time (ages 3 and 5), so it is unclear which G × E interactions could be present in later childhood development, as the effect of environmental factors may vary according to the timing of the experiences.[32]

All in all, the results indicate that the influence of the *MAOA* x Parenting on CU traits is sex- and age-specific. If replicated, our study suggests that early parenting experiences at age 3 might have long-term effects, resulting in a sex-specific G × E interaction for girls in later phases of development. Understanding how genes might interact with parenting practices in early childhood is crucial in preventing early CU symptoms from developing into more severe forms of conduct disorder or antisocial behavior,[45] as parenting strategies are among the most salient risk fac-

tors. Thus, treatment models of CU traits often focuses on cognitive-behavior strategies and parent-child interventions, with treatment outcomes that are generally poor or limited.[2] Underlying G × E mechanisms might contribute to explain why some children show a worse treating response than others, so that children carrying certain risk alleles might be more sensitive than others towards parenting strategies.[7] These G × E mechanisms of CU traits development might need to be considered in the process of designing effective treatment interventions. For example, interventions on parent-child interactions in clinical settings should take into consideration possible sex-differences and specifically address parent-girl relationships when CU behaviors appear already in early childhood.

As G × E interactions might have cascading influences on development and stability of CU traits in older children,[3] longitudinal approaches need further exploration in order to analyze the underlying mechanisms that could shape different developing pathways to CU traits. Thus, our approach might help to identify a group of children who are more vulnerable to their environment in a certain developmental period, providing insight on individual differences in the development of CU traits.

Ethical considerations

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Funding

This work was supported by the Spanish Ministry of Science, Innovation and Universities [Grant PGC2018-095239-B-I00 (MICIU/FEDER)]. We also acknowledge the support of the Comissionat per a Universitats i Recerca, Departament d'Innovació, Universitats i Empresa, Generalitat de Catalunya (2017SGR1577, 2017SGR1271 and 2017SGR33). M Fatjó-Vilas thanks to the Instituto de Salud Carlos III, Spain, for her Sara Borrell contract (CD16/00264).

Conflict of interest

The authors have no conflict of interest to declare.

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