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Association of *OXTR* rs53576 with the Developmental Trajectories of Callous-Unemotional

Traits and Stressful Life Events in 3- to 9-year-old Community Children

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**Abstract**

The objective was to obtain developmental trajectories combining callous-unemotional traits and the number of stressful life-events between ages 3 and 9 years and to ascertain their association with the polymorphism rs53576 at the Oxytocin Receptor gene (*OXTR*). A total of 377 children were assessed yearly from ages 3 to 9 years. Latent class growth analysis for parallel processes was used to identify distinct trajectories for callous-unemotional traits (assessed using the *Inventory of Callous-Unemotional Traits*, ICU) and number of stressful life-events, and then the influence of being an A allele carrier on class membership was included with *OXTR* genotypes as a binary time-invariant predictor, following a 3-step approach. A 3-class model showed the highest entropy (.859) and adequate posterior probabilities of class membership ($\geq$ .884). Class 1 ($n = 226$, 59.9%) included children with low and stable ICU scores and low and descending stressful life-events; class 2 ($n = 127$, 33.7%) included children with high and ascending ICU scores and low and slightly descending stressful life-events; and class 3 ($n = 24$, 6.4%) included children with persistently high profiles both for ICU scores and stressful life-events. Carrying an A allele (genotypes GA/AA) increased the odds of pertaining to class 3 (high and persistent ICU scores and stressful life-events) as opposed to class 2 (OR = 4.27, $p = .034$) or class 1 (OR = 3.81, $p = .042$). The results suggest the importance of considering callous-unemotional traits and stressful life-events in conjunction. In addition, the genetic variability of *OXTR* (rs53576) may help to understand individual differences in early development.

**Keywords:** Callous-Unemotional, Developmental trajectories, Life events, *OXTR,* rs53576

**Association of *OXTR* rs53576 with the Developmental Trajectories of Callous-Unemotional Traits and Stressful Life Events in 3- to 9-year-old Community Children**

Callous-unemotional traits (CU) distinguish a group of children with severely disordered conduct who display a specific affective and interpersonal style characterized by lack of empathy, lack of guilt and constricted emotional expression (Frick, Ray, Thornton, & Kahn, 2014). These characteristics are indicative of difficulties with social cognition and responding to interpersonal cues.

CU traits correspond to the affective dimension of psychopathy and are developmental precursors of adult psychopathy (Patrick, 2010). DSM-5 (American Psychiatric Association, 2013) has included CU traits as a specifier in conduct disorder (conduct disorder with limited prosocial emotions), which identifies a more severe diagnostic picture through development. Similarly, ICD-11 (World Health Organization, 2018) includes this qualifier for oppositional defiant disorder (Evans et al., 2017). CU traits show significant heritability: between 42% and 68% of the variation of CU traits is accounted for by genetic effects (Frick et al., 2014). The traits are moderately stable from preschool to adolescent ages (Ezpeleta, Granero, de la Osa, & Domènech, 2015; Pardini & Loeber, 2008), but significant within-individual variability has also been reported. Fontaine, Rijsdijk, McCrory, and Viding (2010) identified four trajectories of CU from ages 7 to 12 in a sample of 9,462 twins: stable high (3.4%), increasing (9.6%), decreasing (16.9%) and stable low (70.2%). Similarly, in a sample of 503 at-risk boys aged 7 to 15 Byrd, Hawes, Loeber, and Pardini (2018) found five trajectories of CU: early-onset chronic (10.3%), childhood-limited (10.1%), adolescent-onset (11.8%), moderate (17.4%) and low (50.4%). These studies have reported the percentages of children chronically affected by CU traits (between 3 and 10%), showing that these traits present distinct developmental pathways. In Byrd's study, for example, half the boys who initially manifested high levels of

CU maintained these high levels chronically, whereas the others' scores decreased; however, a contrary increasing pathway was also observed and a proportion of the boys with low scores initially became as high as early-onset-chronic through development to adolescence. Therefore, because these traits have the potential for change, their developmental heterogeneity must be taken into account.

**Callous-unemotional traits and stressful life events**

Stressful negative life events refer to a range of life experiences or events that may result in changes in the life of the individuals that necessitate varying degrees of coping and adaptation, and that are susceptible of leading to problems in psychological adjustment (Johnson, 1982). There is a positive association between CU traits and negative life events (Kimonis, Centifanti, Allen, & Frick, 2014; Sharf, Kimonis, & Howard, 2014). It has been proposed that negative life events may have a causal effect on CU traits. In this line, it is postulated that the experience of negative life events and psychosocial adversity may interfere with emotional development, facilitating the expression of CU traits (Ford, Chapman, Mack, & Pearson, 2006; Kerig, Bennett, Thompson, & Becker, 2012; Porter, 1996). The impact of stress on emotional circuitry is also known. Early life stress has been associated with disrupted functional connectivity between the amygdala and medial prefrontal cortex (mPFC), and in turn, weaker amygdala-mPFC has been related to higher levels of aggressive behavior and psychopathic traits (Moul, Killcross, & Dadds, 2012; Park et al., 2018).

On the other hand, the characteristics of CU, such as sensitivity to reward, insensitivity to punishment, impulsivity, disinhibited behavior, fearless and thrill-seeking, and lack of response to others' distress, may evoke more negative life events from the environment (Kimonis, Centifanti, et al., 2014).

The results of Kimonis, Centifanti, et al. (2014) support both possibilities in some cases, suggesting a reciprocal relationship between CU and life events. They found that when reported by children, stressful life events predicted higher CU scores three years apart and that this relationship was reciprocal: higher CU scores predicted higher stressful life events. The reciprocal association was not confirmed when the parents were the reporters. While the directionality of the relationships remains unknown, both variables must be considered simultaneously to fully capture their association.

**Oxytocin Receptor Gene (*OXTR*)**

Oxytocin (Oxt) is a neuropeptide involved in relevant social and affective processes, such as emotional learning and emotion recognition and their affective meaning, through the corticolimbic and amygdala circuits (Aghajani et al., 2018; Jones, Barrera, Brothers, Ring, & Wahlestedt, 2017). Some studies on healthy subjects have shown the positive correlation between endogenous plasma oxytocin levels and social performance (Parker et al., 2014), and the enhancing effect of intranasal oxytocin administration on social cognition (Domes, Steiner, Porges, & Heinrichs, 2013; Rimmele, Hediger, Heinrichs, & Klaver, 2009).

Oxytocin activity depends on an adequate interaction with its unique receptor, the oxytocin receptor (OxtR), which is widely distributed in the brain, including (but not restricted to) areas such as the ventromedial nucleus of the hypothalamus, the amygdala, the lateral septum, the bed nucleus of the stria terminalis, the anterior olfactory nucleus, the preoptic and ventral tegmental areas, and the hippocampus (Jurek & Neumann, 2018).

Evidence on the heritability of social cognitive skills in humans (Scourfield, Martin, Lewis, & McGuffin, 1999) and the Oxt-OxtR signaling role on behavioral response towards stress and social behavior (Jones et al., 2017; Neumann & Landgraf, 2012) have fueled the interest of understanding the role of genetic variability on sociobehavioral domains. In this

regard, the Oxytocin Receptor gene (*OXTR)* has captured much interest as a candidate gene. In general population based studies, one of the most analyzed single nucleotide polymorphisms (SNP) in *OXTR* is the rs53576 (G/A), which has been associated with the regulation of social behavior, trust, sensitive parenting, empathy, positive affective scores and stress response (Gong et al., 2017; Kumsta & Heinrichs, 2013).

When combining the results of multiple studies, meta-analyses have also shown that irrespective of sex, age and ethnicity, individuals with the genotype GG present higher sociality in comparison with A allele carriers (AA/AG), suggesting that this polymorphism influences how individuals tend to respond to other people (Gong et al., 2017; Li et al., 2015). In addition, disorders involving difficulties in social and affective relationships, such as autism, schizophrenia, social anxiety and depression, have also been related to this SNP (Dadds, Moul, Cauchi, Dobson-Stone, Hawes, Brennan, Urwin, et al., 2014; Li et al., 2015; LoParo & Waldman, 2015). However, like many reported genetic associations, not all of these findings have been successfully replicated (Bakermans-Kranenburg & van Ijzendoorn, 2014; Lucht et al., 2009; Wu, Li, & Su, 2012). Such differences have been linked to: i) methodological divergences across studies (sample sizes, or the specific outcomes evaluated), ii) the genetic background differences across populations, iii) the polygenic nature of the traits, and iv) the interaction among genes. This stresses the need of further investigating the role of this SNP on social and affective processes both in healthy and clinical populations. To this end, it must be noted that neuroimaging studies have provided further evidence to support the rs53576 association with brain structure and activity in healthy subjects, specifically in the limbic regions related to empathy, salience processing and mentalizing. In other words, some studies have suggested that the individual genetic make-up at *OXTR* can contribute the brain anatomical and functional differences that underlie cognitive performance variability. For instance, Tost et al. (2010) described a significant allele-load-dependent

decrease of gray matter volume in the oxytocinergic "core" of the brain, the hypothalamus, in rs53576 A allele carriers. At a functional level, they also reported that when processing social information, homozygotes for the A allele show the lowest amygdala activation and an increased coupling of the hypothalamus and the amygdala. In addition, these neural characteristics predicted the level of reward dependence, indicating that the observed impact of *OXTR* on the structure and function of hypothalamic-limbic circuits could be critical for emotion regulation and sociality in humans. In another study, the interaction between the rs53576 and childhood attachment has been shown to modulate brain structure and function also of areas implicated in salience processing and mentalizing (the amygdala, the bilateral temporal pole and the precunei, and the right middle and superior frontal gyri). In this case, rs53576 genotypes are suggested to confer differential susceptibility to childhood social experiences, which in turn would shape brain morphological and activity patterns (Schneider-Hassloff et al., 2016). These neuroimaging genetic studies are in line with the conception of the amygdala, along with its anatomical connections, as a central hub in the social brain network (Bickart, Dickerson, & Barrett, 2014). The amygdala receives information from the hypothalamus about the peripheral body states and it is critical for responses to emotional and stressful stimuli, including expression, regulation, memory and learning of emotional stimuli. Amygdala damage has been shown to affect recognition of negative emotions from faces and social judgements related to threat (Adolphs, Baron-Cohen, & Tranel, 2002). Also, reduced amygdala responsivity and connectivity with regulatory brain areas have been observed in individuals with high CU traits and/or psychopathy (Marsh et al., 2008; Shirtcliff et al., 2009).

Thus, despite rs53576 being a silent polymorphism and taking into account that the pathophysiological significance of its association with brain phenotypes remains to be elucidated, the above mentioned evidences suggest that this SNP could be a marker of the role

of the *OXTR* in the neural mechanism that links the differences in the oxytocinergic system to individual differences in emotional reactivity. This is also supported by the observed effect of other *OXTR* polymorphic variants in amygdala responses to salient social cues (Marusak et al., 2015; Westberg et al., 2016). Considering callous-unemotional traits as an extreme expression of interpersonal and emotional reactivity diversity, this neural mechanism is hypothesized to be also involved in the etiology of CU traits.

**Oxytocin and callous-unemotional traits**

Some findings suggest that abnormalities in the oxytocin neuroendocrinological system may be associated with CU traits (Rice & Derish, 2015) and that the effects appear to be mediated in part by the effect Oxt has on amygdala function (Kanat, Heinrichs, & Domes, 2014). In this regard, Moul et al. (2012) proposed that the cognitive and emotional deficits of psychopathic traits may be associated with an activation imbalance in subregions of the amygdala: the basolateral region (where valence, explicit fear recognition and fear potentiated startle are encoded) would be underactivated, while the central amygdala (where value and attentional shift are encoded ) would be overactivated. According to this model, the authors suggest that in psychopathic individuals low levels of oxytocin would result in greater activation of the central amygdala, leading to a decrease in prosocial behavior in response to social stimuli (Moul et al., 2012).

Few studies, however, have been carried out on child populations. On the one hand, there is evidence to suggest that low salivary oxytocin levels predict higher CU traits in adolescents with CD (Levy et al., 2017) and, on the other hand, there are studies that have found *OXTR* to be influential. Malik, Zai, Abu, Nowrouzi, and Beitchman (2012) studied three SNPs of the oxytocin gene (*OXT*) and five of *OXTR* (one of them rs53576) in a sample of 160 children aged 6-16 with aggressive behavior. They found that rs6770632 and

rs1042778 (but not rs53576) were associated with aggressive behavior, but there was no significant association with CU traits. In the same sample but with different SNPs (three of *OXT* and three of *OXTR* – rs53576 was not included), Beitchman et al. (2012) found that *OXTR* rs237885 AA genotype carriers scored higher on callousness than AC or CC genotype carriers. Dadds, Moul, Cauchi, Dobson-Stone, Hawes, Brennan, Urwin, et al. (2014) studied several polymorphisms of *OXTR* (including rs53576) in two samples of 4-16 year old children referred for behavior problems. They found that rs1042778 genotype TT was associated with higher levels of CU traits. These studies suggest the association of *OXT* and *OXTR* genes with a span of behavior problems related to aggressive and CU traits. The authors interpret that oxytocin facilitates facial recognition and affiliation and, therefore, when oxytocin diminishes the manifested behavior may be coherent with lack of emotions towards others and callousness.

Previous literature shows that rs53576 in children has always been studied in clinical populations and, contrary to the studies on adults (Gong et al., 2017; Kumsta & Heinrichs, 2013), has not been proven to be associated with CU traits. In addition, previous studies have involved small samples, different SNPs, and different populations and age ranges, so consequently they have presented discrepant results, highlighting the need to further explore the role played by *OXTR* and rs53576 on CU traits in healthy children.

**Oxytocin and stressful life events**

In addition to its role in affiliative behavior, oxytocin also has a function to attenuate stress response (anxiolytic effect) in interaction with the hypothalamo-pituitary-adrenal axis (Rodrigues, Saslow, Garcia, John, & Keltner, 2009). In response to stressors, oxytocin decreases cortisol and inhibits cardiovascular response, amygdalar activation and brainstem connectivity (Rice & Derish, 2015). Rodrigues et al. (2009) found that GG homozygous

university students for the rs53576 polymorphism were more empathic and less disposed to stress reactivity (lower heart rate). There are no studies on children with this specific SNP and stressful life events, but Bradley et al. (2011) found a significant interaction effect between rs53576 and childhood maltreatment in predicting emotional dysregulation in adulthood.

The characteristics of CU traits, such as their early presentation, their association with aggressive behavior and poor treatment outcomes (Hawes, Price, & Dadds, 2014), together with their association with stressful life events, suggest that this condition should be studied in combination with the environment in order to understand its biological underpinnings. We used a person-centered approach that groups individuals according to the characteristics of different features and focuses attention on the intra-individual structure of variables, with the advantage that the children are conceived as a whole rather than the sum of isolated features (von Eye & Bergman, 2003; West, Ryu, Kwok, & Cham, 2011). The profiles obtained with this methodology are well-suited for addressing questions concerning group differences in patterns of clinical profiles. The goal was to obtain developmental trajectories combining callous-unemotional traits and the number of stressful life events between ages 3 and 9 years and to ascertain their association with the rs53576 polymorphism in *OXTR*. In line with the results for adults in the literature, we expected to find several latent classes of combined stressful life events and CU traits, at least one of them clustering the most dysfunctional characteristics (higher stressful life events and higher CU scores). We hypothesized that carriers of A allele (AA/AG) of rs53576 would mostly pertain to classes with high callous-unemotional traits combined with a worse environment. This is the first study in young children from the general population to examine this association.

**Method**

**Participants**

The sample comes from a longitudinal study of behavioral problems starting at age 3 years described in Ezpeleta, de la Osa, and Doménech (2014). The initial sample consisted of 2,283 children randomly selected from early childhood schools in Barcelona (Spain). A two-phase design was employed. In the first phase of sampling, 1,341 families (58.7%) agreed to participate (33.6% high socioeconomic status (SES), 43.1% middle SES, and 23.3% low SES; 50.9% boys). To ensure the participation of children with possible behavioral problems, the parent-rated Strengths and Difficulties Questionnaire (SDQ) conduct problems scale (Goodman, 2001) plus four ODD DSM-IV-TR symptoms not included in the SDQ questions were used for screening. Two groups were potentially considered: the first screen-positive, which included all the children with SDQ scores $\geq$ 4, in percentile 90 or with a positive response for any of the 8 DSM-IV ODD symptoms ($N = 417$; 49.0% boys); and the second screen-negative, a random group comprising the 28% of children who did not reach the positive threshold ($N = 205$; 51.2% boys). Refusals in this phase ($n = 135$; 10.6%) did not differ in terms of sex ($p = .815$) or type of school (public or semi-public) ($p = .850$) from the children who agreed to participate (the only difference was in SES, with a higher participation ratio for high socioeconomic levels, 86.2% vs. 73.6%; $p = .007$).

The sample for the follow-up (the second phase of the sampling design) included 622 children (67.0% screen-positive; mean age: 3.77 years; $SD = 0.33$; 96.9% born in Spain) who were followed yearly from age 3 to 9 years (7 assessment points). For 401 of the children there was a biological sample available. No differences in SES [$\chi^2(2) = 0.28$, $p = .869$] or type of school [$\chi^2(1) = 0.07$, $p = .788$] were found when comparing children with and without DNA provided, but there were slightly more boys than girls who agreed to give a biological sample for genotyping [$\chi^2(1) = 6.16$, $p = .013$]. In addition, information was available from only three waves or less for 24 children (6.0%), from four waves for 22 children (5.5%), from five waves for 31 children (7.7%), from six waves for 59 children (14.7%) and from all seven

waves for 265 children (66.1%). We decided to exclude the children with data for less than

half the waves (3 or less waves) and, therefore, the sample used for this study consisted of

377 children [66.0% screen-positive; 201 (53.3%) boys; 35.4% high SES, 46.2% middle-high

or middle SES and 18.3% middle-low or low SES, according to Hollingshead's index

(Hollingshead, 1975); 4.1% living in a one-parent household at age 3 years], all Caucasian

with DNA provided and data from 4 or more of the 7 possible annual follow-ups between

ages 3 and 9 years, representing a 60.6% of the initial sample [59.9% from the positive

screening group and 62.1% from the negative screening group; $\chi^2(1) = 0.30$, $p = .584$]. The

mean age (and *SD*) at each follow-up point was as follows: 3.77 (0.34) for follow-up 1, 4.66

(0.35) for follow-up 2, 5.71 (0.36) for follow-up 3, 6.59 (0.35) for follow-up 4, 7.69 (0.37) for

follow-up 5, 8.64 (0.35) for follow-up 6, and 9.65 (0.35) for follow-up 7.

**Procedure**

The project was approved by the Ethics Committee on Animal and Human

Experimentation of Universitat Autònoma de Barcelona. Families were recruited at the

schools and gave written consent for the assessment and DNA extraction. All the families of

the 3-year-old children from participating schools were invited to answer the screening

questionnaire. The families who agreed and met the screening criteria were contacted by

telephone and interviewed at the school for each annual assessment. The interviewer team

was specifically trained and all interviewers were blind to the screening group. After

obtaining permission from the families, teachers completed the questionnaires.

**Instruments**

The *Inventory of Callous-Unemotional Traits* (ICU; Frick, 2004) includes 24 items

coded on a 4-point Likert-type scale (0: *not at all true* to 3: *definitely true*) structured in three

dimensions: Callousness, Uncaring and Unemotional. The total score, which is the sum of the

raw scores as reported annually by the teachers, was used to obtain the developmental

trajectories of CU traits. Cronbach's alpha for the total scores used ranged through follow-ups

from .88 to .93. Mean scores (and *SD*) at each follow-up were as follows: 20.45 (9.28) at age

3, 20.61 (9.67) at age 4, 19.81 (9.38) at age 5, 20.72 (9.91) at age 6, 20.49 (9.71) at age 7,

20.23 (11.23) at age 8, and 20.38 (10.35) at age 9. No statistically significant differences were

observed in ICU scores in any follow-up by SES or whether there were two vs. one parent

households [$p \geq .055$, after applying Finner's (1993) correction for multiple comparisons].

*Stressful life events* were registered through the *Schedule for Risk Factors* (Unitat

d'Epidemiologia i de Diagnòstic en Psicopatologia del Desenvolupament, 2009), an interview

that lists situations that can have a significant impact on the development of mental disorders.

The interview gathers the occurrence (*yes*/*no*) of 25 stressful life events based on the Life

Events Checklist (Johnson & McCutcheon, 1980) as reported by the mother or caregiver for

the previous year (for example, moving house or school, a new brother/sister, parents' fights,

separation/divorce, a new father/mother, a new member of the household, family illness,

death of a family member, child abuse, somebody hurting a family member, witnessing an

assault, experiencing a disaster/accident, the loss of a friend, parents changing jobs,

unemployment, economic problems, problems with the law, substance abuse). In each follow-

up, a life event was registered as present if the child was exposed to it one or more times

during the previous year (the year between assessments). The total count of stressful events at

each age was used for the analyses. The number of stressful life events ranged from 0 to 11

through the follow-ups, and the mean count of events (and *SD*) at each follow-up was as

follows: 2.5 (1.6) at age 3, 1.3 (1.3) at age 4, 1.2 (1.3) at age 5, 1.3 (1.3) at age 6, 1.2 (1.3) at

age 7, 1.3 (1.4) at age 8, and 1.1 (1.2) at age 9. No statistically significant differences were

observed in stressful life events in any follow-up by SES or whether there were two vs. one

parent households [$p \geq .055$, after applying Finner's (1993) correction for multiple

comparisons]. Only one large effect size based on Cohen's *d* was observed at age 3 years,

with children from a one parent household showing more stressful life events than those from

a two parent household (4.84 vs. 2.42, $d = 1.57$). In addition, correlation values between the

count of stressful life events and ICU scores were null or very low (ranging between $-.01$ at

age 9 years and .10 at age 4 years) (supplementary Table S1).

*Genotype*. Genomic DNA was extracted from the children's buccal mucosa on a

cotton swab using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain). Genotyping

of the intronic SNP rs53576 in the *OXTR* gene (3p25) was performed by means of a

fluorescence-based allelic discrimination procedure (Applied Biosystems Taqman 5'

exonuclease assay) under standard conditions. The genotyping call rate was 100%. After

randomly re-genotyping 10% of the sample, 100% of the genotyping results were

confirmed. The SNP was in Hardy-Weinberg equilibrium [$\chi^2 (2) = 1.09; p = .58$]. The

observed MAF (Minor Allele Frequency) in the present sample (30.4%) was in accordance

with the one in the CEU samples from the 1000 Genomes project (29%). The

rs53576 genotype was used as a binary variable grouping G/A and A/A (200, 53.1%) *vs.* G/G

(177, 46.9%) following the convention in most of the studies (Li et al., 2015). No statistically

significant differences were observed in genotype by SES [$\chi^2 (4) = 7.02, p = .135$] or whether

there were two vs. one parent households [$\chi^2 (2) = 1.58, p = .455$].

**Data Analysis**

Latent class growth analysis (LCGA) for parallel processes was used to identify

distinct groups of individual trajectories for ICU scores and number of stressful life events

jointly, and then the influence of being an A allele carrier on class membership was included

with *OXTR* genotypes as a binary time-invariant predictor. Since some differences on ICU

scores or stressful life events by SES or whether there were two vs. one parent households can

be considered as non-negligible ($p \geq .055$), the convenience of controlling for these

demographic factors was evaluated by comparing if the change in parameters for the crude

models (without covariates) with respect to the adjusted models was > 10% (Maldonado &

Greenland, 1993).

Given the multistage sampling procedure used, analyses with MPlus8 were weighted

by the inverse probability of selection in the second phase of sampling. The Robust Maximum

Likelihood (MLR) method of estimation was used, which enables the inclusion of non-normal

and incomplete data, using the expectation maximization algorithm for missing data with

robust standard errors (i.e., full information method). The growth models for ICU scores and

number of stressful life events considered intercept (I) and slope (S; i.e. linear trends) over the

seven yearly assessments from ages 3 to 9 years, with equal spacing between measurement

occasions. The time was rescaled from 3-9 to 0-6, so the first-year assessment (at age 3 years)

represented the intercept. The influence of rs53576 *OXTR* genotypes (allele A carriers coded

as 1 and non-allele carriers coded as 0) as predictor of between-class variation was

determined using the 3-step approach (R3STEP; AUXILIARY option in the VARIABLE

command) by computing multinomial logit regression coefficients and preserving the class

classification from the unconditional LCGA (i.e., class membership does not change with the

addition of covariates). For the multinomial regression part of the model, the need to include

sex as a potential adjusting variable was evaluated by comparing the OR parameters between

the adjusted model and the crude model (without covariates).

The models with one to five latent classes of growth patterns were compared. The

following criteria was used to determine the best model, plus the best clinical interpretability:

larger decrement in AIC, BIC and sample-size adjusted BIC (aBIC), greater power and more

accurate classification by average posterior probabilities, entropy values equal to or greater

than .70 and more than 20 participants in a class/trajectory. Pairwise mean differences across indicators (ICU scores and stressful life events) and the parameters of the selected LCGA model (intercept and slope for each growth process) at each age between classes were also tested through one-way ANOVA and the Scheffé procedure for post-hoc comparisons.

## Results

Table 1 (left) shows the genotypic distribution of the whole sample by sex. There were no significant differences by sex in the genotypic distribution [$\chi^2 (2) = 1.74$, $p = .420$].

Table 2 shows the goodness-of-fit indices for the LCGA models from one to five classes. Models were adjusted by SES, given that change in some parameter estimates due to the addition of this covariate with respect to the crude models was $> 10\%$. Despite the greater decrement in AIC, BIC and aBIC being found for the 2-class model in comparison with the 1-class model, the former showed differential trajectories for ICU scores, but not for stressful life events. The models with 4 or 5 classes showed some with a low number of participants ($n \leq 20$) and so they were discarded. Regarding the models with 3 or 4 classes, which could be interpreted in a similar way, the former with fewer classes was preferred given that it is more parsimonious. Also, based on the prioritization of clinical interpretability, we consequently selected the 3-class model (Figure 1a and 1b), which showed the highest entropy (.859) and adequate posterior probabilities of class membership ($\geq .884$).

Class 1 ($n = 226$, 59.9%; Figure 1c; 41.0% screen-positive group) included the children with low and stable profile for ICU scores (I = 16.8, $SE_I = 0.93$, $p < .001$; S = −0.33, $SE_s = 0.18$, $p = .069$) and low and descending stressful life events (I = 1.7, $SE_I = 0.12$, $p < .001$; S = −0.15, $SE_s = 0.03$, $p < .001$); estimated ICU means for class 1 corresponded to the 34th percentile of normative Spanish data (Unitat d'Epidemiologia i de Diagnòstic en Psicopatologia del Desenvolupament, 2018). Class 2 ($n = 127$, 33.7%; Figure 1d; 34.4%

screen-positive group) included children with high and ascending ICU scores (I = 24.7, $SE_I$ = 1.19, $p < .001$; S = 0.78, $SE_s$ = 0.27, $p = .004$), but with low and descending stressful life events (I = 1.7, $SE_I$ = 0.16, $p < .001$; S = −0.16, $SE_s$ = 0.03, $p < .001$). And class 3 ($n = 24$, 6.4%; Figure 1e; 54.2% screen-positive group) included children with persistently high profiles both for ICU scores (I = 26.8, $SE_I$ = 3.42, $p < .001$; S = 0.01, $SE_s$ = 0.67, $p = .994$) and stressful life events (I = 3.5, $SE_I$ = 0.47, $p < .001$; S = −0.06, $SE_s$ = 0.14, $p = .676$). Estimated ICU means for classes 2 and 3 corresponded to the 75th percentile of normative Spanish data. Besides, the Monte Carlo study with 1000 replications and sample size of 377 estimated that power for single parameter values ranged between .97 and 1.0.

The mean differences in ICU scores and stressful life events at each age were statistically significant among the three classes ($p < .001$). Post-hoc comparisons with the Scheffé procedure showed that the ICU scores for classes 2 and 3 did not differ ($p \geq .268$), with both higher than for class 1, and that the stressful life events for class 1 and 2 did not differ ($p \geq .847$), both being lower than for class 3. Therefore, class 1 differed from the others due to lower ICU scores ($d \geq 0.79$) and class 3 differed from the others due to higher stressful life events ($d \geq 0.91$). Moreover, intercept estimates for ICU scores and slope estimates both for ICU scores and stressful life events differed between the three classes ($p < .001$, $d \geq 2.25$); the only exception was that the intercept estimate for stressful life events did not differ between classes 1 and 2 ($M = 1.60$, 95% CI mean difference [−0.03, 0.02], $p = .915$, $d = 0.08$), but, as mentioned, the slope estimate did. Taken together, we consider there is support for three distinguishable classes (detailed statistics in supplementary Table S2).

Table 1 (right) shows the genotypic distribution by class. Regarding the effect of *OXTR* genotypes on class membership, an adjusted model including also sex as a covariate did not differ appreciably from the current model and so we present the results of the latter due to their greater parsimony. Being an allele A carrier (genotypes GA or AA) increased the

odds of pertaining to class 3 (high and persistent ICU scores and stressful life events) as compared to class 2 (high and ascending ICU scores and low and descending stressful life events; OR = 4.27, 95% CI [1.11, 16.37], *p* = .034) or class 1 (low and stable ICU scores and low and descending stressful life events; OR = 3.81, 95% CI [1.05, 13.87], *p* = .042).

## Discussion

This is the first study to investigate the joint trajectories of CU traits and stressful life events in young children and to explore the role of the rs53576 *OXTR* polymorphism on such trajectories. The results suggest that genetic variations of *OXTR* may help to understand individual differences in CU early in development. The odds of pertaining to the worst outcome class multiplied significantly for children carrying the A allele by between 3.8 and 4.3; that is, children with a combination of developmentally sustained high CU scores plus high sustained stressful life events compared with those who presented high or low CU but were exposed to fewer stressful life events. These findings are in line with the results of various previous studies that have shown that children homozygous for the G allele present better socio-affective behavior and less reactivity to stress from very early ages (Gong et al., 2017; Li et al., 2015; Rodrigues et al., 2009).

The different classes obtained show three different patterns in the joint evolution of CU traits and stressful life events from ages 3 to 9 years. Group 1 includes the children with a parallel evolution of stable ICU scores and a number of stressful life events both in the lowest range, which would represent the "normative" group (60% of the sample). Group 2 clusters children with sustained and increasing ICU scores above the cut-off score of 24, a point that can be used to identify children at risk for CU traits (Kimonis, Fanti, & Singh, 2014), but with low (and descending) levels of stressful life events. In other words, the CU traits of the children in this group and stressful events develop in a divergent way. Group 3 comprises a

smaller percentage of children with very stable and above the cut-off CU traits running parallel to a sustained high number of life events (from age 3 to 9 years these children were exposed to a mean of 3 stressful life events on average every year). In this community sample, the children were exposed to a mean number of stressful events of about 1.3. It is notable that for group 3 (high CU, high stressful life events) this mean increased to 3.5 and was sustained throughout development. In recent years, there has been increasing interest in describing the heterogeneity of behavior problems, identifying different subtypes and variants in the hope that this may help early detection and prevention, as well as facilitate research into etiological differences. Among this heterogeneity, primary and secondary psychopathy are described and identified in children (Ezpeleta, Granero, de la Osa, & Domènech, 2017; Fanti & Kimonis, 2017). Primary psychopathy refers to the presence of CU traits and secondary psychopathy refers to the presence of CU traits plus emotional dysregulation and anxiety, both showing similar heritability estimates (about .50) (Hicks et al., 2012). Thus, according to Yildirim and Derksen (2015), while primary psychopathy is characterized by an emotionally deficient temperament associated with a constitutional hyporesponsivity of the right-hemisphere fronto-amygdalar complex to socio-affective stimuli, secondary psychopathy might be explained by the interaction between 'genetic liabilities, hormonal imbalances and social experiences' (exposure to harsh and stressful life circumstances) 'that can alter the maturation of affective regulatory systems (vmPFC)' (p.32). However, this is not borne out by all studies. Lee, Salekin, and Iselin (2010), for example, did not find support for these subtypes in young male offenders. So, complexity and controversy exists. Although we are not measuring specifically the CU variants, our groups may in part be capturing these variants, while group 2 would be clustering CU traits that develop independently of environment (primary variant) and group 3 would be grouping CU traits that develop close to the environment (secondary variant).

If A allele differs significantly ($p \leq .042$) between class 3 and the other two classes, what is different in class 3 is mainly the level of stressful life events (which is high). This highlights the putative role of rs53576 polymorphism in the relationship between environment and CU traits. In other words, the higher frequency of A carriers in class 3 observed in our sample seems to suggest the association between this variant and a developmental trajectory with high CU traits and a high number of stressful life events. This finding, together with previous studies showing structural and functional changes in the brains of individuals carrying the A allele (Schneider-Hassloff et al., 2016; Tost et al., 2010), contribute plausible evidence that this polymorphism could account for part of the neurobiological variability underlying CU trait development when stressful life events are present. Such conditional effects are also observed in the study by Schneider-Hassloff et al. (2016), in which it is reported that rs53576 modulates the impact of childhood attachment security on mentalizing-associated neural activity. Alternatively, an evocative gene-environment correlation explanation, which posits that the child's behavior may shape the interpersonal environment (child elicits certain responses from the environment) (Rutter, 2006), would be feasible for children of class 3, in whom the rs53576 may be associated with greater CU behavior and also evokes stressful life events.

However, our approach does not allow for distinguishing the nature of the association, in other words whether A carriers are more prone to both higher CU traits and to being exposed to stressful life events, or whether the A variant increases sensitivity to stressful life events and so a greater impact of life events increases CU trait expression. Accordingly, more research is needed to clarify the underlying gene-environment interplay mechanisms, and also to improve knowledge about the biological roots of the observed role of the rs53576 polymorphism in brain and behavioural phenotypes. In this regard, although the impact of this

polymorphic variant on gene expression or protein function is not known, there is some evidence of interest that can be considered.

First, the effect of genetic variants on gene expression can be evaluated by means of bioinformatic tools such as HaploReg (Ward & Kellis, 2012). For example, despite their intronic position, the alleles (A/G) of rs53576 are predicted to be associated with different affinities for certain transcription factors (AP-2), which could account for certain differences in the gene expression. Similarly, and as described in the introduction, it must be remembered that other variants in the same gene can also help to explain the relationship between the genotype and phenotype variability, clearly highlighting the need for multimarker and epistatic approaches within and between oxytocin related genes. Second, there are other ways in which genomic variability can influence CU traits independently of (or in addition to) the polymorphic DNA sequence variability at/of *OXTR* (rs53576 or other SNPs). As is well established, epigenetic mechanisms are also related to gene transcription regulation. Epigenetic changes in *OXTR* have been associated with a decrease in circulating oxytocin and socio-affective difficulties. In this line, Dadds, Moul, Cauchi, Dobson-Stone, Hawes, Brennan, and Ebstein (2014) reported that adolescent males with conduct problems and high CU traits present higher methylation of the *OXTR* and that this, in turn, correlates with lower levels of oxytocin. Similarly, in youth with CD, Aghajani et al. (2018) reported an interaction between *OXTR* methylation and CU traits associated with brain systems related to processing socioaffective information. These evidences indicate that given that they may affect *OXTR* expression and, therefore, the OxtR density or distribution, genetic and epigenetic variability must be considered to understand the combined effect of environmental variables and CU traits on characterizing early developmental trajectories.

This study has several strengths. First, the SNP analyzed, rs53576, has not been previously associated with CU in children. Second, the design and age of the children,

followed up longitudinally for 7 consecutive years. Third, a 'healthy' community sample participated and the information was collected from several informants through different techniques. Obtaining trajectories that combine two relevant variables enables the phenomenon to be studied more comprehensively and means that the different degrees of severity of these interrelated variables can be empirically identified and the effect of the *OXTR* genotypes on the different combinations determined.

Some limitations should also be considered when interpreting the present results. Our study was carried out on a wide sample of the general population, where high levels of dysfunction or psychopathology are not expected to be found. Nonetheless, the models were able to capture medium-high levels of CU traits and it was shown that even mean levels of CU traits are associated with functional difficulties (Ezpeleta et al., 2015; Fontaine, Hanscombe, Berg, McCrory, & Viding, 2018; Haas, Becker, Epstein, & Frick, 2018). Another limitation is that the study is based on one polymorphism, which does not represent the whole variability gamut of *OXTR.* Also, as the effect of the analyzed polymorphism on gene expression and receptor physiology is unknown, we cannot exclude the possibility that the observed effects reflect the impact of other genetic variants. The polygenic nature of behaviour traits and the minor effect of the common genetic variants (SNPs) limit the power of our sample size, especially if we consider the subclassification of the sample in the three classes. Accordingly, there is a need to replicate the detected effects in larger independent samples including different ethnicities, and also to better screen the polymorphic variability along the *OXTR* gene and other oxytocin related genes.

In view of the fact that CU has serious consequences for the individual with CU traits in terms of antisocial behavior, their social context and poor treatment outcomes (Frick et al., 2014; Hawes et al., 2014), identifying specific environmental factors that may interact with genotypes has important preventive implications. Attempts must be made in all of the child's

developmental contexts (family, school, social environment) to reduce the number of negative stressful life events they are exposed to, as it has been seen that a sustained trajectory of stressful life events covaried with high CU traits. It has also been shown that children with CU respond better to positive reinforcement than to punishment (Hawes et al., 2014), so trying to reduce the number of stressful life events in their environments (removing stressful events from family life, school and peer relationships) may be a target for intervention and may result in greater reward. To this effect, there is a successful experience of an in-school preventive program, after which the 7- to 9-year-old children not only decreased their CU scores, but also experienced greater support from their friends, with parents reporting greater involvement with their children than the control group, which the authors interpret as resulting from the skills taught during the program (Kyranides, Fanti, Katsimicha, & Georgiou, 2018). The program was oriented to developing and enhancing interpersonal skills, such as awareness of own and other's emotions, self-control and emotion regulation, positive self-concept, improving social skills and peer relations, and problem solving and communication skills through cognitive-behavioral therapy. Some of the components included in many cognitive-behavioral interventions, for instance problem solving, may help children high in CU to cope with the different stressful life events they may experience. Currently, however, knowledge about the effects of oxytocin in this social area is weak and inconsistent (Bartz, Zaki, Bolger, & Ochsner, 2011) and further knowledge in the field of oxytocin and CU are needed.

In conclusion, the analysis of CU traits in combination with stressful life events contributes to identifying and better characterizing the developmental pathways of CU traits. Our data also suggest that these developmental trajectories may be better understood when genetic factors, such as *OXTR*, are also considered.

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Table 1
*Unweighted Genotypic Distribution in the Sample by Latent Class; N (%)*

|  | Whole sample | | | Class 1 | Class 2 | Class 3 |
|---|---|---|---|---|---|---|
|  | Females[*] | Males[*] | Total |  |  |  |
|  | (*n* = 176) | (*n* = 201) | (*N* = 377) | *n* = 230 | *n* = 119 | *n* = 28 |
| **GG** | 89 (50.6) | 88 (43.8) | 177 (46.9) | 110 (47.8) | 60 (50.4) | 7 (25.0) |
| **GA** | 73 (41.5) | 95 (47.3) | 168 (44.6) | 120 (52.2) | 59 (49.6) | 21 (75.0) |
| **AA** | 14 (8.0) | 18 (9.0) | 32 (8.5) |  |  |  |

*Sex comparison *p* = .420

Table 2.

*Fitting indices for one to five class LCGAs*

| N. classes | AIC | BIC | aBIC | Class: *N* (weighted) | Class: probability* | Entropy |
|---|---|---|---|---|---|---|
| 1 | 27642.955 | 27737.329 | 27661.183 | 1: 377 | - | - |
| 2 | 26545.362 | 26651.533 | 26565.869 | 1: 239 | 1: .945 | .781 |
|  |  |  |  | 2: 138 | 2: .911 |  |
| **3** | **26360.270** | **26486.102** | **26384.574** | **1: 226** | **1: .951** | **.859** |
|  |  |  |  | **2: 127** | **2: .925** |  |
|  |  |  |  | **3: 24** | **3: .884** |  |
| 4 | 26236.710 | 26382.203 | 26264.811 | 1: 146 | 1: .895 | .811 |
|  |  |  |  | 2: 45 | 2: .933 |  |
|  |  |  |  | 3: 20 | 3: .905 |  |
|  |  |  |  | 4: 166 | 4: .871 |  |
| 5 | 26172.025 | 26337.179 | 26203.923 | 1: 168 | 1: .851 | .824 |
|  |  |  |  | 2: 26 | 2: .865 |  |
|  |  |  |  | 3: 11 | 3: .953 |  |
|  |  |  |  | 4: 120 | 4: .911 |  |
|  |  |  |  | 5: 41 | 5: .941 |  |

Note. aBIC: Sample-Size Adjusted BIC.

*On-diagonal values for posterior probability of class membership. In bold: selected solution of LCGA
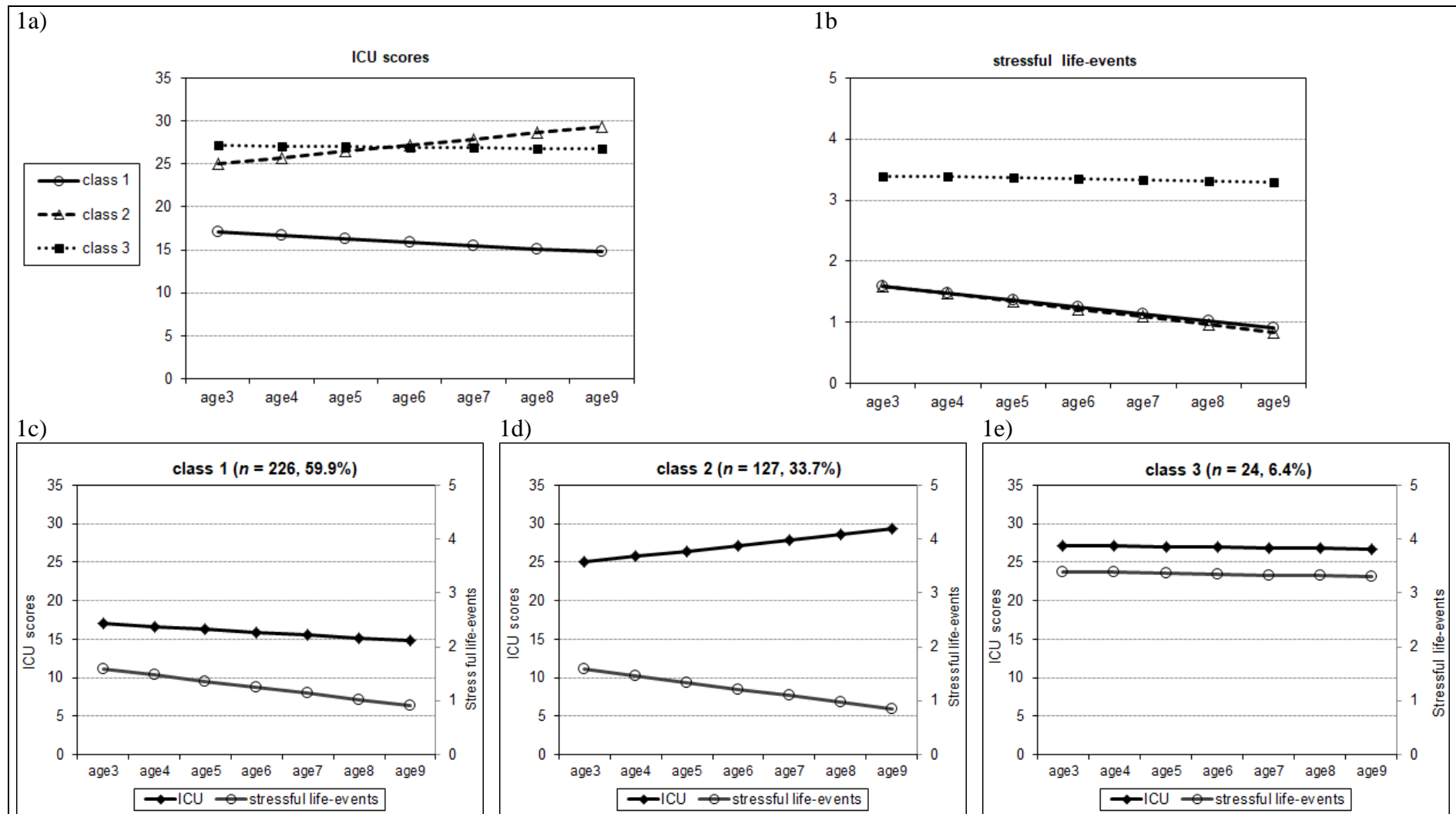
Figure 1. Trajectories for ICU scores and stressful life events by classes (*N* weighted). Above panel shows the three trajectories separately for each measure (ICU scores and stressful life events); below panel shows the trajectories of each resultant class combining both measures.