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ORIGINAL ARTICLE

Developmental Precursors of Primary and Secondary Callous-Unemotional Traits in Youth

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Abstract

There is growing evidence of diverse etiological pathways to the development of callous-unemotional (CU) traits, known as primary and secondary CU variants. The purpose of the present study was to extend previous cross-sectional research and examine theoretical predictors of CU variants prospectively from childhood to adolescence. Participants included high-risk control and normative samples from the Fast Track project ($N = 754$, male = 58%, Black = 46%). Using structural equation modelling, primary CU traits, identified in early adolescence, were associated with higher levels of childhood emotion regulation and lower levels of prosocial behavior. Secondary CU traits were associated with lower levels of childhood emotion regulation and low parental warmth, but not prosocial behaviour. Neither CU variant was related to harsh parenting. Parental warmth moderated emotion regulation and prosocial behavior on secondary CU traits. Results were not moderated by sex. A greater understanding of theoretical developmental precursors of CU variants may better guide intervention efforts.

Keywords Adolescence · Childhood · Callous-unemotional variants · Trauma

The presence of callous-unemotional (CU) traits [e.g., lack of empathy, uncaring; 1] in children and youth has been associated with severe and chronic aggressive and antisocial behavior, and an indifference or lack of responsiveness to others' emotions, particularly fear [2]. Although historically thought to represent a homogeneous and stable group marked by a biological predisposition [3], there is growing evidence of diverse etiological pathways to the development of CU traits, known as primary and secondary CU variants. Karpman's [4] original theory of psychopathy has been extended to CU traits. This theory stipulates that *primary* CU traits stem from a biologically based deficit in emotional processing, which results in low levels of anxiety and diminished sensitivity to others' cues [5]. In contrast, *secondary* CU traits are thought to be due to an affective deficit produced by pathogenic environmental factors. Typically differentiated from primary CU traits on anxiety [e.g., 6], the central premise of this view is that children who are exposed to negative environments or trauma, particularly in the context of relationships with caregivers, become emotionally detached by adopting a "mask" of callousness as a form of coping [7]. There have been a number of studies that have validated these two variants in youth and found some support for the proposed theory [for full review see 8]. However, most studies have been cross-sectional and have used justice-involved male samples [e.g., 9]. Thus, the purpose of the present study was to extend previous cross-sectional research and to examine theoretically relevant predictors of CU variants prospectively from childhood to early adolescence in a mixed-sex sample. Many studies examining

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CU traits include a measure of psychopathy and refer to the broader psychopathy literature; thus, while we do not equate CU traits with psychopathy, we refer to research using both constructs (i.e., CU traits and psychopathy).

There are important precursors that have been proposed for youth who develop primary and secondary CU traits; namely, the development and expression of emotion regulation and low prosocial behavior. Emotion regulation in this paper is defined as the ability for a child to modulate one's emotions, moods, and feelings [10]. Emotion regulation is an acquired process that emerges from both "intrinsic features and extrinsic socioemotional experiences within the context of early parent–child interactions" [11, p. 194]. Effective emotional regulation is associated with optimal physiological arousal [12]. Thus, very low arousal states (i.e., hypoarousal) would be associated with very high levels of emotion regulation, while high arousal states (i.e., hyperarousal) would be associated with low levels of emotion regulation. The normative processing of emotions, or emotion regulation, serves as a prerequisite for adaptive social and moral development [13], which directly influences the development of CU traits.

Children with primary CU traits are thought to be biologically hypoaroused to threat, and thus, they have higher levels of emotion regulation. For example, cross-sectional studies have shown that these youth demonstrate a fearless temperament, as well as impaired recognition of [1], and low psychophysiological reactivity to [14], others' distress cues [15]. Youth with primary CU traits also show lower levels of psychopathology that has been associated with low levels of emotion regulation (e.g., low levels of attention-deficit/hyperactivity disorder [ADHD] symptoms, depression, irritability), compared with secondary variants [8]. In contrast, children with secondary CU traits are conceptualized as being hyperaroused to environmental influences [16], which is thought to interfere with a child's ability to process socialization cues from caregivers, thereby impairing moral development [17]. Secondary CU traits may arise from the active attempt to suppress or avoid hyperarousal caused by negative parental interactions (e.g., harsh parenting), resulting in difficulty processing and regulating emotions [6]. Consistent with this view, children with secondary CU traits have been found to have higher levels of psychopathology [18], reactive aggression [19], and emotionality [20], compared to children with primary CU traits.

Currently, only three longitudinal studies have examined CU variants and indicators of emotion regulation from childhood to adolescence. One study found youth identified as having secondary CU traits at age 13 to have consistently higher rates of ADHD symptoms, emotional difficulties, and oppositionality from ages 7 to 13, compared to youth with primary CU traits or youth without CU traits [21]. Another longitudinal study found that youth identified as having secondary CU traits at age 3 had more psychopathology and higher levels of biological indicators of low emotion regulation at age 15, compared to their primary counterparts [22]. It should be noted that, although secondary variants scored higher than primary variants on these indicators, youth with secondary CU traits did not differ from youth in the low CU traits/high internalizing and externalizing symptom category. Finally, in the same sample as the current study, youth with secondary CU traits in early adolescence were found to have higher levels of psychopathology at age 25 compared to youth with primary CU traits [23]. These studies support the position that youth with secondary CU traits have higher rates of psychopathology and other indicators associated with low levels of emotion regulation.

In addition to emotion regulation, another important individual and clinically relevant difference between primary and secondary CU traits is the expression of early prosocial behavior. CU traits are considered analogous to *limited prosocial emotions*, a specifier used in the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition [DSM-5; 24] for the diagnosis of conduct disorder. Limited prosocial emotions is defined similarly to CU traits as a lack of remorse or guilt, a lack of empathy, shallow affect, and lack of concern about performance [24]. Related to prosocial emotions is prosocial behavior. Prosocial behavior consists of behavior that is intended to benefit others, such as cooperating, sharing, comforting, and helping [25]. CU traits have been found to be negatively correlated with prosocial behavior in elementary school children [26]. Based on Karpman's [4] theory, youth with primary CU traits may be expected to have low prosocial behavior earlier in development, as their etiology is strongly associated with emotional deficits. However, it is unclear from this theory whether secondary CU variants would show a similar early deficit. There is some evidence of low prosocial behavior in both primary and secondary CU variants. In a cross-sectional study, Zwaanswijk and colleagues [27] found that adolescents with both primary and secondary CU traits had lower prosocial behavior than youth with low CU traits. Similarly, in a large epidemiological sample, adolescents identified as having either primary or secondary CU traits at age 13 displayed lower prosocial behaviors than their low CU trait counterparts at ages 7, 10, and 13 [21]. Thus, there is preliminary evidence that adolescents identified as having primary or secondary CU traits display similar levels of low prosocial behavior from middle childhood to early adolescence. However, more research is required to establish whether youth with primary or secondary CU traits have consistently low prosocial behavior or whether prosocial behavior may differ earlier in development between the two CU variants.

In addition to emotion regulation and prosocial behavior, the other primary tenet of CU variant theory is that secondary CU traits arise from pathogenic environmental factors; namely,

negative parenting experiences. In trying to understand the role of negative environments, most of the extant research has focused on abuse perpetrated by parents (e.g., physical, psychological, or sexual abuse, parent–child conflict) or other experiences of trauma (e.g., exposure to community violence or sexual assault). The majority of studies found that youth with secondary CU traits report higher levels of maltreatment by parents [e.g., parental absence; domestic violence; physical, emotional and sexual abuse; 28, 29]. One epidemiological study found that youth who were identified as having secondary CU traits in early adolescence experienced more family adversity, harsh parenting (i.e., physical punishment), and maternal psychopathology in infancy and toddlerhood [21]. Although there appears to be preliminary support for higher rates of negative parenting in youth with secondary CU traits, results are inconsistent across studies. Kimonis, Fanti, Isoma and Donoghue [30] found that youth with primary and secondary CU traits experienced comparable rates of emotional and physical abuse, and youth with primary CU traits reported higher rates of emotional and physical neglect. In another study, Kimonis et al. [31] did not find a significant difference in rates of abuse between youth with secondary CU traits and those with aggressive primary CU traits; however, both of these groups scored higher on trauma exposure than youth with non-aggressive primary CU traits. Additionally, in a longitudinal study, Humayun and colleagues [32] examined CU variants at age 7 and reported negligible differences in rates of negative parenting experiences when the children were 4 years old. Taken together, it is unclear whether youth with secondary CU traits would have higher levels of parental harshness compared to youth with primary CU traits.

Although some researchers have examined the effects of negative parenting longitudinally, they focused on differences in rates of abuse rather than other forms of parenting such as parental warmth. There is evidence that CU traits in general are related to less warm parenting both cross-sectionally [33] and longitudinally [34]. Using the same sample as the current study, Goulter et al. [23] found that broad CU traits and conduct disorder symptoms in early adolescence mediated the association between parental warmth and harshness in childhood and externalizing problems at age 25. Although low parental warmth has been acknowledged as an important contributor to the development or maintenance of CU traits, there have been no studies that have examined differences in parental warmth as a predictor of CU variants in adolescents. Thus, to address this gap, our study includes measures of both parental warmth and harsh parenting.

Current Study

In sum, there is preliminary evidence that there are both individual (i.e., emotion regulation, prosocial behavior) and parenting (i.e., harsh parenting, parental warmth) differences between youth with primary and secondary CU traits. However, much of this evidence is cross-sectional, and thus, it is difficult to determine whether there are distinct etiological pathways to the variants. Thus, we have used data from the large longitudinal Fast Track project [35]. Although prior research has examined CU traits in the Fast Track dataset [34], this study represents a new approach to examining the data, specifically through the differentiation of primary and secondary CU traits. Specifically, this study aims to build on Goulter et al. [36] by examining the differential association between parental warmth and harshness and their interaction with individual factors (i.e., emotion regulation and prosocial behaviour) on the CU variants rather than examining CU broadly. This is particularly important given that the theory that secondary CU are more associated with early environmental influences compared to primary CU traits and the lack of available research to support this hypothesis. There has also been emphasis on experiences of parental maltreatment, with less research on other caregiving environments, such as parental warmth.

The current study aimed to extend prior work by examining early individual and parenting differences in early adolescents with primary and secondary CU traits, through five research questions. First, do childhood emotion regulation and prosocial behavior predict primary and secondary CU variants in early adolescence? Second, do early experiences of negative parenting (i.e., parental harshness and low parental warmth) predict CU variants in early adolescence? Third, when considered together, which childhood individual and parenting factors have the most salient association with CU variants in early adolescence? Fourth, do parenting factors (i.e., warmth and harshness) moderate the effect of individual factors (i.e., emotion regulation, prosocial behavior) on CU variants in early adolescence? Finally, do the models of early individual and parenting factors predicting CU variants differ by child sex?

We hypothesized that primary CU traits would be associated with higher levels of childhood emotion regulation and lower levels of prosocial behaviors. We also hypothesized that secondary CU traits would be associated with lower levels of childhood emotion regulation. We did not hypothesize that secondary CU traits would be associated with childhood low prosocial behaviors as it is unclear from prior research whether secondary CU would be associated with earlier levels of prosocial behaviors. We hypothesized that both primary and secondary CU traits

would be associated with lower levels of parental warmth and that secondary CU traits would be associated with higher levels of parental harshness. No a priori directional hypothesis was made regarding harsh parenting and primary CU traits. Due to the lack of prior research on moderators in CU variants, we explored parenting factors as potential moderators of emotion regulation and prosocial behavior, and thus, did not propose a priori hypotheses. Finally, given most research on CU variants has relied on adjudicated male samples, this study also explored sex differences across the models; we had no a priori directional hypotheses for this research question.

Methods

Participants

The Fast Track project is a longitudinal, multisite (Durham, North Carolina; Nashville, Tennessee; Seattle, Washington; and rural Pennsylvania) investigation of the development and prevention of child conduct problems [35]. Three cohorts of kindergarteners were screened for classroom conduct problems from 1991 to 1993. A total of 9,594 kindergarteners were screened by teachers using the Teacher Observation of Classroom Adaptation-Revised Authority Acceptance Score [37], and a subset were screened for home behavior problems by parents using a 22-item instrument based on the Child Behavior Checklist [CBCL; 38]. Using a multistage screening procedure, children were identified for the high-risk sample (control = 446; intervention = 445) and normative sample ($n = 387$). The present study used data from the high-risk control (65% male; 49% Black, 48% White, 3% other race) and normative (51% male; 42% Black, 51% White, 7% other race) samples; the intervention sample was not included in the present analyses. Seventy-nine of the participants that were recruited for the high-risk control group were included as part of the normative sample, with the total final sample including 754 participants. The present study included data collected from the following periods: covariates in kindergarten ($M_{age} = 6.39$, $SD = 0.54$); predictor variables from kindergarten, grade one, and grade two ($M_{age} = 6.39$, $SD = 0.54$; $M_{age} = 7.39$, $SD = 0.54$; $M_{age} = 8.39$, $SD = 0.54$, respectively); and clustering and validating variables in grade 7 ($M_{age} = 13.39$, $SD = 0.54$). Informed written consent from parents and oral assent from children were obtained. Parent(s) were compensated with $75 for completing each of the summer interviews. All procedures were approved by the Institutional Review Boards of participating universities.

Measures

Predictor Variables

Covariates The covariates, as measured when children entered the study in kindergarten, included sex (male = 58%), race (Black = 46%), and socioeconomic status [SES; M = 25.65; SD = 12.90; 39]. Status of the participant (normative or high risk) was also included as a covariate.

Emotion Regulation and Prosocial Behavior Emotion regulation and prosocial behavior subscales were drawn from the parent and teacher versions of the Social Competence Scale [40]. The emotion regulation subscale includes 6 items rated by parents and 10 items by teachers (e.g., controls temper in a disagreement, expresses needs and feelings appropriately, thinks before acting, calms down when excited or wound up). The prosocial behavior scale also included 6 items rated by parents (e.g., your child is very good at understanding other people's feelings, your child shares things with others, your child is helpful to others). Both scales were scored on a 5-point Likert scale from 0 *Not at all* to 4 *Very well*. Scores from the parent report in kindergarten, grade 1, and grade 2 and teacher report in kindergarten and grade 2 were used to create a latent variable. Teacher scores from grade 1 were not used due to high levels of missing data (64% missing) and the risk of bias in estimating more than 50% missing data. Internal consistencies were excellent across all waves for emotion regulation (α = 0.77–0.82) and prosocial behavior (α = 0.80–0.85).

Harsh Parenting Harsh parenting was measured using the Life Changes questionnaire [41]. The Life Changes questionnaire is a 15-min interview that is completed with the parent and measures a number of constructs including perceptions of the parent–child relationship, childcare history and discipline strategies. Parents were presented with six different situations of children's misbehavior and asked how they would handle each situation (e.g., hitting another child). Parents' responses were coded (0 *Not mentioned*, 1 *Mentioned*, 2 *Typical*) for the following categories: inductive reasoning, withdrawal of privilege, proactive guidance, and physical punishment. For this study, the physical punishment scale was used to represent harsh parenting, with the overall score computed by averaging parents' responses across the six vignettes; αs ranged from 0.41 to 0.55 from kindergarten to grade 2. There was a high interrater correlation coefficient for harsh parenting (0.93), available for a subset of the combined Fast Track high-risk intervention and control samples, supporting the reliability of this measure [42].

Parental Warmth Participants and their mothers completed the Parent–Child Interaction Task [PCIT; 43] at home during the summers following kindergarten (6–7 years old), grade 1 (7–8 years old), and grade 2 (8–9 years old). The PCIT included four tasks: Child's Game (free play; 5 min),

Parent's Game (parent-controlled play; 5 min), Lego Task (completion of a difficult puzzle; 5 min), and Clean-Up (3 min). The Interaction Rating Scale [IRS; 44] was completed by a trained observer after each task. The IRS is rated on a 5-point rating system (1 = low or negative value; 5 = high or positive value). Parental warmth was calculated by using the mean of six items that were coded across the four different tasks (interrater intraclass correlation coefficient = 0.73). Items used for parental warmth were related to maternal gratification (e.g., enjoyment in the interaction with the child), sensitivity (e.g., sensitive responding to the child's cues), and involvement (e.g., time spent interacting with the child). The αs ranged from 0.87 to 0.92 across the three time points.

Grade 7 Clustering Variables

CU Traits CU traits were assessed at the end of grade 7 using parent ratings on the 6-item CU scale ($\alpha = 0.64$) of the Antisocial Process Screening Device [APSD; 45]. Items are rated as 0 (*Not at all true*), 1 (*Sometimes true*), or 2 (*Definitely true*). Example items (reverse-scored) include: "Is concerned about the feelings of others" and "Feels bad or guilty when he/she does something wrong." The mean of the scale was used in the current study. The CU scale of the ASPD has demonstrated good reliability and validity [46].

Anxiety The parent-reported CBCL [38] was administered at the end of grade 7. The anxious/depressed narrow-band scale consisted of 13 items (e.g., cries a lot, feels too guilty, self-conscious or easily embarrassed) and is scored on a 3-point scale ranging from 0 (*never*) to 2 (*always*). A *T*-score of 70 or above indicated clinical range symptoms, whereas a *T*-score of 60–69 indicate sub-clinical range symptoms. Raw scores were used in the analysis while *T*-scores, which are age- and sex-normed, are reported in tables for ease of understanding. This scale has shown excellent psychometric properties in prior studies [$\alpha = 0.88$; 47] and in the current sample ($\alpha = 0.85$).

Grade 7 Validating Variables

Psychopathology The withdrawn (e.g., rather be alone), delinquent behavior (e.g., lies/cheats) and aggressive problems (e.g., physically attacks people) narrow-band *T*-score scales from the CBCL were used to validate the CU variants in grade 7. Internal consistency scores were excellent across all subscales ($\alpha = 0.76$–0.91).

Parent–Child Conflict The Conflict Tactics Scale [CTS; 48] (parent–child and partner-child verbal aggression, hostile-indirect withdrawal, physical aggression, and spanking subscales) was used to examine current levels of parent–child conflict across the variants. This parent-report measure assesses how the parent reacts in a conflict with the child, such as yelling at or insulting the child, stomping out of the room or house, threatening to spank the child, and hitting or trying to hit the child. Items are rated on a 7-point scale ranging from 0 (*never*) to 6 (*almost every day*). Reliability was acceptable for all scales used for both the normative sample (αs = 0.60 to 0.75) and the high-risk control group (αs = 0.57 to 0.81).

Data Analytic Plan

Data analysis proceeded in two stages. First, a two-step cluster analysis in SPSS 26 [49] was conducted to identify primary and secondary variants of CU traits. To examine the research questions regarding the impact of childhood individual and parenting factors on CU variants, a series of structural equation models (SEM) were conducted using Mplus version 7.4 [50]. Details for the analysis plan can be found in Appendix A.

Results

Descriptive Statistics

Descriptive statistics for all study variables can be found in Table 1. Across the three early time points, the female sample was found to have higher levels of prosocial behaviors (t (752) = 1.13, $p < 0.01$, t (710) = 3.86, $p \leq 0.001$, t (686) = 3.00, $p < 0.01$, respectively) and emotion regulation skills (t (752) = 4.49, $p \leq 0.001$, t (710) = 5.41, $p \leq 0.001$, t (686) = 5.30, $p \leq 0.001$, respectively) compared to the male sample across the three predicting time points. The female sample was also found to have higher levels of anxiety symptoms (t (616) = − 2.36, $p < 0.05$) and lower levels of CU traits (t (617) = − 3.87, $p \leq 0.001$). No other sex differences were found.

Cluster Analysis

The two-step cluster analysis indicated a three-cluster solution (Cluster 1, $n = 207$ or 33.5%; Cluster 2, $n = 148$ or 24%; Cluster 3, $n = 262$ or 42.5%) best fit the data. The three-cluster solution had a BIC change of -133.27 between a two- and three-cluster solution, and a ratio distance measure of 2.18 with a silhouette of 0.6. The three-cluster solution was more optimal than a four-cluster solution, which had a BIC change of -47.15 and a smaller ratio distance measure of 1.47 and a silhouette of 0.5. The four-cluster solution had a significantly smaller change in BIC, ratio distance measure, and silhouette below the acceptable range compared to the three-cluster solution indicating that the three cluster was optimal. The first cluster scored relatively high on CU

Table 1 Descriptive statistics for study variables

Variable	*M* (*SD*)	Females *M* (*SD*)	Males *M* (*SD*)	*t*
Anxious/depressed *T*-score	54.29 (6.59)	53.56 (5.57)	54.82 (7.22)	− 2.36*
Callous-unemotional traits	.62 (.37)	.56 (.37)	.67 (.36)	− 3.86***
Prosocial skills Kindergarten	2.50 (.73)	2.60 (.72)	2.43 (.73)	1.13**
Prosocial skills Grade 1	2.57 (.74)	2.69 (.75)	2.48 (.72)	3.86**
Prosocial skills Grade 2	2.52 (.74)	2.62 (.78)	2.45 (.70)	3.00**
Emotion regulation skills Kindergarten	1.89 (.71)	2.02 (.72)	1.79 (.69)	4.49***
Emotion regulation skills Grade 1	1.96 (.73)	2.13 (.75)	1.84 (.69)	5.41***
Emotion regulation skills Grade 2	1.97 (.73)	2.15 (.75)	1.85 (.70)	5.30***
Parental warmth Kindergarten	3.52 (.79)	.80 (.05)	.78 (.04)	ns
Parental warmth Grade 1	3.66 (.82)	.81 (.04)	.83 (.04)	ns
Parental warmth Grade 2	3.65 (.84)	.87 (.05)	.82 (.04)	ns
Parental harshness Kindergarten	.21 (.23)	.24 (.01)	.22 (.01)	ns
Parental harshness Grade 1	.19 (.22)	.20 (.01)	.23 (.01)	ns
Parental harshness Grade 2	.12 (.16)	.16 (.01)	.15 (.01)	ns

ns Nonsignificant

*$p < .05$, **$p < .01$, ***$p \leq .001$

Table 2 Mean Scores for Clustering and Validating Variables at Grade 7 for the Identified Variants

	Low ($n = 262$) *M* (*SD*)	Primary ($n = 207$) *M* (*SD*)	Secondary ($n = 148$) *M* (*SD*)	
CU traits	$.28\ (.18)_b$	$.87\ (.21)_a$	$.90\ (.27)_a$	$F\ (2, 614) = 600.77$***
Anxiety	$52.01\ (3.37)_b$	$50.63\ (1.47)_c$	$63.46\ (6.80)_a$	$F\ (2, 614) = 498.92$***
CBCL				
Withdrawn/ depressed	$51.56\ (3.38)_b$	$51.54\ (3.66)_b$	$60.13\ (8.95)_a$	$F\ (2, 614) = 145.12$***
Delinquency	$53.13\ (4.39)_c$	$55.99\ (6.08)_b$	$64.39\ (8.08)_a$	$F\ (2, 614) = 167.81$***
Aggression	$53.05\ (4.52)_c$	$55.03\ (5.82)_b$	$66.55\ (9.10)_a$	$F\ (2, 614) = 230.54$***
CTS				
Indirect hostile	$.23\ (.44)_c$	$.40\ (.66)_b$	$.77\ (.89)_a$	$F\ (2, 608) = 32.69$***
Physical aggression	$.13\ (.35)_b$	$.28\ (.54)_a$	$.39\ (.68)_a$	$F\ (2, 608) = 13.10$***
Spanking	$.27\ (.61)_b$	$.51\ (.85)_a$	$.64\ (.93)_a$	$F\ (2, 608) = 11.85$***
Verbal aggression	$.86\ (.79)_c$	$1.17\ (.96)_b$	$1.68\ (1.10)_a$	$F\ (2, 608) = 35.77$***

CU Callous-unemotional, *CBCL* Child Behavior Checklist, *CTS* Conflict Tactics Scale

***$p \leq .001$

traits ($M = 0.87$, $SD = 0.21$) and low on anxiety ($M = 50.63$, $SD = 1.47$), and was therefore labelled *Primary CU traits*. The second cluster scored high on CU traits ($M = 0.90$, $SD = 0.27$) and anxiety ($M = 63.46$, $SD = 6.80$), and was labelled *Secondary CU traits*. The third cluster was low on both CU ($M = 0.28$, $SD = 0.18$) and anxiety ($M = 52.01$, $SD = 3.37$), and was labelled *Low*. Table 2 shows the mean differences between the variants on clustering and validating variables. Clusters differed on CU traits with primary and secondary CU traits scoring higher than non-CU trait youth. Primary and secondary CU variants did not differ on CU traits ($p = 0.43$). Secondary youth scored higher than primary and low youth on anxiety.

As shown in Table 2, CU variants' scores differed across the three CBCL narrow-band scales (i.e., depressed/withdrawn, delinquency, aggression).[1] The secondary cluster scored higher than the primary and low clusters on all forms of psychopathology, and was the only cluster to be in the "sub-clinical" range (*T* score 60–69) on the CBCL for depressed/withdrawn, delinquency, and aggression. The variants also differed on various forms of parent–child conflict by the primary caregiver including indirect hostility

[1] Results are presented as *T*-scores in Table 2. Results were the same for *T*-scores and raw scores on the CBCL.

(F (2, 608) = 32.69, $p \leq 0.001$), physical aggression (F (2, 608) = 13.10, $p \leq 0.001$), spanking (F (2, 608) = 11.85, $p \leq 0.001$), and verbal aggression (F (2, 608) = 35.77, $p \leq 0.001$). Based on a post-hoc Tukey's test, youth with secondary CU traits were significantly higher than both primary and low youth on indirect hostility ($p \leq 0.001$) and verbal aggression ($p \leq 0.001$). Youth with primary CU traits were higher than low youth on indirect hostility ($p < 0.01$) and verbal aggression ($p < 0.01$). Primary and secondary youth did not differ from each other on physical aggression and spanking, but both were higher than low youth ($p < 0.01$).

Confirmatory Factor Analysis

We examined whether the latent predictor variables for individual and parenting factors had acceptable fit before including them in the analysis. Starting with the individual variables, the model for emotion regulation fit the data well, X^2 (4) = 2.36, $p = 0.67$, CFI = 1.00, RMSEA = 0, 90% CI [0.00, 0.04]. The model for prosocial behavior was acceptable, X^2 (4) = 10.93, $p = 0.03$, CFI = 0.99, RMSEA = 0.05, 90% CI [0.01, 0.08]. Next, we tested the parenting variables. Parental warmth fit the data well, X^2 (3) = 338.48, $p \leq 0.001$, CFI = 1.00, RMSEA = 0, 90% CI [0.00, 0.00], as did parental harshness, X^2 (3) = 243.15, $p \leq 0.001$, CFI = 1.00, RMSEA = 0 90% CI [0.00, 0.00].

Structural Equation Models

Are Childhood Expressions of Emotion Regulation and Prosocial Behavior Related to CU Variants in Early Adolescence?

The latent emotion regulation and prosocial behavior variables were set to predict the dichotomous primary and secondary CU variables while controlling for normative/high-risk sample, race, sex, and SES. Emotion regulation and prosocial behavior were set to covary, as were primary and secondary CU traits. Modification indices recommended that the residual error for emotion regulation and prosocial behavior covary at each of the three time points; those three additional parameters were included. The model showed a good fit, X^2 (69) = 150.19, $p < 0.001$, CFI = 0.99, RMSEA = 0.04, 90% CI [0.03, 0.05]. Primary CU was predicted by higher levels of emotion regulation ($\beta = 0.33$, $p \leq 0.001$), and lower levels of prosocial behavior ($\beta = -0.37$, $p \leq 0.001$). In comparison, secondary CU traits were predicted by lower levels of emotion regulation ($\beta = -0.36$, $p \leq 0.001$); however, secondary CU traits were not predicted by earlier prosocial behavior ($\beta = 0.06$, $p = 0.47$). The parameters from emotion regulation and prosocial behavior were significantly different for primary and secondary CU traits (Wald's $X^2 = 20.28$, $p < 0.001$, and Wald's $X^2 = 8.82$, $p < 0.01$, respectively).

Are Childhood Experiences of Parental Warmth and Harshness Related to CU Variants in Early Adolescence?

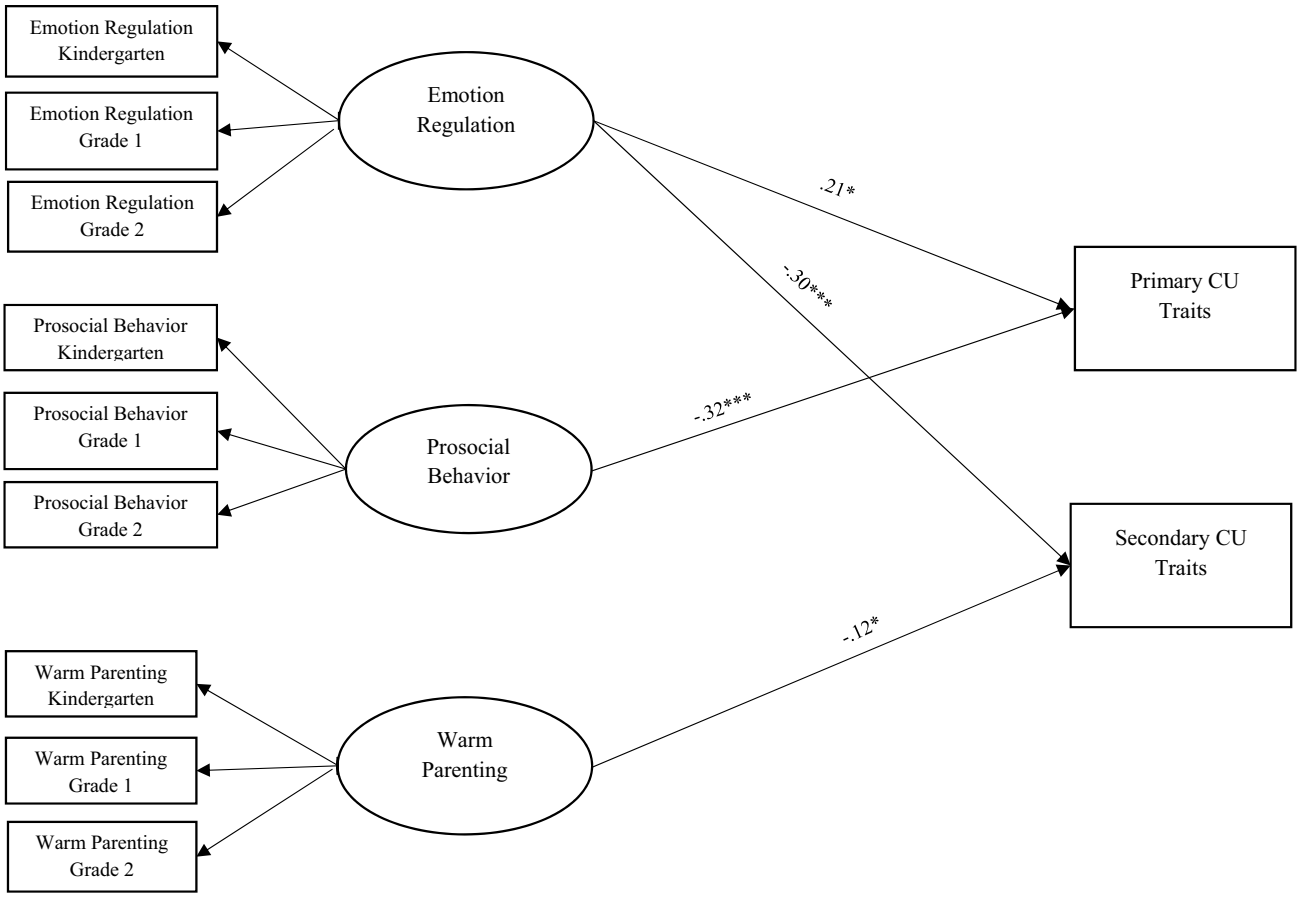
The latent parental warmth and harshness variables were set to predict the dichotomous primary and secondary CU variables. Modification indices recommended that the normative variable covary with the residuals with each indicator from parental warmth and harshness. These six parameters were therefore included. The parental factor model fit the data well, X^2 (29) = 56.66, $p = 0.002$, CFI = 0.97, RMSEA = 0.04, 90% CI [0.02, 0.05]. Parental warmth, but not harshness, predicted secondary CU traits ($\beta = -0.14$, $p < 0.01$). Parental warmth did not predict primary CU traits ($\beta = -0.07$, $p = 0.17$). However, a test of the parameters showed no significant difference between parental warmth and primary and secondary CU traits (Wald's $X^2 = 0.30$, $p = 0.59$) As parental harshness did not predict primary or secondary CU traits in the parsimonious model, it was removed from the final model.

What best predicts CU variants in early adolescence?

The final model included emotion regulation, prosocial behavior, and parental warmth (see Fig. 1). This model showed good model fit, X^2 (102) = 192.40, $p < 0.001$, CFI = 0.99, RMSEA = 0.03, 90% CI [0.02, 0.04]. In the final model, primary CU traits continued to be associated with higher levels of emotion regulation ($\beta = 0.33$, $p \leq 0.001$) and lower levels of prosocial behavior ($\beta = -0.40$, $p \leq 0.001$). Secondary CU traits were predicted by lower levels of emotion regulation ($\beta = -0.38$, $p \leq 0.001$) and lower levels of parental warmth ($\beta = -0.15$, $p \leq 0.001$).

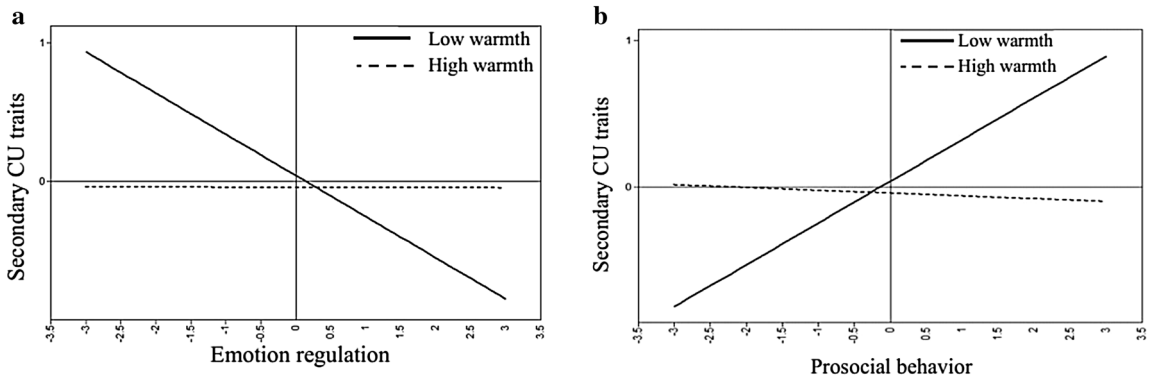
Do Parenting Factors (Parental Warmth and Harsh Parenting) Moderate the Relationship Between Emotion Regulation and Prosocial Behaviors on Primary and Secondary CU Traits?

A latent interaction model was conducted to examine the interaction between emotion regulation and parental warmth, as well as prosocial behavior and parental warmth. Parental warmth did not moderate the effect of emotion regulation or prosocial behavior on primary CU traits. Parental warmth did moderate the effect of emotion regulation on secondary CU traits ($\beta = 0.54$, $p < 0.05$). Upon examining the interaction plot (see Fig. 2a), low levels of emotion regulation appear to be associated with secondary CU traits only at low levels of parental warmth. High levels of parental warmth appear to have a null association with secondary CU traits at both high and low levels of emotion regulation.



Note: CU = Callous-unemotional. Only significant pathways shown. Not pictured: Normative sample, race, and SES used as control variables.
* $p < .05$, ** $p < .01$, *** $p \leq .001$

Fig. 1 Final full structural equation model for all significant predicting factors



Note: CU = Callous-unemotional. Youth who clustered as having secondary CU traits were coded as 1 and youth who did not cluster as having secondary CU traits were coded as 0 as represented on the graph.

Fig. 2 **a** Emotion regulation and parental warmth predicting secondary CU traits. **b** Emotion regulation and parental warmth predicting secondary CU traits

Parental warmth also moderated the effect of prosocial behavior on secondary CU traits ($\beta = -0.53$, $p < 0.05$). Similar to emotion regulation, the interaction plot (see Fig. 2b) revealed that high levels of prosocial behavior are associated with secondary CU traits at low levels of parental warmth and that high levels of parental warmth were not related to secondary CU traits at either high or low levels of prosocial behavior. Taken together, when added to either low emotion regulation or high prosocial behavior, low parental warmth increases the likelihood of secondary CU traits. Additionally, high levels of parental warmth may be a protective factor for secondary CU traits.

Harsh parenting was also examined as a potential moderator between emotion regulation and prosocial behavior on primary and secondary CU traits. However, it did not moderate the effect of emotion regulation or prosocial behavior on either primary or secondary CU traits.

Do Early Individual and Parenting Factors Differ by Child Sex?

The nested sex model revealed no sex differences across the final model, $\Delta X^2\ (22) = 23.25$, $p = 0.39$. Of note, we did not test differences in the control variables across sex. Indicated by modification indices in the nested sex model, the control variables were allowed to vary as the associations between prosocial, emotion regulation, and parental warmth with primary and secondary CU traits were the foci of the analyses.

Discussion

The current study aimed to examine whether childhood individual factors (i.e., emotion regulation and prosocial behavior) and parenting factors (i.e., warm and harsh parenting) were predictive of primary and secondary CU traits identified in early adolescence. We found that primary CU traits were associated with higher levels of emotion regulation and lower levels of prosocial behavior in childhood. Secondary CU traits were associated with lower levels of emotion regulation and lower levels of parental warmth in childhood. Primary and secondary CU traits were not associated with harsh parenting, and primary CU traits were not associated with parental warmth. We also tested for an interaction between childhood individual and parenting factors. We found that low parental warmth moderated the effect of both emotion regulation and prosocial behavior on the development of secondary CU traits, such that low parental warmth combined with low emotion regulation or high prosocial behavior predicted secondary CU traits. Finally, we tested for sex invariance and found that results did not differ by sex.

In terms of individual child factors (i.e., emotion regulation and prosocial behavior), our results are consistent with previous research showing that children and youth with secondary CU traits have more difficulty with emotion regulation [22]. Our findings add to longitudinal research that has found children with secondary CU traits have low levels of emotion regulation across development. For example, Fanti and Kimonis [22] reported that children identified as having secondary CU traits in childhood had low levels of emotion regulation in adolescence. Our results extend these findings by showing that youth identified in early adolescence as having secondary CU traits had low levels of emotion regulation in childhood. The presence of low levels of emotion regulation in childhood supports theory that these youth may have difficulties receiving socialization cues from their parents at an early age [17], thus putting them at risk for the development of CU traits. We also found youth with primary CU traits have higher levels of emotion regulation, which is consistent with research that has found youth with primary CU traits to be hypoaroused, and therefore, at a lesser risk of psychopathology compared to their secondary counterparts [16].

We found that primary (but not secondary) CU traits were associated with lower levels of prosocial behavior in childhood. These results contrast with previous research that found youth with both primary and secondary CU traits have lower levels of prosocial behavior [21, 27]. However, there are some important differences between prior research and the current study. Our study examined childhood indicators of variants identified in early adolescence, whereas previous research in this area has examined the association between prosocial behaviors and CU variants cross-sectionally in childhood [21] or in adolescence [27]. Past research has shown that prosocial behavior is indicative of low levels of CU traits [51], and thus, our finding of lower levels of prosocial behavior in childhood predicting primary CU variants in early-adolescence may demonstrate stability in their expression of CU traits from a young age. Conversely, youth with secondary CU traits did not show the same low prosocial behavior at an early age, indicating that these youth may show an increase in CU traits from childhood to early adolescence. This is consistent with developmental research demonstrating one group that has stable high CU traits from age 7 to 12 and another group that is increasing [52]. It is possible that secondary CU traits emerge later in development around early adolescence; thus, these youth may demonstrate typical levels of prosocial behavior earlier in childhood. Youth with secondary CU traits may also be particularly sensitive to their surroundings, which could present as caring and empathetic towards others in childhood (i.e., prosocial behavior). However, this sensitivity could put them at a higher risk of developing negative coping methods, such as emotional numbing, to deal with

negative environments, such as low parental warmth [7]. This conjecture is supported by our results in that prosocial behavior was predictive of secondary CU traits at low levels of parental warmth, as discussed below. Thus, the relationship between prosocial behavior and secondary CU traits may not be a straightforward association. As this is the first study to examine these childhood indicators of primary and secondary CU variants that were identified in adolescence, further research is required to fully understand the implications of our findings.

Contrary to our hypothesis, neither primary nor secondary CU traits were associated with harsh parenting. Previous findings on the association between harsh parenting and primary and secondary CU traits have been mixed [30, 53]. Our findings differ from two other normative longitudinal studies that found early exposure to harsh parenting to be associated with both primary and secondary CU traits [21, 32]. It is interesting to note that although neither primary nor secondary CU traits were associated with harsh parenting (a measure that included physical punishment) in childhood, both variants were found to experience higher levels of physical and verbal aggression from their parents cross-sectionally in early adolescence compared with low CU youth. Thus, our cross-sectional findings were consistent with previous cross-sectional research that has found both variants have higher levels of harsh parenting or abuse [e.g., 15]. Additionally, youth with secondary CU traits had higher rates of experienced verbal aggression and indirect hostile aggression compared to youth with primary CU traits in early adolescence, supporting the notion that secondary CU traits may emerge as a result of exposure to a negative parenting environment that includes both physical and verbal aggression [54]. As our measure of harsh parenting in childhood contained physical aggression (i.e., physical punishment) and not verbal aggression, it is possible that a broader maltreatment questionnaire, such as the one used in early adolescence, may have yielded different results.

Although not associated with harsh parenting, secondary CU traits were associated with low parental warmth. This finding parallels other research that has found parental warmth to be associated with CU traits in children [e.g., 33], adolescents [e.g., 34], and adults [e.g., 55]. Importantly, Kimonis and colleagues, and Gao and colleagues, found results similar to the present findings in that parental warmth was a significant correlate of CU traits (or psychopathy), even after accounting for childhood history of maltreatment. Thus, there is support for the notion that a lower level of parental warmth is more salient in the development of secondary CU traits compared to harsh parenting or physical abuse. Parental warmth has been thought to be critical in the socialization of children with temperamental styles consistent with CU traits [17], as well as in the development of healthy parent–child relationships. It has been proposed that secondary CU traits are related to the quality of the parent–child relationships [56], which our results support. Research on CU variants has highlighted the role of exposure to negative parenting environments in the development of secondary CU traits but has not consistently found this relationship for youth with primary CU traits [6, 54]. Parenting, particularly warm parenting, may be more salient in the development of secondary CU traits compared to primary CU traits. This finding extends the work of Goulter et al. [36], as the authors found CU traits were broadly associated with CU traits, and by examining the variants more specifically, we found that parental warmth was associated with secondary but not primary CU traits. However, it should be noted that although parental warmth was not significantly associated with primary CU traits in this study, the associations between parental warmth and the two variants were not significantly different. Thus, parental warmth may represent a different association with primary and secondary CU traits compared to maltreatment. More research is required on primary and secondary CU traits in relation to parental warmth to better understand these findings.

The importance of parental warmth in developmental models of CU variants was further supported by our finding that parental warmth moderated the effect of emotion regulation on secondary CU traits. As predicted, low levels of emotion regulation combined with low levels of parental warmth were associated with secondary CU traits. As previously noted, parental warmth also moderated the effect of prosocial behavior on secondary CU traits. Children who are sensitive and caring in childhood (i.e., display higher prosocial behavior) and have difficulties with emotion regulation may be particularly sensitive to their caregiving environment. These results were consistent with the differential susceptibility hypothesis that posits children with difficulties regulating their emotions early in childhood are at a higher risk of developmental psychopathology when exposed to unsupportive or adverse caregivers [57]. In order to cope with their sensitivity to their environment, secondary variants may develop CU traits as a numbing coping mechanism in order to deal with this exposure to negative caregiving environments [7]. However, as this is the first study to examine this moderation, further research is required to examine this hypothesis.

Our results were not moderated by child sex. This is consistent with previous work that has shown that neither the identification of variants [16] nor affective outcomes [58] were moderated by sex. Our findings therefore support the notion of the "significance of affective differences between [CU trait] variants in youth, as they persisted beyond the influence of gender" [58, p. 304].

Implications

The current findings suggest that youth with primary and secondary CU traits may require slightly different treatment targets. It may be important for clinicians to consider assessment of emotion regulation and prosocial behavior earlier in development as potential risk factors for primary and secondary CU traits in adolescence. Adolescents who have primary CU traits, and thus, are more likely to display low prosocial behavior early in development, may respond better to emotion coaching interventions that focus on building empathy skills [59]. In comparison, youth with secondary CU traits may respond better to interventions that are focused on increasing emotion regulation through the parent–child relationship [56]. Although parental warmth was not significantly associated with primary CU traits in the current study, there have been numerous studies that have found it to be an important predictor of CU traits in general [e.g., 33]. Thus, it has been proposed that an important aspect to interventions aimed at children with CU traits is the incorporation of warm parenting [60]. Previous research has found that incorporating empathy skills into the parent–child relationship can help reduce the level of CU traits in young children with conduct problems [59]. Although youth with primary CU traits may benefit from building empathy skills and youth with secondary CU may benefit from emotion regulation skills, it is possible that both variants require the same warm parenting approach to intervention. As no published study to date has examined differences in treatment response across CU variants, this area needs to be further explored in the clinical literature.

Limitations

The present study adds longitudinal support for a number of theories regarding the underlying mechanisms of primary and secondary CU traits [5, 7], contributing significantly to a research base that is largely cross-sectional in nature. However, our findings must be considered within the context of several limitations. First, we assessed primary and secondary CU traits in early adolescence. Thus, our methodology is in contrast to other longitudinal studies that examined outcomes of CU variants identified in childhood [i.e., 22]. As the study did not measure CU traits in childhood, we were unable to determine CU variants during that developmental period. Our results suggest that while primary CU variants appear to display low prosocial behavior earlier in childhood, secondary CU variants do not show the same deficit. Additionally, as the measure of CU traits used in the current study was not repeated in the Fast Track data collection, we were unable to examine CU traits later in development. More longitudinal research is needed to understand the developmental unfolding of CU variants. Second, we chose to differentiate primary and secondary CU traits based on the presence of CU traits and anxiety. These are the most common variables used to cluster the two variants [8]; however, other researchers have also included measures such as trauma exposure and trauma symptoms [6] in their models. Our decision to use anxiety, and not exposure to trauma, was to limit the shared variance between exposure to early harsh parenting and current exposure to parental aggression in our model. Third, although we use multiple reporters in childhood (e.g., observational methods, combined teacher and parent report), the measures in early adolescence were reported by parents. There has been some debate as to whether parent or self-report of CU traits are most accurate; however, research has shown that parents are adequate in their reporting of CU traits [1]. However, researchers may want to consider using multiple reporters for CU traits and psychopathology. Finally, the Fast Track data were collected beginning in 1991; thus, the predictors data from participants in the current study are nearly 30 years old. Data collected over long periods of time permit important prospective modelling, and thus, these data have also allowed us to examine whether the current CU variants are associated with distinct outcomes in adulthood [23]. While we do not anticipate time to be a contributing factor in the present findings, compared to more socially-effected variables that have been shown to change over time [e.g., substance use; 61], it may be important for future research with more recent data collection to replicate our findings.

Conclusion

The different associations with childhood individual and environmental factors across CU variants were consistent with current theories of primary and secondary CU traits. Primary CU traits were associated with early indicators of low prosocial behavior and high levels of emotion regulation, whereas secondary variants were associated with low levels of emotion regulation. Contrary to our hypothesis, neither primary nor secondary variants were related to harsh parenting; however, secondary CU traits were related to low levels of parental warmth. In addition, we examined the moderating role of parental warmth on emotion regulation and prosocial behavior. Our results indicated that low parental warmth may be a risk factor for secondary CU traits for children with low emotion regulation and higher prosocial behavior. Thus, our results highlight the importance of early expressions of hypo- and hyperarousal and the warmth of the parent–child relationship more so than exposure to harsh parenting. By identifying these factors in childhood, we may be better able to identify youth at risk of developing CU traits and intervene such that we divert them from further potential negative outcomes [23].

Summary

There is growing evidence of diverse etiological pathways to the development of callous-unemotional (CU) traits, known as primary and secondary CU variants. Typically differentiated on anxiety, the central premise of this view is that youth with primary CU traits are biologically predisposed to being hypoaroused, while youth with secondary CU traits are typically hyperaroused and develop CU traits in response to being exposed to negative caregiving environments. The purpose of the present study was to extend previous cross-sectional research and to examine theoretically relevant predictors of CU variants prospectively from childhood to adolescence. Participants included high-risk control and normative samples from the Fast Track project. Using structural equation modelling, primary CU traits, as identified in early adolescence, were associated with higher levels of emotion regulation and lower levels of prosocial behavior in childhood (compared with all other participants). Secondary CU traits were associated with lower levels of emotion regulation in childhood (compared with all other participants), but not associated with early prosocial behavior. Secondary CU traits were also associated with low parental warmth, while neither CU variant was related to harsh parenting. In addition, parental warmth moderated the effect of both emotion regulation and prosocial behavior on secondary CU traits. Results were not moderated by sex. Theoretically relevant individual and environmental developmental precursors were related to primary and secondary CU traits. A greater understanding of early indicators of CU variants may better guide intervention efforts.

Appendix A

Data analysis proceeded in two stages. First, a two-step cluster analysis in SPSS 26 [49] was conducted to identify primary and secondary variants of CU traits. The two-step method is an auto-cluster procedure that combines the Bayesian information criterion (BIC), log-likelihood ratio of distances between clusters, and silhouette. A smaller BIC in combination with the largest ratio of distance [62] is considered the best model. In addition, a silhouette that is less than 0.2 is considered poor, one that is between 0.2 and 0.5 is considered fair, and greater than 0.6 is considered a good solution [63]. These combined indicators are used to determine the optimal number of clusters. The cluster analysis included variables consistent with prior research: CU traits (i.e., CU subscale of the APSD) and anxiety[8]. Two-step cluster analysis was selected as the most appropriate analysis as other grouping techniques (e.g., latent profile analysis) require more than two indicators. The groups were then validated against measures found to theoretically differentiate variant groups from each other or from those with low CU; that is, psychopathology (i.e., withdrawn, delinquent, and attention problems scales from the CBCL) and recent exposure to parent–child conflict (i.e., CTS). For the purpose of modelling, groups were coded into two dichotomous variables. In the *primary CU* variable, those in the primary CU trait cluster were coded 1, while all other clusters were coded 0. Likewise, in the *secondary CU* variable, those in the secondary CU trait cluster were coded as 1 while all other clusters were coded as 0. This allowed us to examine the relationship between the predictor variables and primary and secondary CU trait clusters.

To examine the research questions regarding the impact of childhood individual and parenting factors on CU variants, a series of structural equation models (SEM) were conducted using Mplus version 7.4 [50]. Data were considered missing if full scales were not available for participants. Data missing from parent-reported and observational predictor variables from kindergarten, grade 1 and grade 2, included emotion regulation and prosocial behavior (0%, 5.8% and 8.9%, respectively), parental warmth (0.4%, 6.5% and 17.7%, respectively) and parental harshness (0.3%, 5.9% and 9.7%, respectively). Teacher-reported emotion regulation and prosocial behavior missing data included 18.6% for kindergarten measures and 10.4% for grade 2 measures (grade 1 teacher report was removed due to high rates of missing data at 64%). Finally, 18.04% of the data from grade 7 was missing from both the CU traits scale and anxiety scale. All models were estimated using full information maximum likelihood (FIML) with robust standard errors. FIML provides estimates of the variance–covariance matrix for all available data and includes individuals who are missing data on individual measures. Models were evaluated according to the most commonly used critical values for the fit indices. An acceptable model is indicated by a nonsignificant chi-square (X^2), which suggests the observed covariance matrix is similar to the predicted matrix. The root mean square error of approximation (RMSEA) is considered good below 0.05, adequate if between 0.05 and 0.08 [64], and acceptable if between 0.08 and 0.10 [65]. The Comparative Fit Index (CFI) cutoff of 0.90 to 0.95 is suggested for an acceptable fit for the CFI [64]. Parameters were examined using standardized coefficients in Mplus (STDXY). Modification indices were examined to determine whether additional parameters were required in the models.

Prior to conducting full SEM models, assumptions checking revealed all variables of interest had skew and kurtosis within the acceptable range (George & Mallery, 2010). Outliers were identified for the harsh parenting variable,

specifically youth who had experienced more harsh punishment ($n = 22$, 2.9% of the total sample). Analyses were conducted with and without outliers. No difference were found across the models. Given the paper was specifically interested in youth with secondary CU traits, which includes youth who are more likely to have experienced harsh punishment, and the lack of significant difference in the findings, these cases were retained for the final analysis. Predictors (emotion regulation, prosocial behavior, parental warmth, parental harshness) were modelled in separate confirmatory factor models to create predictor latent factors. For emotion regulation and prosocial behavior, parent report from kindergarten, grade 1, and grade 2 were included while teacher report from kindergarten and grade 2 were also included. Teacher report from grade 1 was not included due to high levels of missing data. For parental warmth and harshness, observed variables from kindergarten, grade 1, and grade 2 were included. Latent growth curves were not included due to the stability of all four predictor variables over the 3 years. Next, while controlling for the normative/high-risk sample, race, sex and SES covariates, we examined whether individual (i.e., emotion regulation and prosocial behavior) and parenting (warmth and harshness) latent factors predicted CU variants in separate SEM models. We then included all significant individual and parenting latent factors into a final full model, again controlling for normative/high-risk sample, race, sex, and SES. Next, we examined a moderated model in which the individual and parenting latent factors were set to interact. We examined the interaction between the parental warmth factor and the individual factors (emotion regulation and prosocial behavior) using the loop plot function [50]. Loop plots standardize each predictor into a *z*-score and then plot the interactions two standard deviations above and below the mean for the independent and moderated factors. We also examined the interaction between the harsh parenting factor and the individual factors. We used Wald's test to examine differences in the parameters of each predictor. Finally, we examined potential sex differences in the final non-moderated model by comparing two nested models. The first nested model allowed each parameter to vary across sex; the second fully constrained model held parameters equal across the male and female samples. Models comparing the male and female samples were then compared using a chi-square test. A significant chi-square would indicate the models differ across sex.

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Declarations

Conflict of interest No authors have any conflicts of interest to declare.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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