Title: Inter-relationship between cognitive control, anxiety and restricted and repetitive behaviors in children with 22q11.2 deletion syndrome

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Scientific Abstract

Restricted and repetitive behaviors (RRB) are common in individuals with 22q11.2 microdeletion syndrome (22q11.2DS), yet the underlying mechanisms of these behaviors remain poorly characterized. In the present pilot investigation, we aimed to further our understanding of RRB in 22q11.2DS by exploring their relationship with cognitive control and anxiety as well as with sex, chronological age (CA) and full-scale IQ (FSIQ). Parents of 38 children with 22q11.2DS (17 females; \( M_{\text{age}} = 11.15 \text{ years, } SD = 2.46 \)) completed the Social Communication Questionnaire (SCQ) as a measure of RRB and social and communication (SC) problems and the Behavioral Assessment System for Children-2 (BASC-2) as a measure of anxiety and cognitive control. Higher RRB scores were significantly associated with higher anxiety levels \((r = .44, p = .006)\), more impairments in cognitive control \((r = .56, p < .001)\) and higher SC scores \((r = .43, p = .011)\). In the first step of the hierarchical regression model, anxiety accounted for 24.5% of variance \((F = 10.05, p = .003)\); cognitive control accounted for an additional 18.1% of variance \((F_{\text{change}} = 11.15, p < .001)\) in the second step; SC score accounted for only 0.8% of additional variance in the third step \((F_{\text{change}} = .40, p = .53)\). The final model explained 43.4% of variance \((F = 7.42, p = .001)\), with cognitive control as a unique independent predictor of RRB score \((t = \ldots)\).
2.52, \( p = .01 \). The current results provide the first exploration of the cognitive control—

anxiety—RRB link in individuals with 22q11.2DS and points to cognitive control as a 
potentially viable target for treatments aimed at reducing RRB.

**Lay Summary:** People with 22q11.2 deletion syndrome show high levels of repetitive 

behaviors, however, the previous research has not explored why people with this syndrome 

exhibit high rates of repetitive behaviors. Understanding the reasons for the high levels of 

repetitive behaviors is important given that these behaviors can be highly impairing. Our study 

found that repetitive behaviors were associated with impaired ability to self-regulate and high 

levels of anxiety. These findings need to be further replicated, however, they are important as 

they suggest potentially promising ways of reducing these behaviors.

**Key Words:** 22q11.2DS, repetitive behaviors, anxiety, cognitive control.
Introduction

Chromosome 22q11.2 deletion syndrome (22q11.2DS) results from a microdeletion of a segment of chromosome 22 at the q11.2 band which affects approximately 40-50 genes (Shapiro et al., 2014). 22q11.2DS occurs in approximately 1 in every 3000-6000 live births (Grati et al., 2015). The phenotype of 22q11.2 is highly variable encompassing physical, metabolic and endocrine as well as behavioral and cognitive features. Physical phenotype of 22q11.2DS is characterized by a wide range of features including congenital heart, palatal and pharyngeal abnormalities, mild facial dysmorphism, skeletal anomalies, immune deficiency, and hypoparathyroidism with resultant hypocalcemia (Shprintzen, 2008). At least 40% of individuals with 22q11.2DS are diagnosed with a neurodevelopmental (NDD) and/or neuropsychiatric (NPD) disorders, with schizophrenia, anxiety disorders, attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD) being the most common (Schneider et al., 2014). Importantly, even when symptom presentations and severity do not reach the diagnostic threshold, they can still significantly impair functioning (Esterberg et al., 2013; Kates et al., 2007; Tang et al., 2014). Therefore, over-reliance on the diagnostic categories can lead to clinically meaningful symptoms being discounted. In addition, individuals classified under separate diagnostic categories can exhibit a range of shared symptoms, while those diagnosed with the same disorder can exhibit different deficits (Antshel et al., 2006; Kates et al., 2015; Niklasson et al., 2009). In line with the current dimensional psychopathology frameworks such as the Research Domain Criteria (RDoC; Insel et al., 2010), it has been suggested that
focusing on symptom domains that cut across different categorically defined disorders and their underlying mechanisms is one potentially fruitful approach for better understanding of the diverse and variable phenotypic manifestations that characterize 22q11.2DS (Baker & Vorstman, 2012; Biswas & Furniss, 2014). In this investigation we focus on one such symptom domain—restricted and repetitive behaviors (RRB) that has been reported to be common in 22q11.2DS population, irrespective of the primary diagnosis (Kates et al., 2007; Ousley et al., 2017; Serur et al., 2019), yet remains poorly characterized in terms of underlying mechanisms.

RRB are one of the core diagnostic features of ASD, however, they are not specific to ASD but are also prevalent across a range of other disorders and genetic syndromes (Moss et al., 2009) where they cannot be fully accounted for by either the potential presence of ASD or intellectual disability (Janes et al., 2013; Rodgers et al., 2012; Waite et al., 2015). Importantly, RRB also occur in young typically developing children (Arnott et al., 2010; Evans et al., 1997; Leekam et al., 2007; Uljarević et al., 2017a). Emergence, increase and decrease of RRB during normative development is paralleled by the trajectory of normative fears and anxieties (Brooker et al., 2014; Evans et al., 1999; Gullone, 2000) suggesting that certain RRB serve to constrain the unpredictability of the environment and to ward off fears and anxieties (Evans et al., 1999; Gesell et al., 1974; Leekam et al., 2011; Zohar & Felz, 2001). The developmental period when both RRB and normative fears/anxieties start to reduce in frequency and severity corresponds to the development of different facets of cognitive control such as attentional control, inhibition of dominant and activation of subdominant responses and the ability to shift between multiple tasks.
and/or mental sets. Cognitive control develops gradually, becoming progressively more advanced and complex from early toddlerhood to the school years and beyond (Bridgett et al., 2013; Zelazo et al., 2003; Zhou et al., 2011). Based on these developmental trends, it was suggested (Evans et al., 2004; Uljarević & Evans, 2017; Uljarević et al., 2017b) that as self-regulation capacities develop, children rely less on RRB behaviors for managing fears. On the other hand, in disorders such as ASD, where delays in impairments in cognitive control are well established (Hill, 2004; Poljac & Bekkering, 2012; Solomon et al., 2008), RRB continue to persist, but given their inflexible nature they are ineffective in reducing anxiety and might lead to its worsening. Indeed, in ASD, (i) anxiety is highly prevalent across the life-span (Lever & Geurts, 2016; van Steensel, et al., 2011; White et al., 2009) and associated with elevated RRB levels (Lidstone et al., 2014; Rodgers et al., 2012), (ii) poor cognitive control is separately associated with both elevated anxiety (Hollocks et al., 2014; Schwartz et al., 2009; Wallace et al., 2016) and RRB (Boyd et al., 2009; Lopez et al., 2005; Mostert-Kerckhoffs et al., 2015; South et al., 2007), and (iii) a recent study by Uljarević et al. (2017b) found that the relationship between RRB and anxiety is mediated by poor cognitive control.

Given that both anxiety and cognitive control are transdiagnostic processes that vary across both normative and clinical populations and have been explicitly described within the RDoC framework, their role in relation to RRB is unlikely to be constrained or specific to ASD but rather to be generalizable to other disorders that exhibit impairments across these two domains. Anxiety is highly prevalent in 22q11.2DS with at least 35% of children and adolescents...
and around 30% of adults having a clinical diagnosis and an additional percentage of individuals show elevated symptoms (Schneider et al., 2014; see Kates et al., 2015 and Tang et al., 2015 for recent overviews). It has been reported that individuals with 22q11.2DS consistently perform more poorly than typically developing controls on a range of tasks tapping into different aspects of cognitive control (Antshel et al., 2010; Campbell et al., 2010; McCabe et al., 2014; Shapiro et al., 2014; Maeder et al., 2016) and these impairments have been found to be predictive of poor social skills, adaptive functioning, positive symptoms of psychosis and externalizing problems (Albert et al., 2018; Anthsel et al., 2014; Kiley-Brabeck & Sobin, 2006). Surprisingly, to our knowledge, no studies to date have investigated the relationship between cognitive control and anxiety with RRB in 22q11.2DS. Therefore, our study aimed to characterize this inter-relationship and further explore whether cognitive control and anxiety predict RRB levels over and above the influence of IQ and the presence of social and communication deficits.

Methods

Participants

Thirty-eight individuals with molecularly confirmed 22q11.2DS and their parents took part in the study (17 females; $M_{age} = 11.15$ years, $SD = 2.46$). Participants were recruited through the UC Davis MIND Institute, postings on online discussion groups, foundation newsletters, and by word of mouth. Twenty-nine participants completed the Wechsler Intelligence Scale for Children—4th edition (WISC-IV; Wechsler, 2003) and four completed the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). This research study was approved by
the Institutional Review Board at the UC Davis. The parents of all participants provided written informed consent. See Table 1. for the descriptive statistics of the sample.

Insert Table 1 Here

Procedures and Measures

The Social Communication Questionnaire (SCQ; Rutter et al., 2003). The SCQ is a 40-item parent-report questionnaire designed to index the severity of impairments in social, communication and repetitive behavior domains. It was originally suggested (Berument et al., 1999) that a cut-off score of 15 represents an optimal balance between sensitivity (.85) and specificity (.75) when screening for ASD, however, a recent meta-analysis by Chestnut et al. (2017) suggests a cut-off score of 11 as more optimal (area under the curve = 0.885). Both the cut-off of 11 and 15 were used in this paper.

The Behavioral Assessment System for Children-2 (BASC-2; Reynolds & Kamphaus, 2004). The BASC-2 is a parent report measure that provides an assessment of clinical and adaptive aspects of behavior. The clinically relevant items are grouped into the following 8 specific clinical scales: Aggression, Anxiety, Attention Problems, Atypicality, Depression, Hyperactivity, Somatization, Withdrawal and the following 3 overarching clinical composite scores: Externalizing Problems, Internalizing Problems, Behavioral Symptoms Index. Adaptive items are grouped into Activities of Daily Living, Adaptability, Functional Communication, and Social Skills scales which jointly constitute Adaptive Skills composite scale. For clinical scales,
T scores between 60 and 69 are considered “at-risk” and scores ≥70 as “clinically significant”. Here we focused on the anxiety scale and the executive functioning scale which assesses the ability to control behavior by planning, anticipating, inhibiting, or maintaining goal-directed activity as measures of anxiety and cognitive control, respectively (Karr & Garcia-Barrera, 2017).

Analysis Plan

Statistical analyses were conducted using the statistical package for the social sciences (SPSS v 25.0, New York, New York, USA). Pearson r and point biserial correlations were used to explore the associations between SCQ RRB scale with chronological age (CA), sex, FSIQ, BASC-2 anxiety and cognitive control scores and SCQ Social and Communication score. Variables significantly associated with SCQ RRB score were then entered into the hierarchical regression model.

Results

Twenty-six percent of participants had SCQ total scores above the cut-off of 15 and 47.4% above the cut-off of 11. Thirty-four percent of participants had BASC-2 anxiety T scores in “at-risk” and a further 23.7% were in the “clinically significant” range. The RRB domains score of the SCQ were significantly associated with BASC-2 anxiety ($r = .44, p = .006$) and executive functioning ($r = .56, p < .001$) scores and with SCQ social and communication score ($r = .43, p = .011$). No significant association between SCQ RRB scores and CA, gender and FSIQ.
was found. SCQ social and communication score was significantly associated with BASC-2 anxiety ($r = .37, p = .027$) and executive functioning ($r = .53, p = .001$). Table 2. shows the pattern of associations described above.

Insert Table 2 Here

Before conducting regression we looked for evidence of multicollinearity. The highest correlation was .56, significantly lower than the .7 suggested as the threshold by Tabachnick and Fidel (2014). In addition, the highest Variance Inflation Factor value in the regression model was 1.53, significantly lower than suggested cut-offs of 5 (Sheather, 2009) or 10 (Kutner et al., 2004), further suggesting that multi-collinearity was not a concern. Table 3 summarizes the hierarchical regression model exploring the predictors of SCQ RRB score. In the first step BASC-2 anxiety score accounted for 24.5% of variance ($F = 10.05, p = .003$). In the second step BASC-2 executive functioning score accounted for an additional 18.1% of variance ($F_{change} = 11.15, p < .001$). Although anxiety was a significant predictor in step 1 ($t = 3.17, p = .003$), it was no longer an independent predictor in step 2 ($t = 1.75, p = .091$) after executive functioning was added ($t = 3.08, p = .004$). In the third step SCQ social and communication score accounted for only 0.8% of additional variance ($F_{change} = .40, p = .53$). The final model accounted for 43.4% of variance ($F = 7.42, p = .001$), with executive functioning as a unique independent predictor of SCQ RRB score ($t = 2.52, p = .01$).

Insert Table 3 here
Given that SCQ SC score was also associated with BASC-2 anxiety and depression, we conducted a follow-up regression model to explore the % of variance accounted by these variables. BASC-2 executive functioning and anxiety explained 29.4% of variance ($F= 5.13, p=0.006$) with executive functioning providing unique significant contribution ($t= 2.49, p=.019$), however, once SCQ RRB score was entered in the regression model BASC-2 executive functioning score was no longer a significant predictor ($t= 1.83, p=.076$).

**Discussion**

This study aimed to further our understanding of restricted and repetitive behaviors (RRB) in individuals with 22q11.2 microdeletion syndrome (22q11.2DS) by exploring their relationship with cognitive control and anxiety as well as with sex, chronological age (CA) and full-scale IQ (FSIQ) in a sample of children and adolescents with this syndrome.

We firstly considered the relationship between the RRB and sex, CA and FSIQ and found no significant associations. Across both ASD and normative literature findings on the relationship between sex and RRB have been mixed, with no differences (Evans et al., 1997; Hus et al., 2007; Joseph et al., 2013; Lam et a., 2008), higher levels in males (Arnott et al., 2010; Cevikaslan et al., 2014; Frazier et al., 2014) or in females (Kim & Lord, 2010) being reported. When considering the lack of age and FSIQ effects on RRB two aspects are important to note. Firstly, despite a lack of long-term longitudinal studies, existing findings suggest that the major
of RRB age-related changes in ASD occur during early childhood when RRB steadily increase and then remain somewhat stable during later childhood and adolescence (Esbensen et al., 2009; Richler et al., 2010; South et al., 2005). Therefore given that our sample included children aged 7-15, the lack of a significant association between CA and RRB is not surprising. Secondly, SCQ provides total RRB scores rather than separate ascertainment of the insistence on sameness and repetitive motor behavior domains which have been shown to have a distinct pattern of associations with CA and FSIQ (Bishop et al., 2013; Cannon et al., 2010; Cuccaro et al., 2003). Therefore total score could have potentially masked some of these effects. Previous studies on RRB in 22q11.2DS have not explored the impact of sex, age or FSIQ on RRB, and therefore it will be important for future research to further replicate findings reported here.

Consistent with the literature on RRB in ASD and other disorders (Lidstone et al., 2014; Janes et al., 2014; Rodgers et al., 2012; South et al., 2007), higher levels of anxiety and poor cognitive control were predictive of higher RRB scores. For example, individual variation in cognitive control has been linked with RRB across both normative (Pietrefesa & Evans, 2007; Peleg-Popko & Dar, 2003; Tregay, Gilmor, & Charman, 2009) and ASD samples (Mostert-Kerckhoffs et al., 2015; South et al., 2007) and with anxiety across both normative and clinical groups, including ASD (Eisenberg et al., 2009; Hollocks et al., 2014; Hughes et al., 2011; Murray & Kochanska, 2002; Wallace et al., 2016). Anxiety has been previously reported to be associated with RRB in typically developing individuals (Evans et al., 1999) as well as in individuals with Down Syndrome (Uljarević & Evans, 2017), ASD (Lidstone et al., 2014;
Wigham et al., 2015) and Williams Syndrome (Rodgers et al., 2012). Finally, the only study to date to explore joint effects of cognitive control and anxiety on RRB in ASD (Uljarević et al., 2017b) has found a similar pattern of findings as reported here. Importantly, although both anxiety and executive functioning deficits were also associated with social and communication deficits as measured by SCQ, the comparison between regression models indicated that anxiety and executive functioning accounted significantly more variance in RRB than social and communicative impairments (42.6% vs 29.4%).

Several study limitations are important to note. Although 22q11.2DS has relatively low prevalence and all analyses were supplemented with bootstrapped confidence intervals, findings reported here should be treated as preliminary given the small sample size. The SCQ is a well-validated screening measure for ASD sampling for both social and communication impairments as well as the presence of RRB. However, as noted above, SCQ only provides overall RRB score and does not allow for more fine-grained sampling of different RRB domains such as repetitive motor behaviors and insistence on sameness that might have distinct underlying mechanisms (Langen et al., 2011; Leekam et al., 2011) nor for capturing of more subtle symptom expressions. This study was further limited by the cross-sectional design. Therefore, it is necessary to extend findings reported here and further characterize the dynamics of the cognitive control-anxiety-RRB inter-relationship using the longitudinal designs and comprehensive and sensitive measures of RRB such as for example the Repetitive Behavior Scale-Revised (Bodfish et al., 2000) or the
Repetitive Behavior Questionnaire-2 (Leekam et al., 2007; Barrett et al., 2015; 2018) as well as multimodal assessments of cognitive control.

Despite the limitations, our pilot study provides a significant contribution by establishing for the first time the link between RRB with impaired cognitive control and anxiety in individuals with 22q11.2DS. It has been suggested (see Leekam et al., 2011; Uljarević et al., 2018 for an overview) that insistence on sameness behaviors, which serve as adaptive way of controlling the environment and warding of anxiety earlier in the development, if persistent, can limit exposure to new environments both directly and indirectly (through reinforcing anxiety) and thus deprive an individual from valuable learning opportunities that are necessary for the acquisition of a range of skills and adaptive strategies necessary for functioning across social, education and vocational contexts. Therefore the ability to reduce the insistence on sameness and increase flexibility can have highly beneficial effects for improving daily life functioning of affected individuals. Given that effective treatment approaches targeting RRB across neurodevelopmental disorders are currently lacking (Grahame et al., 2015; NICE, 2013), findings reported in this study, point to the centrality of the cognitive control domain as a candidate mechanism behind the RRB and therefore as a potentially viable target for treatments aimed at reducing these behaviors.


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Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>11.15</td>
<td>2.46</td>
</tr>
<tr>
<td>FSIQ*</td>
<td>68.50</td>
<td>16.86</td>
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<tr>
<td>SCQ Total</td>
<td>10.31</td>
<td>7.50</td>
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<tr>
<td>SCQ RRB</td>
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<td>2.11</td>
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<td>SCQ SC</td>
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<td>6.41</td>
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<tr>
<td>BASC-2 Anxiety</td>
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<td>14.98</td>
</tr>
<tr>
<td>BASC-2 Executive Function</td>
<td>66.11</td>
<td>10.60</td>
</tr>
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</table>

Note: *data available for N= 33 participants; BASC-2: The Behavioral Assessment System for Children-2; FSIQ: Full-Scale IQ; RRB: restricted and repetitive behaviors; SC: Social and Communication; SCQ: Social Communication Questionnaire.
Table 2. Correlations between variables of interest

<table>
<thead>
<tr>
<th></th>
<th>SCQ RRB</th>
<th>CA</th>
<th>Sex</th>
<th>SCQ SC</th>
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<td>SCQ RRB</td>
<td>1</td>
<td>-.22</td>
<td>-.06</td>
<td>.43*</td>
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<td>FSIQ</td>
<td>.02</td>
<td>-.04</td>
<td>.15</td>
<td>-.12</td>
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<tr>
<td>BASC-2 Executive</td>
<td>.56**</td>
<td>-.40*</td>
<td>-.007</td>
<td>.53**</td>
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<tr>
<td>BASC-2 Anxiety</td>
<td>.44**</td>
<td>-.13</td>
<td>.07</td>
<td>.37*</td>
</tr>
</tbody>
</table>

Note: *p< .05; **p< .01; ; BASC-2: The Behavioral Assessment System for Children-2; CA: Chronological Age; FSIQ: Full Scale IQ; RRB: restricted and repetitive behaviors; SC: Social and Communication; SCQ: Social Communication Questionnaire.
<table>
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<tr>
<th>Step</th>
<th>R²Δ</th>
<th>R²Δ Change</th>
<th>B</th>
<th>SEB</th>
<th>β</th>
<th>BCa 95% CI</th>
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<td><strong>Step 1</strong></td>
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<td></td>
<td>-1.98</td>
<td>1.35</td>
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<tr>
<td>BASC-2 Anxiety</td>
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<td>.067</td>
<td>.021</td>
<td>.495</td>
<td>.024; .110</td>
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<td>.181**</td>
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<td>1.762</td>
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<tr>
<td>BASC-2 Anxiety</td>
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<td>.037</td>
<td>.021</td>
<td>.272</td>
<td>-.006; .080</td>
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<td>BASC-2 Executive Functioning</td>
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<td>.089</td>
<td>.029</td>
<td>.481</td>
<td>.030; .148</td>
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<tr>
<td><strong>Step 3</strong></td>
<td>.434**</td>
<td>.008</td>
<td>-5.446</td>
<td>1.962</td>
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</tr>
<tr>
<td>BASC-2 Anxiety</td>
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<td>.033</td>
<td>.022</td>
<td>.245</td>
<td>-.012; .078</td>
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<td>BASC-2 Executive Functioning</td>
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<td>0.032</td>
<td>0.436</td>
<td>0.015; 0.146</td>
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<tr>
<td>SCQ SC</td>
<td>0.036</td>
<td>0.056</td>
<td>0.108</td>
<td>-0.080; 0.151</td>
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Note: *p< .05; **p< .01; ; BASC-2: The Behavioral Assessment System for Children-2; FSIQ: Full Scale IQ; RRB: restricted and repetitive behaviors; SC: Social and Communication; SCQ: Social Communication Questionnaire
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