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Toward better characterization of restricted and repetitive behaviors in individuals with germline heterozygous PTEN mutations

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Title: Towards better characterization of restricted and repetitive behaviors in individuals with germline heterozygous *PTEN* mutations

Running Head: RRB in *PTEN* mutations

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Abstract

This study aimed to further our understanding of restricted and repetitive behaviors (RRB) among individuals with germline pathogenic mutations in *PTEN* by providing multi-method characterization and comparison of key RRB subdomains across individuals with *PTEN* mutations with autism spectrum disorder (ASD) (*PTEN*-ASD), with *PTEN* mutations without ASD (*PTEN*-No ASD) and with ASD and macrocephaly but without *PTEN* mutations (Macro-ASD). Of 86 total research participants, 38 had *PTEN*-ASD (M_{age}= 8.93 years, SD_{age}= 4.75), 25 Macro-ASD (M_{age}= 11.99 years; SD_{age}= 5.15) and 23 *PTEN*-No ASD (M_{age}= 8.94 years; SD_{age}= 4.85). The Repetitive Behavior Scale-Revised (RBS-R) and the Autism Diagnostic Interview-Revised (ADI-R) were used as measures of distinct RRB domains. There were significant group differences in the RBS-R repetitive motor behaviors (RMB; F= 4.52, p= .014, ω^2= .08), insistence on sameness (IS; F= 4.11, p= .02, ω^2= .05) and circumscribed interests (CI; F= 7.80, p= .001, ω^2= .14) scales. Post-hoc comparisons showed that the *PTEN*-No ASD group had significantly lower RMB, IS and CI scores compared to both *PTEN*-ASD and Macro-ASD groups. Importantly, *PTEN*-No ASD group still showed elevated RRB levels. Furthermore, there was a portion of individuals in *PTEN*-No ASD group whose Full-Scale Intelligence Quotient (FSIQ) was >70 that did not show floor level scores in the RMB domain. After adjusting for age and FSIQ scores, group differences were no longer statistically significant. RMB, IS and CI domains showed distinct association patterns with sex, age, and FSIQ. This investigation provides the largest and most comprehensive characterization of distinct RRB domains in individuals with *PTEN* mutations to date. Despite the limitations, our findings have important assessment and treatment implications.

Key Words: *PTEN*, Macrocephaly, Autism, Repetitive Behaviors, Repetitive Motor Behaviors, Insistence on Sameness, Circumscribed Interests

1 Introduction

It has long been recognized that individuals with autism spectrum disorder (ASD) vary widely in terms of onset, configuration, severity and course of core and co-occurring symptoms as well as response to treatments (Bryson et al., 2007; Prior et al., 1998). Given that phenotypic complexity and heterogeneity of ASD likely reflects distinct underlying causes, it has been suggested that focusing on individuals who share genetic risk factors can minimize the noise related to etiological heterogeneity (Berg & Geschwind, 2012; Stessman, Bernier, & Eichler, 2012). Germline pathogenic mutations in the *PTEN* gene, which has an important influence on early neural development, synaptic plasticity and neuronal cytoarchitecture (Sanchez-Puelles et al., 2020; Tilot, Frazier, & Eng, 2015), have been established as important genetic risk factors for ASD (Frazier, 2019; Yehia, Keel, & Eng, 2020). More specifically, at least 23% of individuals with *PTEN* mutations meet the diagnostic criteria for ASD (Ciaccio et al., 2019; Hansen-Kiss et al., 2017; Tan et al., 2011) and pathogenic *PTEN* mutations are identified in approximately 2% of all ASD cases. Furthermore, between 17 and 20% of individuals with ASD and macrocephaly have pathogenic PTEN mutations (Sanchez-Puelles et al., 2020).

The majority of existing research on the *PTEN*—ASD link has adopted a categorical view of ASD (e.g., focusing on % of individuals meeting diagnostic criteria for ASD); however, more recent studies have started to explore whether individuals with *PTEN* mutations exhibit distinct ASD symptom profiles when compared to idiopathic ASD cases (Busch et al., 2019; Frazier et al., 2015). For instance, in a comprehensive molecular and phenotypic comparison between 17 individuals with *PTEN* mutations and ASD (*PTEN*-ASD) and individuals with idiopathic ASD with (Macro-ASD; n= 16) and without (n= 38) macrocephaly, Frazier et al. (2015) reported no significant group differences in terms of ASD symptomatology. Frazier and

colleagues focused on overall ASD symptom severity assessed via total Social Responsiveness Scale (SRS-2; Constantino & Gruber, 2012) score and social interaction, non-verbal communication and restricted and repetitive behaviors (RRB) subscale scores of the Autism Diagnostic Interview-Revised (ADI-R; Rutter, Le Couteur, & Lord, 2003). A recent study from our group (Busch et al., 2019) found no significant differences between *PTEN*-ASD and Macro-ASD groups on total SRS-2 total scores and the Repetitive Behavior Scale-Revised (RBS-R; Bodfish et al., 2000) total scores, but reported significantly lower total Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2012) scores in the *PTEN*-ASD group compared to the Macro-ASD group. In addition, we also found that while the *PTEN*-No ASD group had higher Full-Scale Intelligence Quotient (FSIQ) and lower total SRS-2 and RBS-R scores than the *PTEN*-ASD group, they nevertheless showed significantly higher RBS-R total scores than healthy controls (Busch et al., 2019), suggesting a higher of severity in this domain.

Although based on the studies conducted thus far, it does not appear that *PTEN* mutations are associated with a specific ASD phenotype, these studies have relied on broad, heterogeneous symptom domains such as overall severity of socio-communicative impairments or RRB. Given that each of these two domains is complex and multifactorial, the crucial next step towards a better understanding of the *PTEN*—ASD link is to focus on more discrete symptom domains with distinct trajectories, clinical correlates and hypothesized underpinning mechanisms. Therefore, this study aimed to provide a multi-method, fine-grained characterization and comparison of key RRB subdomains across *PTEN*-ASD, *PTEN*-No ASD and Macro-ASD groups.

RRB are a core diagnostic symptom domain of ASD and one of the earliest predictors of subsequent ASD diagnosis (Canu et al., 2020; Ozonoff et al., 2008). RRB are also observed

across a range of other neurodevelopmental, neuropsychiatric disorders and genetic syndromes including Down Syndrome (Evans & Gray, 2000; Evans et al., 2014; Glenn et al., 2015; Uljarević & Evans, 2017), Fragile X Syndrome (Moss et al., 2009; Oakes et al., 2016), 22q11.2 Deletion Syndrome (Uljarević et al., 2019), Williams Syndrome (Rodgers et al., 2012; Vivanti et al., 2018) and Prader-Willi Syndrome (Flores et al., 2011; Greaves et al., 2006). Regardless of the disorder, RRB represents a complex and heterogeneous cluster of symptoms and behaviors ranging from simple motor mannerisms, such as hand or finger flapping or twisting, to complex routines or rituals. Indeed, factor analytic studies conducted across a range of dedicated RRB measures including the RBS-R, the Repetitive Behavior Questionnaire (Turner, 1995) and the Repetitive Behavior Questionnaire-2 (Leekam et al., 2007) and general diagnostic/symptom severity measures such as the ADI-R (Rutter et al., 2003) have most consistently identified a two- or three-factor solution encompassing Repetitive Motor Behaviors (RMB), Insistence on Sameness (IS) and Circumscribed Interests (CI) (Barrett et al., 2018; Bishop et al., 2013; Honey et al., 2008; Lam et al., 2008; Uljarević et al., 2021a).

Research to date has demonstrated that across ASD and other neurodevelopmental disorders and normative development, RMB, IS and CI RRB domains show distinct trajectories and patterns of association with cognitive functioning and other clinical domains. For instance, longitudinal studies in both ASD (Richler et al., 2010) and normative development (Uljarević et al., 2017a) have suggested that while RMB tend to decrease with age, IS tend to increase. Cross-sectional studies have consistently found age and IQ to be negatively associated with RMB (Bishop et al., 2013; Cuccaro et al., 2003); however, positive (Bishop, Richler, & Lord, 2006), and non-significant (Hus et al., 2007; Lam et al., 2008; South, Ozonoff, & McMahon, 2005) associations with IS and CI have been reported. Preliminary evidence further suggests that RMB,

IS and CI domains might be underpinned by distinct mechanisms. More specifically, RMB are hypothesized to be related to atypical arousal and motor control subserved by a sensory-motor corticostriatal circuit, IS to be related to elevated anxiety and impaired cognitive control subserved by amygdala-ventromedial prefrontal cortex circuitry and associative corticostriatal circuits, and CI to be related to atypical reward processing subserved by the limbic corticostriatal circuit (Langen et al., 2011; Wilkes & Lewis, 2018).

In summary, current evidence from factor analytic, clinical, cognitive and neuroimaging studies suggests that rather than representing a unitary category, RRB are best understood as multidimensional constructs comprising of at least RMB, IS and CI domains. Given that these domains likely have distinct mechanisms (Langen et al., 2011; Leekam, Prior, & Uljarević, 2011), they might require different treatments. Regardless of the primary diagnosis, RRB have a significant negative impact on concurrent functioning and long-term outcomes of affected individuals and their families (Boyd et al., 2012; Grahame et al., 2015; Leekam et al., 2011). Therefore, there is an urgent need to provide a fine-grained understanding of the profile of these clinically impactful symptoms in individuals with *PTEN* mutations. Our study aimed to compare the profile of RMB, IS and CI RRB domains captured by an RRB-specific questionnaire (RBS-R) and gold standard ASD diagnostic instrument (ADI-R) across *PTEN*-ASD, Macro-ASD and *PTEN*-No ASD groups. It further aimed to characterize the effects of age, sex and cognitive functioning on the expression of distinct RRB domains across noted clinical groups. Characterizing the association with age and cognitive functioning was particularly important given that these variables have been previously shown to influence the expression and severity of RRB domains in genetic syndromes (Evans & Gray, 2000; Evans et al., 2014).

2 Methods

2. 1. Research Participants

After informed consent under IRB-approved protocols, research participants were recruited across four sites (Cleveland Clinic, Stanford University Department of Psychiatry and Behavioral Sciences, University of California at Los Angeles and Boston Children's Hospital) as part of an ongoing, multicenter study designed to examine the natural history of ASD and germline heterozygous *PTEN* mutations (clinicaltrials.gov: NCT02461446). All participants underwent a screening assessment by a clinical psychologist to ascertain whether they met DSM-5 ASD diagnostic criteria. In cases where screening indicated the presence of ASD, the diagnosis was confirmed through the administration of the ADI-R and the ADOS-2 (therefore ADI-R was not utilized with individuals in *PTEN*-No ASD group). In addition, all participants completed genetic testing to confirm the presence of *PTEN* mutation and were evaluated for macrocephaly (defined as having occipitofrontal head circumference ≥98th percentile). Informed consent was provided by the patient or a parent or legal guardian. Assent was obtained for all participants aged 7 years or older who were of sufficient cognitive ability. The final sample was comprised of 38 individuals with *PTEN* mutation and ASD diagnosis (*PTEN*-ASD; M_{age}= 8.93 years, SD_{age}= 4.75), 23 individuals with *PTEN* mutation but without ASD diagnosis (*PTEN*-no ASD; M_{age}= 8.94 years; SD_{age}= 4.85) and 25 individuals with ASD and macrocephaly but without *PTEN* mutation (Macro-ASD; M_{age}= 11.99 years; SD_{age}= 5.15). The study was approved by the Institutional Review Boards across all four sites.

2. 2. Measures

The Repetitive Behavior Scale-Revised (RBS-R; Bodfish et al., 2000). The RBS-R is a 43-item parent-report scale designed to provide a comprehensive assessment of different types of restricted and repetitive behaviors (RRB) organized into six subscales. Each item is rated on a 4-

point Likert scale (from 0= behavior does not occur to 3= behavior occurs and is a severe problem). Here, we have primarily focused on the Stereotypic (six items), Sameness (11 items) and Restricted Behavior (four items) subscales as measures of RMB, IS and CI, respectively. We have also conducted supplemental analyses for the self-injurious, compulsive and ritualistic subscales. In this analysis, we have focused on the original 6-factor structure of the RBS-R (Bodfish et al., 2000). We have also conducted a supplemental analysis utilizing the 5-factor structure derived by Bishop et al. (2013) (e.g., stereotypic, ritualistic/sameness, self-injurious, compulsive and restricted behaviors) subscales to explore potential differences with the original factor scores.

The Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 2003). ADI-R is a semi-structured parent interview designed to assess a range of impairments consistent with the ICD-10 and DSM-IV diagnostic criteria for ASD. For the majority of items, both current (behavior during the past 3 months) and ever (behavior in early childhood or at its greatest severity), scores are provided. Although ADI-R originally provided only a total RRB score, subsequent factor analyses of the RRB domain items suggested two- and three-factor solutions (Bishop et al., 2013; Honey et al., 2008; Lam et al., 2008; Uljarević et al., 2021a). The three-factor solution proposed by Lam et al. (2008) including RMB (three items), IS (three items) and CI (three items) was utilized here.

2. 3. Analysis Plan

Statistical analyses were conducted using the Statistical Package for the Social Sciences (IBM Corp, 2017). Diagnostic groups were compared on relevant variables using ANOVAs. Given the age and Full-Scale Intelligence Quotient (FSIQ) differences between groups, these variables were included as co-variates in follow-up univariate models. All comparisons were

supplemented with ω^2 and post-hoc comparisons with Cohen's *d* effect sizes. Given that as noted, ADI-R was not administered to participants in *PTEN*-no ASD group for whom clinical assessment did not indicate the presence of ASD, comparisons for ADI-R RRB scores were conducted only between *PTEN*-ASD and Macro-ASD groups. Pearson *r* and point bi-serial correlations were used to explore the associations between RRB scores and chronological age (CA), sex, and FSIQ.

3 Results

3. 1. Preliminary Analyses

Baseline descriptive statistics stratified by the group are presented in Table 1. There were no sex, race or ethnic differences among the three groups; however, there were significant differences in terms of age (F= 3.45, p= .035, ω^2= .05) and Full-Scale Intelligence Quotient (FSIQ) (F= 14.43, p< .001, $\omega 2$= .27). The Macro-ASD subgroup was older than the *PTEN*-ASD subgroup (p= .045, d= .04), and the *PTEN*-No ASD group had higher FISQ scores than the two ASD groups (p< .001, d range: .13-.18).

Insert Table 1 Here

3. 2. Main Analyses

3. 2. 1. Repetitive Behavior Profiles across Clinical Groups

The distribution of the Repetitive Behavior Scale-Revised scores is presented in Figure 1. There were significant group differences for repetitive motor behaviors (F= 4.52, p= .014, ω^2= .08), insistence on sameness (F= 4.11, p= .02, ω^2= .05) and circumscribed interests subscale scores (F= 7.80, p= .001, ω^2= .14). Post-hoc comparisons indicated that, when compared to

PTEN-ASD and Macro-ASD groups, the *PTEN*-No ASD group had significantly lower repetitive motor behaviors (*d* range: .58-.74) and circumscribed interests (*d* range: .94-1.05) scores. The *PTEN*-No ASD group had significantly lower insistence on sameness scores than the *PTEN*-ASD group (p= .015, d= .76). There were no significant differences between *PTEN*-ASD and Macro-ASD groups nor between *PTEN*-No ASD and Macro-ASD groups. Given noted age and FSIQ differences among groups, a series of univariate models were used to explore the contribution of these variables to the noted differences across the Repetitive Behavior Scale-Revised subscales. After adjusting for the age and FSIQ, there were no longer significant group differences for repetitive motor behaviors (F= .32, p= .73, $\omega 2$= .017; Covariate - FSIQ: p< .001, $\omega 2$= .017, age: p= .76, $\omega 2$= .011), insistence on sameness (F= .77, p= .47, $\omega 2$= .006; Covariate - FSIQ: p= .061, $\omega 2$= .037, age: p= .85, $\omega 2$= .014) and circumscribed interests (F= 1.84, p= .17, $\omega 2$= .022; Covariate - FSIQ: p= .023, $\omega 2$= .058, age: p= .90, $\omega 2$= .013).

Supplemental comparisons showed significant group differences for self-injurious behaviors (F= 3.72, p= .028, $\omega 2$= .85; *PTEN*-No ASD had significantly lower scores than Macro-ASD group [p= .032, d= .77]), compulsions (F= 4.44, p= .015, $\omega 2$= .10; *PTEN*-No ASD showing significantly lower scores than *PTEN* ASD group [p= .011, d= .82]) and for ritualistic behaviors (F= 4.63, p= .012, $\omega 2$=.10; *PTEN*-No ASD had significantly lower scores than Macro-ASD group [p= .012, d= .95]). After adjusting for the age and FSIQ scores, there were no longer significant group differences for self-injurious behaviors (F= 2.10, p= .13, $\omega 2$= .06; Covariate - FSIQ: p= .28, $\omega 2$= .017, age: p= .71, $\omega 2$= .002) and compulsions scores (F= .71, p= .49, $\omega 2$= .021; Covariate - FSIQ: p= .009, $\omega 2$= .098, age: p= .84, $\omega 2$= .001) but significant differences remained for ritualistic behaviors (F= 3.81, p= .027, $\omega 2$= .10; Covariate - FSIQ: p= .925, $\omega 2$= .00, age: p= .998, $\omega 2$= .00). Finally, supplemental comparison utilizing the 5-factor

structure derived by Bishop et al. (2013) showed pattern of findings in line with original 6-factor structure (utilized above), therefore, these findings were not reported here.

Insert Figure 1 Here

The distribution of the Autism Diagnostic Interview-Revised (ADI-R) Current and Ever repetitive behaviors item scores is presented in Figure 2. There were no significant differences between *PTEN*-ASD and Macro-ASD group for ADI-R repetitive motor behaviors (Current: F= .54, p= .45, ω^2= .008; Ever: F= .49, p= .49, ω^2= .009), insistence on sameness (Current: F= .01, p= .90, ω^2= .018; Ever: F= .05, p= .83, ω^2= .017) and circumscribed interests (Current: F= .01, p= .90, ω^2= .018; Ever: F= .18, p= .67, ω^2= .015).

Insert Figure 2 Here

3. 2. 2. Correlates of Repetitive Behaviors Domains

The association between sex, age and FSIQ with the Repetitive Behavior Scale-Revised (RBS-R) and Autism Diagnostic Interview-Revised (ADI-R) repetitive behaviors subscales stratified by clinical group is shown in Supplementary Figures 1 and 2. As is evident across both measures and groups, repetitive motor behaviors were not significantly associated with age. There was a statistically significant association with FSIQ for RBS-R repetitive motor behaviors scores in *PTEN*-ASD (r= -.57, p< .001) and Macro-ASD (r= -.45, p= .034) groups and for ADI-R repetitive motor behaviors scores in *PTEN*-ASD (r= -.57, p< .001) group. RBS-R insistence on sameness and circumscribed interests subscales were not associated with age or FSIQ except for the *PTEN*-ASD group where both subscales were significantly negatively associated with FSIQ (r= -.56, p< .001 and r= -.57, p< .001, respectively). ADI-R circumscribed interests subscale was significantly negatively associated with FSIQ in the Macro-ASD sample (r= -.46, p= .033). Sex

was associated only with RBS-R insistence on sameness subscale in the Macro-ASD sample where male sex was related to higher insistence on sameness scores (r= -.41, p= .047).

4 Discussion

In this study, we sought to develop a more nuanced understanding of restricted and repetitive behaviors (RRB) in individuals with germline pathogenic mutations in the *PTEN* gene. To achieve this goal, we utilized a multi-method approach to compare fine-grained RRB profiles across individuals with germline *PTEN* mutations with (*PTEN*-ASD) and without (*PTEN*-No ASD) ASD diagnosis and individuals with ASD and macrocephaly but without *PTEN* mutations (Macro-ASD). Our findings showed that *PTEN*-ASD and Macro-ASD groups had comparable repetitive motor behaviors (RMB), insistence on sameness (IS) and circumscribed interests (CI) scores and that both groups had significantly higher RRB scores than the *PTEN*-No ASD group. However, after adjusting for age and Full-Scale Intelligence Quotient (FSIQ) scores, differences between groups were no longer significant. Additionally, we evaluated several individual characteristics as potential correlates of distinct RRB domains within and across *PTEN*-ASD, *PTEN*-No ASD and Macro-ASD clinical groups and found that RMB, IS and CI domains showed a distinct pattern of associations with sex and FSIQ.

We first considered group differences in RMB, IS and CI RRB domains captured by a dedicated RRB questionnaire measure (RBS-R) and gold standard ASD diagnostic instrument (ADI-R). No statistically significant differences were found between *PTEN*-ASD and Macro-ASD groups on either of the instruments. Not surprisingly, the *PTEN*-No ASD group had lower RBS-R RMB, IS and CI scores as well as higher cognitive ability when compared to *PTEN*-ASD and Macro-ASD groups. These findings are in line with previous studies suggesting that individuals with *PTEN* mutations without ASD diagnosis generally show a much milder

cognitive phenotype and have fewer behavioral difficulties (Busch et al., 2013; Busch et al., 2019). After adjusting for age and cognitive ability, these differences were no longer meaningful. In addition, as can be seen from Figure 1, there was wide variability in the distribution of RRB scores in the *PTEN*-No ASD group, in particular with regards to IS and RMB domains. This suggests that a portion of these individuals show elevated RRB levels, consistent with our recent finding that *PTEN*-No ASD have significantly higher RBS-R total scores than healthy controls (Busch et al., 2019).

Our second aim was to characterize the association between RMB, IS and CI with age, cognitive functioning and sex. When considering the pattern of association within the RMB domain, it is important to note that although this domain was originally considered as indicative of developmental delay (Prior & McMilan, 1973; Turner, 1999), subsequent empirical studies have provided a more nuanced elaboration of the notion of RMB as "lower-order" RRB. More specifically, although RMB are indeed characteristic of early normative development, sharply declining after children reach 15 months of age (Uljarević et al., 2017a) and tend to be associated with lower IQ in ASD (Bishop et al., 2006; Bishop et al., 2013) and genetic syndromes such as Fragile X Syndrome (Oakes et al., 2016), they are nevertheless prevalent among ASD individuals with IQ in the normative range (Richler et al., 2010; South et al., 2005; Uljarević et al., 2020). This is in line with our findings that while higher RMB scores were associated with lower FSIQ across both measures, particularly among *PTEN*-ASD and Macro-ASD groups, there was still a portion of individuals with *PTEN*-No ASD whose FSIQ was in > 70 range who did not show floor level scores in RMB domain. A number of studies did not find sex differences on any of the RRB domains (Bourreau et al., 2009; Hus et al., 2007; Lam et al., 2008; Sutherland et al., 2017), in line with the lack of significant association between sex and RMB and CI domains

reported here. It will be important for future studies to further investigate the association between male sex and higher severity of IS in the Macro-ASD group reported here.

The general lack of significant association between IS with age and FSIQ reported in our study is in line with a range of studies in ASD samples (Hus et al., 2007; Lam et al., 2008; South et al., 2005). Interestingly, RBS-R IS subscale was negatively associated with FSIQ in the *PTEN*-ASD group. There are two potential interpretations of this finding. First, it has been suggested that individuals with a high degree of IS, due to their rigidity and inflexibility, often limit their exposure to novel situations, thus reducing learning opportunities. This, in turn, has a negative effect on cognitive development (Leekam et al., 2011). Although we did not find a negative IS-FSIQ association in the Macro-ASD group, individuals in this group were significantly older than *PTEN*-ASD individuals and had higher (albeit non-significantly) FSIQ. Given the cross-sectional nature of our study, it will be important to characterize the potential impact of IS on cognitive development using a longitudinal design and explore whether some of the effects might be constrained to particular developmental periods. Second, previous research has suggested a strong shared variance between IQ and executive functioning (EF) at both a behavioral and genetic level (Engelhardt et al., 2016; Friedman et al., 2008). Given the well-established link between IS and EF impairments (South et al., 2005; Uljarević et al., 2017a; Uljarević et al., 2019; Wallace et al., 2016) and EF deficits with the presence of *PTEN* mutations (Busch et al., 2013; Busch et al., 2019; Frazier et al., 2015), it is possible that in our study FSIQ also represented a proxy for EF. Indeed, a recent study by our group has found that more impairments in the set-shifting EF subdomain predicted higher IS severity in individuals with *PTEN* mutations and individuals with macrocephalic ASD (Uljarević et al., 2021b).

Findings regarding the correlates of the CI domain were inconsistent and more complex to interpret. The significant negative association between RBS-R CI subscale with FSIQ is inconsistent with previous ASD studies that have shown either positive (Honey et al., 2008) or the lack of significant association (Hus et al., 2007; Lam et al., 2008; South et al., 2005) between these variables. Although mechanisms and correlates of CI across neurodevelopmental disorders remain under-researched and are generally poorly characterized, CI have been previously linked with EF deficits (Anthony et al., 2013; Faja & Darling, 2019). Therefore, as with IS, it is possible that lower FSIQ might have served as a proxy for impaired EF, which would explain the negative FSIQ—CI association. It is also important to emphasize that the CI domain itself is broad and encompasses behaviors, activities and interests that are (i) unusual in terms of focus and intensity, but otherwise typical/age-appropriate with regards to their content and (ii) unusual in terms of their content, for instance, fascination with numbers, specific objects or events such as telephone poles and fans, and fascination with sensory properties of objects and stimuli. Given the complexity, different facets of CI might be differently associated with FSIQ and other clinical correlates. For instance, CI that are idiosyncratic in terms of content or focused on sensory stimuli might be more prevalent in younger individuals with lower cognitive ability and more readily observed during the diagnostic assessment process when compared to more conceptual CI. Importantly, despite the noted complexity of the CI domain, with the exception of the Yale Special Interests Interview (Klin et al., 2007; South et al., 2005) and the Special Interest Motivation Scale (Grove, Roth, & Hoekstra, 2015), current instruments do not provide a comprehensive assessment of different CI facets. For example, both ADI-R and RBS-R have four or fewer items that capture CI. Therefore, it will be crucial for future studies to utilize

comprehensive CI measures in order to provide a better understanding of the phenomenology and correlates of this RRB domain in *PTEN* mutations.

Several study limitations are important to note. Given the modest sample size, the results reported here should be treated as preliminary. However, it is important to emphasize that all analyses were supplemented with effect sizes and utilized robust statistics. Further, *PTEN* mutations have low prevalence and our study is the largest and most detailed characterization of RRB in this population to date. Although we utilized a multi-method approach combining parent-report questionnaires and interview assessments, ADI-R was not available for the *PTEN*-No ASD group. Finally, findings regarding the associations between RRB domains with age and cognitive functioning are necessarily limited by the cross-sectional design and longitudinal ongoing investigation will shed light on nature and dynamics of these associations.

5 Conclusions

Despite noted limitations, findings reported here have important assessment and treatment implications and offer promising insights into understanding the RRB profiles in individuals with *PTEN* mutations. More specifically, our findings that RMB, IS and CI showed a distinct pattern of associations with age and cognitive functioning across subgroups support the hypothesis that RMB, IS and CI are indeed distinct RRB domains that might, therefore, require different treatment approaches in this population. Further, our findings suggest heterogeneity in the profile of RRB subdomains within each clinical subgroup thus emphasizing the need to individually tailored treatment options. To better inform treatment selection, future studies will need to adopt individual differences statistical approaches such as cluster analysis and factor mixture modeling in order to identify subgroups of individuals characterized by distinct RRB profiles. This would be a crucial initial step towards understanding the individuation of treatment

options and transitioning to precision medicine. Finally, this study emphasizes the need for the development of new quantitative RRB instruments that would enable comprehensive capture of the full range of RRB expressed not only in ASD but also across other neurodevelopmental and genetic disorders.

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Charis Eng, Mustafa Sahin, Thomas W Frazier, and Antonio Y Hardan designed the study. Charis Eng, Mustafa Sahin, Antonio Y Hardan, Robyn M. Busch, Patricia Klaas, Siddharth Srivastava, and Julian A. Martinez-Agosto collected the data. Mirko Uljarević and Gaëlle Rached had full access to the data and conducted the analyses. Mirko Uljarević and Antonio Y Hardan drafted the initial manuscript. All authors critically reviewed and provided the feedback on the initial version of manuscript. All authors approved the final version of the manuscript.

Data Availability Statement

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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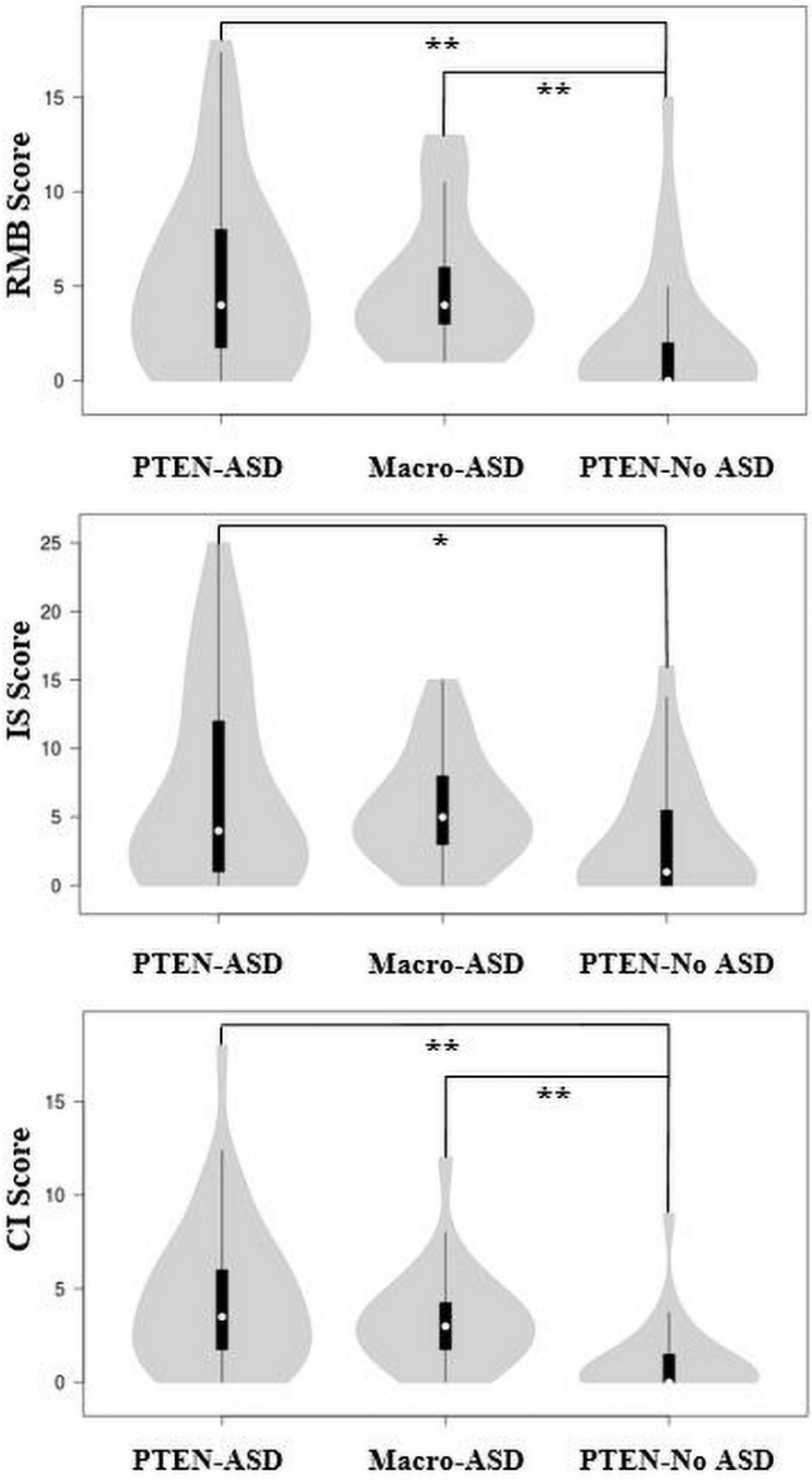

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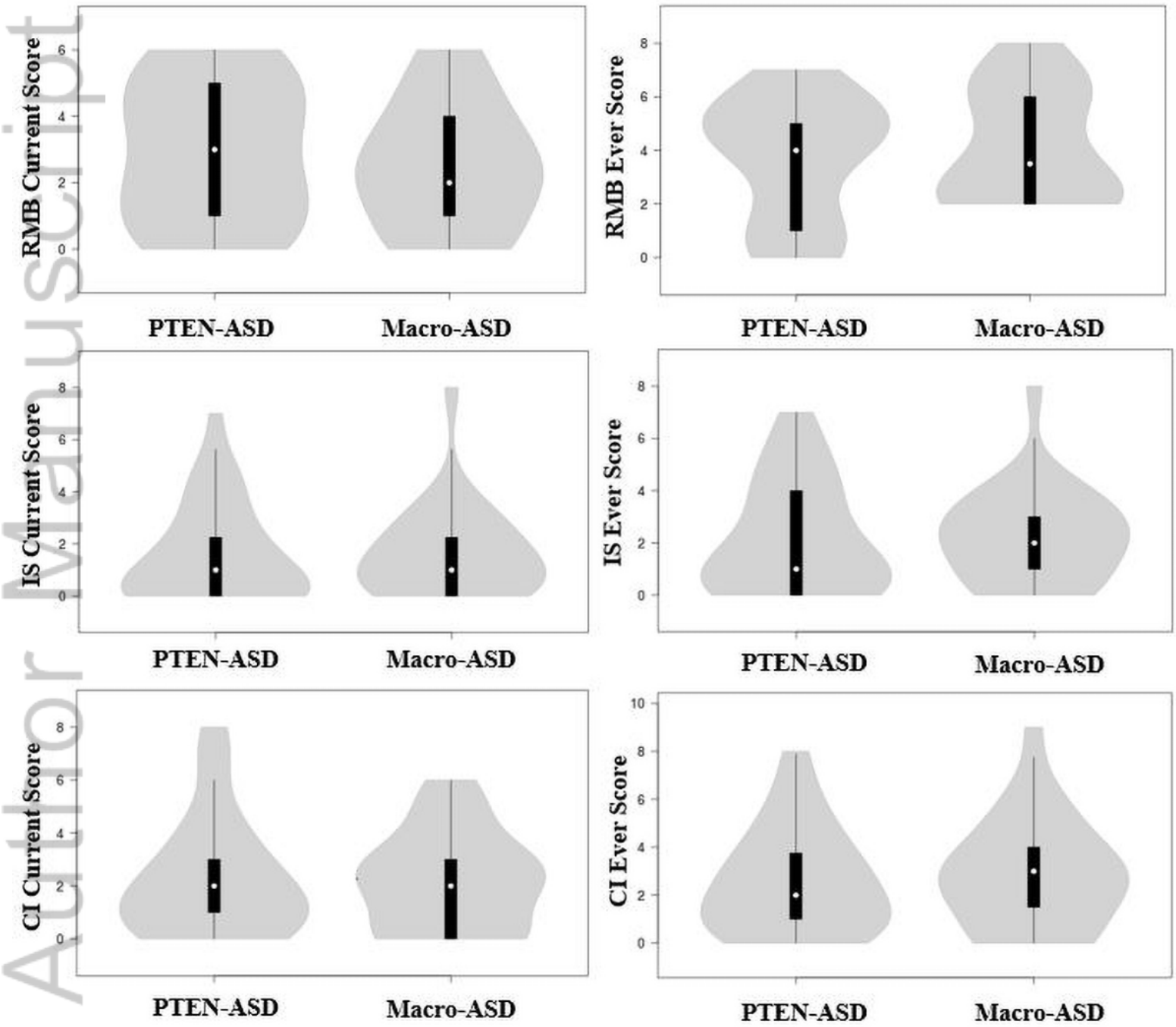

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Table 1. Demographic Characteristics and Descriptives

	***PTEN*-ASD[a]**	**Macro-ASD[b]**	***PTEN*-No ASD[c]**	**Between Subgroup Comparisons**			**BCa 95% CI**		
	N= 38	**N= 25**	**N= 23**	**F/χ2**	**p**	**ω^2/ϕ**	**Contrast a**	**Contrast b**	**Contrast c**
CA (Mean [SD])	8.93 (4.75)	11.99 (5.15)	8.94 (4.85)	3.45	.036	.05	-6.07, -.05	-.33, 6.42	-3.10, 3.07
Sex (Number [%])				2.55	.28	.98	-	-	-
Female	8 (21.1)	4 (16)	8 (34.8)						
Male	30 (78.9)	21 (84)	15 (65.2)						
FSIQ (Mean [SD])	66.32 (22.95)	74.30 (24.50)	99.14 (17.40)	14.43	<.001	.27	-22.79, 6.82	-40.52, -9.15	-47.80, -17.83
Race (Number [%])				16.01	.099	.30	-	-	-
White/Caucasian	30 (78.9)	14 (56)	12 (52.2)						
Black/African American	1 (2.6)		-						
Asian	-	5 (20)	3 (13)						
Multiracial	5 (13.2)	5 (2)	6 (26.1)						
Pacific Islander	-	1 (4)	-						
Unknown/Not Reported	2 (5.3)	-	2 (8.7)						
Ethnicity (Number [%])				3.25	.517	.19	-	-	-
Hispanic	6 (15.8)	2 (8)	1 (4.3)						
Not Hispanic	31 (81.6)	23 (92)	21 (91.4)						
Unknown/Not Reported	1 (2.6)		1 (4.3)						

Note: Contrast a= *PTEN*-ASD vs. Macro-ASD, Contrast b= Macro-ASD vs. *PTEN*-No ASD, Contrast c= *PTEN*-ASD vs. *PTEN*-No ASD; BCa 95% CI= bias-corrected and accelerated bootstrapped confidence intervals; CA= Chronological Age; FSIQ= Full-Scale Intelligence Quotient Macro-ASD= individuals with ASD and macrocephaly but without *PTEN* mutations; *PTEN*-ASD= individuals with *PTEN* mutations with autism spectrum disorder (ASD); *PTEN*-No ASD= individuals with *PTEN* mutations without ASD.