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Emergence of Restricted Repetitive Behaviors in Individuals with Autism Spectrum Disorder and Tuberous Sclerosis Complex

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EMERGENCE OF RESTRICTED REPETITIVE BEHAVIORS IN INDIVIDUALS WITH AUTISM SPECTRUM DISORDERS AND TUBEROUS SCLEROSIS COMPLEX

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EMERGENCE OF RESTRICTED REPETITIVE BEHAVIORS IN INDIVIDUALS WITH AUTISM SPECTRUM DISORDER AND TUBEROUS SCLEROSIS COMPLEX

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MEDICAL/CLINICAL PSYCHOLOGY

ABSTRACT

Genetic disorders are ideal populations through which to study the development of Autism Spectrum Disorder (ASD; Moss, Richards, Nelson & Oliver, 2012). Tuberous Sclerosis Complex (TSC) is a particularly compelling population due to the high prevalence of ASD and prenatal diagnostic ability (Jeste, Wu, Senturk, Varcin, McCarthy, Shimzu, ScM, Vogel-Farley, Sahin & Nelson, 2014; McDonald, Varcin, Bhatt, Wu, Sahin, Nelson & Jeste, 2017; Sundberg & Sahin, 2015). However, findings related to the two core symptom domains of ASD within TSC are mixed, with little research examining restricted repetitive behaviors (RRBs). The current study aimed to define the presence and profile of RRBs in individuals with TSC and ASD. Participants included 196 children and adolescents from the TSC Autism Center for Excellence Network (TACERN, n=111) and Rare Disease Clinical Research Network (RDCRN, n=85) longitudinal studies. Participants in the two studies differed in age, gender, and measures of functioning (p's<.05), leading to separate analyses being conducted. Participants attended up to 7 visits over three years which included neuropsychological and ASD-specific testing once per year. Cognitive ability was measured via the Stanford-Binet-Fifth Edition and Mullen Scales of Early Learning, adaptive ability via the Vineland Adaptive Behavior Scales-Second Edition, and RRBs via the Autism Diagnostic Interview-Revised (ADI-R), as well as the Repetitive Behavior Scale-Revised (RBS-R) for RDCRN. Participants were split into two groups based on their clinical diagnosis (ASD; non-ASD) The ASD group in both studies showed greater

amounts and severity of RRBs, with differential patterns of significance at the item level. Older and more impaired participants with ASD showed greater amounts of all RRBs except compulsions/rituals, while younger and less impaired participants with ASD only showed elevated levels of repetitive use of objects, unusual sensory interest, and hand/finger mannerisms. Results of the current study outline the importance of continued work regarding the profile of RRBs to inform the development of screening tools to identify children at risk for ASD within the TSC population at an earlier age.

Keywords: Autism Spectrum Disorder, Tuberous Sclerosis Complex, Restricted Repetitive Behaviors, Behavior

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABC	Aberrant Behavior Checklist
ADOS-2	Autism Diagnostic Observation Schedule, Second Edition
ADI-R	Autism Diagnostic Interview-Revised
ASD	autism spectrum disorder
CBCL	Child Behavior Checklist
CI	Circumscribed Interests
CR	Compulsions/Rituals
DSC	Developmental Synaptopathies Consortium
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
HM	Hand and Finger Mannerisms
ID	intellectual disability
IS	insistence on Sameness
MRI	magnetic resonance imaging
OM	Other Complex Mannerisms or Stereotyped Body Movements
RBS-R	Repetitive Behaviors Scale-Revised
RDCRN	Rare Diseases Clinical Research Network
RRB(s)	restricted repetitive behavior(s)
RSM	repetitive sensorimotor

RU	Repetitive Use of Objects or Interest in Parts of Objects
SB-5	Stanford-Binet Intelligence Scales, Fifth Edition
SI	Unusual Sensory Interests
SU	Stereotyped Utterances and Delayed Echolalia
TACERN	TSC Autism Center of Excellence Network
TAND	TSC-associated neuropsychiatric disorders
TSC	Tuberous Sclerosis Complex
UP	Unusual Preoccupations
VABS-II	Vineland Adaptive Behavior Scales, Second Edition
VR	Verbal Rituals

Introduction

Autism Spectrum Disorder

Over the past 60 years, what is known about autism has shifted immensely, largely due to the increasing interest of researchers worldwide (Wolff, 2004). Unsurprisingly, the conceptualization and diagnostic criteria of autism have varied significantly over the years as a result of the increasing knowledge regarding its symptoms and phenotypic presentations. The newer term of autism spectrum disorder (ASD) indicates that while individuals with ASD have heterogeneous presentations, they have similar core deficits and symptoms (Kogan, Blumberg, Schieve, Boyle, Perrin, Ghandour et al., 2009). The most recent version of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) categorizes ASD as a neurodevelopmental disorder, which is a disorder which manifests early in life, typically before the child enters school (American Psychiatric Association, 2013).

According to the DSM-5, a diagnosis of ASD requires deficits in social communication and interaction as well as the presence of restricted, repetitive behaviors, interests, or activities (American Psychiatric Association, 2013). These symptoms must be present early in life and cause significant impairment in multiple areas of functioning. They also may be described as one of three levels of severity, based on the level of support the individual requires in their everyday life.

Approximately 1 in 59 children meets criteria for ASD in the United States, with varying rates across other countries (Baio, Wiggins, Christensen et al., 2018). ASD affects individuals of every race, ethnicity, and socioeconomic background. Despite the finding that the majority of parents of children that are diagnosed with ASD have concerns about their child's development before the age of 2 years (Kozlowski, Matson,

Horovitz, Worley & Neal, 2011), an age at which an ASD diagnosis is reliable and stable (Lord, Risi, DiLavore, Shulman, Thurm & Pickles, 2006), the average age of diagnosis in the United States is around 4 years of age (Christensen, Baio, Braun, Dilder, Charles, Constantino, et al., 2016; Filipek et al., 1999). While the majority of children with ASD do begin to manifest symptoms early in development (sometimes referred to as early onset ASD), approximately one third of individuals with ASD have been historically described as experiencing a loss of language and/or skills (a regression) following fairly normal development, typically during the second or third year of life (Al Backer, 2015; Hansen, Ozonoff, Krakowiak, Angukustsiri, Jones, Deprey et al., 2008). However, recent work by Ozonoff and Iosif (2019) suggests that a much greater percentage of individuals with ASD may experience regression, with declines in developmental skills becoming apparent shortly after 6 months of age in prospective studies.

Although not diagnostic, individuals with ASD often have deficits in adaptive functioning, which includes a wide variety of skills needed to function independently in daily life (O'Brien & Pearson, 2004). ASD is also commonly comorbid with intellectual impairment, with approximately half of individuals with ASD having a below-average IQ, and 30% of those individuals having an IQ of 70 or less on standardized assessments, which qualifies as an intellectual disability (ID; Christensen et al., 2016; Kantzer, Fernall, Westerlund, Hagberg, Gillberg & Miniscalco, 2018; McGovern & Sigman, 2005). Individuals with ID have deficits in social, cognitive, and adaptive skills, which makes it unsurprising that comorbid ID is a poor prognostic indicator for individuals with ASD (Matson & Shoemaker, 2009). In addition, when compared to individuals with ASD alone, individuals with comorbid ASD and ID have increased deficits in verbal and non-verbal communication and increased frequency of restricted repetitive behaviors (RRBs),

as well as more general challenging behaviors in comparison to individuals with ASD alone. Individuals with comorbid ASD and ID are also more likely to have challenging behaviors that persist over time and that are less responsive to intervention (O'Brien & Pearson 2004).

ASD is also commonly comorbid with epilepsy (American Psychiatric Association, 2013; Jeste & Tuchman, 2015; Strasser, Downers, Kung, Cross & de Haan, 2017), which can be defined as a predisposition for generating seizures that endures over time (Strasser et al., 2017). Epilepsy has been associated with more severe ID and lower verbal ability in individuals with ASD (American Psychiatric Association, 2013). For individuals with ASD, the lifetime prevalence of epilepsy ranges from 6 to 44%, which is significantly greater than the prevalence in the general population of 0.4-0.8% (Jeste & Tuchman, 2015; Strasser et al., 2017). While many risk factors for developing epilepsy have been discussed, including the presence of regression and the female gender, one of the most strongly identified factors is cognitive ability (Jeste & Tuchman, 2015). For example, individuals with ASD and ID have been suggested to have five times the risk of developing epilepsy than those with ASD alone (Strasser et al., 2017). Similarly, seizures occurring early in life, particularly before the age of two (i.e., infantile spasms), have been associated with overall poor neurodevelopmental outcomes and an increased risk of ASD. Epilepsy has been suggested to disrupt brain development, possibly contributing to developmental deficits that predispose an individual to ASD (Strasser et al., 2017). Despite this controversial theory, little is known about the effect of epilepsy on ASD symptomology (Ko, Kim, Kim, Song & Cheon, 2016).

More recently, there has been increasing interest in examining ASD within genetic disorders (Moss, Richards, Nelson & Oliver, 2012). Up to 15% of cases of ASD

are associated with a known genetic mutation or disorder (American Psychiatric Association, 2013; Cohen, Pichard, Tordjman, Baumann, Burglen, Excoffier, et al., 2005). Similarly, it has been noted that ASD or ASD-like characteristics are seen at high rates in Fragile X, Down Syndrome, and Tuberous Sclerosis Complex (TSC), among others. While the specific prevalence of ASD varies from disorder to disorder, the entire range of prevalence of ASD in genetic disorders of 19-67% is substantially larger than the 1% of individuals with ASD observed in the general population (Clifford, Dissanayake, Huggins, Taylor & Loesch, 2007; Moss et al., 2012; Wulffaert, Berckelaer-Onnes & Scholte, 2009). It is particularly beneficial to study ASD in these populations, as many of these disorders are diagnosed prenatally or shortly after birth, which provides a population in which one can examine the emergence of ASD and its symptoms. It has also been suggested that various genetic syndromes may have distinctive ASD-specific symptom profiles (Bruining, Eijkemans, Kas, Curran, Vorstman, & Bolton, 2014). While these disorders have become popular models for studying ASD, there is little known about the differential presentations of ASD in these disorders (Moss et al., 2012). One such disorder that is particularly compelling, related to ASD, is TSC.

Tuberous Sclerosis Complex (TSC)

TSC is an autosomal dominant genetic disorder characterized by the formation of benign tumor-like growths throughout the body (Bolton, Clifford, Tye, Maclean, Humphrey, Marechal et al., 2015; Hurst, 2016; McDonald, et al., 2017; Northrup & Kreuger, 2013; Prather & de Vries, 2004; Wong 2005). As of 2015, the birth incidence of TSC is 1 in 6000, with approximately one million individuals diagnosed across the globe (Franz, Bissler, & McCormack, 2015). TSC is typically caused by a mutation in the TSC1 and/or TSC2 genes, which are thought to be tumor suppressor genes (Northrup,

Koenig, Pearson & Au, 1999; Cohen et al., 2005; Wong, 2005). Approximately 31% of individuals with TSC have variation in the TSC1 gene, while the remaining 69% have variation in the TSC2 gene (Northrup et al., 1999). It has been suggested that symptom presentation, including physical disease, seizure frequency, and intellectual impairment, may be more severe in individuals with a variation in the TSC2 gene (Dabora, Jozwiak, Franz, Roberts, Nieto, Chung et al, 2001; Lewis, Thomas, Murphy & Sampson, 2004) While TSC is known to be a dominant genetic disorder and therefore can be inherited from parent to child, 60-70% of individuals have a mutation that is considered new or spontaneous (Hurst, 2016; Zaroff et al., 2004). If an individual has TSC, each child they have has a 50% chance of also developing the disorder (Northrup et al., 1999)

Despite the association of TSC with specific genetic mutations, only about 80% of individuals with TSC are identified with genetic testing (Zaroff et al., 2004). Instead, clinical diagnosis of TSC is based on the presence of a combination of 'major' and 'minor' symptoms and is typically qualified as possible, probable, or definite (Northrup & Krueger, 2013; Zaroff et al., 2004). Further adding complexity to diagnosis, many of the distinguishing characteristics of TSC do not become apparent until after the age of three, making early diagnosis more difficult (Curatolo, Bombardieri, & Jozwiak, 2008). Oftentimes the reason that individuals with TSC come to clinical attention is because of seizures (Erol, Savas, Sekerci, Yazici, Erbay, Demir et al., 2015).

The benign growths and other manifestations of TSC often lead to significant health issues, as they can affect the skin, brain, kidneys, heart and lungs. Between 70 and 95% of individuals with TSC have epilepsy, with most patients experiencing two or more types of seizures (Saxena & Sampson, 2015). One of the most common types of seizures in individuals with TSC, other than focal seizures, are early onset seizures, particularly

infantile spasms (Asato & Hardan, 2004; Jeste et al., 2016; Saxena & Sampson, 2015: Zaroff, Devinsky, Miles & Barr, 2004). This type of seizure has been strongly associated with broad developmental and cognitive impairments and is also considered to be a risk factor for ASD in individuals with TSC (Asato & Hardan, 2004; Jeste et al., 2016; Zaroff, Devinsky, Miles & Barr, 2004). Onset of seizures within the first year of life has been shown to be a poor prognostic indicator among individuals with TSC, alongside the presence of multiple types of seizures (Saxena & Sampson, 2015). In addition, increased seizure frequency is associated with lower IQ and poorer adaptive and behavioral outcomes in individuals with TSC (Bolton et al., 2015; Kopp et al., 2008).

The symptoms that are often the most concerning to families of individuals with TSC are cognitive and behavioral rather than physical (Prather & de Vries, 2004). In addition to the physical effects of TSC, affected individuals are at increased risk for developmental disabilities (Prather & de Vries, 2004). It has been suggested that symptomatology involving the brain (e.g., growths in the brain) within children with TSC may lead to an increased risk for ASD and other neurodevelopmental disabilities in this population (Wiznitzer, 2004). Notably, while approximately 90% of individuals with TSC will experience intellectual, behavioral, neuropsychological, psychosocial, academic, or psychiatric difficulties in their lifetime, only approximately 20% have historically received proper evaluation and treatment (de Vries, 2010a; Leclezio, Jansen, Whittemore & de Vries, 2015). As such, the term TSC-associated neuropsychiatric disorders (TAND) was created in an effort to increase identification of these diverse difficulties in individuals with TSC (de Vries, Whittemore, Leclezio, Byars, Dunn, Ess et al., 2015). Approximately 50% of individuals with TSC have global intellectual impairments and developmental psychopathologies (Prather & de Vries, 2004). There is

significant variability in the presentation of TSC, especially in cognitive and behavioral symptoms, with essentially all individuals presenting with structural brain abnormalities, 50-60% having cognitive impairment, 60-80% having hyperactivity, 10-41% having self-injurious behaviors, and at least 10% displaying challenging behavior (Asato & Hardan, 2004; Chung, Lawson, Sarkozy, Riney, Wargon, Shand, et al., 2017; Eden, de Vries, Moss, Richards & Oliver, 2014; Kopp, Muzykewicz, Staley, Thiele & Pulsifer, 2008; Prather & de Vries, 2004). Further complicating behavioral challenges, children with TSC are also at an increased risk for sleep problems in comparison to typically developing individuals (Asato & Hardan, 2004; Zaroff et al., 2004).

While there is no cure for TSC, affected individuals benefit from a variety of treatments targeting various symptoms (Asato & Hardan, 2004; Franz et al., 2015; Wiznitzer, 2004; Zaroff et al., 2004). Maintaining control of seizure activity, which is typically done through antiepileptic medications (e.g., vigabatrin), has been associated with improved outcomes and quality of life (Franz et al., 2010; Saxena & Sampson, 2015; Wang & Fallah, 2014; Zaroff et al., 2004). It has also been shown that the cognitive and behavioral deficits of individuals with TSC can be ameliorated if seizure activity is reduced or controlled early in life (Saxena & Sampson, 2015; Zaroff et al., 2004). In addition, both specific psychological (e.g., applied behavior analysis, social skills therapies) and medical interventions (e.g., medications, surgical seizure control, physical and occupational therapies) can be extremely efficacious in treating the heterogeneous symptoms of TSC (Asato & Hardan, 2004; Wiznitzer, 2004; Zaroff et al., 2004). Regardless of the specific symptoms each individual with TSC exhibits, it is well documented that early detection and intervention improves outcomes drastically (Asato & Hardan, 2004).

TSC has been recognized as an ideal population to study the emergence of ASD as it is one of the most penetrant single gene disorders for ASD, with up to 60% of individuals with TSC meeting criteria for an ASD diagnosis (Jeste, Wu, Senturk, Varcin, McCarthy, Shimzu, ScM, Vogel-Farley, Sahin & Nelson, 2014; McDonald, Varcin, Bhatt, Wu, Sahin, Nelson & Jeste, 2017; Sundberg & Sahin, 2015). As such, children with TSC have been the focus of a multitude of studies focused on the early markers and development of ASD in early life. Intriguingly, despite this increased interest in the emergence of ASD in individuals with TSC, the average age of diagnosis of ASD in individuals with TSC is usually substantially delayed or altogether absent due to the variety of developmental concerns present in affected individuals (Jeste, 2013). This points to an increasing need for further understanding of the early markers of ASD within the TSC population in order to inform earlier diagnosis and treatment for this affected population, as they are likely to benefit from evidence-based interventions specific to both diagnoses.

ASD Symptom Profiles in TSC

As previously discussed, ASD is seen in a large percentage of individuals with TSC (Vignoli et al., 2015; Sundberg & Sahin, 2015). In this population, risk factors for ASD have included epilepsy, particularly early in life (infantile spasms), and TSC2 mutations (Vignoli, La Briola, Peron, Turner, Vannicola, Saccani et al., 2015). Surprisingly, a gender ratio of approximately 1:1 has been reported in individuals with comorbid TSC and ASD, in contrast to the 4:1 ratio in individuals with ASD alone (Wiznitzer, 2004). While TSC is a popular model in which to study the emergence of ASD, there have been mixed findings pertaining to the various symptoms of ASD.

In considering ASD symptomatology in TSC, children with TSC and comorbid ASD have significant impairments and greater deficits in their social communication profiles (e.g., decreased use of absence of gestures, eye contact, shared enjoyment) when compared to children with TSC alone (Jeste, Varcin, Hellemann, Gulsrud, Bhatt, Kasari, Wu, Sahin & Nelson, 2016; McDonald et al., 2017). In addition, the social communication profile of children with TSC and comorbid ASD is essentially identical to individuals with ASD alone (Jeste et al., 2016). Thus, the social communication profile of ASD in TSC resembles ASD in the general population. Despite this focus of a breadth of research on social communication, RRBs, the other core symptom domain of autism, RRBs, has been hugely understudied. Furthermore, it has been noted that despite the presence of ASD-specific behaviors, both caregivers and clinicians frequently do not immediately recognize the proper diagnosis as being ASD (Capal et al., 2017), as it is not well understood what these behaviors look like in individuals with TSC alone versus those with TSC and comorbid ASD. As such, with increased understanding of the types, frequencies, and severities of RRBs seen in individuals with ASD within the TSC population, it may be possible to identify children at risk for ASD in TSC at a younger age, giving them earlier access to ASD-specific interventions and improving their developmental and functional outcomes.

Restricted Repetitive Behaviors

Restricted and/or repetitive displays of behaviors, interests or activities are one of the core symptom domains of ASD (American Psychiatric Association, 2013). This group of symptoms is often broadly referred to as RRBs. These include stereotyped or repetitive speech, movements or use of objects, insistence on strict adherence to daily routines, extreme resistance to change, ritualized behavior, fixated interests of abnormal

intensity or focus, and atypical reactivity to a range of sensory stimuli. While this symptom category has been suggested to be some of the first manifestations of ASD, often presenting prior to delays in language development (Wolff, Botteron, Kager, Elison, Estes, Gu et al., 2014), behavioral difficulties have only been cited as the area of first concern for approximately 16% of children diagnosed with ASD. This is in stark contrast to the almost 50% of concerns that are related to language development (McConachie, Le Couteur & Honey, 2005).

RRBs are also typically seen in normal development (Evans, Leckman, Carter, Reznick, Henshaw, King, et al., 1997; Thelen, 1980; Wolff et al., 2014), which may make it more difficult for parents to identify the level at which they are indicative of a problem (Wolff et al., 2014). In typically developing children, behaviors that are repetitive and ritualistic, such as kicking, waving, and banging (Leekam et al., 2007; Lewis & Kim, 2009; Thelen, 1980), are developmentally appropriate and function to aid children in learning about the world around them (Lewis & Kim, 2009). These behaviors begin in early infancy with repetitive motor movements and peak between 1 and 2 years of age (Lewis & Kim, 2009; Ozonoff, Macari, Young, Goldring, Thompson & Rogers, 2008; Thelen, 1980). As children with typical development age, the RRBs grow in complexity (i.e., ritualistic daily activities, rigid preferences and dislikes, etc.) and may increase in inflexibility and compulsivity while decreasing in overall frequency, until school-age when these behaviors are thought to reduce significantly, if not completely dissipate.

While RRBs can be developmentally appropriate and aid in learning, children across the full range of cognitive and adaptive abilities who go on to be diagnosed with ASD have significantly greater frequency, amounts, and severity of such behaviors

beginning prior to 12 months of age (Ozonoff et al., 2008; Wolff et al., 2014). While almost all children with ASD demonstrate at least one type of RRB, the large majority display three or more (Militerni et al., 2002). In particular, in infancy children with ASD are likely to display repetitive motor movements including rocking, spinning, hand flapping, and unusual posturing (Harrop et al., 2014; Militerni et al., 2002; Ozonoff et al., 2008; Richler et al., 2010; Wolff et al., 2014). By the age of 2 or 3 years, children with ASD are likely to not only display repetitive movements involving their body or objects, but also exhibit unusual sensory preoccupations or interests. Children with ASD who have higher intellectual ability are also likely to demonstrate these more complex RRBs (Militerni et al., 2002). As children with ASD age, they typically display fewer and less severe RRBs regardless of gender, presence of intellectual disability, or medication usage (Esbensen, Seltzer, Lam & Bodfish, 2009). One exception pertains to repetitive movements, as children with ASD who have a comorbid diagnosis of intellectual disability are likely to display more repetitive movements than children with ASD alone. When considering distinct types of RRBs, repetitive movements are the most common type of RRBs in childhood but are the least prevalent in adulthood, while restricted interests remain the most prevalent RRB across the lifespan.

While there is considerable variability within individuals with ASD (Harrop et al., 2014), many studies have supported the existence of two 'clusters' of RRBS in children with ASD that hold true across a range of ages and abilities (Cuccaro, Shao, Grubber, Slifer, Wolpert, Donnelly et al, 2003; Lewis & Kim, 2009; Richler et al., 2010; Szatmari, Georgiades, Bryson, Zwaigenbaum, Roberts, Mahoney, Goldberg & Tuff, 2006; Turner, 1999). The two factors, while described by slightly different terms in varying studies, can be thought of as repetitive sensorimotor and insistence on sameness behaviors (Cuccaro

et al., 2003; Richler et al., 2010; Szatmari et al., 2006). Repetitive sensorimotor (RSM) behaviors include hand, finger and more complex motor mannerisms, the repetitive usage of parts or whole objects, and unusual sensory interests or aversions. Insistence on sameness (IS) behaviors encompass ritualistic and compulsive behaviors, difficulties with changes in daily routines, and resistance to changes in the environment. These two factors appear to have distinct developmental trajectories in individuals with ASD, with RSM behaviors being relatively frequent beginning early in life and staying stable with increasing age, while IS behaviors are typically infrequent in infancy and increase with age, particularly as cognitive ability improves (Richler et al., 2010). While the two-factor model for RRBs in ASD dominates the literature, Lam, Bodfish and Piven (2008) have suggested the existence of the third cluster, related to circumscribed interests, which has been removed from factor analyses of RRBs in the past.

Despite an increased interest in the developmental trajectories of RRBs in ASD, there are still gaps in the literature, including how RRBs may differ in the various genetic disorders that have become popular models in which to study ASD (Moss, Richards, Nelson & Oliver, 2012). Elucidating these profiles may aid in improving early access to ASD-specific interventions for children who are at risk for ASD, ultimately improving long-term outcomes.

Current Study

The current study aimed to examine and define the presence of RRBs in individuals with ASD and TSC. In addition, the emergence of RRBs was examined to determine how the profile of RRBs differs across individuals of varying ages.

Aims and Hypotheses

Aim 1. Determine the number, frequency, and severity of RRBs exhibited by individuals with TSC and comorbid ASD.

Hypothesis 1.1. Based on previous literature involving children with ASD, children with TSC and comorbid ASD will have a greater number, frequency, and severity of RRBs than children with TSC alone at all time points.

Aim 2. Determine which types of and specific RRBs are present in individuals with TSC and comorbid ASD.

Hypothesis 2.1. Based on what is known about ASD, children with TSC and comorbid ASD will exhibit a range of RRBs.

Hypothesis 2.2. As in the ASD population, at 36 months, children with TSC and comorbid ASD will have higher rates of repetitive sensorimotor behaviors than insistence on sameness behaviors.

Hypothesis 2.3. As in the ASD population, older children with TSC and comorbid ASD will have increased rates of repetitive sensorimotor behaviors and insistence on sameness behaviors.

Methods

Participants. Participants included 196 children and adolescents from the TSC Autism Center of Excellence Network (TACERN) and Rare Diseases Clinical Research Network (RDCRN) studies. Participants were recruited by the Developmental Synaptopathies Consortium (DSC) and TACERN to participate in one of two multisite longitudinal studies which aim to characterize the developmental phenotype and identify biomarkers for ASD within a sample of individuals with TSC. Of the current sample, 111 individuals participated in the TACERN study, while the remaining 85 participated in the

RDCRN study. In initial comparisons of the two studies, participants from the RDCRN and TACERN studies significantly differed in age, gender, and measures of cognitive, adaptive, behavioral, and autism-specific functioning (p's<.05, Table 1), likely due to differences in study design and recruitment. Due to these inherent differences between the two groups of participants, they were analyzed separately and will from here on out be referred to as Study 1 (TACERN) and Study 2 (RDCRN).

Power Analysis. Power analysis using GPower revealed that with a sample size of 211 participants and alpha of 0.05, an effect size of 0.25 could be detected using ANOVA with two groups, which is a small effect.

Procedures. Procedures for the larger TACERN and RDCRN studies were approved by the Institutional Review Board. Procedures for the current study fall under the aims of the larger studies and therefore were approved as an amendment by the Institutional Review Board. Participants were invited to participate in the TACERN and RDCRN studies if they had a diagnosis of TSC. If enrolled in one of the larger studies, participants participated in up to 7 visits over the course of three years, which included a blood draw, magnetic resonance imaging (MRI) scans, physical exams, a complete medical history, and extensive neuropsychological assessments.

For Study 1 (TACERN), participants were invited to join the study if they 1) had a confirmed diagnosis of TSC and 2) were between the ages of 3 and 12 months. Exclusion criteria included prematurity, participation in a clinical trial within 30 days of study enrollment, mTOR inhibitor medication, subependymal giant cell astrocytoma which has required medical treatment, prior surgery for epilepsy, and contraindications to MRI. The aim of the TACERN study was to study individuals with TSC longitudinally in early childhood with the aim of identifying early biomarkers of ASD in young children

with TSC. Once enrolled in the study, participants attended up to 7 visits, with neuropsychological and ASD-specific testing occurring at 1 year old, 2 years old, and 3 years old.

For Study 2 (RDCRN), participants were invited to enroll if they 1) had a confirmed diagnosis of TSC, 2) had suspected or confirmed ASD or ID, 3) were between the ages of 3 and 21 years, 4) spoke English as their primary language, and 5) had at least one biological parent who was willing to participate alongside the individual with TSC. Exclusion criteria included participation in a clinical trial, contraindications for MRI, and taking cannabinol (CBD) oil. The aim of the RDCRN study was to follow individuals with TSC and comorbid ASD and/or ID longitudinally. Once enrolled in the study, participants attended up to 5 visits over the course of 2 years, with neuropsychological and ASD-specific testing occurring at baseline, 1-year follow up, and 2-year follow up.

As a part of both larger studies, caregivers completed at least one clinical interview which provided information regarding their child's medical, treatment, and family history, among other variables. To verify ASD diagnosis, the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) and the Autism Diagnostic Interview-Revised (ADI-R) were administered. For Study 1, this occurred at the 24-month and 36-month visits, while for Study 2, this was completed at the baseline visit. The ADOS-2 was administered at each subsequent visit for Study 2, but the ADI-R was not. The clinician also completed the autism certainty rating regarding their level of certainty in the presence of ASD diagnosis at each annual visit for both studies. To measure cognitive ability, the Mullen Scales of Early Learning or the Stanford Binet 5 (SB-5) were administered. To measure adaptive functioning, the Vineland Adaptive Behavior Scales-Second Edition (VABS-II) was given. Caregivers in both studies were also asked to

complete a variety of questionnaires regarding their child's adaptive and behavioral functioning, including the Child Behavior Checklist (CBCL). For Study 2 specifically, caregivers were additionally asked to complete the Repetitive Behavior Scale -Revised (RBS-R). These visits were conducted at one of 5 sites across the country.

For the current study, scores regarding RRBs from the ADI-R, as well as the ADOS and RBS-R were analyzed as outcome variables. Scores from the VABS-II, CBCL, SB-5, and Mullen Scales of Early Learning, as well as information from the clinical interview were used to characterize the overall functioning and developmental profile of the sample. All measures are described in detail below.

Materials.

Clinical Evaluation. During the clinical evaluation, information was acquired from the parents or guardians of participants regarding general demographic information, medical history, interventional history, family history, past and current seizure history, prior and current medications, clinical exam findings, and TSC genotype, if known.

Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). The ADOS-2 is an individually-administered and standardized semi-structured measure utilized in diagnosing ASD (Lord, Rutter, DiLavore, Risi, Gotham & Bishop, 2012). The ADOS-2 is considered one of the two gold standard measures for observational assessment and diagnosis of ASD (Kanne, Randolph & Farmer, 2008). Five modules have been developed for individuals of various ages and communication levels. Scores of the ADOS-2 are based on observations of skills and behaviors (e.g., socially modulated eye contact; repetitive speech or movements; initiation of and response to joint attention) of the individual. Taking into account the entire ADOS-2 assessment, a research-reliable administrator, commonly a psychologist, codes each skill or behavior on a scale from 0 to

3, with 0 meaning the skill is developmentally appropriate, 1 indicating some abnormality, and 2 and 3 indicating levels of impairment commonly seen in individuals with ASD. The ADOS-2 also yields a comparison score which indicates the severity of ASD-related symptomatology compared to other children with ASD of a similar age and language level. The comparison score is on a 1 to 10 scale, with a higher score indicating higher severity of ASD-related symptoms. In the current study, the comparison score and classification were utilized to inform severity of ASD symptomatology and diagnosis of ASD.

Autism Diagnostic Interview-Revised (ADI-R). The ADI-R is a semi-structured, standardized interview conducted by clinicians with caregiver(s) of individuals with ASD (Le Couter, Rutter, Lord, Rios, Robertson, Holgrafer & McLennan, 1989). It is considered one of the two gold standard measures for diagnosing ASD, particularly in combination with the ADOS-2. Scores from the ADI-R were used to examine the presence or absence of RRBs, quantify the RRBs present, and inform the behavioral profile. The main outcome variables were variables regarding RRBs, specifically Stereotyped Utterances and Delayed Echolalia (33; SU), Verbal Rituals (39; VR), Unusual Preoccupations (67; UP), Circumscribed Interests (68; CI), Repetitive Use of Objects or Interest in Parts of Objects (69; RU), Compulsions/Rituals (70; CR), Unusual Sensory Interests (71; SI), Hand and Finger Mannerisms (77; HM), and Other Complex Mannerisms or Stereotyped Body Movements (78; OM). In general, these items are scored by a research-reliable evaluator, often a psychologist, based on caregiver-report of the presence and severity of the given behavior, with a score of 0 meaning the behavior is not present, 1 meaning it is present but not sufficiently severe or frequent, 2 meaning it is present and abnormal, and 3 meaning it is present and severely impacting daily

functioning. When each item is summed to create summary scores and the overall total scores, scores of 3 are converted to 2. The summary scores are Encompassing Preoccupations or Circumscribed Patterns of Interest (C1), Apparently Compulsive Adherence to Nonfunctional Routines (C2), Stereotyped and Repetitive Motor Mannerisms (C3), Preoccupation with Parts of Objects or Nonfunctional Elements of Materials (C4), and the Restricted, Repetitive, and Stereotyped Patterns of Behavior Subscale (CTotal). While C1 and C2 have a range of 0 to 4 and C3 and C4 have a range of 0 to 2, CTotal ranges from 0 to 12, with higher scores on all summary indices indicating greater impairment.

The primary outcome variables for the current study were calculated from the following ADI-R (Figure 1). Specifically, the total number of endorsed items (items with a score of 1, 2, or 3 with a range 0 to 15), the total severity of endorsed items (sum of scores on all endorsed items with a range of 0 to 45), the average severity of endorsed items (average severity of endorsed items with a range of 0 to 3), the number of items scored a 1, 2, or 3 (range of 0 to 15), and the CTotal summary score for each individual participant were calculated and compared between groups.

The frequency of endorsement of each RRB variable on the ADI-R was calculated to determine the frequency of each behavior overall and in each of the two groups. The frequency was determined by the number of individuals with a score of either 1, 2, or 3. The ADI-R RRB items were also split into Repetitive Sensorimotor and Insistence on Sameness behaviors (Figure 2). Two variables were created based on this split which indicated the presence (code of 1) or absence (code of 0) of any of the behaviors in each category. For the Study 2, baseline visit was used to calculate all variables, as the ADI-R was only administered at this time point. For the TACERN study, the 36-month time

point was utilized due to the increased reliability of cognitive and behavioral traits, as well as ASD diagnosis, by this age.

Autism Certainty Clinical Rating. The Autism Certainty Clinical Rating is a ranking scale of the clinician's certainty in the presence or absence of ASD. The clinician first answers a dichotomous yes/no question regarding the presence of ASD, followed by a rating of certainty in the diagnostic category. The evaluator based this rating on all available information from each study visit. This is measured on a scale from one to five, with one being not at all certain, and five being very certain. Alongside the certainty rating, the clinician also indicates any factors that may have impacted their scoring. The dichotomous ASD variable was used to create the groups of ASD and non-ASD, while the rating was used to screen for individuals whom the ASD or non-ASD diagnosis was unclear (i.e., low clinical certainty rating).

Vineland Adaptive Behavior Scales, Second Edition (VABS-II). The VABS-II is a common tool for assessing adaptive behavior in individuals with ASD, among other populations (Perry, Flanagan, Geier & Freeman, 2009). The VABS-II produces an overall Adaptive Behavior Composite score, as well as four adaptive functioning domains: Communication, Daily Living Skills, Socialization, and Motor Skills. The adaptive functioning domain scores, as well as the Adaptive Behavior Composite, are standard scores which have a mean of 100 and standard deviation of 15. The Adaptive Behavior Composite was used to characterize the adaptive functioning level of participants.

Stanford Binet-5 Intelligence Scale, Fifth Edition (SB-5). The SB-5 is a measure that is often used to determine the cognitive abilities of individuals between the ages of 2 to 85 years of age of varying abilities (Madaus, Lynch & Lynch, 2008). It can be used to assess typically developing individuals as well as those with developmental or

intellectual disabilities. The SB-5 yields a Full Scale Intellectual Quotient (FSIQ) standard score (M=100, SD=15) which can be used as a measure of overall cognitive functioning. The FSIQ was used to obtain an IQ score for participants. IQ score was proposed as a covariate for analyses in the current study, however, IQ was closely tied to ASD diagnosis in this population, therefore IQ was used solely as a descriptive.

Mullen Scales of Early Learning. The Mullen Scales of Early Learning is a measure that was designed to measure the development of infants and children aged 0 to 68 months (Shank, 2011). It yields an Early Learning Composite standard score (M=100, SD=15) which can be used as a measure of cognitive functioning in young children. In this study, the Early Learning Composite was used to assess cognitive ability in individuals who could not be administered the SB-5. IQ score was proposed for use as a covariate, however, IQ was closely tied to ASD diagnosis in this population, therefore IQ was used solely as a descriptive.

Repetitive Behaviors Scale Revised (RBS-R). The RBS-R is a questionnaire designed to assess RRBs in individuals with ASD (Bodfish, Symons, Parker & Lewis, 2000). The RBS-R is a 43-item parent-report measure, with each item rated on a 4-point Likert scale from 'behavior does not occur' (0) to 'behavior occurs and is a severe problem' (3). It produces an overall raw score of RRBs which has a range of 0 to 129 and individual scores in the domains of Stereotyped Behavior (defined as apparently purposeless movements or actions that are repeated in a similar manner, e.g., "whole body: body rocking, body swaying") ranging from 0 to 18, Self-Injurious Behavior (defined as "movements or actions that have the potential to cause redness, bruising, or other injury to the body, and that are repeated in a similar manner", e.g., "hits self with body part") ranging from 0 to 24, Compulsive Behavior (defined as "behavior that is

repeated and is performed according to a rule, or involves things being done ‘just so’”, e.g., “arranging/ordering: arranges certain objects in a particular pattern or place”) ranging from 0 to 24, Ritualistic Behavior (defined as “performing activities of daily living in a similar manner”, e.g., “play/leisure: follows a rigid routine during play/leisure”) ranging from 0 to 18, Sameness Behavior (defined as “resistance to change, insisting that things stay the same”, e.g., “becomes upset if interrupted in what he/she is doing”) ranging from 0 to 33, and Restricted Behavior (defined as “limited range of focus, interest, or activity”, e.g., “fascination, preoccupation with one subject or activity”) ranging from 0 12 with higher scores indicating greater impairment. The overall raw score, as well as all of the domains were used to determine the variety and severity of RRBs experienced.

Aberrant Behavior Checklist (ABC). The ABC is a questionnaire utilized to examine a variety of behavior problems (Farmer & Aman, 2017). 58 items are scored on a 4-point Likert scale from 0 meaning the behavior was never a problem, 1 meaning the behavior is a slight problem, 2 meaning the behavior is a moderately serious problem, and 3 meaning the behavior is a severe problem. There is no validated total score for this measure, but there are five subscales which are derived from the individual item scores: Irritability (15 items; e.g., “aggressive to others”, “cries over minor things”), Social Withdrawal (16 items; e.g., “listless, sluggish”, “seeks isolation”), Stereotypic Behavior (7 items; e.g., “recurring body movements”; “odd/bizarre behavior”), Hyperactivity/Noncompliance (16 items; e.g., “excessively active”, “disrupts group activities), and Inappropriate Speech (4 items; e.g., “talks excessively”, “repetitive speech”). Each subscale score is determined by summing the individual item scores that relate to each category with a range of 0 to 45 for Irritability, 0 to 48 for Social

Withdrawal, 0 to 21 to Stereotypic Behavior, 0 to 48 for Hyperactivity/Noncompliance, and 0 to 12 for Inappropriate Speech. The Stereotypic Behavior subscale raw score was used to inform the number, severity, and types of RRBs experienced.

Data Analysis

The original combined dataset included 238 individuals. Participants were retained in the current study if they participated in a baseline visit for Study 2 or a 3-year visit for Study 1 that included an ADI-R and Clinical Certainty Rating. 42 individuals were removed from the dataset due to not having data available for the appropriate visit. An additional 4 participants were removed from the Study 2 dataset due to not having data available from the ADI-R.

Data from the two visits for 22 participants who crossed over between studies were examined. For some individuals, data from Study 2 was incomplete (i.e., ADI-R data had not been entered). All data, except for consensus clinical diagnosis in two cases, were identical between the two visits. Due to this similarity in data between the 3-year Study 1 and baseline Study 2 visits (which were often completed on the same day or within a limited time frame), data from the baseline Study 2 visit was retained and the Study 1 visit was removed, to avoid violating independence of groups. For those individuals whose diagnosis had changed between their 3-year Study 1 visit and baseline Study 2 visit between the ages of 3 and 4 years, the diagnosis from Study 2 was retained, as diagnosis was stable at all time points for Study 2. One individual's diagnosis changed from ASD to non-ASD while the other showed the opposite.

To compare cognitive ability across participants, standard scores from the Mullen and SB-5 were combined into a summary variable. Each participant was administered

either the Mullen or SB-5, therefore scores were transferred into an overall cognitive ability variable.

196 individuals completed either a 3-year Study 1 or baseline Study 2 visit. Of this sample, 111 completed a 3-year Study 1 visit and 85 completed a baseline Study 2 visit at various ages of entry. Data was complete for Clinical Diagnosis, Clinical Diagnosis Level of Certainty, Gender, and the ADI-R. 4.1% of participants had missingness on Race and 0.5% had missingness on Ethnicity. 0.5% of participants had missingness of Focal Seizures. 2.6% of individuals had missingness of ADOS-2 Classification and 5.1% of individuals had missingness on ADOS-2 Comparison Score. In terms of overall measure completion, 3 participants did not receive a cognitive measure and 2 individuals did not receive an ADOS-2. Three individuals from Study 2 did not complete the RBS-R, 4 did not complete the ABC-C, and 1 did not complete the CBCL. In Study 1, 3 individuals did not complete the CBCL. However, participants were retained in the current dataset if they had complete data available on the ADI-R and Clinical Certainty forms.

One-way ANCOVAS comparing RRBs between the ASD and non-ASD groups with IQ as a covariate were planned. However, assumptions of the covariate being linearly related to the dependent variables (e.g., CTotal score, total number and severity of endorsed items on the ADI-R) and homogeneity of regression slopes were violated. Transformations were attempted but did not alleviate the violations. Cognitive ability was also significantly different between the two groups ($p<0.05$), with the ASD group exhibiting significantly poorer cognitive functioning, suggesting that it would not be an appropriate covariate for the analyses. Cognitive ability was found to be closely related to ASD diagnosis in this population, as evidenced by the split in cognitive ability between

ASD and non-ASD groups in both studies, so therefore were not included in analyses. A two-way ANOVA with diagnosis and intellectual disability (defined as cognitive or adaptive score below 70) was considered, but assumptions were violated for this analysis as well. Therefore, Mann-Whitney U-tests, a non-parametric alternative, were used to compare distributions between ASD and non-ASD groups in the two studies. Bonferroni correction was utilized to correct for multiple comparisons.

Study 1 (TACERN; 3-year-olds only)

Participants. Participants in the TACERN study were 111 individuals with an average age of 3.05 years (*SD*=0.14 years). Participants were 50.5% female. The Study 1 sample was largely Caucasian and non-Hispanic/Latino (Table 2). While 35.5% of participants met criteria for either Autism or Autism Spectrum on the ADOS-2, only 19.8% received a consensus clinical diagnosis of ASD during their 3-year study visit. Over 50% of individuals in Study 1 had a reported lifetime prevalence of generalized or focal seizures. Cognitive ability of 83 individuals was obtained and of those individuals, the mean ability level was in the low average range (*M*=83.4, *SD*=23.9) with large variability across participants. Of 108 individuals who had a score on the Vineland-II, the mean ability level was also in the low average range (*M*=82.3, *SD*=15.6). When cognitive and adaptive ability were combined and coded as above or below the cutoff for intellectual disability, including those who could not be administered a cognitive assessment due to functional capacity as falling in the intellectual disability range, 72.8% of individuals in the overall sample had an abilities above this cutoff, suggesting that a large majority of the Study 1 sample are within normal limits of cognitive ability.

Results. To address hypothesis 1.1, Mann-Whitney U tests with Bonferroni adjusted alpha levels of .007 per test were run to determine if there were differences in

the presence and severity of restricted repetitive behaviors on the ADI-R between the ASD and non-ASD groups (Table 3). Dependent variables included the total number of endorsed items, the total severity of endorsed items, the average severity of endorsed items, the number of items scored a 1, 2, or 3, and the CTotal summary score from the ADI-R. As assessed via visual inspection, distributions of the RRB variables for ASD and non-ASD groups were not similar. Total number of endorsed items for the ASD group (mean rank=47.95), was significantly higher than for the non-ASD group (mean rank=31.45), U=802.0, z=3.18, p=.001. Severity of endorsed items for the ASD group (mean rank=50.55), was significantly higher than for the non-ASD group (mean rank=30.32), U=859.0, z=3.84, p<.001. Average severity of endorsed items for the ASD group (mean rank=49.23), was significantly higher than for the non-ASD group (mean rank=30.90), U=830.0, z=3.69, p<.001. Total number items scored a 1 for the ASD group (mean rank=67.23), was not significantly different from the non-ASD group (mean rank=53.22), U=1226.0, z=1.95, p=.051. Total number items scored a 2 for the ASD group (mean rank=86.68), was significantly higher than for the non-ASD group (mean rank=48.42), U=1654.0, z=6.03, p<.001. Total number items scored a 3 for the ASD group (mean rank=56.00), was not significantly different from the non-ASD group (mean rank=56.00), U=979.0, z=0.00, p=1.00. CTotal Summary Score for the ASD group (mean rank=89.75), was significantly higher than for the non-ASD group (mean rank=47.66), U=1721.5, z=5.69, p<.001.

To address hypothesis 2.1, association of endorsement of each RRB item (Table 1) and ASD diagnosis was explored using chi-square tests of independence. Frequencies of endorsement of each RRB variable can be found in Table 4. A chi-square test for association was conducted between clinical consensus diagnosis and endorsement of

individual RRB items on the ADI-R (Figure 1). Expected cell frequencies were greater than five for Repetitive Use of Objects or Parts of Objects (RU), Unusual Sensory Interests (SI), and Hand/Finger Mannerisms (HM). The study design was a 2x2 crosstabulation, therefore Fisher's test was utilized to interpret the results of those variables that did not have expected cell frequencies greater than five. The association between diagnosis and endorsement of Repetitive Use of Objects or Parts of Objects (RU) was significant, $\chi^2(1)=28.78$, $p<.001$. This association was moderately strong, $\varphi = .51$, $p<.001$. The association between diagnosis and endorsement of Unusual Sensory Interests (SI) was significant, $\chi^2(1)=19.42$, $p<.001$. This association was moderately strong, $\varphi = .42$, $p<.001$. The association between diagnosis and endorsement of Hand/Finger Mannerisms was significant, $\chi^2(1)=33.19$, $p<.001$. This association was moderately strong, $\varphi = .55$, $p<.001$.

The association between diagnosis and endorsement of Verbal Rituals (VR), Stereotyped Utterances and Delayed Echolalia (SU), Unusual Preoccupations (UP), Circumscribed Interests (CU), Compulsions/Rituals (CR), and Other Complex Motor Mannerisms (OM) were not significant (p's>.08).

To address hypothesis 2.2, frequencies of Repetitive Sensorimotor (RSM) behaviors and Insistence on Sameness (IS) behaviors were compared between the ASD and non-ASD groups using chi-square tests of independence. All expected cell frequencies were greater than five. The association between diagnosis and endorsement of RSM was significant, $\chi^2(1)=20.15$, $p<.001$. This association was moderately strong, $\varphi = .43$, $p<.001$, with 47.2% of individuals without ASD and 100% of individuals with ASD endorsing at least one RSM behavior. The association between diagnosis and

endorsement of IS was not significant, $\chi^2(1)=5.31$, $p=.021$. This association was low, $\varphi = .22$, $p<.021$, with 21.3% of individuals without ASD and 45.5% of individuals with ASD endorsing at least one IS behavior.

Study 2 (RDCRN; 3 years old and older)

Participants. Participants in the RDCRN study were 85 individuals with an average age of 8.9 years (*SD*=4.8 years). Participants were 37.6% female. The Study 2 sample was largely Caucasian and non-Hispanic/Latino (Table 3). While 53.1% of participants met criteria for either Autism or Autism Spectrum on the ADOS-2, approximately 45.9% received a consensus clinical diagnosis of ASD during their baseline study visit. Over 70% of individuals in Study 2 had a reported lifetime prevalence of generalized or focal seizures. Cognitive ability of 69 individuals was obtained, and of those individuals, the mean ability level was in the well below average range (*M*=60.3, *SD*=17.5) with variability across participants. Of 77 individuals who had a score on the Vineland-II, the mean ability level was also in the well below average range (*M*=65.1, *SD*=14.7). When cognitive and adaptive ability were combined and coded as above or below the cutoff for intellectual disability, including those who could not be administered a cognitive assessment due to functional capacity as falling in the intellectual disability range, only 32.9% of individuals in the overall sample had abilities above this cutoff. Overall, this sample was older and more impaired than the participants in Study 1.

Results. To address hypothesis 1.1, Mann-Whitney U tests with Bonferroni adjusted alpha levels of .003 per test were run to determine if there were differences in the presence and severity restricted repetitive behaviors on the ADI-R between the ASD and non-ASD groups (Table 3). Dependent variables included the total number of

endorsed items, the total severity of endorsed items, the average severity of endorsed items, the number of items scored a 1, 2, or 3, and the CTotal summary score from the ADI-R. As assessed via visual inspection, distributions of the RRB variables for ASD and non-ASD groups were not similar. Total number of endorsed items for the ASD group (mean rank=47.46) was significantly higher than for the non-ASD group (mean rank=19.73), U=1062.5, z=5.74, p<.001. Severity of endorsed items for the ASD group (mean rank=47.28) was significantly higher than for the non-ASD group (mean rank=19.95), U=1055.5, z=5.64, p<.001. Average severity of endorsed items for the ASD group (mean rank=43.92) was significantly higher than for the non-ASD group (mean rank=24.06), U=928.0, z=4.14, p<.001. Total number items scored a 1 for the ASD group (mean rank=59.65), was significantly higher than for the non-ASD group (mean rank=27.63), U=1546.5, z=6.06, p<.001. Total number items scored a 2 for the ASD group (mean rank=53.58), was significantly higher than for the non-ASD group (mean rank=32.90), U=1309.5, z=4.42, p<.001. Total number items scored a 3 for the ASD group (mean rank=49.77), was significantly higher than for the non-ASD group (mean rank=36.20), U=1161.0, z=3.16, p=.002. CTotal Summary Score for the ASD group (mean rank=59.65), was significantly higher than for the non-ASD group (mean rank =27.63), U=1546.5, z=6.05, p<.001.

For further clarification of the RRB profile, Mann-Whitney U tests with Bonferroni adjusted alpha levels of .003 per test were run to examine differences in RRBs between the ASD and non-ASD groups on additional measures. Dependent variables were Overall Items Endorsed on the RBS-R, Overall Total on the RBS-R, RBS-R domain raw scores (i.e., Stereotypic Behavior, Self-Injurious Behavior, Compulsive Behaviors, Ritualistic Behavior, Sameness Behavior, Restricted Interest), and the

Stereotypic Behavior subscale on the ABC-C. Descriptives of these items can be found in Table 5. Overall Items Endorsed on the RBS-R for the ASD group (mean rank=53.57) was significantly higher than for the non-ASD group (mean rank=29.26), U=1279.0, z=4.67, p<.001. Overall Total score on the RBS-R for the ASD group (mean rank=53.82) was significantly higher than for the non-ASD group (mean rank=29.03), U=1288.5, z=4.76, p<.001. On the RBS-R, total number of items and total score for the Stereotypic Behavior (p<.001), Self-Injurious Behavior (p=.001), Compulsive Behavior (p=.002), and Ritualistic Behavior (p<.001) domains were significantly greater for the ASD group than for the non-ASD group (p's<0.05). For Restricted Interests, total number of items endorsed was not significantly different between groups (p=.007), however total score was significantly higher for the ASD group than for the non-ASD group (p<.001). There was no difference between the ASD and non-ASD groups for number of items or total score for the Sameness Behavior subscale (p's>.69). The Stereotypy subscale of the ABC-C for the ASD group (mean rank=56.59) was significantly higher than the non-ASD group (mean rank=25.94), U=1409.5, z=101.6, p<.001.

To address hypothesis 2.1, association of endorsement of each RRB item (Table 1) and ASD diagnosis was explored using chi-square tests of independence. Frequencies of endorsement of each RRB variable can be found in Table 4. A chi-square test for association was conducted between clinical consensus diagnosis and endorsement of individual RRB items on the ADI-R (Figure 1). Expected cell frequencies for all variables were greater than five. The association between diagnosis and endorsement of Stereotyped Utterances and Delayed Echolalia (SU) was significant, $\chi^2(1)=11.03$, p=.001. This association was moderately strong, $\varphi = .36$, p=.001. The association

between diagnosis and endorsement of Verbal Rituals (VR) was significant, $\chi^2(1)=11.06$, $p<.001$. This association was moderately strong, $\varphi = .36$, $p<.001$. The association between diagnosis and endorsement of Unusual Preoccupations (UP) was significant, $\chi^2(1)=10.44$, $p=.001$. This association was moderately strong, $\varphi = .35$, $p=.001$ The association between diagnosis and endorsement of Circumscribed Interests (CU) was significant, $\chi^2(1)=9.36$, $p=.002$. This association was moderately strong, $\varphi = .33$, $p=.002$. The association between diagnosis and endorsement of Repetitive Use of Objects or Parts of Objects (RU) was significant, $\chi^2(1)=15.96$, $p<.001$. This association was moderately strong, $\varphi = .44$, $p<.001$. The association between diagnosis and endorsement of Compulsions/Rituals (CR) was not significant, $\chi^2(1)=3.77$, $p=.052$. The association between diagnosis and endorsement of Unusual Sensory Interests (SI) was significant, $\chi^2(1)=31.59$, $p<.001$. This association was strong, $\varphi = .61$, $p<.001$. The association between diagnosis and endorsement of Hand/Finger Mannerisms (HM) was significant, $\chi^2(1)=24.89$, $p<.001$. This association was strong, $\varphi = .54$, $p<.001$. The association between diagnosis and endorsement of Other Complex Mannerisms (OM) was significant, $\chi^2(1)=32.26$, $p<.001$. This association was strong, $\varphi = .62$, $p=.001$.

To address hypothesis 2.3, frequencies of Repetitive Sensorimotor behaviors and Insistence on Sameness behaviors were compared between the ASD and non-ASD groups using chi-square tests of independence. All expected cell frequencies were greater than five. The association between diagnosis and endorsement of RSM was significant, $\chi^2(1)=24.13$, $p<.001$. This association was strong, $\varphi = .54$, $p<.001$, with 48.9% of individuals without ASD and 97.4% of individuals with ASD endorsing at least one RSM behavior. The relationship between diagnosis and endorsement of IS was also significant,

$\chi^2(1)=16.36$, $p<.001$. This association was moderately strong, $\varphi = .44$, $p<.001$, with 35.6% of individuals without ASD and 79.5% of individuals with ASD endorsing at least one IS behavior.

Discussion

The current study helps to elucidate the profile of ASD-related symptomatology in individuals with TSC. Specifically, this study examines RRBs in individuals with TSC who do and do not have comorbid ASD. As RRBs emerge early in life and may be one of the first manifestations of ASD (Wolff et al., 2014), clarifying the RRB profile may allow for earlier identification of individuals with TSC who have ASD. It is of note that while all participants were recruited based on having a diagnosis of TSC, the enrollment criteria for Study 1 and 2 were largely discrepant, which required analyses to be completed separately for the two groups.

RRBs in TSC/ASD

The main purpose of the current study was to examine the presence and severity of RRBs in individuals with TSC and comorbid ASD. Few studies have examined RRBs in individuals with TSC to date, however, it is well documented that individuals with ASD in the general population showed increased amounts, frequency, and severity of RRBs as compared to typically developing children prior to 12 months of age (Ozonoff et al., 2008; Wolff et al., 2014). Based on this literature, it was hypothesized that children with TSC who have comorbid ASD would have increased number, frequency, and severity of RRBs than children with TSC alone in both studies. Individuals with TSC and ASD were also posited to exhibit a range of RRBs. However, it was expected that in Study 1, individuals with TSC and ASD would have higher rates of RSM than IS behavior, while in Study 2 they would have increased rates of both types of behaviors.

In the current project, individuals with ASD in both studies exhibited a greater total number and severity of RRBs endorsed on the ADI-R in comparison to individuals without ASD. This suggests that on an ASD-specific measure, individuals with ASD within the TSC population are differentiated from those without ASD in terms of the RRBs being endorsed. However, when considered at the item level, there were differences in the patterns of significance between Study 1 and Study 2. In the older and more impaired participants in Study 2, the ASD group had significantly higher scores on verbal rituals, stereotyped utterances and delayed echolalia, unusual preoccupations, circumscribed interests, repetitive use of objects or parts of objects, unusual sensory interests, hand/finger mannerisms, and other complex mannerisms. The only RRB that was not significantly different between groups in Study 2 was compulsions and rituals. This suggests that in older children with greater cognitive and adaptive impairments, individuals with ASD will have significantly greater RRBs of almost all types in comparison to individuals with TSC without ASD. This is in line with previous literature in individuals with ASD within the general population which suggests that as children with ASD age, they begin to exhibit more complex RRBs (Harrop et al., 2014; Militerni et al., 2002; Ozonoff et al., 2008; Richler et al., 2010; Wolff et al., 2014). However, it is also known that as children with ASD get older, they typically display fewer and less severe RRBs regardless of presence of ID (Esbensen, Selzter, Lam & Bodfish, 2009), which is not supported by the results of the current study given the continued presence of many lower order RRBs in the older sample of children. It should also be noted that individuals with ASD in the general population who have a comorbid diagnosis of ID have been shown to exhibit increased amount of repetitive movements (Esbensen et al., 2009), which may help to explain the continued presence of repetitive movements in

older individuals with TSC and ASD, as well as TSC overall. On the other hand, in the younger and less impaired individuals in Study 1, the ASD group only had significantly greater prevalence of repetitive use of objects or parts of objects, unusual sensory interests, and hand/finger mannerisms. This suggests that at a young age, individuals with ASD within the TSC population exhibit a unique profile of RRBs that differs from individuals with TSC alone. In the general population, children with ASD have been shown to exhibit repetitive motor movements and sensory preoccupations and interests by the age of 3 years (Harrop et al., 2014; Militerni et al., 2002; Ozonoff et al., 2008; Richler et al., 2010; Wolff et al., 2014), which reveals similarities in individuals with ASD in the general population and in the TSC population at 3 years of age. Results also may suggest that the profile of RRBs in individuals with ASD within the TSC population may change over time or that the profiles of RRBs between those with and without comorbid ASD may diverge as children age, leading to more RRBs being significant at older ages. It is important to note that while the ASD group in both studies did have significantly greater RRBs overall, the non-ASD group did not have a lack of RRBs, but still exhibited some level of many these behaviors. Overall, more research is needed to hone in on the profiles of RRBs in this population and how they may shift over time.

Within Study 2, it was possible to examine RRBs with non-ASD-specific measures as well as ASD-specific measures. As previously stated, it is well understood that individuals with ASD within the general population exhibit greater amounts and severity of RRBs beginning at a young age (Ozonoff et al., 2008; Wolff et al., 2014). Results from non-ASD specific measures further support similarities between ASD in the general population and in individuals with TSC in that overall number of items and severity of items endorsed were significantly higher for the ASD group as opposed to the

non-ASD group. At the item level, total number of items and severity of items endorsed was significantly different between ASD and non-ASD groups for stereotypic behavior, self-injurious behavior, compulsive behavior, and ritualistic behavior. For restricted interests, total number of items endorsed was not significantly different among groups, but severity diverged, in that the ASD group showed higher severity than the non-ASD group. Surprisingly, there were no differences among groups for sameness behavior. This suggests that RRBs truly differentiate individuals with ASD from those without ASD in the TSC population, even when using non-ASD specific measures. However, it is of note that not all domains of RRBs were significantly different between ASD and non-ASD groups, suggesting that individuals with TSC still have some elevated areas of RRBs. Additionally, the non-ASD group did not exhibit an absence of RRBs, but still demonstrated elevated levels of many types of RRBs. There is potential for screening measures to be developed based on the unique profile of RRBs exhibited.

When considering RSM and IS behaviors more broadly, there were again differences within the two studies. In Study 1 the prevalence of RSM behaviors was significantly higher in the ASD group, while the prevalence of IS behaviors did not differ among groups. In Study 2, the ASD group had significantly higher prevalence of both RSM and IS behaviors. Notably, almost 100% of participants with ASD from both studies were reported to have at least one RSM behavior, indicating that this is an RRB category that is particularly prevalent in this population. Of note, approximately 50% of individuals without comorbid ASD in both studies also reported the presence of at least one RSM behavior. This is unsurprising given that lower order behaviors, which are often broadly categorized as RSM behaviors, are tied closely with ID (Esbensen et al., 2009) and younger ages (Richler et al., 2010). However, this does further suggest a unique

pattern of RRBs in individuals with TSC who do not have a comorbid diagnosis of ASD. This further suggests that the close tie of ASD and ID in this population, as well as the finding that Study 2's participants had significantly lower cognitive and adaptive functioning, made it more likely for RSM behaviors to be endorsed. On a related note, only 45% of individuals with ASD in Study 2 reported the presence of an IS behavior, compared to the 80% of those with ASD in Study 1. Additionally, in individuals with ASD within the general population, distinct developmental trajectories have been suggested, with RSM behaviors beginning early in life and staying stable with age, while IS behaviors are infrequent early in life and increase with age, particularly with increased in cognitive ability (Richler et al., 2010). Study 1 participants with ASD endorsed a much greater percentage of IS behaviors, which may be related to their overall mean ability level being higher than Study 2. However, these individuals were only 3 years of age, which is contradictory to literature that suggests that IS behaviors are associated with older age as well as increased cognitive abilities (Richler et al., 2010). Overall, more research is needed to examine the developmental trajectories of RSM and IS behaviors in this population in both individuals with TSC alone and those with comorbid ASD.

ASD Diagnosis in TSC

While the current literature suggests that the prevalence of ASD within the TSC population is as high at 70% (Vignoli et al., 2015; Sundberg & Sahin, 2015), the current study found much lower rates. In Study 1, a longitudinal study without many inclusion criteria, only 20% of individuals received a consensus clinical diagnosis of ASD prior to age 3 years. On the other hand, in Study 2, where participants were recruited with the goal of having equal numbers of individuals with and without ASD, the rate of ASD consensus clinical diagnosis was around 50%. The results from Study 1 suggest that the

prevalence of ASD may be lower than has been previously suggested, as the individuals presenting for past research studies may have been more impacted and therefore had a greater likelihood of having ASD. Alternately, the children in this sample may have been too young for their ASD symptoms to be clearly distinguished from other aspects of developmental delays. However, in this sample it was also found that ASD diagnosis is fairly stable within the TSC population as early as 3 years of age, as only 2 diagnoses changed from the 3-year Study 1 visit to the baseline Study 2 visit. Additionally, it is of note that the certainty score for the ASD diagnosis was low at both of these time points, suggesting that these two individuals had more complex presentations. Interestingly, in both studies, the percentage of individuals who received a clinical diagnosis of ASD through the study was lower than the percentage of individuals with TSC who were classified as Autism or Autism Spectrum on the ADOS-2. This suggests that the ADOS-2 alone is not enough to diagnosis ASD in this population and different approach may be required to differentiate the general delays related to TSC and more ASD-specific symptomatology that indicates a diagnosis of ASD. Future research is needed to examine children with TSC longitudinally over a greater period of time to determine whether ASD diagnosis is stable throughout childhood and whether the profile of RRBs and other ASD-related symptomatology changes as individuals with TSC age.

Developmental Profiles

In terms of general development, individuals in both Study 1 and Study 2 had cognitive abilities in the low average to well below average range, with large variability. Individuals in Study 1 had abilities in the low average range, indicating that only approximately 30% of the sample met criteria for ID. This is in stark contrast to Study 2 participants who had a mean ability level in the well below average range, with 70% of

individuals meeting criteria for ID. However, it should be noted that Study 2's inclusion criteria included having suspected or confirmed ASD or ID, which may be greatly inflating the true prevalence of comorbid ASD and ID in this population. Participants in Study 1 may be a better representation of the prevalence rates of ASD and ID, as the study had few inclusion or exclusion criteria. These results are contradictory to current literature which suggests that approximately 50-60% of individuals have cognitive impairments (Asato & Harden, 2004; Chung et al., 2017; Eden et al., 2014; Kopp et al., 2008; Prather & de Vries, 2004). However, the link between ASD diagnosis and intellectual disability cannot be denied, as individuals in both studies who met criteria for ASD had significantly worse scores on measures of cognitive and adaptive functioning. In the general population, approximately 50% of individuals with ASD have cognitive impairments (Christensen et al., 2016; Kantzer et al., 2018; McGovern & Sigman, 2005). This discrepancy between the link of ASD and ID in the general population and in individuals with TSC in the current study suggests that further research needs to examine this link between ASD and ID in this population and determine how to best differentiate the two disorders.

Behavioral Profile

In terms of other behavioral difficulties, there were notably higher rates of aggression and self-injury within individuals with ASD in Study 2 in comparison to children with TSC without ASD. This finding adds to past literature that illustrates high rates of self-injurious behavior and aggression within individuals with TSC (Hunt, 1997; Kopp, Muzykewicz, Staley, Thiele & Pulsifer, 2008; de Vries, Hunt & Bolton, 2008; Eden et al., 2014; Staley, Montenegro, Major, Muzykewicz, Halpern, Kopp et al., 2008). However, it should be noted that these behaviors are also closely tied to intellectual

disability (McClinktock, Hall & Oliver, 2003), suggesting that individuals with ASD within the TSC population may be particularly at risk for behavioral difficulties given the close ties of ASD and ID in TSC. These behaviors could not be examined in Study 1 due to the differences in methodology utilized in the two studies, which suggests a need for further research into the behavioral difficulties that this population faces from birth onward.

Seizures

In both studies, there was also a high lifetime prevalence of generalized or focal seizures, with 50% and 70% of individuals reporting one or both types of seizures in Study 1 and Study 2, respectively. It is important to note that within both studies, individuals with comorbid ASD had much higher rates of generalized seizures. However, within Study 2, individuals with and without ASD did not differ on the rates of focal seizures, as they did with generalized seizures, with over 70% reporting lifetime prevalence of focal seizures. Current literature suggests that between 70 and 95% of individuals with TSC have epilepsy (Saxena & Sampson, 2015), therefore Study 1 once again illustrates a lower prevalence of seizures in younger children with TSC than would be expected given previous work. However, it is well recognized that the most common type of seizures in the TSC population are focal seizures (Asato & Harden, 2004; Jeste et al. 2016; Saxena & Sampson, 2015; Zaroff et al., 2004), therefore finding high rates of focal seizures in both studies is unsurprising. Additionally, for individuals with ASD in the general population, epilepsy is present in up to 44% of individuals (Jeste & Tuchman, 2015; Strasser et al., 2016), which may suggest that individuals with TSC and comorbid ASD may be at particular risk for seizure development, explaining the increased prevalence of seizures in both ASD groups. Previous literature also suggests a close link

of epilepsy and ID within the ASD population (Strasser et al., 2017), which may further explain increased epilepsy prevalence in this population.

Limitations and Future Directions

While the current study further illuminates the profile of RRBs present in individuals with TSC with and without comorbid ASD, there are several important limitations to generalizations of findings. Of particular importance was the fact that inclusion and exclusion criteria for the two longitudinal studies utilized were not the same, barring any direct comparison of the two studies. It should be noted that the individuals in Study 1, who were followed closely by the study team for the first three years of life, were likely to have earlier access to intervention than Study 2, possibly limiting the generalization of results. Future research should examine RRBs longitudinally for an increased period of time to clarify the RRB profile further. Furthermore, based on the high prevalence of ID-level functioning in the ASD groups in both studies, as well as previous literature tying ASD and cognitive delays in TSC (Jeste et al., 2014), ASD and ID could not be separated out in this population. Future studies should consider examining RRBs in individuals with TSC with and without ID to determine how RRBs may differ based on ability level, rather than solely ASD diagnosis. It will be particularly important for future studies to have no inclusion criteria regarding ASD or ID, as to more accurately conceptualize the prevalence of ASD within the TSC population and to determine what the ASD-symptom profile looks like across the range of abilities in individuals with TSC with and without ASD.

Overall, the current study adds to the current literature base on RRBs in individuals with TSC. This current study suggests that there are differential profiles of RRBs in individuals with comorbid TSC and ASD compared to TSC alone, information

which may lead to the development of screening tools to improve early detection and diagnosis of ASD within complex medical populations at risk for ASD.

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Tables and Figures

Figure 1.

Data Analysis Variables

Measure	Item	Variables	Use
ADI-R	All items	Total number of items endorsed	Hypothesis 1.1
		Total score of endorsed items (sum of endorsed items)	
		Average score of endorsed items (average across items)	
		Number of items with score of 1	
		Number of items with score of 2	
		Number of items with score of 3	
	C Total	Average CTotal	Hypothesis 1.1
	C1	Average C1	Hypothesis 2.1
	C2	Average C2	
	C3	Average C3	
	C4	Average C4	
	Stereotyped Utterances and Delayed Echolalia (SU)	Frequency of SU endorsement	Hypothesis 2.1
	Verbal Rituals (VR)	Frequency of VR endorsement	
	Unusual Preoccupations (UP)	Frequency of UP endorsement	
	Circumscribed Interests (CU)	Frequency of CU endorsement	
	Repetitive Use of Objects or Interest in Parts of Objects (RU)	Frequency of RU endorsement	
	Compulsions/Rituals (CR)	Frequency of CR endorsement	
	Unusual Sensory Interests (SI)	Frequency of SI endorsement	
	Hand and Finger Mannerisms (HM)	Frequency of HM endorsement	
	Other Complex Mannerisms or	Frequency of OM endorsement	

	Stereotyped Body Movements (OM)		
	Repetitive Sensorimotor	Frequency of endorsement	Hypothesis 2.2, 2.3
	Insistence on Sameness	Frequency of endorsement	Hypothesis 2.2, 2.3
RBS-R	Total Raw Score	Average Total Raw Score	Hypothesis 1.1
	Stereotyped Behavior Domain	Average SB Raw Score	Hypothesis 2.1
	Self-Injurious Behavior Domain	Average SIB Raw Score	
	Compulsive Behavior Domain	Average CB Raw Score	
	Ritualistic Behavior Domain	Average RiB Raw Score	
	Restricted Behavior Domain	Average ReB Raw Score	
ABC	Stereotypic Behavior Subscale	Average Score	Hypothesis 1.1

Figure 2.

Variable Groups

Repetitive Sensorimotor	Hand and Finger Mannerisms (HM)
	Other Complex Mannerisms or Stereotyped Body Movements (OM)
	Repetitive Use of Objects or Interest in Parts of Objects (RU)
	Unusual Sensory Interests (SI)
Insistence on Sameness	Compulsions/Rituals (CR)
	Verbal Rituals (VR)
	Unusual Preoccupations (UP)
	Circumscribed Interests (CU)

Table 1.

Participant Characteristics By Study

	n	ASD *(%)*	Age *(years) M (SD)*	Gender *(% Female)*	Race *(%)* Caucasian	African American	Other	Ethnicity *(% Hispanic)*	Generalized Seizures *(%)*	Focal Seizures *(%)*	Cognitive Standard Score *M (SD)*	Adaptive Standard Score *M (SD)*
Overall	196	31.1%	5.6 (4.3)	44.9%	82.1%	3.1%	10.6%	20.4%	64.8%	66.2%	72.9 (24.1)	75.2 (17.5)
TACERN	111	19.8%*	3.1 (0.2)*	50.5%	81.1 %	0.9%	11.7%	20.7%	57.7%*	58.6%*	83.4 (23.9)*	82.3 (15.6)*
RDCRN	85	45.9%*	8.9 (4.8)*	37.6%	83.5%	5.9%	9.5%	20.0%	74.1%*	76.2%*	60.3 (17.5)*	65.1 (14.7)*

*significantly differed, $p<0.05$

Table 2.

Participant Characteristics by Study and Diagnosis

	n	Age *(years) M (SD)*	Gender *(% Female)*	Race *(%)* Caucasian	African American	Other	Ethnicity *(% Hispanic)*	Generalized Seizures *(%)*	Focal Seizures *(%)*	Cognitive Standard Score *M (SD)*	Adaptive Standard Score *M (SD)*
TACERN	111										
ASD	22	3.08 (0.15)	50%	77.3%	0%	13.6%	27.3%	86.4%*	77.3%*	58.17* (16.61)	66.57* (10.86)
Non-ASD	89	3.05 (0.14)	50.6%	82.0 %	1.1%	11.2%	19.1%	50.6%*	53.9%*	87.63* (22.30)	86.14* (14.19)
RDCRN	85										
ASD	39	9.40 (4.74)	38.5%	87.2 %	0%	10.3%	23.1%	89.7%*	79.5%	54.33* (17.23)	56.54* (11.46)
Non-ASD	46	8.47 (4.89)	37.0%	80.4%	10.9%	8.7%	17.4%	60.9%*	71.7%	64.07* (16.77)	72.21* (13.42)

*significantly differed among groups within each respective study, $p<0.05$

Table 3.

Descriptives for ADI-R RRB Item and Domain Scores

Mean (SD)	SU	VR	UP	CU	RU	CR	SI	HM	OM	CTotal	Aggression to Family	Aggression to Non-Family	Self-Injury
TACERN													
ASD	0.33	0.10	0.41	0.35	1.32	0.36	1.16	1.32	0.63	3.96	-	-	-
	(0.58)	(0.32)	(0.80)	(0.75)	(0.72)	(0.73)	(0.76)	(0.84)	(0.96)	(1.99)			
Non-ASD	0.18	0.14	0.15	0.08	0.31	0.14	0.29	0.23	0.15	1.12	-	-	-
	(0.50)	(0.12)	(0.44)	(0.27)	(0.58)	(0.40)	(0.52)	(0.56)	(0.43)	(1.37)			
RDCRN													
ASD	1.16	1.08	0.85	1.03	1.58	0.64	1.31	1.56	1.46	5.90	1.39	0.95	0.92
	(0.85)	(1.08)	(1.16)	(1.20)	(1.06)	(1.06)	(0.66)	(1.12)	(1.07)	(2.94)	(1.02)	(1.05)	(0.93)
Non-ASD	0.24	0.20	0.11	0.22	0.51	0.29	0.31	0.27	0.18	1.64	0.91	0.32	0.32
	(0.64)	(0.67)	(0.38)	(0.60)	(0.84)	(0.76)	(0.51)	(0.62)	(0.53)	(1.96)	(1.17)	(0.77)	(0.67)

SU: Stereotyped Utterances and Delayed Echolalia, VR: Verbal Rituals, UP: Unusual Preoccupations, RU: Repetitive Use of Objects or Interest in Parts of Objects, CR: Compulsions/Rituals, SI: Unusual Sensory Interests, HM: Hand and Finger Mannerisms, OM: Other Complex Mannerisms or Stereotyped Body Movements

Table 4.

Frequency of ADI-R RRBs

	Frequency Endorsed (%)									
	SU	VR	UP	RU	CR	SI	HM	OM	RSM	IS
TACERN										
ASD	4.50%	4.50%	22.70%	86.40%*	22.70%	68.20%*	77.30%*	22.70%	100%*	45.50%*
Non-ASD	9.00%	1.10%	11.20%	24.70%*	11.20%	20.20%*	15.70%*	9.00%	47.20%*	21.30%*
RDCRN										
ASD	46.10%*	35.90%*	38.50%*	76.90%*	30.80%	89.70%*	71.80%*	71.80%*	97.40%*	79.50%*
Non-ASD	13.10%*	6.50%*	8.90%*	33.33%*	13.30%	28.90%*	17.80%*	11.10%*	48.90%*	35.60%*

SU: Stereotyped Utterances and Delayed Echolalia, VR: Verbal Rituals, UP: Unusual Preoccupations, RU: Repetitive Use of Objects or Interest in Parts of Objects, CR: Compulsions/Rituals, SI: Unusual Sensory Interests, HM: Hand and Finger Mannerisms, OM: Other Complex Mannerisms or Stereotyped Body Movements, RSM: Repetitive Sensorimotor Behaviors, IS: Insistence on Sameness Behaviors

*Significantly differed among groups within each respective study, $p<0.05$

Table 5.

Study 2: Descriptives of RBS-R and ABC-C RRBs

Mean (SD)	ABCC Stereotypy	RBS-R Stereotypic Behavior	RBS-R Self-Injurious Behavior	RBS-R Compulsive Behavior	RBS-R Ritualistic Behavior	RBS-R Sameness Behavior	RBS-R Restricted Interest	RBS-R Overall Items Endorsed	RBS-R Overall Total
ASD	7.89 *	7.81*	3.16*	4.41*	5.46*	6.26	4.14*	20.41*	33.95*
	(4.91)	(4.64)	(2.58)	(4.23)	(4.15)	(5.26)	(2.87)	(9.72)	(18.99)
Non-ASD	1.38*	1.98*	2.26*	2.07*	2.35*	4.33	2.19*	8.91*	13.21*
	(2.96)	(2.58)	(4.46)	(2.96)	(3.35)	(5.10)	(3.08)	(8.84)	(13.89)

*significantly differed, $p<0.05$

Appendix A

IRB Documentation

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

470 Administration Building
701 20th Street South
Birmingham, AL 35294-0104
205.934.3789 | Fax 205.934.1301 | irb@uab.edu

APPROVAL LETTER

TO: Bebin, Martina

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196 (IRB 01)
IORG Registration # IRB00000726 (IRB 02)

DATE: 24-Aug-2018

RE: IRB-141211001
BCH IRB -- Autism Spectrum Disorder (ASD) and Intellectual Disability (ID) Determinants in Tuberous Sclerosis Complex (TSC)

The IRB reviewed and approved the Revision/Amendment submitted on 03-Aug-2018 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review:	Expedited
Expedited Categories:	b2
Determination:	Approved
Approval Date:	24-Aug-2018
Expiration Date:	19-Apr-2019

The following apply to this project related to informed consent and/or assent:

- Waiver of HIPAA

Amendment requesting use of study data for Helen Root's thesis, titled "Emergence of Restricted Repetitive Behaviors in Individuals with Autism Spectrum Disorder and Tuberous Sclerosis Complex."

Documents Included in Review:

- praf.180730

Project Revision/Amendment Form

Form version: June 26, 2012

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

- Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for investigators for additional information.
- Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.

1. Today's Date 7/27/18

2. Principal Investigator (PI)

Name (with degree)	Martina Bebin, MD, MPA	**Blazer ID**	ebebin
Department	Neurology	**Division (if applicable)**	epilepsy
Office Address	CIRC 312	**Office Phone**	934-3866
E-mail	ebebin@uab.edu	**Fax Number**	975-6255

Contact person who should receive copies of IRB correspondence (Optional)

Name	Jennifer Mahaffey	**E-Mail**	jmahaffe@uab.edu
Phone	6-4030	**Fax Number**	
Office Address (if different from PI)	SC 460		

3. UAB IRB Protocol Identification

3.a. Protocol Number F141211001

3.b. Protocol Title Autism Spectrum Disorder (ASD) and Intellectual Disability (ID) Determinants in Tuberous Sclerosis Complex (TSC)

3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- ☐ **Study has not yet begun** — No participants, data, or specimens have been entered.
- ☒ **In progress, open to accrual** — Number of participants, data, or specimens entered: 46
- ☐ **Enrollment temporarily suspended by sponsor**
- ☐ **Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)**
  - Date closed:
  - Number of participants receiving interventions:
  - Number of participants in long-term follow-up only:
- ☐ **Closed to accrual, and only data analysis continues**
  - Date closed:
  - Total number of participants entered:

4. Types of Change

Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- ☐ **Protocol revision (change in the IRB-approved protocol)**
  In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Protocol amendment (addition to the IRB-approved protocol)**
  In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.
- ☒ **Add or remove personnel**
  In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.
  - ☒ **Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication**
    In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).
- ☐ **Change in source of funding; change or add funding**
  In Item 5.c., describe the change or addition in detail, include the applicable OSP proposal number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.
- ☐ **Add or remove performance sites**
  In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

☐ **Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)**
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

☐ **Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)**
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

☐ **Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)**
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

☐ **Revise or amend consent, assent form(s)**
Complete Item 5.d.

☐ **Addendum (new) consent form**
Complete Item 5.d.

☐ **Add or revise recruitment materials**
Complete Item 5.d.

☐ **Other (e.g., investigator brochure)**
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable.
Include a copy of all affected documents, with revisions highlighted as applicable.

5. Description and Rationale

In Item 5.a. and 5.b, check Yes **or** No **and see instructions for** Yes **responses.**
In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.

☐Yes ☒No **5.a. Are any of the participants enrolled as normal, healthy controls?**
If yes, describe in detail in Item 5.c. how this change will affect those participants.

☐Yes ☒No **5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?**
If yes, FAP-designated units complete a FAP submission and send to fap@uab.edu. Identify the FAP-designated unit in Item 5.c.
For more details on the UAB FAP, see www.uab.edu/cto.

5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol.

Previously added personnel, Helen Root, will be working on this project as a research assistant. We are submitting an amendment at this time because she will be using data from this project towards her thesis. The working title is: "Emergence of Restricted Repetitive Behaviors in Individuals with Autism Spectrum Disorder and Tuberous Sclerosis Complex."
Ms. Root's analysis of the data is different but does not change the overall purpose of the study. The overall study aims to characterize the developmental phenotype of autism spectrum disorder (ASD) within a sample of individuals with tuberous sclerosis complex (TSC), with the hopes of improving early detection and diagnosis of ASD within this population. Ms. Root's analysis will examine restricted repetitive behaviors, a single component of the developmental phenotype of ASD. Rather than examining the broader phenotype of ASD within this rare population, Ms. Root will characterize the number, frequency, types, and severity of restricted repetitive behaviors experienced by individuals with TSC and comorbid ASD.

5.d. Consent and Recruitment Changes: In the space below,
(a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them;
(b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and
(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" changes**
- **a revised copy for the IRB approval stamp.**

Signature of Principal Investigator M. Belsky MD Date 7/30/18