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Heterogeneity of the Language Network in Autism Spectrum Disorders: A Data-driven Study of Neurophenotypes

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Clinical Psychology

by

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2020

The Dissertation of Yangfeifei Gao is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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2020

DEDICATION

Dedicated to my family, both here and in China, thank you for instilling the value of education and love of knowledge in me; to my parents for modeling what it's like to be life-long learners; to my JDP family for being the best cohort through rain or shine; and to my soon-to-be husband, Anthony Mance, for his unwavering love and support.

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ABSTRACT OF DISSERTATION

Heterogeneity of the Language Network in Autism Spectrum Disorders: A Data-driven Study of Neurophenotypes

by

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Doctor of Philosophy in Clinical Psychology

University of California San Diego, 2020
San Diego State University, 2020

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Autism Spectrum Disorders (ASD) are heterogeneous developmental disorders associated with atypical functional connectivity (FC) and neuroanatomy. Language impairments affect individuals with ASD, but the neural underpinnings remain elusive, partly due to the heterogeneity across the ASD population. The current studies utilized multimodal neuroimaging to explore 1) differences in language network intrinsic FC (iFC) between children diagnosed with ASD and typically developing (TD) children; 2) whether there are distinguishable ASD subgroups based on language network iFC patterns, and 3) how these iFC subgroups relate to ASD subgroups derived from anatomical features of the language network. Study 1 (Gao et al., 2019): Seed-to-whole brain iFC analyses revealed that school-age children with ASD (*n*= 52) had increased iFC of language regions with posterior cingulate cortex and visual regions, in

comparison to TD peers (*n*=50). Study 2: An expanded sample of 69 children with ASD showed atypically increased within-group heterogeneity of language network iFC. Latent profile analysis (LPA) of language iFC dimensions revealed three distinct ASD subtypes, each with lower language abilities than their TD peers (*n*= 60): one subgroup that was similar to the TD group in iFC, and two subgroups that exhibited broad under- and overconnectivity, respectively. Study 3: LPA of anatomical dimensions of combined cortical thickness (CT) and local gyrification index (lGI) in a cohort of 104 ASD children uncovered three distinct subgroups (sASD1-3): sASD1 characterized by increased lGI and lower language scores, sASD2 composed of older ASD participants with lower sociocommunicational symptoms, and sASD3 showed greater CT in left hemisphere language regions. Subgroup membership based on iFC- and morphology-based LPA was not clearly related. Neither subgroup type (iFC or structural) was related to diffusion indices of language-related white matter tracts. Overall, our findings expand on previous reports of increased heterogeneity and atypical language network iFC in ASD. Language network ASD subtypes and membership differed across imaging modalities. The existence of ASD subtypes with distinct iFC and anatomical patterns may explain conflicting results in the ASD imaging literature. Our findings underscore the need to focus on individual variability in ASD beyond conventional group-level analyses.

INTRODUCTION OF DISSERTATION

Language abilities in ASDs

Autism Spectrum Disorders (ASDs) are a group of heterogeneous neurodevelopmental disorders first described in the 1940s by Leo Kanner (1943) and Hans Asperger (1944). The Austrian researchers separately identified children with insistence on sameness and difficulties relating to others. Even in their initial conceptualization of the disorders, Asperger and Kanner described affected children who displayed difference of language abilities, intelligence, and sensorimotor symptoms. Since then, the diagnostic criteria for ASDs have gone through considerable changes, most recently being defined by core symptoms of persistent deficits in social communication, and restricted, repetitive patterns of behavior or interests (American Psychiatric Association, 2013). The expression of these symptoms varies greatly in frequency and severity amongst diagnosed individuals.

The large variability in language abilities exhibited by individuals on the spectrum is a salient example of the overall behavioral heterogeneity that is characteristic of ASDs (Tager-Flusberg, Paul, & Lord, 2013). Although absence or delay in development of spoken language is no longer a criterion for the diagnosis of ASD based on the current Diagnostic Statistical Manual of Mental Disorders (American Psychiatric Association, 2013), language impairments are pervasive. In fact, a recent naturalistic language study found 3.7% of children diagnosed with an ASD to be nonverbal, and 34% of affected children only have minimal verbal abilities at age 3 (Bacon, Osuna, Courchesne, & Pierce, 2018). Those who develop functional verbal language exhibit qualitative differences in spoken language compared to typically developing (TD) individuals (Boucher, 2012; Eigsti, de Marchena, Schuh, & Kelley, 2011). Language is a particularly important domain of study in ASDs because a delay in language development is one

of the best identifiers for the later diagnosis of ASDs in children (Mitchell, Cardy, & Zwaigenbaum, 2011; Stone et al., 1999). Language abilities are also among the best predictors of outcomes (e.g., adaptive skills) later in life for individuals with ASDs (Mawhood, Howlin, & Rutter, 2000; Sallows & Graupner, 2005; Szatmari et al., 2015; Szatmari, Bryson, Boyle, Streiner, & Duku, 2003). Better characterization of language impairments and the mechanisms underlying these deficits would improve the development of treatments for this highly prevalent set of disorders, which is currently estimated to affect 1 in 59 children aged 8 years in the United States (Baio et al., 2018).

Behavioral studies of language show a broad spectrum of abilities in ASDs. Some verbal individuals may display echolalia, neologisms, and idiosyncratic or inappropriate use of words and phrases (Eigsti et al., 2011). There is also individual variability in the trajectories of language development, with a majority showing delay in age of first word utterance by up to 18 months compared to TD children (Howlin, 2003). However, language onset and rate of language acquisition differs widely between ASD children. Across linguistic and functioning levels, all individuals with ASDs display difficulties with social aspects of communication including pragmatic language skills (e.g., turn-taking), nonverbal communication (e.g., gesturing, eye-contact, emotional expression), and prosody (Eigsti et al., 2011).

In a review of structural language in ASDs, Boucher (2012) reported on multiple studies supporting a receptive-expressive language profile that is unique to ASDs, with higher expressive than receptive abilities. However, other studies have shown mixed or opposite patterns (Kjelgaard & Tager-Flusberg, 2001; Luyster, Kadlec, Carter, & Tager-Flusberg, 2008), and a recent meta-analysis (Kwok, Brown, Smyth, & Oram Cardy, 2015) found no evidence supporting expressive-over-receptive language advantages in ASDs. Instead, this meta-analysis

revealed overall reduced levels of both receptive and expressive language in ASDs, with negligible differences between production and comprehension.

Brain organization for language in ASDs

Functional neuroimaging techniques (e.g., functional magnetic resonance imaging (fMRI) and positron-emission topography (PET)) allow for the in-vivo observation of neural activity associated with cognitive processes, such as reading and sentence construction. While considered to be restricted to left perisylvian regions (Broca's and Wernicke's area) in the classic neurological model (N. Geschwind, 1970), imaging studies in recent decades have shown a more expansive language system that includes regions such as the dorsal striatum, precuneus, inferior parietal lobule, and parts of the cerebellum (Berl et al., 2014; Price, 2010; Rodd, Vitello, Woollams, & Adank, 2015). Imaging studies have revealed that language function is predominantly lateralized to the left hemisphere in typical adults (McAvoy et al., 2016; Nielsen, Zielinski, Ferguson, Lainhart, & Anderson, 2013). However, language processing in TD children also involves homologous regions in the right hemisphere (Gaillard et al., 2000), with increased left hemisphere lateralization with age (Berl et al., 2014). In addition to covering localization of cognitive functions in healthy participants, neuroimaging also permits precise comparisons of functional neuroanatomy of language between diagnostic groups.

Imaging research in ASDs has revealed several anomalies in language processing. A first set of findings comes from activation studies implementing language tasks. Evidence includes atypical activation in visual cortex during language tasks across multiple studies (Gaffrey et al., 2007; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Knaus, Silver, Lindgren, Hadjikhani, & Tager-Flusberg, 2008; Pang et al., 2016). In addition, language comprehension and production have been found to be associated with unusual levels of activity in homologous language regions

of the right hemisphere in ASDs (Anderson et al., 2010; Eyler, Pierce, & Courchesne, 2012; Groen et al., 2010; Herringshaw, Ammons, DeRamus, & Kana, 2016; Kleinhans, Müller, Cohen, & Courchesne, 2008; Knaus et al., 2010; Müller et al., 1999; Nielsen et al., 2014; Williams, Goldstein, & Minshew, 2006).

A second set of findings relates to network organization and connectivity. The complex range of symptoms observed in ASDs is thought to reflect impairment in multiple, distributed neural networks (Geschwind & Levitt, 2007; Müller, 2007; Rippon, Brock, Brown, & Boucher, 2007). Functional connectivity (FC) MRI examines functional network organization by observing low frequency (<0.1Hz) blood-oxygen level dependent (BOLD) signal fluctuations that are synchronized between distributed brain regions. These BOLD signal fluctuations can be detected not only during task performance, but also during rest. In the latter case of resting state fMRI, correlations of slow, spontaneous BOLD signal fluctuations between brain regions, interpreted as intrinsic functional connectivity (iFC), are detected while subjects are either awake but not performing a tasks or while asleep (Van Dijk et al., 2010). Venkataraman and colleagues (2015) employed data-driven functional connectomics on a large dataset from the Autism Brain Imaging Data Exchange (ABIDE; Di Martino et al., 2014) and found that two functional networks with regions implicated in language function (e.g. left middle temporal gyrus, left posterior cingulate, left supramarginal gyrus, left middle superior temporal sulcus) differentiated ASD from TD children. However, characterization of FC of the language network in ASDs has been disputed, with some studies showing general underconnectivity between language regions (Just, Cherkassky, Keller, & Minshew, 2004; Kana et al., 2006; Knaus et al., 2008; Verly et al., 2014) and others showing mixed effects or overconnectivity (Lee, Park, James, Kim, & Park, 2017; Shen et al., 2012). The conflicting results have been attributed to methodological

differences in 'co-activation' FC and iFC MRI, data processing, and differences in sample characteristics (e.g., language level of ASD participants; Hull, Jacokes, Torgerson, Irimia, & Van Horn, 2017). In addition, previous studies with small sample sizes lacked the power to detect reliable effects (Button et al., 2013). Furthermore, results may be affected by outliers or overrepresentation of groups of ASD participants (e.g., those with a specific symptom presentation) sampled within the small cohorts.

A third body of evidence supporting atypical language processing in ASDs comes from the structural imaging literature. Related to the functional findings of abnormal lateralization, structural asymmetry of grey matter volume is also seen in areas of the extended language network (e.g., inferior parietal lobule, supramarginal gyrus, primary auditory cortex) in adult men with ASDs (Floris et al., 2016). Joseph et al. (2014) found that increased grey matter rightward asymmetry of the pars opercularis (a part of Broca's area; Dronkers, Plaisant, Iba-Zizen, & Cabanis, 2007) was also associated with greater verbal abilities in a group of ASD children aged 4-7 years. Structural connectivity between language regions has also been reported to be affected in ASDs (Travers et al., 2012). Diffusion Tensor Imaging (DTI), which maps the diffusion of water molecules within and across white matter fibers (Mori & Zhang, 2006), can be used to characterize the volume, orientation, and integrity of white matter tracts between brain regions. One DTI study (Moseley et al., 2016) found bilaterally decreased volume of the arcuate fasciculus (the white matter tract that connects frontal and temporal language regions; Geschwind, 1970) in adults with ASDs compared to typical controls, but no group differences in fractional anisotropy (FA) and mean diffusivity (MD). Another study found no difference in FA, but pronounced increase in MD of the left arcuate fasciculus, compared to TD participants, in ASD individuals with language impairments, and less so in those without language impairments

(Nagae et al., 2012). Differences in findings further illustrate that certain observed effects may be driven by ASD subtypes and differential subtype composition in limited samples.

Similarly, when describing the variable language abilities in individuals with ASDs, Kwok and colleagues (2015) suggested that subgroups are likely to exhibit different expressive and receptive language profiles. The authors postulated that studies averaging across large heterogeneous samples of individuals with ASDs may not be able to detect such differences. Their reasoning was supported by a recent study of language phenotypes, which identified 3 ASD subgroups that differed in their spontaneous spoken language (Wittke, Mastergeorge, Ozonoff, Rogers, & Naigles, 2017).

Another important aspect of heterogeneity in ASDs is the evolution of symptomatology across the lifespan. For example, individuals with severe repetitive, ritualistic behavior in childhood may show a reduction in stereotypies in adulthood (Shattuck et al., 2007). However, developmental changes in symptom expression also differ *between* individuals with ASDs (Fountain, Winter, & Bearman, 2012; Pelphrey, Shultz, Hudac, & Vander Wyk, 2011; Szatmari et al., 2015). In a longitudinal study of expressive language development, Tek and colleagues (2014) found two distinct language profiles: a high language ASD group with developmental trajectories similar to those seen in typically development, and a lower language ASD group with less improvement over time. Similarly, Pickles et al. (2014) combined both expressive and receptive language in a latent growth-curve analysis and discovered 7 different developmental trajectories. Given the distinct patterns of functional language abilities and language development within ASDs, it is plausible that these various groups would also show neurobiological differences in language processing.

Heterogeneity in ASDs: Etiology and environmental effects

The vast differences in linguistic abilities and diversity of neuroimaging findings in ASDs may, in part, be due to varied etiologies. Twin and family studies have demonstrated the high heritability of ASDs, with a monozygotic concordance rate greater than 50% (Constantino et al., 2013; Hallmayer et al., 2011; Ronald et al., 2006). Recent reviews of genetic research in ASDs, report advances that have led to the identification of hundreds of genetic variants and mutations that may contribute to ASDs (Jeste & Geschwind, 2014; Vorstman et al., 2017). The reviews noted differences in the penetrance of genotypes, gender ratio, and comorbidities (e.g., epilepsy, motor impairments, schizophrenia), all of which suggest diverse underlying biological mechanisms that give rise to the ASD phenotype. Some studies have also revealed differences in the mode of transmission between simplex autism (one member within a family diagnosed with an ASD) and multiplex autism (multiple members within a family diagnosed with ASDs) as well as between females and males (Leppa et al., 2016; Sanders et al., 2015; Virkud, Todd, Abbacchi, Zhang, & Constantino, 2009). Leppa and colleagues found that even within multiplex families, there were differences in ASD risk copy-number variants between affected siblings, further supporting the complex etiological heterogeneity of ASDs. In addition to genetic contributions toward the risk of ASDs, environmental influences such as perinatal factors, maternal immune response, neuroinflammation, and the interaction between genetic and environmental factors (e.g., parental age, folate intake) have also been found to affect ASD risk (Garay & McAllister, 2010; Larsson et al., 2005; Mandy & Lai, 2016; Patel et al., 2017; Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005).

Individuals with ASDs vary in etiological origin of symptoms and in experiential factors such as the age of onset, types, and severity of symptoms, and subsequently, also in the type and

length of treatments they have received. The types of interventions sought out by parents and caregivers for the treatment of ASD-related symptoms differ widely from dietary restrictions (Sathe, Andrews, McPheeters, & Warren, 2017) to highly controversial, non-empirically supported therapies such as Chelation (Green et al., 2006; Hess, Morrier, Heflin, & Ivey, 2008). Even within the wide range of evidence-based therapies (EBTs) received by individuals diagnosed with ASDs, the targeted symptoms or behaviors differ greatly, along with the degree of demonstrated efficacy of each EBT (Wong et al., 2015). For example, speech therapy may be employed to address specific articulation and structural language concerns, while Applied Behavioral Analytic interventions may be used to build appropriate social behavior (Virués-Ortega, 2010). In addition, pharmacotherapies are often utilized in conjunction with behavioral interventions for the treatment of comorbid conditions such as hyperactivity and anxiety or to address difficult behaviors (e.g., risperidone to mitigate irritability and self-harm behavior, Broadstock, Doughty, & Eggleston, 2007; Howes et al., 2018; Jesner, Aref-Adib, & Coren, 2007). The type, start time, and duration of each intervention, and numerous combinations of multiple therapies all have differing effects on symptoms and long-term outcome of the ASD patient (Bradshaw, Steiner, Gengoux, & Koegel, 2015; Helt et al., 2008; Klintwall, Eldevik, & Eikeseth, 2015; Wong et al., 2015). Taken together, the evidence suggests highly heterogeneous etiologies that are further compounded by experiential and interventional variability in ASDs, all of which impact patterns of aberrant functional and structural connectivity.

The myriad of possible complex interaction of etiologies, differences in behavioral symptoms, developmental trajectories, treatment responses, and conflicting findings have raised questions about the validity of ASD as a single neurobiological construct (Waterhouse, London, & Gillberg, 2016). As an alternative to the assumption of a singular diagnostic group, researchers

have suggested studying more homogeneous subsets within the larger category of ASDs (Haebig & Sterling, 2017; Jeste & Geschwind, 2014; Sahin & Sur, 2015; Szatmari et al., 2015; Tager-Flusberg & Joseph, 2003). This may improve the detection of more nuanced differences within ASDs and thus enable the field to move toward better characterization of deficits and the design of more targeted treatments.

Subtype studies of ASDs

The studies of ASD subtypes has been mostly limited to behavioral data (DeBoth & Reynolds, 2017, 2017; Feczko et al., 2018; Haebig & Sterling, 2017; Kjelgaard & Tager-Flusberg, 2001; Pickles et al., 2014; Szatmari et al., 2015; Tager-Flusberg & Joseph, 2003; Tek et al., 2014). In a review of sensory-based subtypes in ASDs, DeBoth and Reynolds (2017) reported that studies found 3 to 5 different subtypes of sensory-processing profiles based on parent-report questionnaires and one standardized observational assessment (Baranek, Boyd, Poe, David, & Watson, 2007). The numbers of subtypes differed between studies depended on sensory response style, severity, and whether sensory domains were combined. In pursuit of specific cognitive phenotypes of ASDs, Feczko et al. (2018) utilized random forest and community detection techniques to identify patterns in neurocognitive profiles in children with and without ASDs diagnoses. The group found 3 ASD and 4 TD groups that differed in their performance in different cognitive domains, but also exhibited significantly difference in iFC of several function networks. Investigating language-related subtypes in ASDs, Tager-Flusberg and colleague (2001, 2003, 2006) identified a subgroup of children with ASD that also exhibit language impairments (ASD-LI), similar to non-ASD children with the diagnosis of specific language impairment (SLI). The authors also observed a shared reverse asymmetry (greater cortical volume of homologous frontal language area in the right hemisphere) in both ASD

children and SLI children (Tager-Flusberg & Joseph, 2003). Similarly, Lombardo et al. (2015) followed the developmental progress of infants and toddlers. They found that groups of children that were later diagnosed with ASDs and had poor language development also showed decreased left superior temporal activation to speech-language stimuli (around 12 months of age) compared to ASD and non-ASD children with good language scores. The results of these studies support the existence of stratified groups within ASDs that differ in their basic neurobiology.

Other groups have attempted to identify ASD phenotypes based on these neurobiological characteristics instead of behavioral symptoms. Amaral and colleagues (2017) reported a neurophenotype that is defined by disproportional large head-to-body size in boys with ASDs (ASD-DM). The team reported that the ASD-DM group is associated with higher rates regression, lower expressive language abilities, and slower acquisition of adaptive skills than ASD children with normal head size and TD children.

Recent advances in statistical learning techniques have precipitated a new wave of studies that utilize neuroimaging data to identify subgroups in clinical populations including schizophrenia, ADHD, and ASDs (Cauda et al., 2017; Costa Dias et al., 2015; Easson, Fatima, & McIntosh, 2017; Gates, Molenaar, Iyer, Nigg, & Fair, 2014; Hong, Valk, Di Martino, Milham, & Bernhardt, 2017; Sun et al., 2015; Van Dam et al., 2017; Yang et al., 2012). Cauda et al. (2017) utilized a combination of meta-analytic and machine learning techniques to differentiate schizophrenia spectrum disorders (SCZD), ASDs, and obsessive-compulsive spectrum disorders (OCSD) based on grey and white matter morphology across 203 studies. The authors found 2 clusters: the first was mostly specific to SCZD and consisted of grey matter alterations of frontal, insular, and anterior cingulate regions in the cognitive control system, and a second cluster that was mostly specific to OCSD and involved alterations in the auditory-visual, premotor, and

somatic systems. Grey and white matter alteration patterns seen in ASD studies were distributed across the two clusters. Hong et al. (2017) used hierarchical clustering of ABIDE participants with four neuroanatomical variables for each of 20,484 vertices: cortical thickness, grey and white matter boundary contrast, cortical surface area, and geodesic distance. They identified 3 anatomical ASD subtypes that also differed in iFC and symptom severity. Easson and collaborators (2017) identified 2 ASD subtypes using k-means clustering of static and dynamic iFC. The 2 groups did not differ in behavioral characteristics (e.g., ASD symptom severity, IQ) but both differed from TD participants in their static and dynamic FC of default mode, visual, and sensorimotor networks. No neuroimaging study has employed data-driven subtyping methods based on the functional connectivity and neuroanatomy of the language network in ASDs.

General aims

The over-arching goals of this modified staple dissertation are to (1) better characterize language network iFC in a large cohort of ASD youths using an extensive set of language regions derived from multiple language studies, (2) describe the heterogeneity of language network iFC within this sample of ASD participants in comparison to their TD peers, (3) utilize data-driven techniques to distinguish ASD subgroups based on their pattern of language network iFC, and (4) combine multimodal (functional, diffusion, and anatomical) imaging and behavioral measures to better improve the categorization of language subgroups.

Chapter 1. Study 1

The language network in autism: Atypical functional connectivity with default mode and visual regions

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ABSTRACT

Autism Spectrum Disorders (ASDs) are neurodevelopmental disorders associated with atypical brain connectivity. Although language abilities vary widely, they are impaired or atypical in most children with ASDs. Underlying brain mechanisms, however, are not fully understood. The present study examined intrinsic functional connectivity (iFC) of the extended language network in a cohort of 52 children and adolescents with ASDs (ages 8-18 years), using resting state functional magnetic resonance imaging. We found that, in comparison to typically developing (TD) peers (n=50), children with ASDs showed increased connectivity between some language regions. In addition, seed-to-whole brain analyses revealed increased connectivity of language regions with posterior cingulate cortex (PCC) and visual regions in the ASD group. Post-hoc effective connectivity analyses revealed a mediation effect of PCC on the iFC between bilateral inferior frontal and visual regions in an ASD subgroup. This finding qualifies and expands on previous reports of recruitment of visual areas in language processing in ASDs. In addition, increased iFC between PCC and visual regions was linked to lower language scores in this ASD subgroup, suggesting that increased connectivity with visual cortices, mediated by default mode regions, may be detrimental to language abilities.

Keywords: Autism spectrum disorders, default mode, language, resting state functional magnetic resonance imaging, visual cortex

INTRODUCTION

Autism spectrum disorders (ASDs) are neurodevelopmental disorders with high and increasing prevalence, recently estimated at 1 out of 45 children in the United States (Zablotsky, Black, Maenner, Schieve, & Blumberg, 2015). Closely related to core symptoms in the sociocommunicative domain is language impairment. Linguistic ability serves as one of the best predictors for ASD diagnoses and functional outcome (Lombardo et al., 2015; Szatmari et al., 2015). This highlights the importance of understanding the neurological bases of language processing in ASDs and their relation to behavioral symptomatology.

Up to 25% of children who receive ASD diagnoses never develop functional verbal language skills (Luyster, Kadlec, Carter, & Tager-Flusberg, 2008; Tager-Flusberg, Paul, & Lord, 2013). Among those who acquire functional language, the age at which first words are spoken is on average delayed by 12-18 months compared to typically developing (TD) children (Howlin, 2003), and a wide range of verbal abilities can be observed later in life. Some individuals exhibit severe problems, such as repetitive neologisms and echolalia (speech parroting; Eigsti, de Marchena, Schuh, & Kelley, 2011). However, even highly verbal individuals with ASDs may find some aspects of communication challenging, such as pragmatic language skills (e.g., turn-taking), nonverbal communication (e.g., gesturing, facial expression), and prosody (Eigsti et al., 2011). In a review of structural language characteristics of ASDs, Boucher (2012) suggested that the ASD language profile is highly heterogeneous and that verbal individuals display more impairment in receptive than expressive language.

Early evidence on the neural basis of language came from the study of brain lesions (Broca, 1861). This led to the identification of two language areas with gross functional characterization: Broca's area (left inferior frontal gyrus) for speech production, and Wernicke's

area (left posterior superior temporal cortex) for comprehension (Price, 2000). Connected by the arcuate fasciculus (Catani, Jones, & ffytche, 2005), these regions constitute the language network in the traditional neurological model (N. Geschwind, 1970), although the precise anatomical location of these regions has been questioned (Mesulam, Thompson, Weintraub, & Rogalski, 2015). Recent neuroimaging studies have shed light on a more extensive language system that includes the dorsal striatum, insula, precuneus, inferior parietal lobule, and cerebellum in addition to the classic language regions (Berl et al., 2014; Price, 2010; Rodd, Vitello, Woollams, & Adank, 2015). Concordant with lesion findings, imaging studies have provided further support that language function is mostly lateralized to the left hemisphere in right-handed TD individuals (McAvoy et al., 2016; Nielsen, Zielinski, Ferguson, Lainhart, & Anderson, 2013).

Neuroimaging has also contributed to the understanding of biological bases of language impairments in ASDs over the past decades. Among anatomical studies, Herbert et al. (2002) found atypical asymmetries in frontal and temporal language regions in boys with ASDs. Others have reported white matter anomalies including decreased volume of the arcuate fasciculus (Moseley et al., 2016) in adults with ASDs. One study reported evidence of potential white matter compromise (increased mean diffusion) in the left superior longitudinal fasciculus, detected only in ASD individuals with language impairments (Nagae et al., 2012). Another study (Peeva et al., 2013) found weaker structural connectivity (number of streamlines) between areas involved in speech production (left ventral premotor cortex and left supplementary motor area) in ASD adults with average language abilities.

Functional imaging studies have revealed further anomalies of language processing in ASDs, including recruitment of visual regions during language tasks (Gaffrey et al., 2007; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Knaus, Silver, Lindgren, Hadjikhani, & Tager-

Flusberg, 2008; Pang et al., 2016), increased activation in homologous language regions of the right hemisphere (Anderson et al., 2010; Eyler, Pierce, & Courchesne, 2012; Groen et al., 2010; Herringshaw, Ammons, DeRamus, & Kana, 2016; Kleinhans, Müller, Cohen, & Courchesne, 2008; Knaus et al., 2010; Müller et al., 1999; Nielsen et al., 2014; Williams, Goldstein, & Minshew, 2006), and reduced connectivity of left inferior frontal cortex and right cerebellum with other language regions (Verly et al., 2014).

In the past decade, there has been increasing awareness that symptomatology and cognitive-behavioral impairments in ASDs require explanation at the level of distributed neural networks (Geschwind & Levitt, 2007; Müller, 2007; Rippon, Brock, Brown, & Boucher, 2007). A method of choice in the study of functional network organization is functional connectivity MRI. Functional connectivity (FC) is inferred from synchronized low frequency (<0.1Hz) blood-oxygen level dependent (BOLD) signal fluctuations and can be measured during rest, referred to as intrinsic functional connectivity (iFC). IFC has been used to examine the language network in healthy adults, using traditional perisylvian regions (Broca's and Wernicke's area) as seeds (Tomasi & Volkow, 2012; Zhu et al., 2014). Findings from these studies have shown extensive short-range left-lateralized FC for both seeds with more long-range bilateral connectivity for posterior Wernicke's area.

The few available functional connectivity MRI studies of the language network in ASDs have generated conflicting findings. Task-based studies reported reduced FC of the language network in ASDs (Just, Cherkassky, Keller, & Minshew, 2004; Kana et al., 2006; Knaus et al., 2008). One small-sample iFC study found decreased language network iFC in ASD children with language impairment (Verly et al., 2014), whereas others (using iFC methods) observed mixed effects (Lee, Park, James, Kim, & Park, 2017) or even extensive overconnectivity with regions

outside canonical language networks, including visual cortices (Shen et al., 2012) in individuals with ASD with and without comorbid language impairment. While methodological differences between co-activation FC and iFC may account for some inconsistencies (Müller et al., 2011; Nair et al., 2014), the evidence from previous, mostly small-sample studies (including ≤20 participants per group) remains overall inconclusive.

The present study investigated iFC of a comprehensive language network in children and adolescents with ASDs and their TD peers. We hypothesized that in ASD participants 1) iFC of the language network would be partially increased (compared to TD peers), within and outside the network, including visual cortex; 2) iFC of language regions would be less left lateralized; and 3) altered connectivity would be related to language abilities and symptom severity.

METHODS

Participants

A total of 163 participants, ages 8-18 years, were recruited from the community and through ongoing collaborations with local clinicians. TD participants had no family history of ASDs or any other neurological, developmental, or psychiatric disorder. For the ASD group, only individuals with idiopathic ASDs were recruited (i.e., excluding any syndromic forms such as Fragile X or Rett syndrome). ASD diagnoses based on DSM-5 (American Psychiatric Association, 2013) criteria were confirmed using the Autism Diagnostic Interview-Revised (ADI-R; Rutter, Le Couteur, & Lord, 2003), the Autism Diagnostic Observation Schedule (ADOS or ADOS-2; Lord, Rutter, DiLavore, & Risi, 2001; Lord et al., 2012), and expert clinical judgment (co-author IF). Participants were tested on IQ using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), handedness using the Edinburgh Handedness Inventory (Oldfield, 1971), and language abilities using the Clinical Evaluation of Language Fundamentals

(CELF-4; Semel, Wiig, & Secord, 2004). Groups were matched on age, nonverbal IQ, head motion, handedness, gender, and handedness by gender (Table 1.1). The study protocol was approved by the Institutional Review Boards of San Diego State University and University of California San Diego. Assent and informed consent were obtained from all participants and their caregivers.

After enrollment, 61 participants were excluded based on demographic or diagnostic information, or image quality. Four recruits for the ASD group did not meet full diagnostic criteria, while two TD participants were excluded for meeting diagnostic criteria for ADHD. Participants were also excluded based on unusual neuroanatomical findings (3 ASD, 1 TD), presence of seizures or history of in-utero drug exposure (2 ASD), siblings with neurological conditions (2 TD), excessive drowsiness during the scan (1 TD), or excessive motion during MRI scanning (26 ASD, 10 TD; see below). Ten subjects (5 ASD, 5 TD) were removed to optimally match ASD and TD groups for age, sex, handedness, non-verbal IQ, head motion, and handedness by gender (Table 1.1). The final sample included 52 ASD and 50 TD participants.

Data Acquisition

Imaging data were acquired on a General Electric 3T Discovery MR750 scanner with an 8-channel head coil at the University of California San Diego Center for Functional Magnetic Resonance Imaging. High-resolution structural images were collected using a standard Fast Spoiled Gradient-Echo T1-weighted sequence (172 slices; repetition time [TR] = 8.136; echo time [TE] = 3.172ms; field of view [FOV] = 256 x 256mm; flip angle = 8°; $1mm^3$ resolution). Functional T2*-weighted images were obtained using a single-shot gradient-recalled, echo-planar pulse sequence of 180 whole brain volumes (TR = 2000ms; TE = 30ms; FOV = 220mm; flip angle = 90°, 64 x 64 matrix, $3.4mm^3$ resolution, 42 axial slices covering the whole brain).

During the 6-minute resting-state scan, participants were shown a white crosshair centered on a black background and instructed: “Keep your eyes on the cross, relax, but please stay as still as you can. Do not fall asleep.” In-bore MRI-compatible video monitoring was used to verify compliance with instructions and wakefulness.

Functional Magnetic Resonance Imaging Data Preprocessing

Functional MRI data were preprocessed and analyzed using Analysis of Functional NeuroImages software (AFNI 16.2.13; R. W. Cox, 1996). We used a standard pipeline to unwarp, field map correct, slice-timing correct, motion correct, and spatially smooth the image using a Gaussian kernel of 6mm FWHM. FMRI software library (FSL; Smith et al. 2004) was used to normalize structural images to MNI-152 template space and segment structural images into white matter, grey matter, and cerebrospinal fluid. The segmented white matter and CSF maps were eroded by 1 voxel. Functional images were co-registered to the preprocessed structural image and transformed to 3mm isotropic resolution. Functional time-series were bandpass filtered at 0.008-0.08Hz using a Butterworth filter. Root-mean-squared-difference (RMSD) was calculated from 6 motion parameters (3 translational and 3 rotational) to estimate in-scanner head motion. The 6 motion parameters and time-series from white matter and CSF, as well as their first temporal derivatives were equally bandpass filtered (Hallquist, Hwang, & Luna, 2013) and used as nuisance regressors in AFNI’s 3dDeconvolve to remove motion and noise from the functional signal.

Primary analyses were performed without global signal regression (GSR), a processing step that remains contentious. While GSR is recognized for its strengths in denoising fMRI time series (Power, Plitt, Laumann, & Martin, 2017), it is also known to generate anti-correlations that may not be biologically meaningful (Schölvinck, Maier, Ye, Duyn, & Leopold, 2010) and has

been found to distort group differences in some studies (Abbott et al., 2016; Gotts et al., 2013; Saad et al., 2012). However, analyses including GSR are additionally presented in the Supplement (Supplementary Figure 3, Supplementary Table 6-7).

Time-points with frame-wise displacement >0.5mm were censored including two subsequent time-points. Blocks of time-series between censored time-points with <10 time-points remaining were also removed. All included participants retained more than 80% of their original time-points. Groups were tightly matched for head motion RMSD (Table 1.1).

Functional Connectivity Analyses

The language network was defined by regions adopted from an Activation Likelihood Estimation (ALE) analysis of 54 functional neuroimaging studies of language comprehension in both spoken and written modalities (Rodd et al., 2015). Statistical maps from the ALE combining all 54 studies were obtained from the authors and thresholded to reduce differences in cluster volumes (to a range of 32-101 voxels). This produced a total of 14 distinct clusters used as regions of interest (ROIs) or seeds (Table 1.2, Supplementary Figure 1).

To measure within-network connectivity, average time-series data were extracted from each of the 14 ROIs in each participant and correlated with time-series from every other ROI, using Pearson's correlation, resulting in a 14 x 14 language network connectivity matrix. The resulting coefficients were then normalized using Fisher z-transformation and entered into one-sample t-tests to examine iFC within ASD and TD groups, separately, and two-tailed t-tests to identify group differences. The results were adjusted for multiple comparisons using local FDR-correction (Efron, 2007). Local FDR was preferred to traditional FDR due to non-uniform distribution of the obtained *p*-values.

Laterality of iFC in the extended language network was examined through two separate analyses. First, ipsilateral connectivity was computed by averaging each ROI-ROI pair within the same hemisphere. Next, laterality indices were calculated for each participant using the following equation: (Left – Right)/(Left + Right). Positive laterality indices signified leftward asymmetry, negative indices signified rightward asymmetry. The laterality indices were then compared between groups. In a second analysis, contralateral iFC was examined by averaging between-hemisphere ROI-ROI connections (e.g., left inferior frontal gyrus and right superior temporal sulcus) and compared between groups.

Connectivity outside the language network was examined using whole-brain iFC analyses, by correlating average time-series from each ROI with time-series from all other grey matter voxels. In an effort to reduce the number of comparisons, only ROIs that showed group differences in the within-network analyses were used for whole-brain iFC analyses (Figure 1.1B, Figure 1.1C, Table 1.2). The resulting whole-brain iFC maps were directly compared between groups and corrected for multiple comparisons using AFNI's 3dttest++ -Clustsim permutation tests (Cox, Chen, Glen, Reynolds, & Taylor, 2017). For each group, mean *z*-scores extracted from significant clusters of between-group effects were correlated with CELF-4 Core Language, Receptive Language, and Expressive Language scores, as well as ADI-R Social and Communication scores, ADOS Communication and Social Interaction, and ADOS Total scores, while controlling for effects of age and in-scanner head motion. Results were corrected using false discovery rate (FDR).

RESULTS

Within-network analyses

One-sample t-tests showed generally high connectivity between language network ROIs in both TD and ASD groups (Figure 1.1A). After correcting for multiple corrections, 69 ROI pairs in the ASD group and 61 ROI pairs in the TD group showed significant difference from zero (qs<0.05).

Two-sample t-tests for group comparisons of within-network matrices revealed 7 connectivity differences between language ROIs that survived local FDR adjustment (Cohen's ds>0.44, ps<0.03 uncorrected, qs<0.27; Figure 1B-C). All 7 pairs showed more positive correlations between language ROIs in the ASD group compared to the TD group. Such effects were seen for connections of both left and right inferior frontal ROIs with parietal ROIs (i.e., inferior parietal lobule, angular gyrus, and precuneus/posterior cingulate cortex). Higher correlations were also seen in the ASD group between the bilateral dorsal precuneus and left supramarginal gyrus, and between right inferior parietal lobule and the left pericentral region. Except for one connection between right inferior frontal and inferior parietal ROIs, these effects reflected correlations close to or below zero in the TD group and more positive correlations in the ASD group (Figure 1.1D).

Laterality analyses

We examined between group differences in language network lateralization by calculating laterality indices and by examining the average connectivity of between-hemisphere language regions. We found that the laterality indices between ASD participants and TD participants did not differ significantly (t(100)=0,69, p=0.51). Similarly, we did not find a

significant group difference in between-hemisphere language network iFC ($t(100)=0.25$, $p=0.80$).

Whole-brain (outside network) analyses

We selected 5 ROIs that were part of two or more significant connections (indicated by asterisks in Table 1.2) as seeds for further exploration in whole-brain connectivity analyses. Three of these showed significant whole-brain iFC group differences. For inferior frontal seeds bilaterally, there was increased connectivity in the ASD group with PCC (Figure 1.1E). Additionally, for the left precuneus/PCC seed, four clusters of greater iFC in the ASD group were detected in occipital lobes bilaterally, including middle occipital and lingual gyri (Supplementary Table 1).

Post-hoc analysis of effective connectivity

Findings of overconnectivity with visual cortex from whole-brain analyses were remarkable in view of several previous ASD reports of atypical engagement of visual areas in language processing (Gaffrey et al., 2007; Kana et al., 2006; Knaus et al., 2008) and increased functional connectivity between inferior frontal gyrus and visual cortices (Shen et al., 2012). However, none of these explored the role of PCC. As our findings suggested that PCC might mediate connectivity between IFG and visual regions in ASDs, we performed follow-up analyses of effective connectivity, using Group Iterative Multiple Models Estimation (GIMME; Gates & Molenaar, 2012). GIMME utilizes unified structural equation modeling in a data-driven approach. Its ability to model contemporaneous and lagged relationships between regions allows for the study of effective FC in resting state MRI (Gates, Molenaar, Iyer, Nigg, & Fair, 2014).

We conducted four semi-confirmatory GIMME analyses that estimated pre-specified directional relationships between variables and added paths as needed for each subject until the

best-fit model was found. Analyses were run using the average time-series extracted from the three seeds with significant whole-brain group differences (left IFG, right IFS, and left prec/PCC ROIs), and from the corresponding significant group-effect clusters in PCC and the occipital lobes (Figure 1.1E, Supplementary Table 1). The clusters in PCC (overconnectivity with inferior frontal seeds) and the left prec/PCC seed (which also showed significant overconnectivity with left IFG and right IFS in the within-network analyses) were in close proximity and were merged. The four clusters in occipital cortex (overconnectivity with left prec/PCC seed) were also merged. We tested whether connectivity between inferior frontal regions and visual cortex was mediated by PCC. In view of atypical language-related asymmetries of IFG reported in several ASD studies (Kleinhans et al., 2008; Knaus et al., 2010), right and left inferior frontal ROIs were modeled separately. We tested 4 mediation models that varied in directionality of connectivity, each containing 4 factors: left IFG, right IFS, PCC, and visual cortex (Supplementary Figure 2). Each subject's iterative path estimates and their fit for the pre-specified model were then used to determine whether an indirect, or mediated, relationship exist for each model. Across the 4 models, we found that 24 out of 52 ASD participants showed significant PCC mediation of connectivity between frontal language areas and visual cortex (with 18 of the 24 showing this effect for the right inferior frontal ROI). The subgroups did not differ in age, head motion, IQ, or language abilities (ps>0.30, uncorrected; Supplementary Table 2). A marginal difference was found in parent-reported restricted and repetitive behaviors, as measured by the ADI-R ($t(43.5)=2.16$, $p_{uncorrected}=0.04$).

Relation between connectivity and behavioral measures

Next, we explored the relationship between connectivity (mean z) of the 7 within-network connections with significant group differences (Figure 1.1B-C) and measures of

symptom severity and language ability. In the ASD group, iFC between right inferior parietal lobule (IPL) and left pericentral region was negatively correlated with scores of the ADI-R Social subscale (r=-0.51, q=0.006; Figure 1.2A; Supplementary Table 3), with higher iFC linked to lower social symptom severity (controlling for age and head motion). No significant correlations between iFC pairs and language measures were found in either group (Supplementary Table 4).

For whole-brain iFC results, we found no significant relationship with behavioral language measures or symptomatology in the ASD group. However, in the TD group, there was a significant negative correlation for CELF Receptive Language score and iFC between right IFS and PCC (red cluster in Figure 1.1D), controlling for age and motion (r=-0.55, q_{FDR}=0.009; Supplementary Table 5). Similarly, higher connectivity between the PCC/Precuneus ROI and left medial occipital gyrus was linked to lower CELF Core Language scores (r=-0.56, q_{FDR}=0.009) and Expressive Language scores (r=-0.59, q_{FDR}=0.008), after correcting for multiple comparisons.

Finally, we examined the relationship between language function and whole-brain iFC in the subgroup of ASD participants who showed PCC mediation in GIMME analyses. Similar to the TD group, we found that connectivity between the PCC/precuneus ROI and visual cortex were all negatively correlated with CELF scores in this ASD subgroup. After controlling for age and head motion, and correcting for multiple comparisons, we found a significant relationship between iFC of left Precuneus/PCC with right lingual gyrus and CELF Receptive Language (r=-0.74, q_{FDR}=0.022; Figure 1.2B; Supplementary Table 5). No correlations were observed between iFC results and CELF measures in the ASD subgroup without significant PCC mediation.

DISCUSSION

Language deficits are commonly observed in ASDs, but the underlying neurobiology is not fully understood. We examined intrinsic functional connectivity of the language network in children with ASDs, using resting state fcMRI, and found atypically increased connectivity between some language regions, in addition to increased connectivity of language ROIs with PCC and visual regions. Although several right-hemisphere ROIs showed overconnectivity in the ASD group, quantitative analyses of asymmetry yielded no group differences. Effective connectivity analyses revealed a subgroup of ASD youths whose connectivity between inferior frontal language regions and visual cortex was mediated by PCC. In this subgroup, increased iFC between PCC and visual cortex was also associated with lower language abilities.

Predominant overconnectivity of the language network in ASDs

FC within the language network (adopted from a large meta-analysis; Rodd et al., 2015) was predominantly greater in the ASD compared to the TD group. While seemingly at odds with some previous underconnectivity findings (Just et al., 2004; Kana et al., 2006; Knaus et al., 2008), inconsistency may be largely explained by methodological differences between co-activation FC (testing for task-driven BOLD correlations) and iFC (Müller et al., 2011; Nair et al., 2014). One recent iFC study of language also reported underconnectivity in ASDs (Verly et al., 2014), which was, however, observed in ROIs (including right cerebellum) that largely differed from those implemented in the present study. In addition, Verly and colleagues specifically selected 19 ASD participants with a history of significant language delay and impairment, whereas the present study included 52 ASD participants with a wide range of linguistic abilities.

For all but one of the ROI-ROI pairs with increased connectivity in ASDs, group differences reflected low levels of iFC in the TD group, contrasting with more positive iFC in the ASD group (Figure 1.1C). These connections may represent an extended language network of regions that do not consistently co-activate during language processing and therefore show low levels of intrinsic synchronization in the TD brain. Only one ROI pairing (right IFS and right IPL) showed positive BOLD correlations in the TD group, which were even more pronounced in the ASD group. Note, however, that overconnectivity between ROIs of the extended language network was mostly only of medium effect size, surviving only a relatively lenient local FDR correction.

For a few ROI pairings, overconnectivity in the ASD group was associated with lower sociocommunicative symptom severity (Supplementary Table 3). Specifically, atypically increased connectivity between left IFG and angular gyrus in the ASD group was associated with *lower* ADOS Social Communication scores. This reflects a connection between core language regions in the dominant hemisphere crucial for semantic processing, lexical selection, and other language subprocesses (Price, 2010). However, this medium-sized effect (r=-0.32) did not survive FDR correction. More robust was an effect of increased iFC between right IPL and left pericentral cortex was associated with decreased ADI-R Social scores. Right IPL, which has traditionally been linked to spatial processing and selective attention (Husain & Nachev, 2007), has also been noted for its role in distinguishing between self and others (Ruby & Decety, 2004; Uddin, Molnar-Szakacs, Zaidel, & Iacoboni, 2006). Left pericentral cortex is important for speech production (Price, 2010), but has also been found to activate during speech comprehension (Adank, 2012). Findings of overconnectivity between nodes of an extended language network in ASDs may be indicative of a history of increased co-activation (Crossley et

al., 2013; Lewis, Baldassarre, Committeri, Romani, & Corbetta, 2009). More specifically, relatively mild levels of social deficits in ASD could reflect a history of high levels of effortful processing in these non-core language regions, with some compensatory effect and associated relatively mild symptomatology.

Connectivity between language, visual, and default mode networks

Whole-brain analyses revealed overconnectivity in the ASD group bilaterally between IFG and PCC. The PCC has been described as a "hub" with dense connectivity for information integration (Ray et al., 2014; van den Heuvel & Sporns, 2011). Traditionally associated with the default mode network (DMN), PCC is activated during self-reflection, mentalization, and episodic memory (Hull, Jacokes, Torgerson, Irimia, & Van Horn, 2017; Washington et al., 2014). Increased FC between DMN and language-related networks in ASDs has been previously reported in an ICA study by Zhao and colleagues (2016), who found right hemisphere homologous language regions (e.g., IFG, AG, and supramarginal gyrus) to be overconnected with the DMN. This may suggest reduced segregation between the DMN and the language network, possibly consistent with findings of atypical crosstalk between DMN and other functional networks (Abbott et al., 2016; Fishman, Keown, Lincoln, Pineda, & Müller, 2014; Ray et al., 2014; Rudie et al., 2013; Rudie et al., 2012; Yerys et al., 2015).

The finding of increased iFC between PCC and visual cortex is consistent with previous reports of overconnectivity between the DMN and the visual network in ASDs (Washington et al., 2014; Yerys et al., 2015). Yerys and colleagues (2015) found increased PCC connectivity with bilateral occipital pole, LG, and fusiform gyri. Together with the overconnectivity of the PCC and DMN discussed above, this is of special interest to language function as studies have

reported increased activation of visual cortex in ASD participants during language processing (Gaffrey et al., 2007; Kana et al., 2006; Knaus et al., 2008; Pang et al., 2016).

An effective connectivity analysis of the language network in ASDs by Shen et al. (2012) detected an atypical path between left IFG and right extrastriate cortex; however, PCC was not considered in their model. In the present study, whole-brain iFC analysis revealed PCC to be overconnected with both bilateral IFG and visual cortex. Therefore, PCC was tested as a potential mediator of iFC between the frontal and visual regions – a mediation that was confirmed in almost half of the ASD participants. A negative association of robust effect size between CELF-4 scores and brain connectivity as seen in this ASD subgroup with PCC mediation suggests that connectivity between PCC and visual regions may be detrimental to language functioning. As concordant effects were seen in the TD group, this link may not be specific to ASDs. However, whereas PCC-visual connectivity was generally low in TD children, it was robust in many children with ASDs, indicating that the detrimental effect on language is common in ASDs, but uncommon in TD children. Functionally, it may indicate over-engagement of internal reflection and mental imagery in early development, contributing to increased synchronization between DMN and visual networks (Spreng, Mar, & Kim, 2009). Accompanying overconnectivity between DMN and frontal language regions may further be associated with a history of reduced vigilance and performance efficiency during language processing (Anticevic, Repovs, Shulman, & Barch, 2010; Götting et al., 2017; Hinds et al., 2013).

Limitations

IFC findings were not robustly associated with behavioral language measures of the CELF-4. Although commonly used, CELF subtests additionally engage non-language cognitive

functions such as working memory (e.g., Recalling Sentences), which may have limited our ability to detect links between iFC and language abilities. As with most fMRI studies of ASDs, data collection was limited to relatively high-functioning individuals who were able to lie almost motionless throughout the scan. While our aim was to include a wide range of linguistic abilities to better represent the ASD population, inclusion of children with normal-level language abilities may have weakened group-level effects and differences of brain-behavior relationships.

Conclusions

We found the intrinsic functional organization of the language network in high-functioning children and adolescents with ASDs to be characterized by partial overconnectivity, mostly involving regions of an extended network that do not show robust signal correlations in TD peers. Atypical connectivity was distinct for a triad of inferior frontal, default mode, and visual regions. While overconnectivity with DMN (PCC) was seen for the entire ASD cohort, mediation of connectivity between inferior frontal and visual regions by PCC was seen only in an ASD subgroup, where high level of visual connectivity was associated with relatively low language abilities. Findings suggest that atypical connectivity in ASDs may predominantly affect regions of an extended network (rather than traditional regions such as Broca's and Wernicke's), with great heterogeneity even within the fully verbal and high-functioning segment of the spectrum.

Chapter 1, in full, is a reprint of the material as it appears in the Journal of *Autism Research, 12*, 1344 -1355. **Gao, Y.**, Linke, A. C., Jao Keehn, R. J., Punyamurthula, S., Jahedi, A., Gates, K., Fishman, I., and Müller, R.-A., Wiley, 2020. The dissertation author was the primary investigator and author of this paper.

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Table 1.1. Participant information

	ASD (n = 52)		TD (n = 50)		
Gender	8 female		8 female		
Handedness	7 left (0 female)		7 left (0 female)		
	Mean (SD)	Range	Mean (SD)	Range	T, *p*-value
Age in years	13.7 (2.6)	9.2-18.0	13.6 (2.6)	8.0-17.6	0.29, *p*=0.77
Head Motion					
RMSD pre-censoring	0.065 (0.030)	0.019-0.148	0.064 (0.032)	0.017-0.148	-0.20, *p*=0.84
RMSD after denoising	0.003 (0.002)	0.001-0.008	0.003 (0.003)	0.001-0.014	-0.94, *p*=0.34
Post-censoring TP	178 (4.2)	158-180	177 (3.9)	166-180	0.25, *p*=0.80
WASI					
Verbal IQ	102 (17.1)	70-147	108 (9.1)	87-126	-2.14, *p*=0.04
Nonverbal IQ	106 (17.2)	53-140	105 (13.3)	62-137	0.34, *p*=0.73
Full-scale IQ	104 (16.4)	66-141	107 (11.0)	79-130	-1.12, *p*=0.26
CELF-4*					
Core Language	99 (17.5)	56-120	110 (9.1)	91-126	-3.18, *p*=0.00
Receptive	99 (16.1)	60-131	104 (11.2)	76-127	-1.22, *p*=0.23
Expressive	97 (17.1)	55-120	107 (8.9)	91-124	-2.96, *p*=0.00
ADOS-2†					
Social Affect	10.2 (3.7)	5-20	--	--	
Repetitive Behavior	3.4 (1.7)	0-8	--	--	
Total	13.5 (4.2)	5-24	--	--	
Severity	7.5 (1.9)	3-10	--	--	
ADI-R					
Social Interaction	18.4 (4.9)	7-28	--	--	
Communication	13.4 (5.1)	2-24	--	--	
Repetitive Behavior	6.1 (2.3)	1-12	--	--	

TP: Time-points (180 total time-points before censoring). * CELF-4 scores were not available for 11 ASD and 12 TD participants. † 38 ASD participants were assessed with Module 3 and 14 participants were assessed with Module 4 of the ADOS.

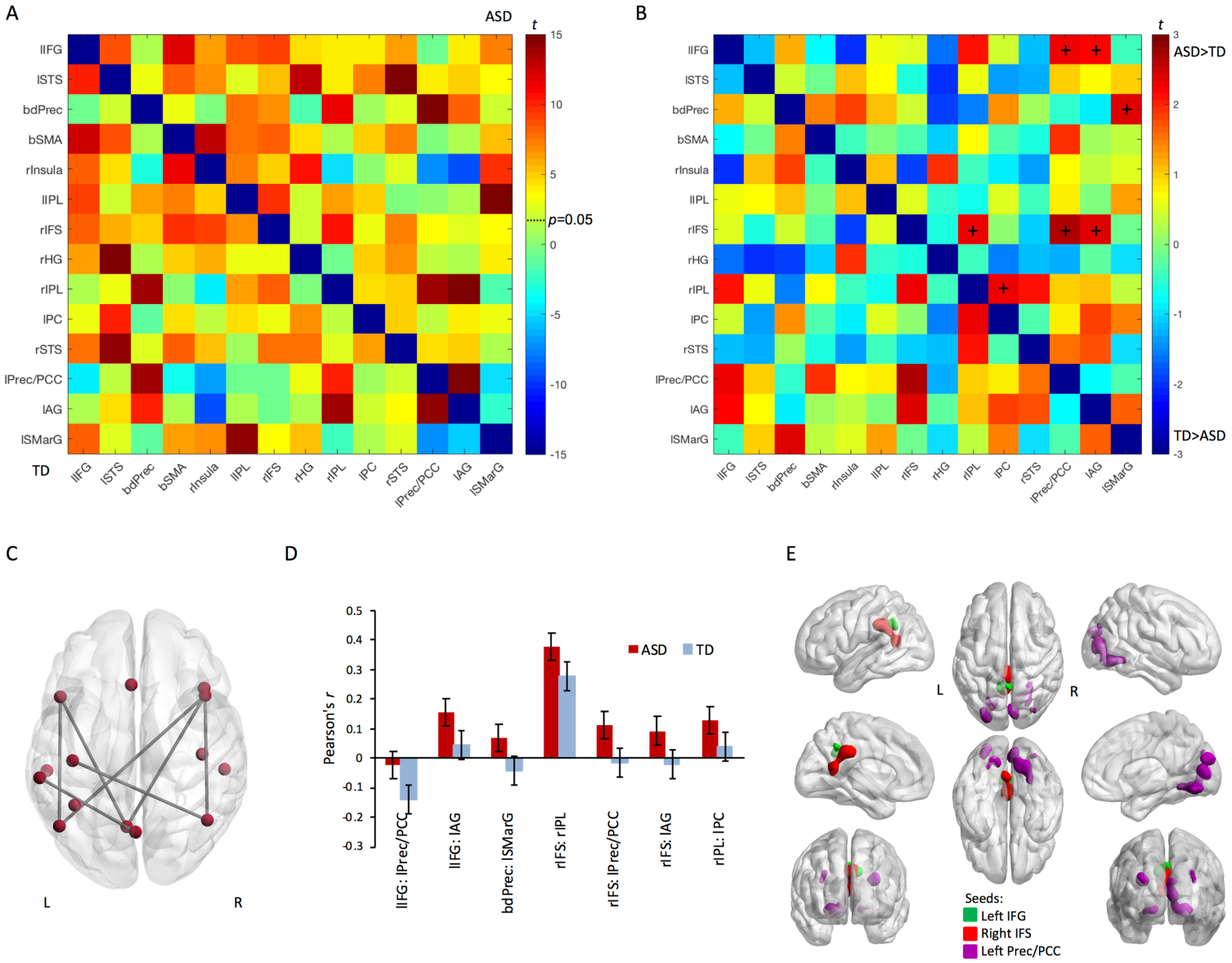
Table 1.2. Regions of interest

Cluster	Anatomic Label	Abbreviation	Voxels	CM x	CM y	CM z
1	Left inferior frontal gyrus*	lIFG	101	52	14	17
2	Left superior temporal sulcus	lSTS	100	56	-28	2
3	Bilateral dorsal precuneus	bdPrec	99	0	-67	44
4	Bilateral supplementary motor area	bSMA	91	2	16	52
5	Right insula	rInsula	82	-37	23	6
6	Left inferior parietal lobule	lIPL	94	38	-50	46
7	Right inferior frontal sulcus*	rIFS	91	-43	17	30
8	Right Heschl's gyrus	rHG	72	-42	-19	6
9	Right inferior parietal lobule*	rIPL	70	-45	-60	37
10	Left pericentral region*	lPC	65	39	-23	54
11	Right superior temporal sulcus	rSTS	57	-55	-28	-1
12	Left precuneus/ posterior cingulate cortex*	lPrec/PCC	44	5	-64	24
13	Left angular gyrus	lAG	34	47	-64	34
14	Left supramarginal gyrus	lSMarG	32	59	-34	37

Regions of interests (ROI) are derived from statistical maps taken from an Activation Likelihood Estimate analysis of 54 language comprehension studies (Rodd et al., 2015). All coordinates are listed in MNI space. CM: Center of mass of each cluster. *ROIs used for whole-brain iFC analyses

Figure 1.1. Language network iFC

(A) Matrix showing results from within-group *t*-test of language network connectivities (warm colors represent positive t-values; cool colors represent negative *t*-values; critical *t*-value of 1.68 is marked by p=0.05). The ASD group (top right) showed 69 connections above this threshold and the TD group showed 62 significant connections (bottom left). After multiple comparison corrections (qs<0.05), the TD group retained 61 of the 62 significant connections, while the ASD group retained all 69 significant connections. **(B)** Group difference matrix for connectivities between the 14 language ROIs. + symbolizes local FDR-corrected significant difference between the groups (Cohen's ds>0.44, ps<0.03 uncorrected, qs<0.27). **(C)** Glass brain rendering of significant between-group differences in language network connectivities after local FDR-correction, corresponding to cells labelled + in panel B. **(D)** Between-group differences for ASD (red) and TD (blue) in correlations between language ROIs. Error bars signify standard error of the correlations. lIFG: left inferior frontal gyrus; lSTS: left superior temporal sulcus; lIPL: left inferior parietal lobule; lPC: left pericentral region; lPrec/PCC: left precuneus/posterior cingulate cortex; lAG: left angular gyrus; lSMarG: left supramarginal gyrus; bdPrec: bilateral dorsal precuneus; bSMA: bilateral supplementary motor area; rInsula: right insula; rIFS: right inferior frontal sulcus; rHG: right Heschl's gyrus; rIPL: right inferior parietal lobule; rSTS: right superior temporal sulcus. **(E)** Clusters of between-group difference from whole-brain iFC analyses for seeds in left inferior frontal gyrus (green cluster) and right inferior frontal sulcus (red cluster). Both ROIs show higher connectivity with the posterior cingulate cortex (PCC) in the ASD group (voxel-level threshold p=0.001, α<0.05). For the left precuneus/PCC seed extensive overconnectivity is found in occipital cortex (purple clusters) in the ASD relative to the TD group (voxel-level p=0.001, α<0.01).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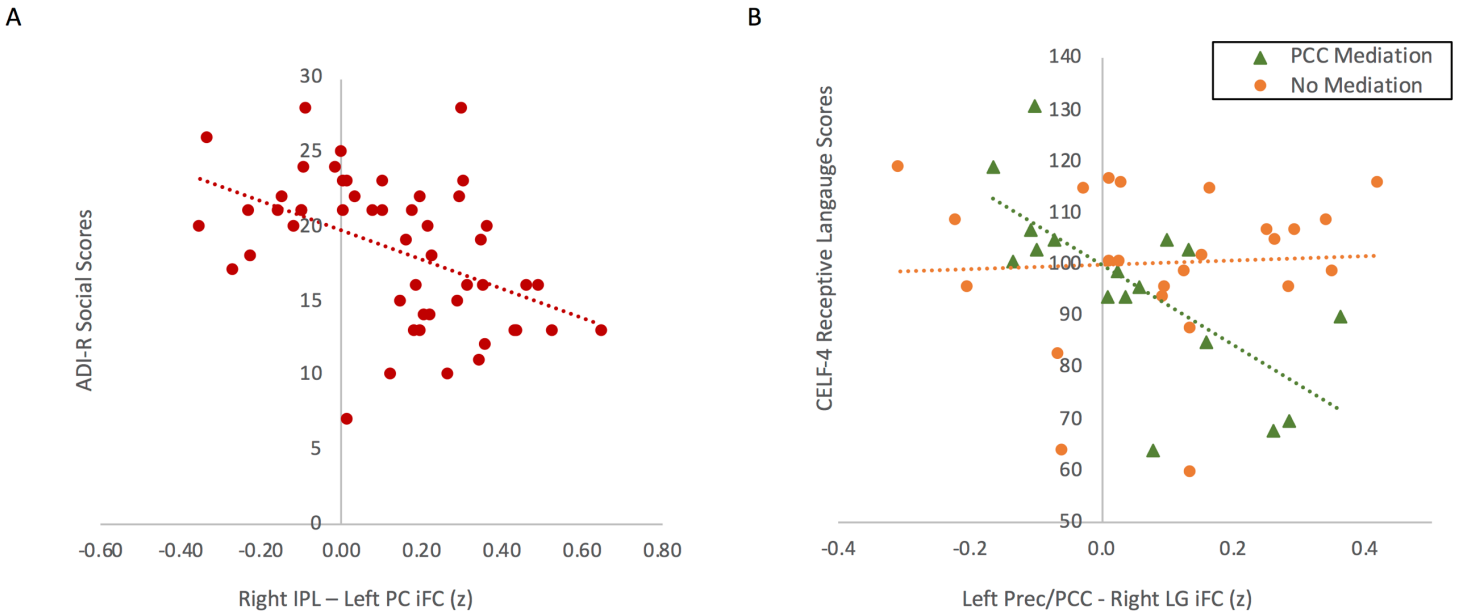

Figure 1.2. Correlations between brain connectivity and behavioral measures

(A) Correlation of ADI-R Social scores with iFC between right inferior parietal lobule and left pericentral region in the ASD group (r=-0.51, q_{FDR}=0.006), after controlling for age and in-scanner head motion. **(B)** Correlation of CELF-4 Receptive Language scores with iFC between the PCC/Precuneus ROI and right lingual gyrus in ASD subgroups with PCC-mediated connectivity between inferior frontal ROIs and visual cortex (shown in green [r=-0.74, q_{FDR}=0.022]) and without PCC mediation (shown in orange [r=0.09, q=0.955]), after controlling for age and in-scanner head motion, and multiple comparison correction

Chapter 2. Study 2

Distinct Subtypes of Language Network Connectivity in Children with Autism Spectrum Disorders

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ABSTRACT

Autism spectrum disorders (ASD) are a group of highly heterogeneous developmental disorders associated with atypical brain connectivity. Closely related to sociocommunicative core symptoms is language ability, which varies greatly between affected individuals. However, the neural substrates underlying the variability of language abilities in ASD are poorly understood. This study explored the heterogeneity as well as distinct patterns of intrinsic functional connectivity (iFC) within the language network (using data-driven regions of interest) in children with ASD. Resting state fMRI scans from 69 ASD and 60 typically developing (TD) youths (ages 7-18) were included. At the whole-group level, no significant group differences in language network iFC were found; however, heterogeneity was greater in the ASD than the TD group. Latent profile analysis of iFC dimensions revealed three distinct ASD subtypes of language network. While the first subtype did not differ significantly from the TD group in iFC despite poorer language abilities, the two other subtypes showed broad under- and over-connectivity of the language network, respectively. Existence of distinct iFC subtypes may account for some conflicting results in the ASD connectivity literature. Our findings underscore that focus on individual variability in ASD is needed, beyond conventional group-level analyses.

Keywords: Autism spectrum disorder, heterogeneity, subtyping, language, functional connectivity.

INTRODUCTION

Autism Spectrum Disorders (ASD) are a set of heterogeneous developmental disorders characterized by persistent sociocommunicative deficits and repetitive, restricted behaviors and interests (APA; American Psychiatric Association, 2013), currently estimated to affect 1 in 54 children aged 8 years in the United States (Maenner, 2020). Although absence or delay in development of spoken language is no longer a diagnostic criterion (APA, 2013), language impairments are common. A recent naturalistic language study found that close to 40% of children diagnosed with an ASD are nonverbal or have minimal verbal abilities at age 3 years (Bacon et al., 2018). Those who develop functional language exhibit qualitative differences in spoken language (e.g., neologisms) compared to typically developing (TD) individuals (Boucher, 2012; Eigsti et al., 2011). In addition, language abilities are among the best predictors of outcomes (e.g., adaptive skills) later in life in ASD (Mawhood et al., 2000; Sallows & Graupner, 2005; Szatmari et al., 2003, 2015). Improved understanding of atypical language development and underlying neurobiology is therefore important.

In a review of structural language in ASD, Boucher (2012) highlighted multiple studies supporting a language profile with higher expressive than receptive abilities considered unique to ASD. However, other studies have shown mixed or opposite patterns (Kjelgaard & Tager-Flusberg, 2001; Luyster et al., 2008), and a meta-analysis (Kwok et al., 2015) revealed overall reduced levels of both receptive and expressive language in ASD (with no expressive-over-receptive advantage). The large variability in language abilities across the autism spectrum (Tager-Flusberg et al., 2013) is a salient example of the overall behavioral heterogeneity that characterizes ASD (Lombardo et al., 2019).

Neuroimaging Studies of Language Processing

Imaging has indicated potential neural substrates of atypical language processing in ASD. Evidence includes atypical activation in visual cortex during language tasks across multiple studies (Gaffrey et al., 2007; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Knaus, Silver, Lindgren, Hadjikhani, & Tager-Flusberg, 2008; Pang et al., 2016). In addition, language comprehension and production have been found to be associated with unusual activity levels in right hemisphere regions homologous to canonical left hemisphere language areas (Anderson et al., 2010; Eyler, Pierce, & Courchesne, 2012; Groen et al., 2010; Herringshaw, Ammons, DeRamus, & Kana, 2016; Kleinhans, Müller, Cohen, & Courchesne, 2008; Knaus et al., 2010; Müller et al., 1999; Nielsen et al., 2014; Williams, Goldstein, & Minshew, 2006).

A second set of findings relates to network organization and connectivity. Functional connectivity (FC) MRI examines functional network organization by observing low frequency (<0.1Hz) blood-oxygen level dependent (BOLD) signal fluctuations that are synchronized between distributed brain regions during task performance and at rest. In resting state fMRI, correlations of slow, spontaneous BOLD signal fluctuations between brain regions are interpreted as intrinsic functional connectivity (iFC; Van Dijk et al., 2010). Findings on FC of the language network in ASD have been diverse, with some studies showing underconnectivity between language regions (Just et al., 2004; Kana et al., 2006; Knaus et al., 2008; Verly et al., 2014) and others showing mixed effects or overconnectivity (Lee et al., 2017; Shen et al., 2012). However, small samples may have provided insufficient statistical power to detect reliable effects in these previous studies (Button et al., 2013). In addition, conflicting results have been attributed to methodological differences in ‘co-activation’ FC during task performance vs. iFC MRI (Nair et al., 2014), data processing, and differences in sample characteristics (e.g., language

level of ASD participants; Hull, Jacokes, Torgerson, Irimia, & Van Horn, 2017). Furthermore, the common practice of group-wise comparisons (ASD vs. TD) in small samples neglects the known heterogeneity across the autism spectrum, which may be associated with greater interindividual variability of connectivity patterns (Byrge et al., 2015; Nunes et al., 2019). Notably, in our recent study (Gao et al., 2019) language network iFC differences at the whole-group level (TD vs. ASD) were modest and unrelated to language abilities, but a *subgroup* of ASD participants exhibited distinctly atypical connectivity patterns associated with lower receptive language scores.

Heterogeneity

Variability of linguistic abilities in ASD and inconsistent neuroimaging findings may, in part, be due to diversity of etiologies. Recent reviews, for example, suggest that hundreds of genetic variants and mutations may be risk factors for ASD (Nakanishi et al., 2019; Vorstman et al., 2017). The reviews noted differences in the penetrance of genotypes, gender ratio, and comorbidities (e.g., epilepsy, motor impairments, schizophrenia), all of which suggest diverse underlying biological mechanisms that give rise to the ASD phenotype. As an alternative to the assumption of a singular diagnostic group, some researchers have suggested studying more homogeneous subsets within the larger category of ASD (Haebig & Sterling, 2017; Jeste & Geschwind, 2014; Sahin & Sur, 2015; Szatmari et al., 2015; Tager-Flusberg & Joseph, 2003) while others have emphasized the need to study subgroups of ASD within larger cohorts (Easson et al., 2019).

Language subtypes of ASD

Kwok and colleagues (2015) suggested that studies averaging across heterogeneous samples of individuals with ASD cannot detect differences in subgroups with different

expressive and receptive language profiles. This is supported by a recent study of language phenotypes, which identified 3 ASD subgroups that differed in their spontaneous spoken language (Wittke et al., 2017). In a longitudinal study of expressive language development, Tek and colleagues (2014) found two distinct language profiles: a high language ASD group with developmental trajectories similar to those seen in typically development, and a lower language ASD group with less improvement over time. Pickles et al. (2014) combined both expressive and receptive language in a latent growth-curve analysis and discovered 7 different developmental trajectories. Given the divergent patterns of functional language abilities and language development within ASD, neurofunctional differences in language processing are likely.

There have been attempts to identify ASD subtypes based on neurobiological characteristics instead of behavioral symptoms. Amaral and colleagues (2017) reported a neurophenotype with disproportional large head-to-body size in boys with ASD, associated with higher rates of regression, lower expressive language abilities, and slower acquisition of adaptive skills. Other groups have employed data-driven techniques to explore ASD neurophenotypes derived by neuroanatomic (Hong et al., 2017), and fcMRI (Easson et al., 2019; Kernbach et al., 2018; Tang et al., 2019). While Kernbach and colleagues explored subtypes through FC of the default mode network across diagnostic groups, none of the studies to date have used statistical learning techniques to distinguish ASD subtypes of language network iFC and to examine how these relate to differences in language skills.

The present study aimed to (1) test the heterogeneity of language network iFC within a sample of children and adolescents with ASD in comparison to their TD peers, (2) utilize data-driven techniques to distinguish ASD subgroups based on their pattern of language network iFC,

and (3) use behavioral measures of ASD symptoms and language abilities to characterize language network iFC subgroups.

METHODS

Participants

A total of 218 (135 ASD, 83 TD) participants, ages 7-18 years, were recruited from the community and through ongoing collaborations with local clinicians. For the ASD group, only individuals with idiopathic ASDs were recruited (excluding any syndromic forms of ASDs such as Fragile X or Rett syndrome). ASD diagnoses were confirmed using the Autism Diagnostic Interview-Revised (ADI-R; Rutter, Le Couteur, & Lord, 2003), the Autism Diagnostic Observation Schedule (ADOS or ADOS-2; Lord, Rutter, DiLavore, & Risi, 2001; Lord et al., 2012), and expert clinical judgment based on DSM-5 diagnostic criteria (APA, 2013). For the TD group, participants with no known family history of ASDs or any other developmental, neurological, or psychiatric disorder were recruited. Of the 218 recruited participants, a total of 89 were excluded due to: excessive motion (28 ASD, 8 TD), low data quality (7 ASD, 7 TD), incidental neuroanatomical findings (6 ASD, 2 TD), additional clinical information obtained after enrollment (6 ASD, 5 TD), and attrition between study sessions (15 ASD), with another 4 ASD and 1 TD participants were excluded to optimize between-group matching on age, nonverbal IQ, in-scanner head motion, handedness, and gender (Table 2.1). The final sample included 69 ASD and 60 TD participants. Of the 69 ASD participants, 23 had co-morbid diagnoses (i.e., ADHD, depression, anxiety), and 25 were taking psychotropic medications at the time of study enrollment (Table 2.4). All participants were given a battery of behavioral tests, including Wechsler Abbreviated Scale of Intelligence (WASI-II; Wechsler, 2011) as a measure of IQ, Edinburgh Handedness Inventory (Oldfield, 1971) for handedness, Clinical Evaluation of

Language Fundamentals (CELF-4; Semel, Wiig, & Secord, 2004) for language abilities. The study protocol was approved by the Institutional Review Boards of San Diego State University and University of California San Diego. Assent and informed consent were obtained from all participants and their caregivers.

MRI Data Acquisition

Imaging data were acquired at the University of California San Diego Center for Functional MRI using a GE 3 Tesla Discovery MR750 scanner with an 8-channel head coil. A standard Fast Spoiled Gradient-Echo T1-weighted sequence (172 slices; repetition time [TR] = 8.136; echo time [TE] = 3.172ms; field of view [FOV] = 256 x 256mm; flip angle = 8°; $1mm^3$ resolution) was used for the collection of high-resolution structural images. Functional T2*-weighted images were acquired using a single-shot gradient-recalled, echo-planar imaging pulse sequence of 180 whole brain volumes (TR = 2000ms; TE = 30ms; FOV = 220mm; flip angle = 90°, 64 x 64 matrix, $3.4mm^3$ resolution, 42 axial slices covering the whole brain). During the 6-minute resting state fMRI scan, participants were shown a white crosshair centered on a black screen. They were instructed to fixate on the crosshair, relax, and let their mind wander. Participants were video monitored for wakefulness and compliance with instructions.

fMRI Data Preprocessing

Functional MRI data were preprocessed using Analysis of Functional NeuroImages (AFNI v16.2.13; R. W. Cox, 1996) following a standard pipeline for reconstruction, slice-time-, motion-, and field-map-correction. Structural images were normalized to template space from Montreal Neurological Institute (MNI-152) and segmented into white matter, grey matter, and cerebrospinal fluid using FreeSurfer (v5.3; Dale, Fischl, & Sereno, 1999). The segmented maps were then eroded by 1 voxel. Functional images were co-registered to preprocessed structural

images then transformed to 3mm isotropic voxels. Functional data were spatially smoothed using a Gaussian kernel of 6mm full-width half-maximum. Time-series were high-pass filtered ($f >$ 0.008Hz) using a Butterworth filter. Six rigid-body motion parameters, white matter, CSF, and their first-order temporal derivatives were also high-pass filtered and used as nuisance regressors. Root-mean-squared-displacement (RMSD), calculated from the six motion parameters, was used as an estimate of in-scanner head motion. Time-points with frame-wise displacement greater than 0.5mm and the two subsequent time-points were censored. Blocks of time-series with fewer than 10 consecutive time-points were also censored. All included participants had at least 80% of remaining time-points. Data were visually assessed for quality at each step of preprocessing.

Language Network Identification

Several steps were taken to identify data-driven regions of interest (ROIs) related to the language network. First, the meta-analysis association map for the term "language" was obtained from NeuroSynth.org. Next, to ensure fit with our sample, a group independent component analysis (ICA) of preprocessed resting state fMRI data, combining both ASD and TD groups, was carried out using FSL's Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC). MELODIC automatically estimated 36 components from the combined resting state data. For ICA components with spatial correlation $r > 0.2$ with the NeuroSynth language map, areas of 100% grey matter overlap between the ICA component and NeuroSynth language map were extracted and thresholded to a maximum of 100 voxels. This resulted in eight ROIs (Figure 2.1A). Average BOLD time-series were extracted from each ROI and Pearson correlated with the time course from each other ROI. Resulting values were

transformed to Fisher's z scores, for a total of 28 ROI-ROI FC estimates reflecting each participant's language network iFC pattern.

Heterogeneity Analyses

First, between-group language FC comparisons were carried out using independent-samples t-tests, with correction for multiple comparisons using false discovery rate (FDR; Benjamini & Hochberg, 1995). Next, within-group similarity for ASD and TD groups was evaluated by correlating each participant's full set of iFC estimates with those of every other subject. The resulting similarity matrix was averaged across columns, yielding a single measure of within-group language network similarity for each participant. Mean similarity scores were then compared between groups using independent-samples t-tests.

Subgroup Analysis

To conserve power for subgroup analysis, each participant's language network pattern was dimensionally reduced using T-Distributed Stochastic Neighbor Embedding (T-Sne; van der Maarten & Hinton, 2008). This method was chosen as it allows for nonlinear dimension reduction while preserving local structure. MPlus (version 8; Muthén & Muthén, 1998-2011) was then used to conduct a latent profile analysis of the T-Sne transformed language network connectivity patterns from each ASD participant to derive ASD language subgroups. The ASD group was split into smaller subgroups iteratively until a best fit solution was found. Subgroup solutions were evaluated with the following fit indices: Akaike Information Criterion (Akaike, 1974), Bayesian Information Criterion (Schwarz, 1978), sample size-adjusted BIC (Sclove, 1987), and entropy (Ramaswamy et al., 1993). In addition, Lo–Mendell–Ruben Adjusted Likelihood Ratio Test (Lo et al., 2001) and Bootstrapped Likelihood Ratio Test (Arminger et al., 1999; McLachlan et al., 2000) were used to compare fit between models (i.e., *k* subgroups vs *k*-1

subgroups) to ensure parsimony in model solution. Language network iFC matrices of the ASD subgroups were then each compared to the TD group using one-way analysis of variance (ANOVA) to identify the differences in network organization between ASD subgroups. Finally, the subgroups were characterized with respect to intellectual abilities, symptom severity, and language abilities. The subgroups and TD group were compared on behavioral measures using one-way ANOVA, with multiple comparison adjustment using Tukey's honest significant difference test (Tukey's HSD) and, when appropriate, Games-Howell post-hoc test (GH).

RESULTS

Heterogeneity Analysis

Between-group comparisons of language network iFC did not reveal significant differences for any of the 28 ROI-ROI pairings [all $p_{FDR}>0.84$]. However, the ASD group exhibited significantly lower within-group language network similarity than the TD group [Figure 2.1B, $t(127)=-3.08$, $p=0.003$, Cohen's $d=0.55$], meaning that heterogeneity across ASD participants was greater than across TD participants.

Subgroup Classification

Latent profile analysis of the dimensionally reduced language network iFC resulted in a best-fit solution that consisted of three ASD subgroups (Table 2.2). The subgroups (ASD1-3) included 20, 30, and 19 participants, respectively. Classification probability for the most likely latent class membership within each subgroup varied between 83%-95%. The subgroups did not differ on head motion, age, gender, handedness, IQ, or medication status (all ps>0.32; Table 2.3). The subgroups displayed different iFC patterns in comparisons between each another and with the TD group for 19 out of 28 language ROI pairs [Fs(3, 125)>4.2, ps<0.01]. Post-hoc tests

showed that while ASD1 did not differ significantly in iFC pattern from the TD group, after correcting for multiple comparisons, ASD2 and ASD3 were characterized by broad patterns of underconnectivity and overconnectivity, respectively, compared to the TD group (Figure 2.1C).

Behavioral Characteristics

Subgroups differed significantly on CELF Expressive Language scores [$F(3, 97)=6.63$, $p<0.001$], which were driven mostly by difference between the TD group and ASD1 and ASD3 [GH $p=0.013$, $p=0.01$, respectively, Figure 2.1D]. There were also marginally lower Receptive Language scores in ASD3 when compared to TD participants [GH $p=0.083$]. None of the post-hoc comparisons between the ASD subgroups yielded significant findings [Fs$(2, 66)<1.43$, ps>0.25; Table 2.3].

DISCUSSION

The present study examined language network iFC and heterogeneity using data-driven methods. While there were no significant group differences in iFC, the ASD group showed atypically increased within-group heterogeneity. Absence of iFC findings at the whole-group level was elucidated by latent profile analysis, which revealed three ASD subgroups with distinct language network iFC. Two of these subgroups showed robust differences from the TD group, characterized by broad under- and over-connectivity, respectively. Language abilities did not differ significantly between ASD subgroups, but were overall reduced in comparison with the TD group.

No FC differences at the whole-group level

Despite significant differences in CELF scores and VIQ, we found no differences in language network iFC at the level of whole groups. This contrasts with some previous reports in

smaller samples (<20 participants per group; Gaffrey et al., 2007; Knaus et al., 2008; Shen et al., 2012; Verly et al., 2014). The lack of group findings in the current study may be explained, in part, by methodological differences (e.g., in location and size of language network ROIs). For instance, between-group differences were previously observed in connections between language regions and areas outside the canonical language network such as visual areas (Gaffrey et al., 2007; Gao et al., 2019; Kana et al., 2006; Knaus et al., 2008; Shen et al., 2012) and default mode regions (Gao et al., 2019; Zhao et al., 2016) that were not included in the current analyses. No iFC differences were found between language regions in this study, despite the use of data-driven ROIs and the larger sample size. While this may be in part related to methodological advances, including improved motion control and tight group matching, interindividual variability of language network organization in ASD likely contributed to the lack of robust findings at the whole-group level.

Increased heterogeneity of language network FC in ASD

Language network heterogeneity was significantly greater within the ASD group than within the TD group. The severity and nature of presenting symptoms as well as the method and timing of interventions vary greatly within the ASD population. There is also growing evidence of a diversity of potential genetic and epigenetic causes as well as etiological pathways in ASD (Geschwind & State, 2015). It is therefore no surprise to also find neurobiological differences within this highly heterogenous population. Some studies have reported that individuals with ASD are more likely to exhibit individually unique or 'idiosyncratic' connectivity patterns (Hahamy et al., 2015; Nunes et al., 2019). Our findings suggest that while the ASD population exhibits higher within-group FC heterogeneity and idiosyncrasy than their neurotypical peers, there are clusters of relatively homogenous FC patterns that characterize ASD subgroups.

Distinct subtypes of language FC

Some recent studies have pursued FC-derived ASD neurophenotypes (Easson et al., 2017; Kernbach et al., 2018; Tang et al., 2019; Urchs et al., 2020), using a variety of supervised and unsupervised data-driven techniques across a range of functional networks. However, none have specifically examined the language network. The present study utilized latent profile analysis of language network FC dimensions and revealed three ASD subgroups: one that was similar to the TD group, and two that exhibited broad under- and overconnectivity compared to each other, as well as in comparison to the TD group. The FC patterns of these latter subgroups are reminiscent of the longstanding debate of general underconnectivity vs. overconnectivity in the ASD FC literature. While diverse accounts of the inconsistencies have been proposed, relating to developmental trajectories (Nomi & Uddin, 2015; Uddin et al., 2013), motion artifact (Deen & Pelphrey, 2012), and other methodological differences (Müller et al., 2011; Nair et al., 2014), our findings suggest that the existence of subgroups with divergent FC patterns may further contribute to conflicting results. This may specifically apply to studies with small samples that have dominated the ASD literature on language FC, due to chance variations in cohort composition.

ASD subgroups with broad under- vs. over-connectivity

FC-derived subgroups characterized by under- or overconnectivity have been identified by other research groups. Easson and colleagues (2019) identified two ASD subgroups using k-means clustering of combined static and dynamic FC. They found one subgroup that displayed greater within network and lower outside-network static FC with increased temporal stability of dynamic FC, while the second subgroup displayed the opposite pattern. Both subgroups showed greater within network static FC (i.e. in the occipital network) compared to TD participants.

However, this study did not focus on language networks. Another group (Urchs et al., 2020) utilized hierarchical agglomerative clustering of dissimilarity matrices for FC network maps and found 11 FC subtypes that were then divided into "risk" and "protective" with respect to ASD diagnosis. The protective subtypes were characterized by overconnectivity in unimodal sensory and motor networks (including auditory network) and greater convergence of FC alterations. The risk subtypes displayed pervasive underconnectivity and more variability between subtypes. Our findings support the existence of subgroups characterized by broad overconnectivity and underconnectivity specifically for language networks. However, we found no significant differences in diagnostic scores between subgroups that would indicate autism-specific protective or risk factors associated with the divergent FC patterns.

ASD subgroups with distinct FC profiles are similar behaviorally

Despite distinct language network FC patterns, the three ASD subtypes did not differ in symptom severity or behavioral language measures. They did however differ in language abilities from the TD group. The lack of differences between ASD subtypes may be attributed to insufficient power from small subgroup sample size. Others have also found ASD subgroups with distinct FC that did not differ in symptomatology (Easson et al., 2019). This suggests that diverse network FC patterns (as identified in our three ASD subgroups) may converge at the behavioral level with respect to sociocommunicative core symptomatology and reduced language abilities.

Limitations

In order to preserve power with a large number of FC variables, we utilized dimensional reduction (T-Sne). Although this technique is able to maintain local structure at high dimensionality (Maaten, 2009), it may not preserve all relevant information included in full FC

matrices. In addition, stringent criteria of data quality and group matching reduced our total sample, and relatively small subgroup sample sizes may have prevented detection of clinical and language differences. Finally, the present study divided participants into categorical subtypes while other studies have suggested the existence of dynamic or continuous models of FC subtypes (Tang et al., 2019; Urchs et al., 2020). The use of soft clustering or membership criteria could be explored in future studies.

Conclusions

We find evidence of heterogeneity in functional connectivity of the language network within ASD. Two ASD subgroups characterized by broad language network under- vs. overconnectivity were identified, utilizing unsupervised statistical learning. However, distinct FC profiles were not linked to significant differences in language ability or symptom severity between subgroups.

Chapter 2, in full, is currently being prepared for submission for publication. **Gao, Y.**, Linke, A. C., Mash, L. E., Fong, C. H., Alemu, K., Pastrana, J., Helm, J. L., Fishman, I., and Müller, R.-A. The dissertation author was the primary investigator and author of this paper.

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Table 2.1. Participant demographics

	ASD (n = 69)		TD (n =60)		Statistic	*p*-values
Gender	11 female		14 female		X^2 (1) = 1.122	0.289
Handedness	9 left		8 left		X^2 (1) = 0.002	0.961
	Mean (SD)	Range	Mean (SD)	Range		
Age in years	13.2 (2.7)	8.0-18.0	13.1 (2.8)	6.9-17.6	*t*(127)= 0.34	0.73
Head Motion						
RMSD pre-censoring	0.076 (0.03)	0.02-0.16	0.081 (0.09)	0.02-0.16	*t*(127)= -0.47	0.64
RMSD after censoring	0.003 (0.002)	0.00-0.01	0.003 (0.002)	0.00-0.01	*t*(127)= 0.71	0.48
Post-censoring TP	175.4 (6.4)	150-180	176.7 (6.2)	146-180	*t*(127)= -1.04	0.29
WASI-II						
Verbal IQ	100 (18)	67-147	108 (10)	78-133	*t*(127)= -2.82	0.005
Nonverbal IQ	103 (17)	53-140	106 (13)	62-137	*t*(127)= -1.17	0.242
Full-scale IQ	102 (16)	64-141	108 (11)	79-132	*t*(127)= -2.18	0.031
CELF-4*						
Core Language	93 (21)	40-120	110 (13)	62-127	*t*(99)= -4.732	<0.001
Receptive	93 (20)	42-131	104 (14)	60-128	*t*(99)= -3.09	0.003
Expressive	90 (21)	29-120	108 (12)	67-130	*t*(99)= -4.83	<0.001
SRS-2 Total	77 (9)	60-101	43 (4)	37-58	*t*(99)= 24.88	<0.001
ADOS-2†						
Social Affect	10.2 (3.8)	4-20	--	--		
Repetitive Behavior	3.0 (2.0)	0-8	--	--		
Total	13.2 (4.3)	5-24	--	--		
Severity	7.3 (2.0)	3-10	--	--		
ADI-R						
Social Interaction	17.9 (4.8)	6-28	--	--		
Communication	13.5 (5.1)	2-24	--	--		
Repetitive Behavior	5.9 (2.1)	1-12	--	--		

ASD: autism spectrum disorders; TD: typically developing; RMSD: root-mean-squared displacement; TP: Time-points (180 total time-points before censoring). * CELF-4 scores were only available for 56 ASD and 45 TD participants. † 23 ASD participants were assessed with Module 3 and 3 participants were assessed with Module 4 of the ADOS.

Table 2.2. Latent profile analysis subgroup model fit

	AIC	BIC	n-Adj BIC	Entropy	LMRT	BLRT
2-class Solution	801	816	794	0.775	$p = 0.035$	$p = 0.030$
3-class Solution	794	817	785	0.770	$p = 0.048$	$p < 0.001$
4-class Solution	797	826	785	0.838	$p = 0.446$	$p = 0.667$

AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; n-Adj BIC sample size-adjusted Bayesian Information Criterion; LMRT: Lo–Mendell–Ruben Adjusted Likelihood Ratio Test; BLRT: Bootstrapped Likelihood Ratio Test.

Table 2.3. Subgroup demographics

	ASD1 (n=20)		**ASD2** (n=30)		**ASD3** (n=19)	
Gender	4 female		3 female		4 female	
Handedness	2 left		4 left		3 left	
Medications	7 on; 13 off		11 on; 19 off		6 on; 13 off	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Age in years	13.6 (3.0)	8.5-17.8	13.5 (2.7)	8.0-17.7	12.5 (2.4)	9.2-18
Head Motion						
RMSD pre-censoring	0.074 (0.03)	0.03-0.15	0.078 (0.04)	0.02-0.16	0.07 (0.03)	0.02-0.12
WASI						
Verbal IQ	100 (14.1)	70-118	101 (19.6)	69-147	102 (18.1)	67-131
Nonverbal IQ	104 (19.8)	53-140	107 (14.7)	84-140	99 (18.0)	66-134
Full-scale IQ	103 (14.8)	66-123	104 (17.0)	76-141	101 (17.6)	64-127
CELF-4*						
Core Language	91 (22.7)	52-120	96 (24.3)	40-120	93 (16.3)	50-117
Receptive	94 (21.8)	54-131	93 (23.1)	42-119	94 (13.4)	54-107
Expressive	87 (22.2)	51-118	93 (24.2)	29-120	92 (12.4)	55-114
SRS-2 Total	78 (9.2)	60-95	77 (10.0)	62-101	76 (7.5)	62-89
ADOS-2						
Social Affect	10.4 (3.9)	6-20	10.7 (3.5)	5-16	9.5 (4.4)	4-17
Repetitive Behavior	2.6 (1.8)	0-5	3.5 (2.2)	0-8	2.6 (1.9)	0-5
Total	12.9 (5.2)	6-24	14.1 (3.7)	5-21	12.1 (4.2)	6-18
Severity	6.9 (1.9)	3-10	7.7 (1.8)	3-10	6.9 (2.1)	3-10
ADI-R						
Social Interaction	17.6 (4.1)	10-22	18.7 (5.2)	6-28	16.7 (4.9)	7-25
Communication	13.0 (5.3)	4-22	14.1 (5.6)	2-24	13.0 (4.4)	4-19
Repetitive Behavior	5.3 (1.8)	2-9	6.2 (2.1)	3-11	6.2 (2.6)	1-12

ASD1-3: autism spectrum disorders subgroups; RMSD: root-mean-squared displacement; *CELF-4 scores were only available for 17 participants in ASD1, 22 in ASD2, and 17 in ASD3. There were no significant between subgroup differences in the above variables.

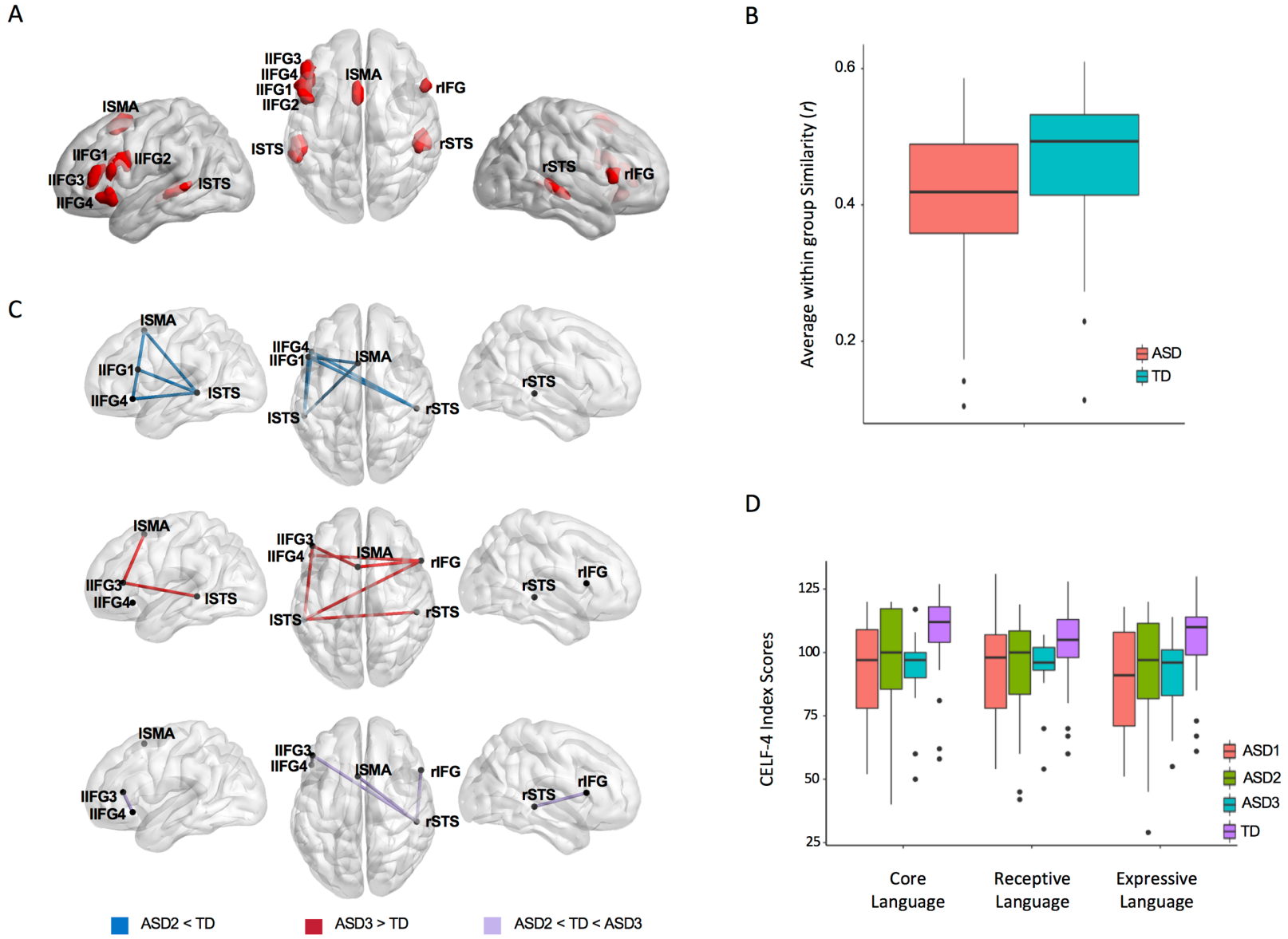
Table 2.4. Current psychotropic medication of Autism Spectrum Disorders (ASD) group

Participant	Stimulants	Mood Stabilizers [a]	Antidepressants	Anxiolytics/ Others [b]	List of Medications
1	+				Amphetamine/dextroamphetamine
2		+		+	Aripiprazole, alprazolam, hydroxyzine, risperidone
3	+		+		Methylphenidate, sertraline
4			+		Fluoxetine
5			+		Paroxetine
6	+	+	+		Sertraline, risperidone, methylphenidate
7	+			+	Amphetamine/dextroamphetamine, guanfacine
8	+	+	+		Methylphenidate, citalopram, risperidone
9	+		+		Amphetamine/dextroamphetamine, sertraline
10		+	+	+	Aripiprazole, oxcarbazepine, clonidine, fluoxetine
11			+		Fluoxetine
12		+		+	Ziprasidone, guanfacine
13	+			+	Lisdexamfetamine, guanfacine
14	+		+		Lisdexamfetamine, venlafaxine
15	+		+		Sertraline, lisdexamfetamine, fluvoxamine
16	+	+	+		Divalproex sodium, methylphenidate, fluoxetine, lamotrigine
17	+		+	+	lisdexamfetamine, guanfacine, sertraline
18			+		Escitalopram
19				+	Clonidine
20		+			Aripiprazole
21	+		+	+	Guanfacine, methylphenidate, fluoxetine
22				+	Unnamed anxiolytic
23	+	+			Aripiprazole, lisdexamfetamine
24	+				Dexmethylphenidate
25		+	+	+	Escitalopram oxalate, guanfacine, aripiprazole
Total	14	9	15	10	

[a] Includes antipsychotics and anticonvulsants; [b] Includes sympatholytic and antihistamine used as an anxiolytic; Medication information was not available for 5 ASD participants.

Figure 2.1. Language network iFC

(A) Language network regions of interest derived from spatial overlap between statistical parametric maps from NeuroSynth (term-based meta-analysis for "language") and group ICA using FSL MELODIC. lIFG1: left inferior frontal gyrus/ anterior Brodmann Area (BA) 44; lIFG2: left inferior frontal gyrus/ posterior BA 44; lIFG3: left inferior frontal gyrus/ BA 45; lIFG4: left inferior frontal gyrus/ BA 47; lSTS: left superior temporal sulcus; rSTS: right superior temporal sulcus; lSMA: left supplemental motor area; rIFG: right inferior frontal gyrus/ BA44. **(B)** Average within-group similarity boxplots for ASD (red) and TD (teal) groups. Midline represents median, hinges of the box denote first and third quartile, whiskers extend from quartile to the most extreme value within 1.5x of distance between first and third quartile (inter-quartile range). Values outside of the 1.5x inter-quartile range are shown as outliers. **(C)** Differences in language network iFC between subgroups and TD group. Glass brain depictions of connections of language ROI pairings with significantly lower connectivity in subgroup ASD2 than in the TD group (blue; upper row); with significantly greater connectivity in ASD3 than in the TD group (red; middle row); and with reduced connectivity in ASD2, but increased connectivity in ASD3, compared to TD group (purple; lower row). **(D)** CELF-4 Core Language, Receptive Language, and Expressive Language index scores by ASD subgroup, in comparison to TD group.


A
lIFG3
lIFG4
lIFG1
lIFG2
lSMA
rIFG
lSTS
rSTS
rSTS
rIFG
B
Average within group Similarity (r)
0.6
0.4
0.2
ASD
TD
C
D
CELF-4 Index Scores
125
100
75
50
25
ASD1
ASD2
ASD3
TD
Core Language
Receptive Language
Expressive Language
ASD2 < TD
ASD3 > TD
ASD2 < TD < ASD3

Chapter 3. Study 3

Distinct Variants of Anatomical Network Organization for Language in Autism Spectrum Disorders

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INTRODUCTION

Autism spectrum disorders (ASD) are a group of heterogeneous developmental disorders associated with atypical brain morphology (Carper et al., 2002; Ecker et al., 2013) and structural connectivity (Travers et al., 2012). Numerous genes involved in neuronal proliferation, differentiation, and migration have been implicated in atypical cortical organization and developmental heterogeneity in ASD (for review see Courchesne et al., 2019; Ecker & Murphy, 2014). Recent interest in ASD subtypes has pointed to stratification of more homogeneous subgroups of individuals with ASD to better account for variability in neuroanatomy and clinical heterogeneity. Some studies have found morphologically distinct ASD subgroups such as individuals that exhibiting macrocephaly (Amaral et al., 2017). In a recent neuroimaging-driven ASD subtyping study, Hong et al. (2017) identified subgroups characterized by differences in cortical thickness, surface area, grey-to-white matter tissue blurring, and geodesic distance. However, no group thus far has considered ASD subgroups based on language-related morphometry and gyrification. The aims of the present study were to 1) examine language-related morphological and structural connectivity differences between ASD and typically developing (TD) children; 2) identify homogeneous subgroups of ASD participants based on morphological features including cortical thickness and local gyrification index of language regions; 3) test for subgroup differences in diffusion indices of language-related white matter tracks; 4) characterize subgroups by demographic and clinical measures of language and ASD symptomology; and 5) explore the relationship between functionally and structurally derived ASD subgroups.

METHODS

Participants

Study 3 used neuroimaging and behavioral data that have been continuously collected for the same study protocol as Studies 1 and 2, however, with additional participants added since the initial study. A total of 177 participants (104 ASD, 73 TD) with structural MRI (sMRI) data and 138 (73 ASD, 65 TD) with diffusion-weighted imaging (DWI) data were included in this study (Table 3.1). All participants completed behavioral measures of intellectual abilities (WASI-II; Wechsler, 2011), handedness (Edinburgh Handedness Inventory; Oldfield, 1971), and language abilities (Clinical Evaluation of Language Fundamentals, CELF-4; Semel, Wiig, & Secord, 2004). In addition, caregivers completed a questionnaire regarding ASD-related symptoms (Social Responsiveness Scale- Second Edition, SRS-2; Constantino & Gruber, 2012).

Imaging Specifics

All anatomical and diffusion images were acquired using the same 3 Tesla GE Discovery MR750 scanner and 8-channel head coil, as described in Studies 1-2. Anatomical images were collected using a T1-weighted inversion recovery fast spoiled gradient echo sequence. DWI data were collected using an echo planar imaging (EPI) pulse sequence, encoded for 61 non-collinear diffusion directions at b = 1000 s/mm^2, and one at b=0 s/mm^2 (TR= 8,500 ms; TE= 84.9 ms; flip angle= 90; FOV= 240 mm; 1.88 × 1.88 × 2 mm^3 resolution). Field map images were collected with the same spatial resolution to correct for geometric distortions induced by field inhomogeneities.

Anatomical processing including cortical reconstruction, normalization to MNI space, segmentation of gray/white matter boundaries (white matter surface) and gray matter/cerebrospinal fluid boundaries (pial surface), and polygonal tessellation (Fischl et al.,

2001) were completed using FreeSurfer (Dale et al., 1998). All FreeSurfer output was examined on a slice-by-slice basis to identify inaccuracies in surface placement, which were corrected with white matter control points as needed and then reassessed for accuracy. Scans that showed persistent inaccuracies were excluded, as were those with major artifacts, such as ghosting or ringing.

DWI processing included automatic reconstruction of white matter tracts and extraction of tensor-based indices (e.g., mean diffusivity) using TRActs Constrained by UnderLying Anatomy (TRACULA; Yendiki et al., 2011), FMRIB Software Library (FSL; Smith et al., 2004), and FreeSurfer. Susceptibility distortions caused by phase encoding direction were corrected using a fieldmap (fugue). FSL's eddy_correct was used to correct for eddy current-induced distortions and inter-volume head movement. Participant motion were measured by average volume-by-volume rotation and average volume-by-volume translation (Yendiki et al., 2014). TRACULA used each participant's FreeSurfer anatomical parcellation and segmentation, probabilistic tractography (FSL BEDPOSTX; Behrens et al., 2003, 2007), as well as a prior anatomical learning dataset to label and reconstruct 18 white matter tracts. Average diffusion indices for each tract were weighted by the tract's per-voxel probability. Each participant's reconstructed white matter tracts were visually inspected by at least two independent raters for severity of signal dropout and proper vector orientation. Tracts that failed to reconstruct or were severely malformed were removed from analyses.

Language Anatomical Variables

Language network region of interests (ROIs) derived from a combination of group independent component analysis (ICA) of resting state fMRI data and NeuroSynth meta-analysis association map for the term "language" from Study 2 were adopted for sMRI measures in the

present study. These volumetric ROIs were transformed into surfaces, resampled onto fsaverage, and registered to each individual participant's native surface space to extract morphometric variables. Anatomical variables included cortical thickness (CT) and local gyrification index (lGI), a ratio of surface area within sulcal fold relative to the surface area of the outer cortical hull, extracted from the aforementioned ROIs.

Diffusion features included fractional anisotropy (FA), as well as mean, radial, and axial diffusivity (MD, RD, AD) of two major tracts considered critical for language processing, the arcuate and uncinate fasciculi (Catani & Mesulam, 2008; Parker et al., 2005). In addition, the inferior longitudinal fasciculus was included, given connections between temporal and visual regions, which have previously been implicated in language processing in individuals with ASD (Gaffrey et al., 2007; Kana et al., 2006). All structural and diffusion variables were normalized to z-scores and compared between ASD and TD groups using independent-sample t-tests. False discovery rate (FDR) was used to correct for multiple comparisons.

sMRI Subgroup Analysis

As in Study 2, a combination of CT and lGI of each ROI was dimensionally reduced using T-Sne (van der Maarten & Hinton, 2008) to conserve power for subgroup analysis. Latent profile analysis (LPA) of these T-Sne language morphology dimensions was carried out using MPlus (version 8; Muthén & Muthén, 1998-2011) to derive ASD language morphology subgroups. The number of ASD subgroups was iteratively increased until a best fit solution was found. Subgroup solutions were evaluated based on Akaike Information Criterion (AIC; Akaike, 1974), Bayesian Information Criterion (BIC; Schwarz, 1978), sample size-adjusted BIC (nBIC; Sclove, 1987), and entropy (Ramaswamy et al., 1993), Lo–Mendell–Ruben Adjusted Likelihood Ratio Test (LMRT; Lo et al., 2001), and Bootstrapped Likelihood Ratio Test (BLRT; Arminger

et al., 1999; McLachlan et al., 2000). Multivariate analysis of covariance (MANCOVA) was used to identify specific language network morphological differences between subgroups and TD participants, while controlling for effects of Total Brain Volume (TBV). TBV was used as a covariate to control for individual variability in brain size. Similarly, four different MANCOVA models were used for each of the diffusion indices (FA, AD, RD, MD) for the three bilateral white matter tracks, while controlling for the effects of motion. False Discovery Rate was used to correct for multiple comparisons across models. Finally, the subgroups were characterized with respect to intellectual abilities, symptom severity, and language abilities using one-way ANOVA, with multiple comparison adjustment using Tukey's honest significant difference test (Tukey's HSD) and, when appropriate, Games-Howell post-hoc test (GH).

Comparisons with Functional Subgroups

To further explore relationships between functional subgroups and anatomical language features, multinomial logistic regressions were employed to statistically predict iFC subgroup membership in Study 2 from language morphology dimensions (T-Sne reduced dimensions of CT and lGI). Associations between functionally- and structurally derived subgroup memberships were assessed using cross-tabulation Chi-squared tests. The iFC-derived subgroups (fASD1-3) were further examined by direct between-subgroup comparisons of individual anatomical (i.e., CT, lGI) and diffusion indices using MANCOVA, while controlling for the effects of TBV and motion, respectively.

RESULTS

Comparison at the whole-group level (ASD vs. TD) did not reveal significant differences in cortical thickness or lGI ($p_{FDR} > 0.95$) in any of the language ROIs. There were also no

significant group differences in diffusion indices of the uncinate, arcuate, or inferior longitudinal fasciculi, after controlling for motion (p_{FDR}> 0.98).

ASD subgrouping based on anatomical features

Latent profile analysis of T-Sne-derived structural dimensions of language ROIs (including lGI and cortical thickness) revealed a best fit solution consisting of three ASD subgroups (ΔAIC= 8.7; ΔBIC= 0.8; ΔnBIC= 10.3; entropy= 0.69; LMRT=13.8, p= 0.08; BLRT= 14.8, p< 0.001; Table 3.2). The subgroups (sASD1-3) included 27, 36, and 41 participants, respectively. The classification probability of most likely latent class membership for each subgroup ranged from 85% to 88%. The subgroups differed in cortical thickness from each other and from the TD group in all six left hemisphere language regions (Table 3.3, p_s< 0.01), after controlling for total brain volume (TBV). The differences were mostly driven by higher cortical thickness in sASD3 in five of six regions, and significantly lower cortical thickness in sASD1 in Brodmann Area 47 (BA 47; Figure 3.1A). Similarly, there was a significant effect of subgroup membership on lGI in six out of eight language regions (Table 3.3, p_s< 0.05). In all six regions, sASD1 displayed significantly higher gyrification than the other subgroups and TD peers (Figure 3.1B).

Comparison of Subgroups between Imaging Modalities

There was no significant association of subgroup membership between functionally- and morphologically-derived ASD subgroups ($\chi^2(4)$= 1.44, p= 0.84). Structural dimensions did not significantly predict functional subgroup membership (ps> 0.12). However, the iFC-derived subgroups did exhibit some differences in language region morphology. The functionally-derived subgroups showed a significant difference in cortical thickness in BA 44 ($F(2, 55)$= 4.16, p= 0.02) and BA 45 ($F(2, 55)$ = 3.25, p= 0.05), after controlling for TBV. In both cases, fASD3

exhibited higher cortical thickness. There was also a significant between-subgroup effect on lGI of BA 47 ($F(2, 55)= 4.60$, $p= 0.01$), after controlling for TBV; largely driven by the difference in gyrification between fASD1 and fASD3. The morphologically-defined ASD subgroups did not differ in iFC between language regions ($p_{FDR}> 0.92$). There was no relationship between ASD subtypes (iFC or structural) and diffusion indices of language-related white matter tracts.

Behavioral Characteristics

The subgroups displayed trending difference in communicational symptoms (SRS-2 Social Communication subscale score, $p= 0.06$; Table 3.4), mostly driven by lower scores in sASD2. In addition, the subgroups showed a marginal difference in language abilities (CELF-4 Core Language, $F(2, 74)= 2.62$, $p= 0.08$; CELF-4 Receptive Language, $F(2, 74)= 2.64$, $p= 0.08$; CELF-4 Expressive Language, $F(2, 74)= 2.41$, $p= 0.10$) with marginally lower language abilities in sASD1 (Table 3.3). The subgroups did not differ in gender, non-verbal intelligence, or medication status ($ps> 0.27$; see Table 3.5 for participant medications). However, the participants in sASD2 were significantly older than the participants in the two other subgroups ($ps< 0.05$).

CONCLUSION

Our study examined language-related neuroanatomical and structural connectivity in ASD. We did not find differences between ASD and TD cohorts at the whole-group level. However, when ASD participants were divided into more homogeneous subgroups, three distinct patterns of cortical morphology emerged: sASD1 characterized by greater gyrification and worse language abilities, sASD2 composed of older participants with less severe sociocommunicative symptoms, and sASD3 differentiated by increased cortical thickness. Our results suggest increased gyrification (which could be caused by regionally diverging rates of neuroproliferation as well as differing cortical architecture and degree of axonal tension (Ronan & Fletcher, 2015))

within language regions could be detrimental to development of language abilities. The existence of sASD2 indicates a need for further investigation into age-related trajectories of structural brain development in children and adolescents with ASD. Our finding of sASD3 was consistent with a previous report by Hong et al. (2017) of an ASD subgroup with greater cortical thickness, suggesting that such a variant may be relatively prevalent within the larger ASD population. These subgroups of independent neuroanatomical features in language regions indicate divergence of cortical development such as neuroproliferation, dendritogenesis (Shaw et al., 2008), and myelination (Deoni et al., 2015) within the ASD population.

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Table 3.1. Participant characteristics

	ASD (n = 104)		TD (n = 73)		Statistic	*p*-values
Gender	20 female		15 female		X^2 (3) = .74	.69
Handedness	13 left		8 left		X^2 (3) = 2.29	.52
	Mean (SD)	Range	Mean (SD)	Range		
Age in years	12.7 (2.9)	7.3-18.1	12.9 (2.8)	6.9-18.5	*t*(175)= -.35	.73
Head Motion[a]						
Average Translation	.88 (.23)	.41-1.81	.83 (.22)	.47-1.75	*t*(130)= 1.25	.22
Average Rotation	.005 (.003)	.002-.014	.004 (.002)	.002-.014	*t*(130)= 1.63	.11
WASI-II[b]						
Verbal IQ	98 (20)	45-147	107 (12)	73-147	*t*(173)= -3.71	< .001
Nonverbal IQ	102 (18)	53-145	105 (13)	62-137	*t*(173)= -1.39	.17
Full-scale IQ	100 (18)	59-141	107 (12)	79-141	*t*(173)= -2.82	.005
CELF-4[c]						
Core Language	91 (24)	40-127	109 (14)	58-129	*t*(128)= -5.43	<.001
Receptive	91 (22)	42-131	104 (14)	60-128	*t*(128)= -3.87	<.001
Expressive	90 (23)	29-130	106 (14)	61-130	*t*(128)= -4.95	<.001
SRS-2 Total[d]	77 (11)	55-101	45 (6)	37-79	*t*(152)= 23.74	<.001
ADOS-2						
Social Affect	10.7 (4.3)	1-20	--	--		
Repetitive Behavior	3.0 (2.1)	0-11	--	--		
Total	13.6 (4.9)	3-25	--	--		
Severity	7.3 (2.1)	1-10	--	--		
ADI-R						
Social Interaction	18.7 (4.9)	6-28	--	--		
Communication	14.3 (5.3)	2-25	--	--		
Repetitive Behavior	6.0 (2.1)	1-12	--	--		

ASD: autism spectrum disorders; TD: typically developing; **a** Head Motion measures were only available for 73 ASD and 59 TD participants; **b** IQ scores were available for 102 ASD and 73 TD participants; **c** CELF-4 scores were available for 75 ASD and 55 TD participants; **d** Social Responsiveness Scale-2 Total scores were available for 92 ASD and 62 TD participants.

Table 3.2. Latent profile analysis subgroup model fit

	AIC	BIC	n-Adj BIC	Entropy	LMRT	BLRT
2-class Solution	904	922	900	0.580	$p = 0.170$	$p = 0.267$
3-class Solution	895	922	890	0.687	$p = 0.079$	$p < 0.001$
4-class Solution	898	932	891	0.713	$p = 0.412$	$p = 0.667$

AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; n-Adj BIC sample size-adjusted Bayesian Information Criterion; LMRT: Lo–Mendell–Ruben Adjusted Likelihood Ratio Test; BLRT: Bootstrapped Likelihood Ratio Test.

Table 3.3. Between-group differences in morphological variables

	ROIs	sASD1 (n= 27)		sASD2 (n= 36)		sASD3 (n= 41)		TD (n= 73)		Post-hoc Comparisons
		M	SD	M	SD	M	SD	M	SD	
Cortical Thickness (z)	**lIFG1**	-0.31	0.89	-0.55	0.86	0.70	0.78	0.04	0.95	*sASD3,* TD > sASD2
	lIFG2	-0.63	0.89	-0.28	0.85	0.71	0.71	0.01	1.01	*sASD3*, TD > sASD1
	lIFG3	-0.25	0.71	-0.37	1.20	0.48	0.78	0.00	1.01	*sASD3*
	lSTS	-0.07	0.96	-0.61	0.69	0.63	0.84	0.04	0.95	*sASD3*, TD > sASD2
	rSTS	-0.14	0.83	-0.22	1.15	0.29	0.93	0.00	1.01	-
	lIFG4	-0.69	0.80	-0.03	0.94	0.49	0.91	0.02	0.99	*sASD3*
	lSMA	-0.26	1.06	-0.50	0.68	0.66	0.81	0.04	0.96	*sASD3*, TD > sASD2
	rIFG	-0.01	1.10	-0.38	0.88	0.35	0.93	0.02	1.00	-
Local Gyrification Index Cortical (z)	**lIFG1**	0.38	0.44	-0.29	0.56	-0.20	0.50	0.01	1.00	*sASD1*
	lIFG2	0.11	0.24	-0.20	0.29	-0.13	0.23	0.01	1.00	-
	lIFG3	0.49	0.67	-0.35	0.79	-0.18	0.49	0.01	1.00	*sASD1*
	lSTS	0.57	0.78	-0.41	0.93	-0.08	0.95	0.00	1.01	*sASD1*
	rSTS	0.39	0.72	-0.34	0.92	-0.12	0.55	0.01	1.01	-
	lIFG4	0.98	0.79	-0.30	0.82	-0.42	0.81	0.00	1.01	*sASD1*
	lSMA	0.73	1.17	-0.44	0.90	-0.13	0.64	-0.06	0.85	*sASD1*
	rIFG	0.79	0.86	-0.38	1.05	-0.14	0.70	-0.05	0.89	*sASD1*

Bolded ROIs represent significant group effect after controlling for total brain volume ($ps<0.05$). *Italicized* groups in post-hoc comparisons signify significantly greater value than all other groups ($ps<0.05$), after correcting for multiple comparisons. Non-italicized groups denote significant difference between specified groups ($ps<0.05$), after multiple comparison correction. ROIs: Region of interests; lIFG1: left inferior frontal gyrus/ anterior Brodmann Area (BA) 44; lIFG2: left inferior frontal gyrus/ posterior BA 44; lIFG3: left inferior frontal gyrus/ BA 45; lSTS: left superior temporal sulcus; rSTS: right superior temporal sulcus; lIFG4: left inferior frontal gyrus/ BA 47; lSMA: left supplemental motor area; rIFG: right inferior frontal gyrus/ BA44.

Table 3.4. Clinical characteristics between subgroups

	sASD1 (*n*=27)		sASD2 (*n*=36)		sASD3 (*n*=41)		
Gender	4 female		5 female		11 female		$p = 0.24$
Handedness	4 left		2 left		7 left		$p = 0.22$
Medications	6 on; 21 off		10 on; 26 off		15 on; 26 off		$p = 0.47$
	Mean	SD	Mean	SD	Mean	SD	Post-hoc
Age in years*	12.3	2.8	14.1	2.6	11.9	2.8	sASD2+
WASI-II							
Verbal IQ	96	25	101	20	97	17	
Nonverbal IQ	102	18	100	19	104	18	
Full-scale IQ	99	22	101	18	101	17	
CELF-4							
Core Language°	80	29	95	23	94	19	sASD1<sASD2†
Receptive°	81	24	95	22	94	20	
Expressive°	80	28	94	21	93	20	
SRS-2							
Total	78	10	74	9	78	12	
Social Communication°	77	11	72	9	78	11	sASD2<sASD3†
ADOS-2							
Social Affect	12.1	4.4	10.4	4.0	10.2	4.3	
Repetitive Behavior	3.1	1.7	3.3	2.2	2.8	2.2	
Total	15.4	4.9	13.4	4.9	12.7	4.8	
Severity	8.1	1.6	7.2	2.2	6.9	2.3	
ADI-R							
Social Interaction	17.9	4.7	18.4	5.8	19.5	4.2	
Communication	13.9	6.3	13.8	5.0	15.1	4.8	
Repetitive Behavior	5.8	2.3	6.2	1.8	6.0	2.2	

sASD1-3: structurally derived ASD subgroups 1-3. * Main effect of subgroup significant at $p < 0.05$; ° Marginally significant effect of subgroup at $p < 0.10$. sASD2+ participants were significantly older than participants from the two other subgroups at ps < 0.05, after correcting for multiple comparisons. † Marginally significant difference between subgroups ($p < 0.10$) in post-hoc comparison, after correcting for multiple comparisons.

Table 3.5. Current psychotropic medication of Autism Spectrum Disorders (ASD) group

Participant	Stimulants	Mood Stabilizers[a]	Antidepressants	Anxiolytics/ Others[b]	List of Medications
1	+				Amphetamine/dextroamphetamine
2		+		+	Aripiprazole, alprazolam, hydroxyzine, risperidone
3	+		+		Methylphenidate, sertraline
4			+		Paroxetine
5	+	+	+		Sertraline, risperidone, methylphenidate
6	+			+	Amphetamine/dextroamphetamine, guanfacine
7	+	+	+		Methylphenidate, citalopram, risperidone
8	+		+		Amphetamine/dextroamphetamine, sertraline
9		+	+	+	Aripiprazole, oxcarbazepine, clonidine, fluoxetine
10			+		Fluoxetine
11	+	+	+		Sertraline, amphetamine/dextroamphetamine, lamotrigine
12		+		+	Ziprasidone, guanfacine
13	+		+		Lisdexamfetamine, venlafaxine
14	+		+		Sertraline, lisdexamfetamine, fluvoxamine
15	+	+	+		Divalproex sodium, methylphenidate, fluoxetine, lamotrigine
16	+		+	+	Lisdexamfetamine, guanfacine, sertraline
17			+		Escitalopram
18		+			Aripiprazole
19	+		+	+	Guanfacine, methylphenidate, fluoxetine
20				+	Unnamed anxiolytic
21				+	Guanfacine
22	+	+			Aripiprazole, lisdexamfetamine
23	+				Dextroamphetamine
24		+			Lamotrigine, aripiprazole
25				+	Guanfacine
26	+		+		Methylphenidate, citalopram
27			+	+	Sertraline, guanfacine
28		+			Aripiprazole, risperidone, quetiapine
29		+		+	Lithium, buspirone, risperidone
30	+				Dexmethylphenidate
31		+	+	+	Escitalopram, guanfacine, aripiprazole
Total	16	13	17	12	

[a] Includes antipsychotics and anticonvulsants; [b] Includes sympatholytic and antihistamine used as an anxiolytic; Medication status information were not available for 12 of 104 ASD participants.

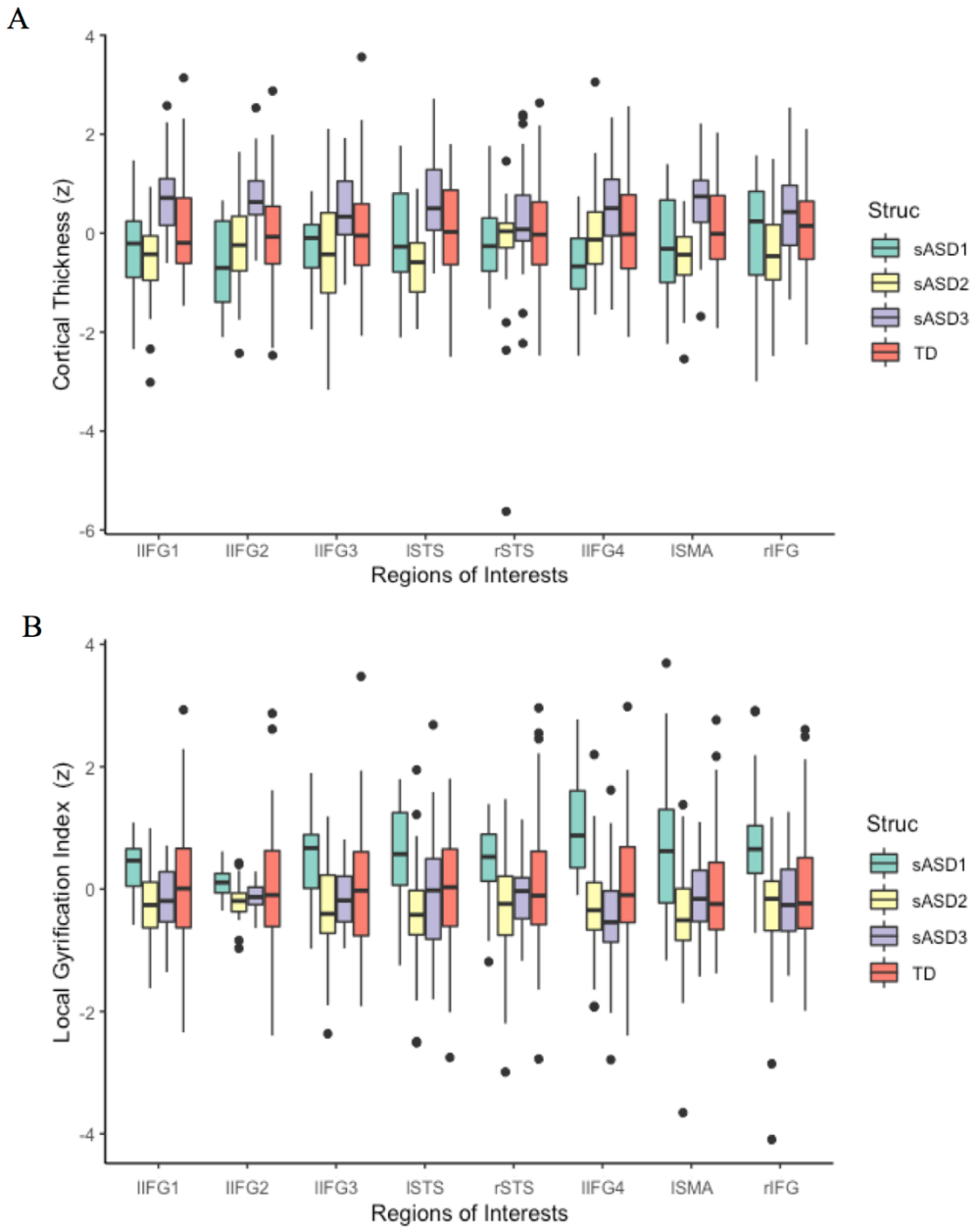

Figure 3.1. Between group morphological differences

(A) Cortical thickness boxplots for sMRI-derived ASD subgroup 1 (sASD1; teal), subgroup 2 (sASD2; yellow), subgroup 3 (sASD3; lavender), and TD group (red). Boxplot midline denotes median with hinges of the box representing first and third quartile. Trailing dots represent outliers for each region outside of 1.5x inter-quartile range. lIFG1: left inferior frontal gyrus/ anterior Brodmann Area (BA) 44; lIFG2: left inferior frontal gyrus/ posterior BA 44; lIFG3: left inferior frontal gyrus/ BA 45; lIFG4: left inferior frontal gyrus/ BA 47; lSTS: left superior temporal sulcus; rSTS: right superior temporal sulcus; lSMA: left supplemental motor area; rIFG: right inferior frontal gyrus/ BA44. **(B)** Local gyrification index boxplots.

DISSERTATION CONCLUSION

Summary of findings

The three studies of this dissertation explored the functional, and structural neuroanatomy of the language network, with specific focus on heterogeneity and interindividual variability, in children with ASD, using multimodal neuroimaging. Study 1 examined iFC within the language network and between language areas and other brain regions (seed-to-whole brain analysis) in children with ASD, using an extensive set of language regions adapted from a meta-analysis of 54 neuroimaging studies of language comprehension (Rodd et al., 2015). We found overall increased iFC within the language network in addition to increased connectivity between language regions, PCC, and visual regions in the ASD group, in comparison to TD peers. While effects of diagnostic status were modest at the whole-group level, a *subgroup* of ASD participants showed a distinctive pattern of PCC-mediated iFC between frontal language region and visual cortex, identifying the need for further investigation of heterogeneity of the language network in ASD.

This investigation was pursued in Study 2. The language regions in Study 2 differed from Study 1 for the purpose of developing data-driven ROIs from a wider range of neuroimaging studies (beyond language comprehension ROIs from Study 1), and those that better fit school-age participants. Although Study 2 included a larger sample than Study 1, no significant iFC differences at the whole-group level were detected, which is remarkable given several previous studies with ASD smaller samples reporting significant language FC differences from TD peers. However, we found significantly higher within-group heterogeneity of language network iFC in the ASD group. LPA of iFC dimensions resulted in three ASD subgroups, with distinct patterns of language network iFC. Two of the subgroups showed opposite patterns of connectivity

(under- vs. overconnectivity), which likely explains overall absence of effects at the whole-group level. While all three subgroups exhibited lower language abilities than TD participants, no significant differences in symptom severity or language skills between the subgroups were detected, possibly due to limited statistical power in relatively small subgroups.

In Study 3, we combined sMRI and DWI to better understand ASD language subtypes. Similar to Study 2, at the whole-group level, ASD participants did not differ significantly from their TD peers in CT or lGI of language regions. However, after the ASD group was partitioned using LPA of morphological dimensions, three subgroups emerged that differed from one another as well as from the TD group. These subgroups differed in age and marginally in language abilities and sociocommunicative symptoms. Interestingly, diffusion indices of language-related white matter tracts did not differ between subgroups in either the functionally-derived nor the morphologically-derived subgroups.

General conclusions

Taken together, the three studies expanded on previous reports of atypical increased FC of language network with visual regions (Knaus et al., 2008; Pang et al., 2016) and DMN (Zhao et al., 2016) as well as increased FC variability (Hahamy et al., 2015; Nunes et al., 2019) in ASD. Strikingly, even with an improved ROI scheme and expanded sample size, we were not able to detect between-group differences in language network iFC, morphology, or structural connectivity. Conversely, when the ASD group was parsed into more homogeneous subgroups, a clear picture of varied language network ASD subtypes emerged. The existence of ASD subgroups composed of markedly different patterns of iFC and morphology may explain some of the inconsistencies seen in the ASD imaging studies, such as the longstanding debate of underconnectivity (Just et al., 2004; Verly et al., 2014) vs. overconnectivity (Lee et al., 2017;

Supekar et al., 2013) in ASD FC literature. These findings indicate that by using a one-size-fits-all approach to ASD research, much of the intricacies in underlying neurobiology of language is lost to the noise within a heterogeneous population. While there has been an interest in studying distinct ASD subsets, the existing literature has mostly relied on traditional group-level comparisons (Hull et al., 2017), thus failing to account for the known heterogeneity and likely missing critical differences that account for symptomatology in ASD individuals.

Comparing the findings from Studies 2 and 3, fMRI and sMRI modalities yielded different subgroup clusterings of the ASD cohort. Our findings also did not demonstrate a clear link in membership between the two imaging modalities, which suggests that heterogeneity of brain organization of language in ASD may differ between functional and anatomical dimensions. As such, it was unsurprising to see a lack of relationship between subgroup membership and diffusion measures of the language-related white matter tracts. Continuation of the current study could combine DWI with sMRI and fMRI for more comprehensive neuro-subtyping. However, care must be taken to avoid collinearity and differential weight assignment of various imaging features (as some features maybe more informative than others; Eill et al., 2019).

In addition, sMRI-derived subgroups showed behavioral differences while iFC-derived subgroups did not. This distinction may be due to differences in neurodevelopmental history captured by the two imaging modalities (Eill et al., 2019). On the one hand, morphological features are likely to reflect atypicality predominantly with respect to development history (Hazlett et al., 2017). Furthermore, lGI has been shown to be more sensitive to group differences and developmental changes in ASDs (Kohli, et al., 2018). On the other hand, iFC patterns – although often considered to reflect ‘intrinsic’ functional network architecture (Van Dijk et al.,

2010) are equally affected by cognitive state and online processing (Buckner & Krienen, 2013; Mason et al., 2007), as well as transient state-based changes (Mash et al., 2019; Rashid et al., 2018) and impact of learning (Lewis et al., 2009).

The lack of robust links between brain-based subgroupings and language abilities or symptom severity indicates complexity in ASD heterogeneity that is beyond what was explored in this dissertation. Previous studies have found reduced differentiation between neural networks in the ASD population (Hong et al., 2019; Keown et al., 2017). Subgrouping derived solely based on language network FC may be missing important features of network organization, such as segregation or differentiation from other networks, that may be crucial to account for differences in language abilities. This was partially supported by Study 1, which revealed a subgroup of participants with posterior default mode region (PCC)-mediated FC between frontal language regions and visual regions that was linked to lower language abilities. As this dissertation focused on the language network, the FC and morphology of other functional domains may provide additional evidence for behavioral language and symptom variability in ASD.

In addition, our participant sample mostly consisted of high-functioning children in middle to late childhood. Thus, the ASD participants had completed years of schooling and may have had various interventions. As learning and experiences has been shown to have an impact on FC (Lewis et al., 2009), it is possible that more robust links between in language abilities and distinct language FC patterns may be detectable in younger cohorts of ASD participants. Another possible explanation for the weak link between language differences and subgroup membership could be the lack of sensitivity from the behavioral measure used. While the CELF-4 provides general index scores in expressive and receptive language, more subtle variabilities within those

language abilities may be lost. Future studies with a more thorough neuropsychological battery may be able to detect more salient language and cognitive differences between subgroups. Finally, while neuropsychological assessments enable researchers to examine various cognitive abilities, the ecological validity of the currently available measures to truly capture language abilities in individuals with autism may be questionable (Chaytor & Schmitter-Edgecombe, 2003).

The present dissertation included three cross-sectional neuroimaging studies of language in ASD. In Studies 1 and 2, neither diagnostic groups (ASD, TD) nor ASD subgroups differed in age. However, in Study 3 there was a difference in age between the sMRI-derived subgroups. Our participants span between ages 7 and 18 years, a period characterized by extensive maturational changes in the brain (Luna et al., 2001; Steen et al., 1997; Whitford et al., 2007). Age-related morphological changes between childhood and adolescents including gradual increases in cortical volume and gyrification in childhood followed by decrease in cortical thickness and gyrification in adolescence (Kohli et al., 2019; Paus et al., 2008; Tamnes et al., 2010). The three studies utilized participants age-matching and covariates including total brain volume to reduce age-related confounds. However, there may be differences in developmental trajectory in language related regions and network connectivity between participants. Without longitudinal data, we are limited in our ability to account of developmental changes that occur from childhood to adolescence.

While the sample size of this dissertation is relatively large compared to earlier neuroimaging studies of the language network in ASD, it is relatively small compared to other statistical or machine learning studies (Hong et al., 2017). Measures were taken to conserve power (i.e. dimension reduction); however, a larger sample size may improve neuroimaging

subtyping. For example, the entropy of the structural 3-subgroups model was relatively low (Entropy = 0.68). It is possibly that a larger sample size would improve the statistical fit of the model. Increased statistical power may also improve clinical characterization of the subgroups. Relatedly, while detection of subgroups in this dissertation corroborates heterogeneity of language neurobiology within ASD, the exact composition of subgroups may not be readily generalizable. Definitive studies of neurophenotypes will require large data repositories. While multisite repositories such as Autism Brain Imaging Data Exchange (ABIDE; Di Martino et al., 2014; Di Martino et al., 2017) are becoming available, site variability remains an issue. Currently available large ASD repositories also do not encompass rich sets of multimodal imaging, clinical, and phenotyping data that are likely indispensable for conclusive findings of ASD subtypes. More generally, the findings from the three studies presented underline that ASD studies using conventional group-level analysis, which have dominated the literature, must be interpreted with caution and can only be considered gross or first pass-approaches to detection of ASD-specific brain anomalies, which must be followed up by analyses for the detection of more homogeneous subgroups or clusters of individuals with ASD.

ASD are a highly prevalent set of disorders, currently estimated to affect 1 in 54 children aged 8 years in the United States (Maenner, 2020), with an estimated lifetime economic impact of $3.2 million per capita (Ganz, 2007). As language abilities are the best predictors of future outcome for individuals with ASD (Mawhood et al., 2000; Szatmari et al., 2015), it is essential to gain a better understanding of language processing in this vulnerable population. However, the heterogeneity across the autism spectrum in etiology (Betancur, 2011; Jeste & Geschwind, 2014; Mandy & Lai, 2016), symptom presentation (Constantino & Charman, 2016; Georgiades et al., 2013), and interventions (Bradshaw et al., 2015; Helt et al., 2008; Wong et al., 2015) has made it

difficult to elucidate language processing in ASD. To date, this dissertation is the first study aimed to both address language network heterogeneity and to identify language-focused ASD subtypes using statistical learning of neuroimaging data. The empirical identification of these subtypes highlighted a need to address individual variability in ASD research and for a more representative nosology for this group of disorders. We hope that this will eventually translate to the development of more precise interventions that target language impairments in subsets of the ASD population.

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