An Examination of the Broader Autism Phenotype in Simplex and Multiplex Families

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Abstract

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Non-inherited genetic mutations are more prevalent in families with only one individual diagnosed with autism spectrum disorder (ASD; simplex) whereas inherited genetic risk factors may play a greater role in families with more than one affected individual (multiplex). Behavioral genetic studies examining the broader autism phenotype document increased presence of BAP traits in first-degree relatives from multiplex versus simplex families. Mothers, fathers, and siblings from 87 multiplex and 41 simplex were included in analyses. In general, findings supported a differential presentation of behavioral features of the broader autism phenotype in family members from multiplex families compared to simplex families. This was particularly relevant in social communication skills. Multiplex family members showed decreased social interest, more impaired nonverbal communication abilities, and less flexible conversation skills compared to simplex family members. Effects were moderate to large across skill areas and were most consistently observed in siblings, followed by fathers, and then mothers.

Cognitive profiles presented differently in parents compared to siblings. Specifically, differences between multiplex and simplex families in cognitive measures such as cognitive variability, face memory, and phonological processing were absent in parents, but were apparent in siblings. Multiplex siblings showed greater cognitive variability and were more likely to have impaired face memory and phonological processing skills compared to simplex siblings. Thus, multiplex siblings showed a number of similar elements of the cognitive phenotype in ASD in addition to social communication difficulties compared to simplex siblings.

No relationship between the symptoms of the affected children and family member traits were found in the current study. Thus, increased symptom severity in affected children did not predict greater BAP traits in either simplex or multiplex families.

Despite reports that macrocephaly appears to be a familial trait in ASD, head circumference measurements did not differ between multiple-incidence and single-incidence families. However, a positive relationship between head circumference and increased BAP traits was more pronounced in simplex siblings compared to multiplex siblings. Given the variability in predictions of increased head circumference and ASD symptoms in affected individuals, this finding was not predicted and it is difficult to interpret it in isolation. Further work is necessary to replicate this relationship.

The decreased number and intensity of BAP traits observed in parents and siblings within simplex families provide behavioral evidence consistent with findings of increased de novo genetic events since ASD-related behavioral traits were observed less frequently in simplex compared to multiplex families. These behaviorally-based findings suggest that multiplex families may be more vulnerable to ASD symptoms given shared genetic variance.

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DEDICATION

To my father…

who inspired me to pursue higher education through his example and who encouraged me to always do my best.

CHAPTER 1

BACKGROUND AND RATIONALE

1. Introduction

Autism spectrum disorder (ASD) is a term that has been adopted widely to describe an array of diagnoses, including autistic disorder, Asperger's disorder, and pervasive developmental disorder - not otherwise specified (PDD-NOS). ASD is a neurodevelopmental disorder that is biologically based and has an onset of symptoms prior to age three. Males are more often affected with ASD than females with a sex ratio of approximately 4:1. Impaired social interactions are a common feature among all three of the ASD diagnoses, and social impairments are considered to be the core area of functioning that is affected in ASD. Current diagnostic criteria put forth in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*; American Psychiatric Association, 1994) for ASDs include impairments in three domains: reciprocal social interactions, communication skills, and restricted and/or repetitive interests and behaviors. Individuals with ASD often present with a unique array of symptoms within these domains.

Examples of impaired social skills include a limited ability to develop appropriate peer relationships, decreased shared enjoyment with others, and challenges with nonverbal interaction skills such as inconsistent eye contact and limited use of facial expressions. Diagnostic criteria in the communication domain include difficulty in maintaining reciprocal conversations and presence of unusual speech patterns such as overly formal or repetitive speech. The last area of impairment is often the most outwardly striking and involves a pattern of behavior, interests, and activities that are unusually repetitive or restricted in quality. Examples of symptoms in this domain include hand flapping, insistence on maintaining routine, and intense interests.

The development of the *DSM-V* is underway and significant revisions to the ASD diagnosis are currently under discussion. Some of the potential modifications include combining the social and communication domains of impairment to reflect one focal area of challenge in social communication and replacing the three separate diagnoses (autistic disorder, Asperger's disorder, and PDD-NOS) with one general diagnosis of ASD with a severity specification. The final outcome of these impending modifications to the diagnosis remains to be seen. However, given the forward motion to eliminate the diagnostic boundaries among ASDs, the term ASD will be used hereafter to refer to all three groups.

Although the general areas of challenge are similar across individuals with ASD, ASD is a heterogeneous disorder and children and adults with ASD vary significantly in their phenotypic presentations. Epidemiological studies suggest that although approximately 65% of individuals with ASD have a co-occurring intellectual disability (previously known as *mental retardation*), the remainder have general cognitive abilities in the average to above average range (Fombonne, 2003). Language abilities vary widely as well. Although one-third to one-half of those diagnosed with autistic disorder have severely impaired language and/or remain nonverbal through adulthood, many other individuals are verbally fluent and may develop language early (Howlin, Goode, Hutton, & Rutter, 2004).

In the United States, the average age of diagnosis for ASDs ranges from 3.5 – 5.0 years of age, depending on the state (Autism and Developmental Disabilities Monitoring Network, 2009). Factors such as lower IQ, being male, and the presence of a regression in skills contribute to a younger age of diagnosis (Shattuck et al., 2009). Despite an average age of diagnosis in the preschool years, parents of children with ASD often report symptom onset between 12 – 18

months of age (De Giacomo & Fombonne, 1998; Rogers & DiLalla, 1990). Early markers of ASD in infancy have received considerable research attention (e.g., Zwaigenbaum et al., 2005).

In 2007, the Centers for Disease Control and Prevention reported a prevalence rate in the United States of 1 in every 150 children, which is a dramatic increase from reported prevalence rates of approximately 1 in 2,500 reported in decades prior (e.g., Lotter, 1966). Two years later, the prevalence rate rose to 1 in every 110 (Autism and Developmental Disabilities Monitoring Network, 2009) and 1 in 90 parents reported that their child had a diagnosis on the autism spectrum (Kogan et al., 2009). The exact causes for this increase are under discussion.

Much research has been conducted on differences in brain function and structure between typically developing individuals and individuals with ASD. Individuals with ASD have an unusually large amygdala, which is associated with emotional functioning and processing (Mosconi et al., 2009). Cellular abnormalities in the cerebellum have also been noted (Allen & Courchesne, 2003; Courchesne, 1997). The cerebellum is involved with complex motor activities, attention, language, and integration of brain functions. These are all areas of challenge in ASD. In addition to specific brain regions that are unusual in ASD, there is poor connectivity between different brain regions, particularly between the frontal cortex and other regions (Minshew & Williams, 2007).

Most cases of ASD are currently considered to be idiopathic, meaning that they do not have a known cause such as brain injury or genetic syndrome. However, there is clearly a genetic influence in ASD as individuals are at increased risk if they have a positive family history of the disorder. Monozygotic (MZ) twin concordance rates are substantially higher than dizygotic (DZ) twin rates (Folstein & Rutter, 1977), reaching 96% when diagnostic boundaries are broadened (Bailey et al., 1995). Although this suggests a strong role of genetic factors in the

development of ASD, it also implies that environmental factors (such as prenatal exposure to a number of different teratogens) are involved to a lesser degree in some individuals since MZ twin concordance rates are not 100%. In a review of environmental influences in ASD, Landrigan (2010) concludes that many chemicals exist in the environment that are known to be developmental neurotoxicants and may be causal in some cases of ASD.

Many options exist for treating individuals with ASD. Early intensive behavioral intervention is the recommended treatment to address the core symptoms (Dawson & Osterling, 1997). A behavioral intervention approach called Applied Behavior Analysis (ABA) has been studied extensively with promising results in improving outcomes for individuals with ASD (e.g., Dawson et al., 2010; Lovaas, 1987; McEachin, Smith, & Lovaas, 1993).

2. Genetics of Autism Spectrum Disorders [1]

A. Foundations

Prior to the 1970s, autism was not considered to be a biologically based disorder. Psychoanalytic theory, which dominated mid-twentieth century psychological thought, viewed autism as rooted in parental rejection of the child. Followers of this *refrigerator mother theory* purported that cold, aloof parenting was the cause of autism. Therefore, work during this time period was not focused largely on determining genetic factors. Research on the genetic bases of autism and ASD began to emerge in the 1970s. In 1977, Susan Folstein and Michael Rutter published a seminal paper describing MZ and DZ twins with ASD and concluded that ASD is a genetically-based disorder not caused by poor parenting (Folstein & Rutter, 1977).

[1] A portion of this section has been published in: Gerdts, J., Ackerman, S., & Bernier, R. (in press). Genetics. In: M. Martins (Ed.), *Encyclopedia of autism*. Greenwood Press: Santa Barbara, CA.

Using same-sexed twin pairs, Folstein and Rutter (1977) found that of 11 MZ and 10 DZ twins, 36% of MZ pairs were concordant for strict autism compared to 0% of DZ twins. Concordance rates further increased to 82% in MZ twins and 10% in DZ twins when criteria were expanded to include broader cognitive abnormalities and social/communication challenges and repetitive behaviors falling short of formal diagnostic criteria (Folstein & Rutter, 1977). These different concordance rates signified the first empirical evidence for the role of genetics in ASD. Several subsequent twin studies have replicated these initial discrepant concordance rates with ranges from 60-96% in MZ twins compared to 0 – 23% in DZ twins depending upon the sample and diagnostic boundaries (Bailey et al., 1995; Ritvo, Freeman, Mason-Brothers, Mo, & Ritvo, 1985; Steffenburg et al., 1989).

In the largest population-based twin study of autism to date (n = 192), twins underwent a thorough diagnostic and cognitive examination using current gold standard diagnostic tools (Hallmayer et al., 2011). This study confirmed early reports of discrepant concordance rates between MZ and DZ twins. Probandwise concordance rates were 58 - 60% for strict autism in MZ twins versus 21 - 27% for same-sex DZ twins, depending on sex of the children. Probandwise concordance rates using broader diagnostic criteria were 77% for MZ male twins and 50% for MZ female twins versus 31% and 36%, respectively, in DZ twin pairs. Additionally, authors suggest that environmental factors common to twins explained about 55% of the liability to autism, which is higher than earlier estimates (Hallmayer et al., 2011).

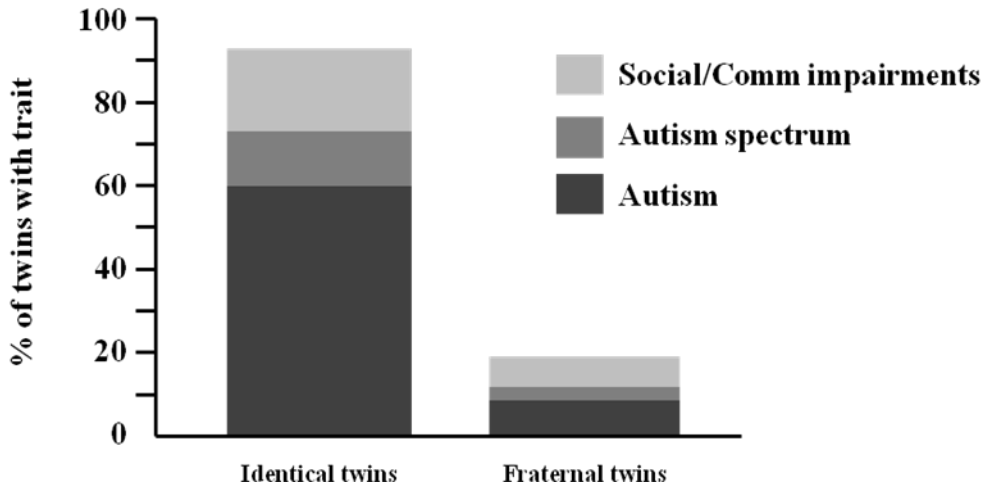


Figure 1. Adapted from Bailey et al., 1995. Monozygotic and dyzygotic twin concordance rates

The sibling risk rate for idiopathic autism in an early epidemiological survey was estimated to be approximately equal to concordance rates of DZ twins in early reports: 8.5% (Ritvo et al., 1989), with an average of 5% reported in a meta-analysis (Simonoff, 1998). This was predicted given that siblings share, on average, the same amount of genes as DZ twins. More recent estimates using updated clinical diagnostic criteria to include diagnoses of Asperger's Disorder and PDD-NOS in smaller, clinically ascertained samples suggest sibling risk rates similar to the DZ twin estimates reported by Hallmayer and colleagues (2011): 20 - 36% (Goldberg et al., 2005; Zwaigenbaum et al., 2005). These rates are elevated compared to the current general prevalence rate in the United States of 1 in 110 or 0.9% (Autism and Developmental Disabilities Monitoring Network, 2009). Additionally, first-degree relatives of an individual with ASD are more likely to exhibit ASD-related traits compared to relatives of individuals with either no abnormalities or other developmental disorders such as Down syndrome (e.g., Bishop, Maybery, Wong, Maley, & Hallmayer, 2006; Landa, Piven, Wzorek, & Gayle, 1992; Piven, Palmer, Landa et al., 1997). Taken together, these behavioral genetic

studies suggest that ASD risk rises as the level of shared genes increases and provide solid ground for exploring specific genetic mechanisms at play in ASD.

B. Gene Identification Methodologies

Early behavioral genetic studies such as Folstein and Rutter's twin study (1977) were restricted by the technology of the day in terms of the ability to identify genes involved in ASD. Increasingly better technology for genetic analysis has allowed a more detailed picture of ASD molecular genetics to emerge and tremendous advances in such technology over the past decade (and especially the past several years) have revamped our understanding of genetic mechanisms in ASD. The terrain is ever changing and undoubtedly will be transformed even further in upcoming years.

Gene identification methods common in the 1990s include both linkage and gene association studies. Linkage refers to the tendency of certain genes to be inherited together because they are on the same chromosome. Thus, combinations of characters from parents are more common in their offspring than combinations from unrelated individuals. Linkage is measured by the percentage recombination between genetic loci and utilizes an unbiased approach without a predetermined genomic candidate region. Analyses examine the correlation between the pattern of inheritance of genomic markers and the pattern of inheritance of a phenotypic trait within a family. Most linkage studies in ASD have focused on affected sibling pairs in multiplex ASD families.

In contrast, gene association studies explore the relation between a candidate gene and a given phenotype. The decision to investigate particular candidate gene markers is generally made based on signals from promising chromosomal regions identified through linkage studies, or based on theoretical rationale of, for instance, other genes purported to be involved in similar

pathways. Findings from gene association studies produced several potential promising ASD-risk loci.

Standard karyotyping detects sizeable cytogenetic abnormalities, including large segments of missing or added genetic material (e.g., the extra copy of chromosome 21 that is causal in Down syndrome). Genetic mutations such as these differ from the expected number of copies of genetic material, but with karyotyping only very large differences are observable. Of recent importance, the completion of the Human Genome Project in 2003 and the International HapMap in 2005 has allowed researchers to compare most of the genome of persons with ASD to a reference human genome sequence (National Human Genome Research Institute, 2010). This is referred to as a genome-wide association study, and contemporary genetic technologies allow detection of much smaller mutations than previously possible with karyotyping.

Increased resolution of genetic sequencing used in today's analyses allows researchers to identify increasingly smaller copy number variations (CNVs). A CNV is a type of genetic mutation involving a DNA segment that is longer than 1 kb and differs from reference DNA in terms of the number of copies present (Freitag, Staal, Klauck, Duketis, & Waltes, 2009). A CNV can refer to DNA that is missing (deletion), repeated (duplication), or added (insertion). CNVs can be inherited or may arise *de novo* and are considered pathogenic whey they affect gene(s) involved in the development or maintenance of the human body (Freitag et al., 2009). It is estimated that humans in the general population have several hundred CNVs, both common and rare, and the majority do not cause known impairment. In fact, population geneticist Matthew Hurles of the Wellcome Trust Sanger Institute in Cambridge, England stated "it's not normal to be walking around with the perfect genome." (pg. 25, Wenner, 2009). Although genetic

mutations are common in the population, a specific CNV is considered individually rare if it occurs in less than 1% of the population.

C. Genetic Models of ASD

Introduction

Recently some researchers have suggested that approximately 10-20% of ASD cases are likely to have a genetic cause through an identifiable genetic syndrome, observable genetic mutation, or *de novo* CNV (Abrahams & Geschwind, 2008). Authors of a review in 2008 concluded that 20 mutations relevant in ASD had been identified at that time (Abrahams & Geschwind, 2008). Over 200 candidate genes have been documented on AutDB, a public, curated, web-based database for autism research (Basu, Kollu, & Banerjee-Basu, 2009).

Despite the large amount of genetic findings associated with ASD, there still is no single genetic mutation which explains a large number of ASD cases and the presence of any one genetic event does not necessarily prescribe a diagnosis. Indeed, individuals in the general population can have the same mutations as individuals with ASD (albeit at a lesser rate), yet do not present with ASD. It is clear that ASD is not a classic Mendelian disorder resulting from a single gene mutation. Rather, its genetic origins are quite likely heterogenous and complex.

Two primary genetic models have dominated the field in recent years. One model views ASD as adhering to the *common disease-common variant* (hereafter referred to as *common variant*) framework. The common variant model suggests that ASD is a polygenic and oligogenic disorder, meaning that multiple genes interact with one another to produce the disease. Several commonly varying genes (defined as occurring in at least 1% of the population) are thought to act together to generate the disease.

A second model that has grown increasingly popular in recent years is the *common disease-rare variant* (hereafter referred to as *rare variant*) model, which suggests that ASD results from rare, highly penetrant genetic mutations. Recent reports of single mutations causing functional disruption hypothesized to cause ASD in an individual provide support for the rare variant model, and potentially provide insight into genetic variation in ASD.

Technological approaches generally correspond to these two philosophies: linkage and gene association studies across unrelated individuals tend to adhere to the common variant view, whereas identification of mutations in candidate genes and *de novo* CNVs are rooted in the rare variant framework. Although many researchers may fall in one camp or the other, it is possible that the two models both contribute to genetic variation in ASD.

Finally, epigenetic models of ASD have begun to emerge as well. A recent review demonstrates that specific epigenetic mechanisms associated with ASD may be important in understanding its etiology (Grafodatskaya, Chung, Szatmari, & Weksberg, 2010).

Common Variants in ASD

Linkage studies.

Suggestive linkage signals have been reported on nearly every chromosome in family studies of individuals with ASD. However, as with many other psychiatric disorders, replicating these findings has proved to be very difficult. One of the largest linkage studies to date failed to produce the identification of a common variation (Szatmari et al., 2007). In their review, Abrahams and Geschwind (2008) suggest that difficulty in replication may be due to the small effect size attributable to any given gene and to the heterogeneity in both diagnostic procedures and the disorder itself. To counteract these complications, subtyping efforts have been made to encourage more homogeneous samples. For example, stratifying a sample by sex, presence of

regression, cognitive variability, and variables regarding language development have all served to increase linkage signals (e.g., Chapman et al., 2011; Schellenberg et al., 2006).

Suggestive linkage signals reported previously in ASD were subjected to a meta-analysis by Trikalinos and colleagues (2006). Areas with significant evidence for linkage at a genome-wide level were identified at region 7q22–32, with suggestive evidence for linkage to 10p12–q11.1 and 17p11.2–q12 (Trikalinos et al., 2006). Linkage signals may suggest that a risk gene for a given disorder is present in the chromosomal region implicated. For instance, common variation in the CNTNAP2 gene (7q35) and age at first word was found because of linkage signals identified in the 7q34-7q36 region (Alarcon et al., 2008).

To further investigate positive linkage signals, quantitative trait locus (QTL) mapping is often applied using biomarkers rather than affected status of the individual. Endophenotypes are components of the phenotype that are quantifiable and theorized to be more closely tied to underlying genetic influences (Gottesman & Gould, 2003). QTL-based analyses investigate traits that vary in both affected and unaffected family members. Examples in ASD are particular aspects of social behavior, language onset, conversational skills, and discrepancies in cognitive abilities. For instance, using a measure of ASD-related characteristics in affected and unaffected family members across nuclear ASD families, social motivation and range of interest/flexibility were found to have high heritability using QTL mapping (Sung et al., 2005). A recent report examining families with affected individuals who had a significant discrepancy between verbal and nonverbal IQ provided strong evidence for a QTL on chromosome 10, and suggestive evidence on chromosome 16 (Chapman et al., 2011).

Gene association.

Many common variants have been implicated through gene association studies, including met proto-oncogene (MET) promoter variant at 7q31 (Campbell et al., 2007; Campbell et al., 2006), the serotonin transporter gene SLC6A4 at 17q11 (e.g., Sutcliffe et al., 2005; Wassink et al., 2007), and gamma-aminobutyric acid receptor subunit beta-3 (GABRB3) gene at 15q11–15q12 (Buxbaum et al., 2002; Cook et al., 1998). However, genes identified through these studies are not replicated consistently and many are associated with intronic or intergenic single-nucleotide polymorphisms (SNPs). Therefore, their role in modulating phenotypes, if any, continues to be elusive.

Genome-wide association studies with sufficiently large samples may begin to address the potentially false positive findings that plagued earlier gene association studies. Three relatively large studies of this type have recently been published. First, using large numbers of markers, a genome-wide association study identified common genetic variants on 5p14.1 associated with ASD diagnosis and replicated this finding in an independent sample (Wang et al., 2009). This was followed by a later genome-wide association study that noted a common variant on 5p15 (Weiss, Arking, Daly, & Chakravarti, 2009). Potentially relevant genes in the 5p14.1 and 5p15 regions include CDH9, CDH10, and SEMA5A, which are implicated in axonal guidance as well as shaping the physical structure and functional connectivity of the brain (Freitag et al., 2009). A third genome-wide association study demonstrated evidence for association at the locus MACROD2 (Anney et al., 2010). Unfortunately, none of these genome-wide studies confirmed the findings of the others. This likely speaks to three issues: larger sample sizes may be needed, the genetic etiology of ASD is quite heterogeneous, and there is great difficulty in identifying variants of incomplete penetrance and variable expressivity.

Overall, common variation in genes found to be associated with ASD and related conditions do not thus far appear to overlap with findings of rare mutations in genes related to ASD. However, one example of an exception is the CNTNAP2 gene, which has been found to be associated with ASD in both a common and a rare variant (Alarcon et al., 2008; Strauss et al., 2006).

Rare Variants

Genetic syndromes related to ASD.

Rare variants in ASD susceptibility were first implicated through examinations of large chromosomal abnormalities (as reviewed in Vorstman et al., 2006). In early studies, these large abnormalities in the chromosome (i.e., several million basepairs) could be identified through karyotyping. Causal genetic mutations for several single-gene syndromes were identified using these methods, including Down syndrome and Fragile X syndrome. Reports of the co-occurrence of ASD with some of these single-locus disorders (Blomquist et al., 1985), first propagated the field to explore the relationship between genetic abnormalities and ASD. Since then, several genetic syndromes have been found to co-occur with ASD more often than would be expected by chance.

Abrahams and Geschwind (2008) reviewed rates of comorbid genetic syndromes with ASD and reported that ASD presents in more than 40% of individuals with Angelman syndrome (15q duplication), 70% of individuals with Cortical Dyspalisa-Focal Epilepsy syndrome, 25% of males and 6% of females with Fragile X syndrome, 25% of individuals with Joubert syndrome, 20% of individuals with Tuberous Sclerosis, and many individuals with the 16p11 and 22q deletion. Although none of these single-locus disorders individually account for more than 1-2% of ASD cases, collectively they explain a relatively large proportion of ASD cases. Genes

affected by these various cytogenetic abnormalities are often involved in synaptic function and brain connectivity.

Examination of syndromes such as these involving large genetic abnormities can be enlightening in terms of etiology. However, chromosomal anomalies alone cannot advance understanding of particular molecular functions impaired in ASD given the large regions involved. Previously very expensive, it is now relatively cost-effective to resequence areas of the genome and isolate molecules within these regions to identify particular genes affected. Risk genes associated with these syndromes have been revealed through these improved methods, including SHANK3 in the 22q deletion, TSC1 and TSC2 in Tuberous Sclerosis, and FMR1 in Fragile X.

Building on cytogenetic studies through resequencing in search of specific candidate genes in ASD has revealed three general areas of gene influence thus far: (1) cell–cell relations and synaptic function, including dendritic spine growth, (2) neuronal migration and growth, and (3) excitatory and inhibitory neurotransmission (Freitag et al., 2009; Zoghbi, 2003). Although genes potentially involved in ASD and related syndromes likely have multiple molecular functions and do not necessarily affect brain functioning alone, it seems plausible that the syndromes share biological pathways or brain circuits (Abrahams & Geschwind, 2008). For instance, genes affecting receptors for neurotransmitters (GABRB3) and an enzyme for a protein expressed in the brain (UBE3A) are reduced in Angelman syndrome, Rett syndrome, and idiopathic autism (Samaco, Hogart, & LaSalle, 2005).

ASD rare variants.

Due to technological advances mentioned above, geneticists are now able to detect small cytogenetic abnormalities previously unidentifiable via standard karyotyping. Although it was

formerly thought that a small percentage of base pair changes accounted for genetic variation from one individual to the next, increased resolution of array-based approaches has illuminated significant individual variation in the number of copies of each DNA segment contained in the genome. Thus, several additional ASD risk genes have been clarified in recent years.

The most common cytogenetic abnormality identified through a rare variant approach is an inherited duplication involving the 15q11-13 chromosomal region, generally maternally inherited (Abrahams & Geschwind, 2008; Freitag et al., 2009). This mutation alone may account for 1-2% of ASD cases. Using higher resolution sequencing methods within the 15q11-13 locus, the genes GABRB3 (e.g., Samaco et al., 2005) and UBE3A (e.g., Peters, Beaudet, Madduri, & Bacino, 2004) were implicated, both affecting neural functioning. Mutations in the genes NLGN3 at locus Xq13 and NLGN4X at locus Xp22 involved with neuroligin, a postsynaptic protein used to bind neurons at the synapse, have also been documented (Jamain et al., 2003). Further work on deletions involving 22q13 locus identified the important role of SHANK3, a synaptic adaptor protein (Durand et al., 2007; Moessner et al., 2007). Microdeletions and microduplications found at 16p11.2 also implicated genes involved with brain functioning (Kumar et al., 2008; Weiss et al., 2008). Of course many more rare variants have been identified, including duplications or deletions on 2q37, 1q21, 11p14.1, 16p13.11, 16q21, 17q12, 22q11, and Xp22.11, among many others (Bucan et al., 2009; Filges et al., 2011; Moreno-De-Luca et al., 2010; Pagnamenta et al., 2011; Ramalingam et al., 2011; Shinawi et al., 2011). Again, none of these genes individually explains ASD, but they can inform our understanding of disease mechanisms.

De Novo rare variants.

De novo CNVs are non-inherited genetic mutations that occur in affected individuals, but are not present in parents. These events are more likely to occur in individuals with ASD than controls and may play a causal role in the development of the disorder. For instance, the recurrence of a *de novo* deletion involving 30 genes on chromosomal region 16p11 (Marshall et al., 2008; Weiss et al., 2008) is associated with ASD and may explain ~1% of ASD cases (Weiss et al., 2008).

De novo CNVs elucidate the possibility of distinctive genetic causal mechanisms because they are more often present in simplex (i.e., those families with just one clinically diagnosed individual) compared to multiplex (i.e., those families with more than one clinical diagnosed individual) families of individuals with ASD. Sebat and colleagues (2007) first reported *de novo* CNV variation in 1% of controls, 3% of multiplex ASD families, and 10% of simplex ASD families. This finding was replicated in an independent sample by Marshall and colleagues (2008) who reported that 2% and 7% of multiplex and simplex ASD families had *de novo* CNV mutations, respectively. Furthermore, of the 7% of simplex families, 11% had two or more *de novo* events (Marshall et al., 2008). These are likely underestimates since many smaller *de novo* CNVs, indels, and point mutations exist that could only recently be detected using whole genome sequencing technologies. Similarly, a different research group reported significantly increased rates of *de novo* genetic events present in autism probands from carefully phenotyped simplex families compared to their unaffected siblings (Sanders et al., 2011). These findings suggest that *de novo* CNVs are more common risk factors in sporadic compared to familial ASD. Exploration of potentially differing phenotypes in affected individuals and family members within these family-types may complement these findings.

Future Clinical Implications of ASD Genetics

The average age of diagnosis in the United States is currently estimated to be 3 - 5 years of age and many children remain undiagnosed until well into elementary school years (Autism and Developmental Disabilities Monitoring Network, 2009). However, ASD is a developmental disorder present at birth and atypicalities in social and language development begin much earlier than age of diagnosis (Osterling, Dawson, & Munson, 2002; Rogers & DiLalla, 1990; Zwaigenbaum et al., 2005). Additionally, neural differences (McCleery, Akshoomoff, Dobkins, & Carver, 2009) and abnormal brain growth (Courchesne, Carper, & Akshoomoff, 2003; Elder, Dawson, Toth, Fein, & Munson, 2008) in infants at risk for ASD have been detected within the first year of life. Identifying genetic risk factors very early in life, possibly at birth, could lead to even earlier identification of ASD risk and possibly improved outcomes for children.

Earlier behavioral intervention leads to better outcomes for individuals with ASD such as improved language, social relationships, and adaptive functioning (McEachin et al., 1993; as reviewed in Rogers, 1998; Sallows & Graupner, 2005) likely because it capitalizes on brain plasticity early in life (Dawson, 2008). Dawson (2008) describes a risk model for ASD in which early intervention serves to alter the abnormal developmental trajectory of young children with ASD by targeting social interactions between the child and social partner. If these risk processes could be identified very early in life, possibly at birth, interventions focusing on improving social interactions could be implemented at much younger ages, theoretically resulting in better outcomes. Since the majority of parents do not become concerned about ASD symptoms in their child until 18 months of age (Rogers & DiLalla, 1990), the identification of risk genes in ASD before any behavioral differences are detectable may allow for earlier detection than is currently

possible. Earlier identification of children at risk can lead to earlier intervention, which can lead to better outcome and possibly even prevention of ASD diagnosis for some children.

The ultimate goal for the genetic study of ASD is to help elucidate the etiology of the disorder. If researchers can understand which genes are involved, then it may be possible to comprehend the underlying pathophysiology. Once the disease process of ASD is known, perhaps then definitive therapeutic treatments can be established.

However, genetic findings in ASD must be interpreted with caution. Effect sizes in molecular genetics studies are generally very small and many individuals with ASD do not have currently identifiable genetic mutations. Further, no mutations or molecules identified to date are specific to ASD and are instead related to a variety of phenotypes, including intellectual disability and other neuropsychiatric disorders. Additionally, the presence of rare variants does not necessarily indicate a causal mechanism since they also may be present in some family members without a diagnosis and control subjects to some degree. It is important to determine how a CNV or any rare mutation affects gene function and expression before assuming it is causal.

Due to the rapid development of genetic technologies over the past decade, it is an exciting and promising time for the study of ASD genetics. Much has been discovered and new genetic findings are published nearly every day. Both the common variant and rare variant approaches have proved to be intriguing. However, they are thus far incomplete and significant work will be required to make complete sense of findings to date. At this stage, several conclusions can be drawn: ASD has an undeniably strong genetic component and it most likely does not obey the principles of classical Mendelian genetics. Instead ASD is a complicated

puzzle that in many cases may result from multiple genetic variants on multiple genes. Sorting out this puzzle will require many additional studies to come.

3. Broader Autism Phenotype [2]

A. Introduction

Psychological characteristics of relatives of children with ASD were first noted in Leo Kanner's 1943 paper in which he noted, "For the most part, the parents, grandparents, and collaterals are persons strongly preoccupied with abstractions of a scientific, literary, or artistic nature, and limited in genuine interest in people (pg. 250, Kanner, 1943)." Empirical studies following this line of research initially focused on rates of cognitive disorders, such as intellectual disability, learning disabilities, and language disorders, in family members without a diagnosis of ASD. Elevated rates of such disorders were noted in siblings of individuals with ASD (August, Stewart, & Tsai, 1981; Minton, Campbell, Green, Jennings, & Samit, 1982). However, later reports suggested that these cognitive deficits may instead have been markers of intellectual disability rather than autism per se as they were generally present in relatives of individuals with ASD *and* intellectual disability, but not in relatives of children with ASD with normal intelligence (Baird & August, 1985). Since then, the majority of studies have failed to document increased rates of cognitive dysfunction in relatives, regardless of the intelligence level of the affected child (Folstein et al., 1999; Freeman, Ritvo, Mason-Brothers, & Pingree, 1989; Szatmari, Jones, Tuff, & Bartolucci, 1993).

Studies do consistently describe a milder phenotype in relatives of individuals with ASD that is qualitatively similar to the defining features within the three domains of ASD: social,

[2] A portion of this section has been published in: Gerdts, J.A., & Bernier, R. (2011). The broader autism phenotype and its implications on the etiology and treatment of autism spectrum disorders. *Autism Research and Treatment. 2011*.

communication, and restricted interests and behaviors (e.g., Bishop et al., 2006; Bolton, Macdonald, Pickles, & Rios, 1994; Landa et al., 1992; Piven, Palmer, Jacobi, Childress, & Arndt, 1997). These subclinical differences in social skills and traits, communication abilities, and personality traits are generally considered to constitute the *broader autism phenotype* (BAP). Studies have generally compared parents of children with ASD (hereafter termed *ASD parents*) and unaffected siblings of individuals with ASD (hereafter termed *ASD siblings*) to family members of control subjects. Subsequent studies have identified variations in social cognition abilities (e.g., Baron-Cohen & Hammer, 1997), neurocognitive functioning (e.g., Hughes, Plumet, & Leboyer, 1999), and biological dimensions (e.g., Elder et al., 2008) as well that perhaps relate to or explain the clinical presentation of the BAP. Early markers of the BAP are currently under investigation in infancy (e.g., Christensen et al., 2010; Gamliel, Yirmiya, & Sigman, 2007; Zwaigenbaum et al., 2009).

Studies examining social abilities, communication skills, and personality traits will be reviewed as well as measures available to assess the BAP in ASD parents and siblings. In subsequent sections, neurocognitive functioning in relatives will be explored as well as studies conducted on biological features, such as head circumference and neural functioning, of ASD parents and siblings.

The BAP itself is not a diagnostic entity. In general, difficulties and differences evidenced in ASD parents and siblings are much milder than those of their child/sibling with ASD and do not fall in the clinically significant range. ASD parents and siblings may demonstrate more BAP features than controls, but the traits, by definition, do not cause enough functional impairment to justify a clinical diagnosis. Therefore, while a number of statistically

significant differences are summarized between family members and controls within this line of investigation, these features do not generally warrant clinical concern.

Like the diagnosis of ASD, BAP traits tend to aggregate more often in male relatives than female relatives (Bolton et al., 1994; Pickles et al., 2000; Piven, Palmer, Jacobi et al., 1997; Schwichtenberg, Young, Sigman, Hutman, & Ozonoff, 2010; Wheelwright, Auyeung, Allison, & Baron-Cohen, 2010), although this finding is not universal (e.g., Landa et al., 1992; Piven, Palmer, Landa et al., 1997). Informant report of social, communication, and repetitive behaviors in grandparents, aunt/uncles, and first cousins of individuals with ASD have suggested that these relatives exhibit similar, but less pronounced differences (Pickles et al., 2000; Piven, Palmer, Jacobi et al., 1997; Szatmari et al., 2000). Importantly, most studies find that at least half of relatives do *not* have quantifiable impairments (e.g., Landa et al., 1992; Piven, Palmer, Landa et al., 1997; Whitehouse, Barry, & Bishop, 2007; Wolff, Narayan, & Moyes, 1988), implying that differences may be present in only a subset of family members.

B. Social Abilities, Communication Skills, and Personality Traits

The most consistent evidence for the BAP emerged from studies directly assessing social communicative skills, personality traits, and histories in relatives. Early criticisms of such studies centered on the use of relatives of typically developing children as control groups. Critics noted that the differences found in relatives of children with ASD might have resulted from the environmental effects of living with and/or raising a child with a severe disability rather than genetic similarity. Therefore, many researchers changed the nature of their control groups to include family members of children with a developmental disability, often Down syndrome, in order to control for the effects of having a child with disability as a family member. While including families of children with a non-familial disability such as Down syndrome addresses

the environmental influences of living with a child with a significant disability, some researchers have preferred to include a second control group to account for other familial aspects of ASD. For example, developmental language disorders often have a strong genetic component and are included in some studies to control for genetic influences in ASD (e.g., Pilowsky, Yirmiya, Shalev, & Gross-Tsur, 2003).

Social traits, such as decreased interest in reciprocal social interactions and a focus on special interests as a conversational topic, identified using clinical interviews have been noted more often in ASD parents and siblings compared to parents and siblings in control groups, (Bolton et al., 1994; Piven, Palmer, Jacobi et al., 1997; Wolff et al., 1988). Personality types relating to decreased interest in social interactions ("aloof"), showing a restricted range of affective expression ("undemonstrative"), and using behavior that is interpreted to be off-putting ("untactful") are also more often more common in ASD parents and siblings compared to controls (Murphy et al., 2000; Piven, Palmer, Landa et al., 1997; Piven, Wzorek, Landa, & Lainhart, 1994). Other personality traits ("rigid") relating to decreased flexibility and difficulty adjusting to changes (e.g., altered routines) have also been reported in ASD parents compared to controls (Hurley, Losh, Parlier, Reznick, & Piven, 2007; Piven, Palmer, Landa et al., 1997). Self-report generally corroborates findings from clinical evaluations and interviews (Bishop, Maybery, Maley et al., 2004; Briskman, Happé, & Frith, 2001).

ASD parents and siblings also consistently show differences compared to control groups in social communication skills using clinical interview (Bishop, 2006; Di Michele, Mazza, Cerbo, Roncone, & Casacchia, 2007; Landa et al., 1992; Piven, Palmer, Landa et al., 1997). Self-report of pragmatic language, the social use of language in the context of verbal or nonverbal exchanges, in parents supports these findings (Whitehouse et al., 2007). Table 1

provides an overview of the chief findings from BAP studies conducted to date examining social abilities, communication skills, and ASD-related personality traits in ASD parents and siblings.

Table 1. Investigations of Social Abilities, Communication Skills, and Personality Traits in ASD Parents and Siblings

	Sample	Control Group	Measures of Social Comm. Skills	Key Findings
Wolff, Narayan, & Moyes, 1988	- Affected children all had useful language - ASD parents (21 mothers, 14 fathers) - Control Parents (21 mothers, 18 fathers)	Parents of children with variety of developmental delays, including DS, Prader-Willi Syndrome, and idiopathic delays	Personality interview developed by author (Wolff & Chick, 1980)	- 46% of ASD parents, particularly fathers, versus 0% of controls had "schizoid" personality traits, meaning impaired rapport with interviewers, suspiciousness, low emotional responsiveness, difficulties with communication (either over or under), guardedness, and excessive discussion of special interests - Most ASD parents did not meet full criteria for a personality disorder
Landa, Piven, Wzorek, & Gayle, 1992	- 43 ASD parents (sex breakdown not reported) - 21 Control Adults (sex breakdown not reported)	DS parents and adults with no children with ASD	PRS	- Using blindly rated scores, 42% of ASD parents had some pragmatic language deficit compared to 2% of controls - No sex differences and scores were not significantly correlated with IQ
Szatmari, Jones, Tuff, & Bartolucci, 1993	- Affected children had a variety of cognitive levels, evenly sampled across severity levels (IQ<50, 51-70, and 70+) - ASD parents (51 mothers, 46 fathers) - Control Parents (30 mothers, 24 fathers) - 72 ASD siblings (aged 6 – 18 years) - 46 Control siblings (aged 6 – 18 years)	DS parents (to control for low IQs) and parents of very low birth weight children (to control for higher IQs)	- Relative's Screening Interview (develop-mental history) - VABS	- Primarily examined cognitive functioning and results are described in text - No differences on the social and communication domains of the VABS in ASD siblings compared to control siblings or across IQ of the affected child (all were in average range) - No differences per developmental history of social and communication delays between the groups (i.e., 18.8% of ASD siblings had social problems compared to 15.9% of control siblings)
Bolton, Macdonald, Pickles, & Rios, 1994	- Affected children were oversampled for females. Sampled evenly for IQs between 30-49, 50-69, and 70+. - ASD Relatives (198 parents, 137 siblings) - Control Relatives (72 parents, 64 siblings)	DS parents and siblings	FHI	- 20.4% of ASD siblings demonstrated either communication atypicalities, social impairments or restricted behaviors compared to 3.1% of control siblings - Parents showed this same pattern of results, to a lesser degree - More common among males - Severity of *lesser variant* was related to severity of symptoms in child with ASD only for those children with speech

Piven, Wzorek, Landa, & Lainhart, 1994	- Affected children had IQs evenly distributed across severity levels. - ASD parents (45 mothers, 42 fathers) - Control Parents (19 mothers, 19 fathers)	DS parents	M-PAS	- 24% of ASD parents were rated to be aloof (decreased interest in social interaction) compared to 8% of DS parents - 22% of ASD parents were rated to be undemonstrative (restricted range of affective expression) compared to 11% of DS parents - 10% of ASD parents were rated to be untactful (behavior that was interpreted to be off-putting) compared to 3% of DS parents - 16% of ASD parents were rated for at least 2 of these traits compared to 0% DS parents - IQ of children was unrelated to M-PAS ratings in parents
Fombonne, Bolton, Prior, Jordan, & Rutter, 1997	- Same ASD sample as Bolton et al., 1994 - ASD parents (74 fathers, 86 mothers) - Control Parents (19 fathers, 23 mothers) - ASD siblings (62 males, 58 females) - Control siblings (16 males, 23 females)	DS relatives	FHI	- Primarily examined cognitive functioning in family members and results are described elsewhere - BAP traits were unrelated to IQ scores
Piven, Palmer, Landa, Santangelo, Jacobi, & Childress, 1997	- Multiplex ASD families, 51% of affected children had IQs over 70 - ASD parents (25 mothers, 23 fathers) - Control Parents (30 mothers, 30 fathers)	DS parents	-M-PAS-R -PRS	- 25-50% of ASD parents showed aloof, hypersensitive to criticism, anxious, and rigidity (difficulty adjusting to change) traits compared to 3-5% of DS parents - No consistent evidence for sex differences - Quality and quantity of friendships was rated lower in ASD parents (particularly fathers) - Significantly more ASD parents had some measure of pragmatic language deficits compared to controls (impairments in 18 - 25% of ASD parents versus 0-6% of DS parents) - Unrelated to IQ scores
Piven, Palmer, Jacobi, Childress, & Arndt, 1997	- Same ASD and parent sample as Piven, Palmer, Landa, et al., 1997 - 12 ASD siblings - 53 Control siblings - ASD Extended Family Members (96 grandparents, 145 aunts/uncles) - Control Extended Family Members (120 grandparents, 168 aunts/uncles)	DS relatives	FHI	- social deficits: 57% of ASD fathers versus 13% of DS fathers; 36% of ASD mothers versus 13% of DS mothers - communication deficits: 20% of ASD mothers versus 0% of DS mothers; nonsignificant difference in fathers - stereotyped behaviors: 26% of ASD fathers versus 3% of DS fathers; 12% of ASD mothers versus 0% of DS mothers - ASD siblings and extended relatives demonstrated more social deficits and restricted/repetitive interests on the FHI than controls (more often in male relatives); differences were less pronounced than parents
Folstein et	- Affected children stratified to	DS relatives	- FHI	- Primarily examined cognitive functioning and results are described

al, 1999	include ~equal numbers across IQ severity levels (<30, 30-50, 50-70, and 70+) - 166 ASD parents - 75 Control Parents - ASD siblings (42 males, 45 females) - Control siblings (28 males, 36 females)		- PRS - Friendship Interview - PAS ("Aloof")	elsewhere - ASD parents with early cognitive difficulties on the FHI (e.g., late onset of speech, reading/spelling difficulties) showed greater pragmatic language deficits despite average Verbal IQ compared to control parents - This relationship was insignificant on the "aloof" item of the PAS and the friendship interview
Murphy et al., 2000	- Same ASD sample as Bolton et al., 1994 - ASD Relatives over 18 years old (195 parents and 97 siblings) – Control Relatives over 18 years old (72 parents and 52 siblings)	DS relatives	- M-PAS - FHI	- In ASD parents, the traits anxious and conscientious were prominent compared to DS Parents - In ASD adult siblings, the traits aloof, shy, undemonstrative, impulsive, sensitive, self-conscious and eccentric were significantly elevated compared to DS adult siblings - Factor analysis revealed three factors on the M-PAS: "Withdrawn," "Tense," and "Difficult". In ASD parents and siblings, the item total for each factor was more than twice that of DS parents and siblings - One SD increase in symptom severity of the affected child on the ADI led to a 17% increase in "withdrawn" factor on M-PAS
Pickles et al., 2000	- Combined two study samples to allow for even distribution of IQ of the affected child (including an oversampling of IQs <50) - 285 ASD parents - 72 Control Parents - 189 ASD siblings (also 30 half siblings) - 64 Control siblings (also 1 half sibling) - ASD Extended Family Members (527 grandparents, 543 aunts/uncles, 774 first cousins) - Control Extended Family Members (139 grandparents, 166 aunts/uncles, 277 first cousins)	DS relatives	FHI	- 7.5% of all ASD relatives were classified as BAP by the FHI compared to 2.7% of controls - Differences in extended relatives were less significant than in ASD parents and siblings - More often in males - No evidence for simple X-linked or imprinted X-linked inheritance patterns - Severity of BAP traits on the FHI was related to severity of symptoms in the affected child only for those affected children with speech
Briskman, Happé, & Frith, 2001	- Affected children had IQ >65 and were only male - ASD parents (21 mothers, 21 fathers) - Dyslexia Parents (14 mothers,	- Relatives of individuals with Dyslexia - Relatives without any	- Interview developed by authors - Parent or self-report	- ASD parents (particularly fathers) had significantly higher scores than parents in both the dyslexia and typically developing groups - 62% of ASD fathers obtained high total scores compared to 15% of the dyslexia and 0% of the control fathers - Differences not found in siblings

	13 fathers) - Control Parents (14 mothers, 14 fathers) - 19 ASD siblings (males only) - 13 Dyslexia siblings - 20 Control siblings	family members with developmental disorders	of social functioning and nonsocial features related to ASD	
Bishop et al., 2004	- Affected children (59 met for autism, 21 met for PDD-NOS) - ASD parents (65 mothers, 46 fathers) - Control Parents (48 mothers, 37 fathers)	Parents of children without ASD	AQ	- Social and communication skills significantly lower in ASD parents (particularly fathers) compared to control parents - Scores on other categories (attention to detail, attention switching, and imagination) did not differ between groups
Bishop, Maybery, Wong, Maley, & Hallmayer, 2006	- Same ASD sample as Bishop et al., 2004 - 43 ASD siblings - 46 Control children	Typically developing children of a variety of IQ levels	CCC-2	- 23.8% of ASD siblings scored 2 SD below the control mean compared to 2.2% of controls - ASD siblings with low scores on the CCC-2 tended to have fathers with evidence for the BAP via self-report on the AQ - VIQ of the affected child was unrelated to CCC-2 scores in ASD siblings - Differences in structural language skills also noted
Di Michele, Mazza, Cerbo, Roncone, & Casacchia, 2007	- Average FSIQ of affected children was 88.9 - ASD parents (12 mothers, 11 fathers) - DS Parents (10 mothers, 2 fathers) - Control Parents (9 mothers, 14 fathers)	- Parents of "healthy" children matched for mental (but not chronological) age of affected child - Parents of children with DS	Gricean conver-sational maxims tasks - Methods described elsewhere (Surian, Baron-Cohen, & Van der Lely, 1996)	- ASD parents detected fewer pragmatic language errors (2 SD below the norm) in recorded conversations (e.g., failing to perceive redundant, irrelevant, or uninformative information) compared to parents in both control groups
Losh & Piven, 2007	- Affected children were "high functioning" and were either simplex or multiplex - ASD parents (enriched for those with BAP traits as measured by M-PAS-R; 23 fathers, 25 mothers) - Control Parents (9 fathers, 13	DS parent or parent of typically developing children	- M-PAS-R - PRS - Friendship Interview - Reading the Mind from the Eyes test	- Only those parents with aloof personalities had significant difficulty recognizing emotion/mental states - Performance predicted difficulties in pragmatic language and lower quality of friendships for aloof parents only - Offers evidence for distinct subtypes of ASD relatives

	mothers)			
Ruser et al., 2007	- Affected children had "sufficient language to complete battery"; average FSIQ was 86.63 - ASD parents (23 fathers, 24 mothers) - SLI Parents (21 fathers, 26 mothers) - DS Parents (10 fathers, 11 mothers)	- SLI parents (score <13th percentile on standardized language test or <9th percentile on nonword repetition) - DS parents	PRS-M	~ 15% of both SLI and ASD parents (particularly fathers) had significant conversation challenges compared to < 5% of DS parents - No significant differences in overall score between SLI and ASD parents on the PRS-M
Whitehouse, Barry, & Bishop, 2007	- Affected children had NVIQ >85 - ASD parents (20 mothers, 10 fathers) - SLI Parents (22 mothers, 8 fathers) - Typical Parents (23 mothers, 7 fathers)	- SLI parents (scores <10th percentile on at least 2 standardized language tests) - Parents of typically developing children	AQ	- Primarily examined structural language functioning and results are described elsewhere - Self-report of pragmatic language abilities differentiated SLI from ASD parents on the AQ - 20% of ASD parents showed BAP compared to 3.3% of SLI parents
Scheeren & Stauder, 2008	- Affected children had FSIQ >70 - ASD parents (12 mothers, 13 fathers) - Control Parents (12 mothers, 13 fathers)	Parents of typically developing child without family history of ASD	AQ	- No group differences (except control mothers scored higher than ASD mothers on one subtest "attention to detail")
Losh et al., 2009	- Affected children were >16 years and had NVIQ > 80 - 83 ASD parents - 32 Control Parents	- Parents of typically developing child without family history of ASD	- M-PAS-R - Several social cognition tasks	- 27% of ASD parents had social features of BAP (more often in fathers) while 41% had rigidity traits on the M-PAS-R (equal % mothers and fathers) - Only the group of ASD parents with social features of the BAP were less accurate in several social cognition tasks - ASD parents without the social BAP performed similarly to controls on these tasks
Schwichten-berg et al., 2010	- Affected children diagnosed with autism, Asperger's, or PDD-NOS via ADOS and	- Parents, infant, and additional siblings of	SRS	- At 36 months, infant siblings were categorized as typical, ASD, or "other developmental concerns" - Greater BAP traits in ASD fathers and additional siblings (but not mothers)

	clinical evaluation (either simplex or multiplex) - All families included mother, father, "proband," and infant sibling (portion of families had an additional sibling) - 124 Simplex ASD Families - 11 Multiplex ASD Families - 82 ASD Controls	typically developing child		compared to control fathers (No differences between ASD simplex and multiplex fathers, but multiplex siblings had greater SRS scores than simplex siblings) - ASD multiplex infant siblings more likely (64%) to develop ASD than ASD simplex (9%) and control (4%) - 27% of ASD simplex infant siblings had other developmental concerns versus 11% of typical infant siblings - Neither parent, affected child, or additional sibling severity predicted infant sibling diagnostic status - Father (but not mother) severity predicted affected child severity and affected child severity predicted additional sibling severity
Wheelwright et al, 2010	- Affected children (parent-report of diagnosis on the autism spectrum) - 90% simplex, 10% multiplex - ASD Parents (1429 mothers, 571 fathers) - Control Parents (558 mothers, 349 fathers)	Parents of typically developing children	AQ	- ASD parents had greater AQ scores in 4 of 5 domains than control parents, with males overall showing greater impairment than females in both groups - 33% of ASD fathers and 23% of ASD mothers versus 22% control fathers and 9% of control mothers had AQ scores at least 1 SD above mean
Bernier, Gerdts, Munson, Dawson, & Estes (in press)	- Affected children assessed using ADOS, ADI-R, and DSM-IV - 39 Multiplex ASD Parents - 22 Simplex ASD Parents - 20 DD Parents - 20 Typical Parents	- Parents of typically developing child without family history of ASD - Parents of children with variety of developmental delays	BPASS	- In the Social and Conversation BPASS domains, multiplex parents scored significantly higher than the three other family groups (no differences among the simplex, DD, and typical parent groups). - In the Restricted Interests BPASS domain, multiplex ASD parents had greater impairment than the typical parent group, but not between simplex and DD groups.

Abbreviations: ADOS = Autism Diagnostic Observation Schedule; AQ = Autism Spectrum Quotient; ASD parents = undiagnosed parents of individuals diagnosed with ASD; ASD siblings = undiagnosed siblings of individuals diagnosed with ASD; BPASS = Broader Phenotype Autism Symptom Scale; CCC-2 = Children's Communication Checklist-2; DS = Down syndrome; FHI = Family History Interview; M-PAS = Modified Personality Assessment Schedule, M-PAS-R = Modified Personality Assessment Schedule, Revised; PAS = Personality Assessment Schedule; PRS = Pragmatic Rating Scale; SD = Standard Deviation; SLI = Specific Language Impairment; VABS = Vineland Adaptive Behavior Scales

C. BAP in Infancy

The majority of BAP studies have focused on parents and school age siblings of children with ASD. However, prospective studies following infant siblings of older children with ASD are ongoing to determine the nature and timing of the BAP in early childhood. Although the precise presentation of the BAP in infancy and its relation to later development remains unclear, several studies have documented social and communication differences in infant/toddler-aged younger siblings of children with ASD who do not go on to be diagnosed ASD (hereafter termed *non-ASD sibs*) (e.g., Stone, McMahon, Yoder, & Walden, 2007; Toth, Dawson, Meltzoff, Greenson, & Fein, 2007; Zwaigenbaum et al., 2005).

A study examining the BAP in toddler-aged non-ASD sibs reported decreased social communication, cognitive, adaptive, and language abilities via both observational and parent report measures (Toth et al., 2007). The use of pointing, directed facial expressions, and the quality of social overtures discriminated non-ASD sibs from control infant siblings in a different sample at 18 months of age (Brian et al., 2008). Hutman and colleagues (2010) reported that while response to name and showing appropriate response to distress was impaired in infant siblings who *were* diagnosed with ASD, these variables did not distinguish non-ASD sibs from typical infants. This suggests that response to name and response to distress are not early signs of the BAP, but may be part of the early presentation of ASD (Hutman et al., 2010). Gamliel, Yirmiya, and Sigman (2007) reported that some non-ASD sibs with cognitive delays at 14 and 24 months were no longer delayed cognitively in preschool years. However, expressive and language delays remained in a portion of these non-ASD sibs. The language-related delays

continued into school age years and emerged as language-related learning and academic difficulties in 40% of ASD siblings versus 16% of control children, as reported in a follow-up study (Gamliel, Yirmiya, Jaffe, Manor, & Sigman, 2009). Therefore, there may be variability in the developmental trajectory of non-ASD sibs, with delays from some early challenges resolving by later childhood and others continuing into school age years.

Christensen and colleagues reported fewer functional play skills and increased repetitive non-functional play in non-ASD sibs compared to controls (Christensen et al., 2010). Increased repetitive interests and atypical sensory behaviors were also observed in non-ASD sibs versus typical infants in a separate longitudinal study (Brian et al., 2008). A number of studies on infant siblings have reported decreased visual attention to social stimuli, increased attention to non-social stimuli, and difficulty disengaging from one stimuli to another (Bhat, Galloway, & Landa, 2010; Ibanez, Messinger, Newell, Lambert, & Sheskin, 2008; Zwaigenbaum et al., 2005). However, diagnostic outcomes have not yet been reported in these studies, so it is unclear if these traits are early markers of ASD itself or an early manifestation of the BAP.

Overall, ASD-related traits may be present at a very early age in relatives of individuals with ASD; however, follow-up of these infants and toddlers are necessary as certain findings have been inconsistent across studies and some of these differences have resolved later in life.

D. Measures of ASD-Related Symptoms in Family Members

Researchers have developed many different measures of social and communication traits in relatives of individuals with ASD using a variety of

methodologies. Formats include self-report via questionnaire, parent report via questionnaire and interview, semi-structured interview, and behavioral observation. These measures are summarized in Table 2.

Table 2. Measures Developed and Utilized to Assess the BAP

	Measure Name	Reference	Intended Population	Measure notes
Questionnaires	Autism-Spectrum Quotient (AQ)	Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001	Typical adults and ASD parents	- A brief, self-report questionnaire assessing 5 domains: social skills, communication, attention to detail, attention switching, and imagination - Differentiates high-functioning individuals with ASD from controls, males from females, and college students majoring in mathematics versus humanities - Identifies higher rates of BAP in ASD parents compared to controls
	Broader Autism Phenotype Questionnaire (BAPQ)	Hurley, Losh, Parlier, Reznick, & Piven, 2007	ASD parents	- A self- and informant-report questionnaire of social personality, rigidity, and pragmatic language deficits (intended to parallel the 3 domains of impairment in ASD) - Each question is rated on a 6-point scale, allowing a range of possible responses to maximize variability - Validation has shown high sensitivity and specificity when BAPQ scores are used to predict direct clinical observations of BAP traits
	Children's Communication Checklist, 2nd Edition	Bishop, 2003	Children aged 4-16 years of age	- A parent-report questionnaire assessing various communication skills with age-based scaled scores and a general communication composite - Scales include structural aspects of language, pragmatic language, and social relations and interests - Composite scores differentiate children with communication challenges (both SLI and ASD) from typically developing children (Norbury, Nash, Baird, & Bishop, 2004) - ASD siblings were overrepresented in the lower range of scores compared to typically controls (Bishop et al., 2006)
	Social Responsiveness Scale (SRS)	Constantino, Przybeck, Friesen, & Todd, 2000	Children and adults both with and without ASD	- An informant-report (generally parent, teacher, or spouse) questionnaire measuring autism-related traits along one continuum of social reciprocity, which purportedly reflects a single underlying vulnerability to ASD-related traits (Constantino et al., 2004; Constantino & Todd, 2000) - Widely used in many genetic studies and researched in both clinically ascertained and population-based samples (Constantino et al., 2004; Constantino et al., 2006; Ho, Todd, & Constantino, 2005) - Scores from the SRS have been linked to specific areas of the genome through QTL-based analyses (Duvall et al., 2007)

Interviews and Direct Behavioral Observation	Broader Phenotype Autism Symptom Scale (BPASS)	Dawson et al., 2007	Children and adults both with and without ASD	- Assesses autism-related traits in 4 domains (social motivation, social expressivity, conversational skills, and flexibility/range of interests) via both direct clinical observation and interview - Adults are interviewed about themselves; parents are interviewed about their children - Ratings for items within domains range from impaired to non-impaired, with some questions identifying those with a level above the norm (designed to increase statistical power to detect evidence for genetic effects in QTL analyses) - Two domains (social motivation and flexibility) have the positive QTL findings (Sung et al., 2005) - Discriminated between multiplex ASD parents and simplex ASD parents, DD parents, and parents of typical children on 3 of 4 domains (Bernier et al., in press).
	Family History Interview (FHI) or Family History Schedule (FHS)	Bolton et al., 1994 ; Piven, Palmer, Jacobi, et al., 1997	Children and adults both with and without ASD	- First measure developed to directly measure autism-related traits in family members via a semi-structured interview and provides categorical (not continuous) information - Informant rates social and communication skills and range of interests of immediate and extended family members - An algorithm determines whether there is a presence or absence of BAP traits in 3 domains: social, communication (primarily assessing a history of language/reading delays), and repetitive behaviors
	Modified Personality Assessment Schedule-Revised (M-PAS-R)	Piven, Palmer, Landa, et al., 1997 ; Piven et al., 1994	Adults	- A semi-structured interview measuring ASD-related personality traits - Participants are interviewed about themselves while an informant (generally a spouse) is asked similar questions in a separate interview - Participants are rated by trained examiners based on behavioral examples given by either the subject or the informant - Originally including 18 domains, it was later revised to focus on 8 traits particularly applicable to ASD: conscientious, rigidity, aloof, undemonstrative, anxious, hypersensitive to criticism, unresponsive, and untactful - Rigidity and aloof traits have been the most valid and reliable discriminators (Losh et al., 2009; Piven, Palmer, Landa et al., 1997; Piven et al., 1994).
	Pragmatic Rating Scale (PRS)	Landa et al., 1992	Adults	- Intended to assess verbal and nonverbal pragmatic language via 15 minutes of conversational exchange and perhaps time spent in direct assessment of parents - Assesses skills in maintaining a flow of conversation. - Subscales: disinhibited social communication (e.g., abrupt topic change and overly detailed descriptions), awkward/inadequate expression (e.g., vague and inadequate clarification), and odd verbal interaction (e.g., odd humor and inappropriate topics) - Successfully differentiated ASD parents from controls in many studies (e.g., Folstein et al., 1999; Piven, Palmer, Landa et al., 1997)

E. Depression and Anxiety

Piven and colleagues (1990) first reported increased rates of depression and anxiety in adult siblings of individuals with ASD based on direct assessments. Additional studies corroborated these early reports, with major depression, obsessive compulsive disorder, and social phobia being the most commonly observed psychiatric diagnoses in first degree relatives (Bolton, Pickles, Murphy, & Rutter, 1998; Micali, Chakrabarti, & Fombonne, 2004; Piven & Palmer, 1999; Smalley, McCracken, & Tanguay, 1995). Depression and anxiety diagnoses seem to be more prevalent in female relatives, particularly mothers, compared to male relatives (Micali et al., 2004). It is reasonable to assume that having a child with autism significantly impacts mood and anxiety levels. However, studies have indicated that affective episodes in relatives had an onset prior to the birth of their child(ren) with ASD and suggest that the stress of raising a child with autism did not necessarily cause the psychopathology (Bolton et al., 1998; Piven & Palmer, 1999). Additionally, mood and anxiety symptoms are largely unrelated to the presence of BAP traits in an individual (Bolton et al., 1998; Piven & Palmer, 1999), although a more recent study did report a relationship between depressive symptomotology and BAP traits via self-report on the AQ (Ingersoll & Hambrick, 2011).

F. Cognition

Intelligence and General Cognitive Abilities

Intelligence and general cognitive abilities in individuals with ASD can range from significantly below average in the intellectual disability range to above average in the intellectually gifted range. Given these variable intelligence levels, researchers have examined intellectual functioning in relatives of children with ASD. Although early reports suggested below average intellectual functioning and cognitive disabilities in ASD siblings (August et al.,

1981; Minton et al., 1982), many subsequent studies have failed to support these initial findings (Folstein et al., 1999; Freeman et al., 1989; Szatmari et al., 1993). Full scale IQ in ASD parents and siblings has since been found to be in the average to high average range in most research studies (e.g., Dawson et al., 2007; Fombonne, Bolton, Prior, Jordan, & Rutter, 1997b; Freeman et al., 1989; Gokcen, Bora, Erermis, Kesikci, & Aydin, 2009; Lainhart et al., 2002; Szatmari et al., 1993).

Another often-reported finding in individuals with ASD is a pattern of significant variability in cognitive abilities, such as enhanced visual spatial and nonverbal reasoning skills compared to knowledge of vocabulary and comprehension of language (Happé, 1999; Lincoln, Courchesne, Kilman, Elmasian, & Allen, 1988). Studies of ASD parents and siblings have similarly documented increased variability in general cognitive abilities as measured by intelligence tests compared to control groups suggesting a possible association of cognitive variability to the BAP (Folstein et al., 1999; Fombonne et al., 1997b; Pilowsky et al., 2003; Piven & Palmer, 1997; Schmidt et al., 2008).

Fombonne and colleagues (1997b) noted overall high average cognitive abilities, but greater variability among subdomain scores in ASD parents and siblings compared to relatives of children with Down syndrome on an intelligence test. The trend in this study was higher verbal as compared to nonverbal scores. The cognitive profile pattern was evidenced similarly in ASD parents both with and without markers of social and communication impairments on the FHI. In studies of parents of multiple children with ASD (Piven & Palmer, 1997) and just one child with ASD (Schmidt et al., 2008), ASD parents were also found to score significantly lower on nonverbal IQ measures as compared to controls, despite overall IQs in the average to high average range.

Another study examining cognitive functioning in ASD parents and siblings compared to relatives of children with Down syndrome generally replicated these findings. The authors noted overall high average IQ in ASD Parents, but greater variation in IQ subdomain scores with significantly lower nonverbal than verbal IQ compared to controls (Folstein et al., 1999). The same finding of increased variability among scores was noted in ASD siblings compared to siblings of individuals with Down syndrome. A greater discrepancy between nonverbal and verbal IQ in ASD Siblings compared to controls was also noted by Pilowsky and colleagues (2003). Again, all of these findings highlight variability among general areas of cognitive functioning, not overall deficits in intelligence. In general, it seems that enhanced verbal as compared to nonverbal skills may typify general cognitive abilities in ASD parents and siblings.

Central Coherence

There are several cognitive theories that attempt to explain the triad of impairments in ASD. One such theory involves a cognitive process called *central coherence*. Central coherence is a model of cognitive processing style ranging from a tendency to focus on smaller details with a weakness in seeing more global perspectives (local processing bias) to a propensity for seeing the gestalt while sacrificing details (global processing bias). Weak central coherence results in an over-focus on the parts rather than the whole while strong central coherence involves a good understanding of the larger picture, but less attention to details. The block design subtest included in many intelligence tests has been considered a proxy for central coherence since breaking down the picture to be replicated into its parts is the most efficient strategy for completing the task. Individuals with ASD have been reported by some groups to have weak central coherence (Shah & Frith, 1983, 1993) while others have reported intact central coherence in children with ASD (Mottron, Burack, Stauder, & Robaey, 1999; Ozonoff, Strayer, McMahon,

& Filloux, 1994; Plaisted, Swettenham, & Rees, 1999). Thus, the validity of the central coherence theory in ASD remains unclear.

In a two-part exploration of central coherence in relatives, Briskman and colleagues (Briskman et al., 2001; Happé, Briskman, & Frith, 2001) compared central coherence among parents and siblings of individuals in three groups: no developmental abnormality, dyslexia, and ASD. Fathers of children with ASD demonstrated differential performance suggestive of weak central coherence on all measures (Happé et al., 2001). There were no differences between mothers of children with ASD and controls on any of the tasks, suggesting sex differences in central coherence in relatives. On self-report assessing the social and nonsocial (e.g., special interests, sensory sensitivities, preference for routines) aspects of the BAP, those parents (particularly fathers) with greater nonsocial scores tended to have weaker central coherence (Happé et al., 2001). A separate research group has also documented weak central coherence compared to controls (Baron-Cohen & Hammer, 1997).

However, weak central coherence was reported to be absent in ASD siblings, suggesting no apparent differences in central coherence in ASD siblings, male or female (Briskman et al., 2001). A number of additional studies have reported intact central coherence and failed to document differences between ASD relatives and controls in tasks purported to tap into central coherence, such as block design (Losh et al., 2009; Nydén, Hagberg, Goussé, & Rastam, 2011; Piven & Palmer, 1997; Scheeren & Stauder, 2008). Therefore, like the debate about the validity of the central coherence theory in individuals with ASD, the relation of weak central coherence to the BAP remains to be determined.

Executive Functioning

Another cognitive theory of the underlying processing mechanism in ASD involves a primary deficit in executive functioning. Executive functioning encompasses abilities that underlie goal-directed behavior, including working memory, inhibition, cognitive flexibility, and planning. Individuals with ASD often have difficulty with executive functioning tasks (McEvoy, Rogers, & Pennington, 1993; Ozonoff, Pennington, & Rogers, 1991). However, there is substantial debate over the exact nature of executive functioning challenges in ASD, and differences noted may be measure-specific.

Differences in executive functioning are documented in ASD parents and siblings as well compared to controls. Compared to parents of children with learning disabilities and typically developing children, ASD parents, particularly fathers, showed challenges on computerized tasks of four components of executive functioning: attentional flexibility, planning, spatial working memory, and spatial short-term memory (Hughes, Leboyer, & Bouvard, 1997). Attentional flexibility was particularly difficult with half of ASD parents showing an impaired ability to shift problem-solving strategies when necessary compared to a small percentage of control parents. In another study, parents within multiple-incidence ASD families demonstrated poorer performance compared to parents of children with Down syndrome on the Tower of Hanoi task, a measure of planning (Piven & Palmer, 1997).

Studies of ASD siblings have showed the same general trend. Hughes and colleagues (1999) found that a greater percentage of ASD siblings demonstrated increased difficulty with attentional flexibility and more advanced stages of planning on a computerized executive functioning task compared to siblings of children with severe to moderate developmental delays and neurotypical children. In another study, there was a significant difference between ASD

siblings' performance on the Tower of Hanoi task compared to siblings of children with various other learning disabilities (Ozonoff & Rogers, 1993). Precursors to executive functioning abilities have recently been examined in infancy. Latency in disengaging from one stimulus to attend to another has been reported in a subset of infant siblings of children with ASD compared to control infants (Holmboe et al., 2010).

Wong and colleagues (2006) suggest that poor planning may reflect an executive functioning challenge in generativity in ASD, meaning difficulty generating more than one strategy to solve a problem (also called *fluency*). In their study comparing ASD parents and siblings to relatives of children with mild intellectual disabilities, they found differences in several measures of verbal and nonverbal generativity/fluency between index family members and controls (Wong et al., 2006).

Working memory challenges that are often present in individuals with ASD are also present to a lesser degree in ASD parents. One study reported that ASD parents scored significantly lower on a verbal working memory task compared to parents of typically developing children (Gokcen et al., 2009). ASD parents made more errors in a delayed oculomotor response task assessing spatial working memory via tracking of saccades (or fast movements of the eye) compared to control adults (Koczat, Rogers, Pennington, & Ross, 2002). However, many of these same executive functioning studies have not documented differences in every measure in their battery (Delorme et al., 2007; Gokcen et al., 2009; Hughes et al., 1999; Szatmari et al., 1993; Wong et al., 2006) and some have found no differences on any measure of executive functioning (Losh et al., 2009).

Social Cognition

An additional theory purporting to explain the underlying social and communication difficulties observed in ASD involves a primary deficit in social cognition: specifically a construct called *theory of mind* (ToM). ToM involves the ability to understand other's emotions, motivations, and intent. It allows individuals to take another person's perspective and intuit his or her mental state.

Mild ToM challenges in using facial cues and other features to determine mental states have been noted in relatives of individuals with ASD. For instance, Baron-Cohen and Hammer (1997) found that ASD parents, particularly fathers, were slightly less able to determine the thoughts and feelings of people based solely on photographs of their eyes (*Reading the Mind from the Eyes* test) compared to adult controls. Another study using this task found a trend toward differences in ASD parents compared to parents of typically developing children (Gokcen et al., 2009); a similar pattern of results has been noted in ASD siblings compared to control children using the same measure (Dorris, Espie, Knott, & Salt, 2004). Significant differences in an advanced ToM task were detected in ASD parents' ability to reason about emotions in order to correct inconsistencies in emotional expressions. Additional studies have reported similar differences using advanced ToM experimental tasks (Di Michele et al., 2007) and self-report of emotion processing abilities, particularly emotion identification (Szatmari et al., 2008), in ASD parents compared to control parents. However, several studies have reported no difficulties in ToM tasks in ASD siblings compared to siblings of individuals with other developmental disabilities (Ozonoff & Rogers, 1993; Shaked, Gamliel, & Yirmiya, 2006; Szatmari et al., 1993).

The relationship between social and communication skills and ToM abilities in ASD parents has also been examined. Findings from two studies suggest that impairments on specific

aspects of social cognition may only be relevant for subgroups of ASD parents with social features of the BAP: namely, those with "aloof" personality styles involving social dispositions relating to preference for alone time and infrequent use of social chat during social exchanges (Losh et al., 2009; Losh & Piven, 2007). ASD parents without this personality style did not demonstrate difficulties on ToM tasks.

G. Language Abilities

Phonological Processing and Reading Abilities

Phonological processing is the manner by which written and spoken words are processed and can be crucial for strong reading and writing abilities. These skills are heritable (e.g., Bishop et al., 1999; Bishop, North, & Donlan, 1996) and some children with ASD show marked impairments in measures of phonological processing (Kjelgaard & Tager-Flusberg, 2001). Evidence suggests that only children with ASD who have a language impairment (and not those without a language impairment) have challenges in phonological processing and reading-related skills, similar to children with a specific language impairment (SLI) without ASD (Kjelgaard & Tager-Flusberg, 2001; Lindgren, Folstein, Tomblin, & Tager-Flusberg, 2009; Roberts, Rice, & Tager-Flusberg, 2004; Whitehouse, Barry, & Bishop, 2008). Therefore, it is possible that reading-related challenges and phonological processing skills are not specific to the ASD phenotype per se, but related to overall language challenges.

Compared to age- and sex-matched adults, ASD parents scored lower on the nonword repetition task (a measure of phonological processing; Schmidt et al., 2008). This was a particular challenge for nonwords of three syllables or more, suggesting that phonological short-term memory, rather than perception of sounds or sound production, may be a specific area of interest for future study. A similar report of increased difficulties in reading of nonwords longer

than three syllables in a much larger sample was noted in ASD parents (particularly those with a history of language-related challenges) compared to Down syndrome parents (Folstein et al., 1999). In a study of multiplex families, ASD parents demonstrated weaker performance on some reading measures (e.g., passage comprehension and rapid automatized naming) compared to parents of individuals with Down syndrome (Piven & Palmer, 1997). Rapid automatized naming skills in ASD parents and their high-functioning children were related, with both groups showing longer latencies than Down syndrome parents (Losh, Esserman, & Piven, 2010). Rapid automatized naming skills in ASD parents (particularly fathers) related to retrospective reports of early language delay and were associated with a socially reticent personality style.

However, many studies have failed to report difficulties in phonological processing and reading abilities in relatives. For example, Bishop and colleagues (2004) found no differences between parents and siblings of children with ASD versus those of typically developing children of a variety of different IQ levels on measures of phonological processing (nonword repetition and reading of nonword passages). Additionally, no differences between ASD siblings and control siblings were noted in other studies examining word fluency, rapid automatized naming, and pragmatic language (Pilowsky et al., 2003; Shaked et al., 2006). An additional study compared language, reading, and phonological processing abilities in ASD parents and siblings of children both with and without language impairment to parents and siblings of children with SLI (Lindgren et al., 2009). While parents and siblings of children with ASD who had a language impairment scored lower than relatives of children with ASD who did not have an impairment, neither groups' scores were in the impaired range. Additionally, SLI relatives scored significantly lower than both groups, suggesting separate genetic etiologies of language impairments in affected children within these two disorders (Lindgren et al., 2009). Therefore,

research suggests that challenges in reading abilities and phonological processing may be absent in ASD siblings and unclear in ASD parents.

Structural Language

ASD parents and siblings are more likely than controls to have had an expressive language delay in childhood as well as other language-based learning deficits, such as dyslexia (Bailey, Palferman, Heavey, & Le Couteur, 1998; Bolton et al., 1994; Fombonne et al., 1997a; Piven, Palmer, Jacobi et al., 1997). However, a number of other studies have failed to report difficulties in structural language problems in relatives. Whitehouse, Barry, and Bishop (2007) compared ASD parents to parents of children with language and/or literacy impairments and typically developing children found that structural language related challenges only presented in parents of children with language/literacy impairments, but not those of children with ASD. In ASD siblings, Pilowsky and colleagues (2003) found that ASD siblings and siblings of children with intellectual disability had better developed receptive and expressive language skills and higher verbal IQs than siblings of children with language disorders. Therefore, it is unclear the extent of these reported language difficulties and it is possible that some early language challenges may resolve by later childhood and adulthood.

H. Biological Dimensions

Head Circumference and Brain Volume

Macrocephaly is a consistent physical finding reported in individuals with ASD. Many studies have found increased head circumference in both children and adults with ASD compared to age-based normative measurements (Bailey et al., 1995; Courchesne et al., 2003; Dementieva et al., 2005; Deutsch & Joseph, 2003; Lainhart et al., 2006). Magnetic resonance imaging (MRI) studies find increased cerebral volume in children with ASD compared to

typically developing children and those with other developmental delays (Piven et al., 1995; Sparks et al., 2002).

Despite this well-replicated finding of increased head size and brain volume in ASD, macrocephaly does not appear to define a specific subgroup of clinical features. Some studies have found that increased head size in ASD is advantageous and is related to higher IQ (Sacco et al., 2007) and better social skills (Dementieva et al., 2005) whereas others have reported negative effects, such as delayed onset of words (Lainhart et al., 2006), more impaired social cognition (Sacco et al., 2007), and increased stereotyped behaviors (Fidler, Bailey, & Smalley, 2000). Still other studies have found no direct associations between head circumference and ASD-related symptoms (Deutsch & Joseph, 2003). Children later diagnosed with more severe ASD demonstrated a faster and greater rate of brain overgrowth during infancy, compared to children with less severe symptoms (Courchesne et al., 2003).

Less research has been conducted on head circumference/brain volume among family members of individuals with ASD. The limited research available does suggest that head size is increased in family members. In first-degree relatives, 18.9% of ASD parents and 11.4% of ASD siblings had head circumference measurements exceeding the 97th percentile (Fidler et al., 2000). These numbers were significantly elevated compared to normative measurements, but were not different from the comparison group, consisting of family members of individuals with tuberous sclerosis and seizure disorders. In other family samples, head circumference of ASD parents was skewed toward macrocephaly (Lainhart et al., 2006; Miles, Hadden, Takahashi, & Hillman, 2000). The trait appears to be familial as 35-45% of affected children with macrocephaly had a macrocephalic parent (Lainhart et al., 2006; Miles et al., 2000). However,

in another sample, brain volume of ASD parents was not different from controls (Palmen et al., 2005).

Two studies have examined the relation between head circumference and BAP traits in ASD relatives. Elder and colleagues (Elder et al., 2008) reported that young siblings of children with ASD with a faster rate of growth in head circumference in the first year of life followed by slowing of growth in the next year had a greater number of BAP traits. In contrast, Constantino and colleagues (Constantino, Majmudar et al., 2010) failed to find a relationship between ASD traits as measured by the SRS and rate of head growth in infant male siblings of children with ASD.

Neural Functioning and Structure

Neural studies using technologies such as electroencephalography (EEG) and functional MRI (fMRI) provide further support for biological differences between ASD parents and siblings and control groups. In a small pilot study, Baron-Cohen and colleagues (2006) compared ASD parents to adult controls using fMRI on a visual search task and an advanced emotion recognition task (both of which differentiate controls from individuals with ASD using the same technology). Findings corroborated an earlier report from the same group of differences in behavioral outcomes on both of these tasks (Baron-Cohen & Hammer, 1997) as well as atypical brain response compared to controls. Additionally, mothers and fathers processed the tasks differently at a neural level, providing further support for sex differences in the presence of the BAP (Baron-Cohen et al., 2006).

Facial processing also appears to be different in ASD parents and siblings. Dawson and colleagues (2005) reported that ASD parents showed a delay in the face-related event related potential (ERP) component, the N170, when looking at faces, although not when looking at

objects, suggesting a neural difference in face processing (Dawson et al., 2005). In a second facial processing study using eye tracking and imaging, ASD siblings spent significantly less time looking at eyes in photographs compared to controls and instead spent the same amount of time looking at eyes as their siblings with ASD (Dalton, Nacewicz, Alexander, & Davidson, 2007). The ASD siblings showed greater activation to faces in the right posterior fusiform gyrus but not the left, which was largely accounted for by eye fixation (Dalton et al., 2007). Emerging evidence for an atypical neural response to direct eye gaze in infant siblings has also been reported (Elsabbagh et al., 2009).

Total brain and brain structure volume has been explored in ASD parents and ASD siblings. Kates and colleagues (2004) found that MZ twin pairs who were concordant or discordant for ASD had similar cerebral gray and white matter volumes. However, only the concordant twin pairs had similar *cerebellar* gray and white matter volumes. Compared to control subjects, both diagnosed and undiagnosed twins within discordant twin pairs exhibited lower frontal, temporal, and occipital white matter volumes, suggesting that this neural difference is also present in undiagnosed relatives (Kates et al., 2004). Compared to controls, ASD siblings show decreased amygdala volume (Dalton et al., 2007). Conversely, in ASD parents, left hippocampal volume was found to be larger compared to typical adults (Rojas et al., 2004). ASD parents have also shown increases and decreases in regional gray matter volume using voxel-based morphometry--a finding similar to observed variations in brain structure in individuals with ASD (Peterson et al., 2006).

I. Moderators of the BAP

Traits of Affected Children

Various aspects of the BAP appear to be present in relatives across subtypes of individuals with ASD. ASD parents whose children experience a regression in skills demonstrate characteristics of the BAP at the same rates as those whose children do not experience a regression (27.8% versus 32.9%; Lainhart et al., 2002). The sex of the child also does not appear to be related to the degree of expression of the BAP in relatives (Bolton et al., 1994; Szatmari et al., 2000). Moreover, the cognitive ability of children with ASD is unrelated to the degree of expression of the BAP in parents (Bishop, Maybery, Maley et al., 2004; Folstein et al., 1999; Freeman et al., 1989; Piven et al., 1994). However, Szatmari and colleagues (2000) reported that social and communication impairments were more common in immediate and extended relatives of affected children with IQs above versus below 60.

The severity of ASD symptoms in affected children appears to be related to BAP traits in family members. Pickles and colleagues (2000) found that the severity of symptoms in verbal children with ASD was related to the degree of expression of the BAP in family members, but was unrelated in nonverbal affected children. Similarly, self- and informant-reports of autism-related traits in the typical population indicate that families in which both parents show subthreshold social reciprocity challenges are more likely to have children with scores in the impaired range of social behavior suggestive of an ASD diagnosis (Constantino & Todd, 2005). Additionally, while social responsiveness (assessed via the SRS) in parents, affected children, and siblings did not predict *diagnostic status* of infant siblings in a one study, social responsiveness in fathers predicted social responsiveness in the affected child and affected child responsiveness predicted sibling responsiveness (Schwichtenberg et al., 2010). Therefore, there

is some evidence that the severity of autism-related traits is correlated within some families, particularly in males.

Family Structure

The number of affected children within a family also relates to BAP traits in family members. A number of studies to date have compared relatives within simplex and multiplex ASD families on measures of the BAP. Szatmari and colleagues (2000) screened nearly 2,000 immediate and extended relatives and found that social impairments, but not communication challenges or presence of restricted behaviors, were more common in multiplex relatives as compared to simplex relatives. Bölte and Poustka (2003) found that simplex ASD parents and siblings demonstrated superior performance in emotion recognition compared to ASD parents and siblings within multiplex families. Virkud and colleagues (2009) found that mean scores on the SRS in ASD male siblings from simplex families were substantially lower, suggesting fewer ASD-related traits, than mean scores in ASD siblings from multiplex families. There was a trend in the same direction on spouse-report SRS for fathers, but not mothers (Virkud et al., 2009). The finding of different SRS scores in male siblings from multiplex versus simplex families was recently replicated in an independent sample (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010). Unlike Virkud and colleagues (2009), this study reported a similar pattern of results in female siblings.

An additional study by a different group examining relatives within multiplex and simplex ASD families and relatives of typically developing children replicated findings of increased BAP severity in siblings (Schwichtenberg et al., 2010). Siblings within multiplex families had significantly greater SRS scores than siblings within simplex families, with both groups showing greater BAP severity than typical siblings. No differences between multiplex

and simplex fathers or mothers were reported (Schwichtenberg et al., 2010). Infants from multiplex families in this study were more likely (64%) to develop ASD than simplex (9%) and control families (4%).

In the most extensively phenotyped sample to date, Losh and colleagues (2008) found a nearly consistent linear trend across measures of personality traits associated with the BAP, friendship preferences, and pragmatic language between simplex and multiplex ASD parents and Down syndrome parents. The multiplex ASD parents had more BAP traits than simplex ASD parents and the simplex ASD parents had more traits than Down syndrome parents. Additionally, it was more common in multiplex than simplex ASD families for both parents to show features of the BAP (Losh et al., 2008).

A recent study at the University of Washington using the BPASS reported that multiplex ASD parents scored higher on the BPASS Social and Conversation Skills domains (suggesting greater impairment in social communication skills) than simplex ASD parents, DD parents, and parents of typically developing children (Bernier, Gerdts, Munson, Dawson, & Estes, in press). There were no differences among the simplex ASD, DD, and typical parent groups, which the authors suggest signifies that simplex ASD parents do not possess a greater number or intensity of social BAP traits than the population at large. Scores on the BPASS Restricted Interests domain of multiplex ASD, simplex ASD, and DD parents were all higher than the typical control parents. Therefore, this study suggests that restricted patterns of behavior may not be specific to ASD and instead may generally be associated with having a child with a disability (Bernier et al., in press).

Overall, these findings lend support to the theory that families containing multiple children with ASD families carry a higher loading for ASD-related traits given that the presence

of such traits is more common in this family-type than in families containing just one child with ASD. Additionally, a number of genetic studies have found that *de novo* or sporadic CNVs are more common in affected individuals from simplex ASD families as compared to both multiplex ASD families and families without any history of ASD (Marshall et al., 2008; Sanders et al., 2011; Sebat et al., 2007; Weiss et al., 2008). Therefore, it is possible that the types of genetic causes of ASD may vary between single-incidence and multiple-incidence families.

If affected children from simplex families are more likely than those from multiplex families to develop ASD as a result of a *de novo* genetic event occurring only in that individual, then findings of an increased presence of ASD-related traits in multiplex families may suggest that such family members are more vulnerable to ASD symptoms given shared genetic variance.

However, the validity of this phenomenon requires future research attention. The majority of the studies published in this area of research did not use or specify strict criteria to minimize the likelihood that a simplex family member had ASD. Studies with carefully obtained pedigrees and strict exclusionary criteria regarding suspected ASD in relatives are necessary to better ensure true simplex status. These stringent criteria have not been used widely in previous research. Most report that affected children in simplex families are the "only child in the family with ASD" without careful descriptions of criteria by which this was determined. Additionally, many general studies of the broader autism phenotype do not mention whether or not there were multiple affected children in the family. Studies specifically examining multiplex families have tended to be more specific in describing their inclusionary and exclusionary criteria.

An often-discussed inherent limitation of simplex studies is that the diagnostic status of future children, of course, cannot be determined. Therefore, it is impossible to say for certain that the family is truly simplex. Given the 5-10% sibling recurrence risk rate and a 4:1 ratio of

affected males to females, families with only a single affected child might become multiplex if more (particularly male) children were subsequently born into the family. Many families decide to stop having children after receiving an ASD diagnosis in their youngest child, which further complicates the household diagnostic picture. Therefore, additional criteria such as ensuring that at least one other undiagnosed child is in the family and conducting assessments of broader phenotype traits in family members may help to clarify simplex status.

Furthermore, findings are not universal. Self-report broadband inventories in one study assessing symptoms and personality traits in parents of individuals with ASD, OCD, schizophrenia, and a variety of developmental disorders did not differentiate groups (Bölte, Knecht, & Poustka, 2007; Sanders et al., 2011). An additional study reported no differences in emotion processing abilities between simplex and multiplex ASD parents (Szatmari et al., 2008). Therefore, continued research in the examination of ASD-related traits in relatives within simplex and multiplex ASD families coupled with further genetic analysis to expand upon early reports (e.g., Sebat et al., 2007) is necessary to help determine the validity of this pursuit.

J. Summary

A number of domains have been studied in ASD parents and siblings. Mild differences in social and communication skills related to the core deficits in ASD are present in a subset of relatives and are reported nearly consistently across studies. Therefore, social and communication impairments are likely the most familial of autism-related traits. Variable cognitive abilities, differences in theory of mind and executive functioning skills, increased head circumference, and differences in neural functioning and structure have all been documented in ASD parents and siblings. However, many studies have reported contradictory findings in other

areas (e.g., central coherence and phonological processing), making their association with the BAP questionable.

4. Study Rationale

The presence of mild social and communication difficulties (e.g., Bishop et al., 2006; Landa et al., 1992; Piven, Palmer, Landa et al., 1997), social cognition challenges (Baron-Cohen & Hammer, 1997; Dorris et al., 2004), impairments in phonological processing (Folstein et al., 1999; Schmidt et al., 2008), variability among cognitive abilities (Folstein et al., 1999; Pilowsky et al., 2003), difficulty in other cognitive processes such as central coherence (Briskman et al., 2001; Happé et al., 2001) and executive functioning (Hughes et al., 1997; Hughes et al., 1999), different facial processing and memory skills (Dalton et al., 2007; Dawson et al., 2005), and larger head circumferences (Lainhart et al., 2006; Miles et al., 2000) have all been documented in a substantial proportion of ASD parents and siblings compared to controls. These differences suggest a genetic liability for ASD-related traits in families.

Affected individuals in simplex families are more likely to experience sporadic genetic mutations as compared to affected individuals in multiplex families and controls (Marshall et al., 2008; Sebat et al., 2007; State, 2011; Weiss et al., 2008). This suggests that genetic influences in affected individuals who are the only persons in their family with ASD may be different than in affected individuals who have a family history of the disorder. The majority of studies examining the BAP have either exclusively examined first-degree relatives in multiplex samples, in which genetic vulnerability for such traits may be higher, or have not specified information about a family history of the disorder. Given the potentially differing genetic mechanisms involved in ASD in simplex versus multiplex families, it is plausible that differences in broader phenotype traits exist as well.

A limited number of studies have directly compared BAP traits in ASD parents and siblings from multiplex and simplex families (Bernier et al., in press; Constantino, Zhang et al., 2010; Losh et al., 2008; Schwichtenberg et al., 2010; Szatmari et al., 2000; Virkud et al., 2009). These studies as a whole document increased presence of BAP traits in multiplex ASD parents and siblings compared to simplex first-degree relatives. Uneven broader phenotype presentations offer support for sporadic versus familial ASD and provide insight into the behavioral manifestations of these recent genetic findings.

Although studies comparing the BAP in simplex and multiplex ASD families are significant and the methodology relatively novel, there are a number of potential limitations regarding generalizability of findings. The authors' reports of study criteria in place to help ensure simplex status have generally been minimal. Diagnostic procedures in the affected child(ren) have either not been thoroughly explained or have been confirmed from a review of medical records rather than direct evaluation. Additionally, no study to date has completed research-administered cognitive testing in relatives; direct, comprehensive assessments of the BAP in relatives within the two family types is lacking. The majority of studies have exclusively examined social and communication traits via informant report (generally the SRS). Although mild social and communication deficits are a major component of the BAP, there are many other areas of functioning impacted in relatives that merit attention.

A number of different measures of ASD-related social and communication skills have been used in studies comparing the BAP in simplex and multiplex families. Losh and colleagues (2008) administered the M-PAS-R, which is a promising instrument with much behavioral research support. However, administration and scoring are not manualized and genetic studies

linking traits assessed on the M-PAS-R to the genome have yet to be reported. Additionally, the M-PAS-R is only appropriate for adults and therefore was only administered with ASD parents.

The FHI was used in another study to assess ASD-related traits (Szatmari et al., 2000). The FHI was widely used in early studies of the BAP, but has more recently been replaced with other measures, such as the M-PAS-R, BPASS, and SRS, which provide continuous rather than categorical outcomes. Questions about communication on the FHI largely focus on communication disorders rather than pragmatic use of language, which is more often impaired in relatives than structural language.

The SRS was used in three additional studies examining the BAP in ASD simplex and multiplex siblings and parents (Constantino, Zhang et al., 2010; Schwichtenberg et al., 2010; Virkud et al., 2009). The SRS is a short questionnaire that has grown increasingly popular in ASD genetic studies due to the ease of administration and interpretation as well as links to genetic findings. However, the SRS relies on informant-report rather than interview and direct behavioral observations by a trained interviewer to gauge relative presence and absence of traits. Additionally, the SRS in adults only yields a single overall severity score whereas other measures of the BAP provide scores in separate domains that may be more useful in subtyping.

Finally, one study in press from the University of Washington used the BPASS to measure ASD-related traits in simplex and multiplex ASD parents as well as DD parents and parents of typically-developing children (Bernier et al., in press). However, this was a small study consisting largely of mothers, combined mothers and fathers into a single parent group, and did not include siblings. Since research has shown that the BAP presents differently in females versus males, a larger and more representative sample is needed to generalize these findings.

In my dissertation, I intended to augment findings of differences in BAP traits in simplex versus multiplex families by addressing these possible criticisms. The BPASS (Estes, Munson, Bernier, & Dawson, 2004) was used to assess the social and communication skills as well as range of interests and flexibility in mothers, fathers, and siblings without a diagnosis of ASD. The BPASS is administered by a trained interviewer, uses behavioral observation in addition to direct questioning, is designed to assess a wide range of ages, and has positive QTL findings (Sung et al., 2005). Additionally, there is preliminary evidence that it discriminates between simplex and multiplex ASD families in a number of different domains (Bernier et al., in press).

Direct evaluations of phonological processing and face memory was administered in addition to an IQ assessment, providing estimates of cognitive variability and central coherence via the block design subtest. Head circumference was also measured and its association with the broader phenotype measures was examined. Scores in all areas were obtained from family members within carefully phenotyped simplex and multiplex ASD families.

5. Aims

1) To obtain a multifaceted assessment of the broader autism phenotype in unaffected parents and siblings within simplex and multiplex families.

The primary aim was to assess and compare a number of different aspects of the BAP in ASD parents and siblings in simplex versus multiplex families. Areas of assessment were limited to those that have been shown in past research to be affected in first-degree relatives of individuals with ASD. Measures included assessments of general cognitive abilities, social communication skills and range of interests, face memory, and phonological short-term memory. Given the potential for differing genetic mechanisms underlying simplex and multiplex ASD, these traits were compared among family members within these family structures. Careful

attention was paid to fathers as male relatives have nearly consistently presented with more pronounced features of the BAP in previous research, particularly in social and communication challenges (e.g., Bolton et al., 1994; Smith et al., 2009; Wolff et al., 1988). Additionally, many studies examining the BAP report that only a subset of ASD parents and siblings present with ASD-related traits; the majority do not demonstrate noticeable differences compared to controls. Therefore, proportions of family members showing evidence for impairment were also compared among simplex and multiplex families. Due to shared genetic vulnerability for ASD and based on findings from related studies (e.g., Losh et al., 2008; Virkud et al., 2009), ASD parents and siblings (particularly male relatives) in multiplex families were predicted to demonstrate greater impairment and be overrepresented in the lower range of scores on variables of interest compared to relatives in simplex families.

2) *To increase an understanding of head circumference in unaffected parents and siblings within simplex versus multiplex families*

A secondary aim was to add to the scant knowledge base on head circumference in family members of individuals with ASD. Despite highly replicated findings of increased head circumference and rates of macrocephaly in affected children (Bailey et al., 1995; Courchesne et al., 2003; Dementieva et al., 2005; Deutsch & Joseph, 2003; Lainhart et al., 2006), there have been few reports of head circumference in unaffected relatives. However, the limited studies conducted on head circumference in relatives do suggest that head size is increased in ASD parents and siblings (Fidler et al., 2000; Lainhart et al., 2006; Miles et al., 2000). No study to date has compared head circumference measurements in ASD parents and siblings in multiplex and simplex families. Therefore, this was the first report of its kind and provides insight into the potential link between genetic findings and physical characteristics associated with ASD. Given

shared genetic vulnerability, mothers, fathers, and siblings without a diagnosis of ASD in multiplex families were predicted to have larger measurements of head size as compared to relatives in simplex families.

3) To assess the relation between head circumference and degree of expression of the broader autism phenotype in family members within simplex and multiplex families

One exploratory aim was to examine the relationship between head circumference and degree of expression of the BAP in ASD parents and siblings. The two other studies examining this research question report contradictory results regarding an association between severity of ASD-related traits and increased rate of head growth early in life in young ASD siblings (Constantino, Majmudar et al., 2010; Elder et al., 2008). The relation between head size/brain volume in affected individuals also does not appear to predict a consistent phenotype. While a number of studies have reported negative effects of increased head size in ASD (Courchesne et al., 2003; Fidler et al., 2000; Lainhart et al., 2006), others have found no association (Deutsch & Joseph, 2003) and still more report improved outcome (Dementieva et al., 2005; Sacco et al., 2007). Given the minimal research conducted in this area in relatives and inconsistent reports in affected children, the examination of the relationship between broader phenotype traits and head circumference in ASD parents and siblings in simplex and multiplex families was exploratory.

4) To determine the relation between affected child(ren) characteristics and broader autism phenotype traits in family members within simplex and multiplex families

Lastly, the relationship between characteristics of the affected children and BAP traits in ASD parents and siblings within simplex and multiplex ASD families was explored. Severity of autism symptoms, number of social communication impairments and amount of restricted/repetitive behaviors were examined. There is preliminary evidence that the severity of

autism-related traits is correlated within some families (Constantino & Todd, 2003; Murphy et al., 2000; Pickles et al., 2000; Schwichtenberg et al., 2010). Genetic theory would predict that in families whose child developed ASD as a result of a *de novo* genetic event, the relation between symptoms of the affected child and BAP traits in ASD parent and siblings would be minimal given that ASD resulted from a genetic mutation not shared by other family members. Therefore, the relationship between traits of the affected child and ASD parent and sibling characteristics was expected to be weaker or insignificant for simplex families as compared to multiplex families.

CHAPTER 2

METHODS

Participants

Participants were recruited from two genetic studies of ASD conducted at the University of Washington Autism Center: the Family Study of Autism (FSA) and the Simons Simplex Collection (SSC). Data collection for FSA took place between 1998 - 2007 while data collection for the SSC occurred between 2007 - 2011. The University of Washington Institutional Review Board approved both studies and informed consent was obtained.

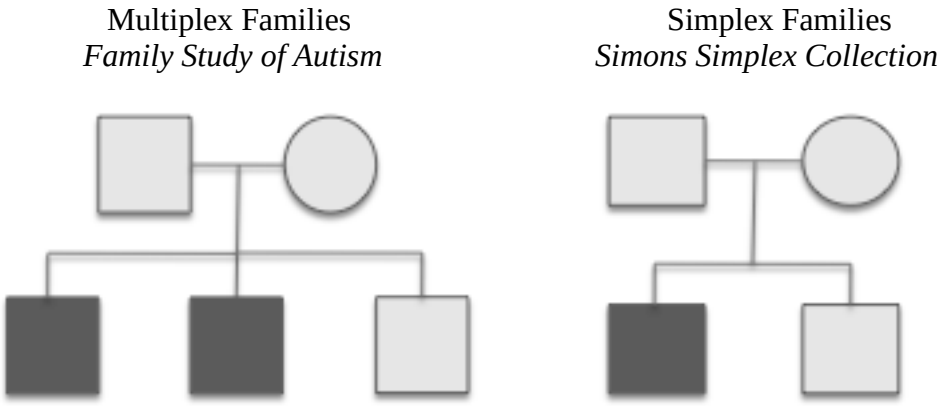


Figure 2. Example of family structure of participants.

Study procedures, protocols, measures, and inclusionary/exclusionary criteria were comparable for the FSA and SSC studies and will be described in detail. Similarly trained staff and at times identical clinicians worked on both studies. In general, demographic information for the subset of families included in these analyses was similar in terms of age at evaluation, race, ethnicity, and parent education level. See Table 3. There were two exceptions: 1) Mothers and fathers in the SSC sample were slightly (but significantly) older than mothers and fathers in the FSA sample, $t(125) = 3.49$, $p < 0.001$ and $t(110) = 2.21$, $p = 0.03$, respectively, and 2Fathers

in the SSC sample obtained a higher level of education than FSA fathers, $\chi^2(4, N = 109) = 15.98$, $p = .003$. Overall, although the samples were collected during different time points and were not specifically designed to answer the research questions put forward, there are ample similarities among the samples to allow for valid comparisons.

Table 3. Demographic Information for Simplex and Multiplex Family Members

		Mother		Father		Affected Child		Unaffected Child	
		Simplex	Multiplex	Simplex	Multiplex	Simplex	Multiplex	Simplex	Multiplex
Age *	Mean (Standard Deviation)	42.56 (5.32)	38.99 (5.42)	44.18 (6.08)	41.17 (7.33)	9.89 (3.68)	9.28 (3.76)	11.51 (3.59)	10.20 (4.20)
Sex+	M	0 (0%)	0 (0%)	40 (100%)	87 (100%)	35 (85%)	149 (81%)	20 (49%)	58 (50%)
	F	41 (100%)	87 (100%)	0 (0%)	0 (0%)	6 (15%)	35 (19%)	21 (51%)	59 (50%)
Race+	American Indian/Alaska Native	1 (2%)	3 (4%)	1 (3%)	1 (1%)	0 (0%)	5 (3%)	0 (0%)	2 (2%)
	Asian	3 (7%)	2 (2%)	1 (3%)	2 (2%)	1 (2%)	4 (2%)	1 (2%)	2 (2%)
	Black/African American	1 (2%)	1 (1%)	1 (3%)	3 (4%)	1 (2%)	2 (1%)	1 (2%)	3 (3%)
	More than one race	0 (0%)	5 (6%)	0 (0%)	3 (4%)	3 (7%)	16 (9%)	3 (7%)	12 (11%)
	White	36 (88%)	74 (87%)	37 (93%)	76 (89%)	36 (88%)	152 (85%)	36 (88%)	90 (83%)
Ethnicity+	Non-Hispanic	40 (98%)	75 (99%)	38 (97%)	75 (99%)	39 (95%)	151 (99%)	39 (95%)	91 (99%)
	Hispanic/Latino	1 (2%)	1 (1%)	1 (3%)	1 (1%)	2 (5%)	2 (1%)	2 (5%)	1 (1%)

	Some High School	0 (0%)	0 (0%)	0 (0%)	1 (1%)		
	High School	1 (2%)	9 (13%)	1 (3%)	6 (9%)		
Education Level[+**]	Some College	12 (29%)	27 (38%)	4 (11%)	25 (35%)		
	College Graduate	20 (49%)	28 (39%)	14 (37%)	26 (37%)		
	Graduate Degree	8 (20%)	7 (10%)	19 (50%)	13 (18%)		

+ presented as total *N* in each group and percentage of sample

* Difference between simplex and multiplex samples is significant for *mothers*, $p < .05$, and *fathers*, $p < .001$

** Difference between simplex and multiplex samples is significant for *fathers*, $p < .001$

Importantly, behavioral measures for family members had already been collected as part of the FSA protocol. Parents, siblings, and affected children included in the current analyses were seen simultaneously during one family visit between the years of 1998 and 2007. In the SSC, head circumference and height for all family members as well as all proband measures were collected during the initial SSC diagnostic visit. This initial diagnostic visit for the subset of families participating in the current project occurred between 2007-2010. However, family behavioral data was not part of the initial diagnostic visit of the SSC. Therefore, SSC families were invited back to participate in a more complete behavioral assessment for parents and siblings. This second visit occurred between 2009 - 2011.

Multiplex Families

Data for the multiplex family sample was obtained from the FSA, which collected data from family members within multiplex ASD families. The study was conducted as part of the Collaborative Program of Excellence in Autism (CPEA) Family Study of Autism at the University of Washington Autism Center. The National Institute of Child Health and Human Development (NICHD) and National Institute on Deafness and Other Communication Disorders (NIDCD) funded the FSA. Approximately one-half of families were from the Puget Sound region and were seen at the University of Washington Autism Center. The remainder lived throughout the United States, with a small number of families from Canada, Britain, and New Zealand. Teams of clinicians traveled to evaluate non-local multiplex families at private homes, universities and other community agencies. Five hundred fifty families were screened and self-identified as having more than one child with ASD. These families were then screened further using the ADI-R (Lord, Rutter, & Le Couteur, 1994) to identify families with a high probability of having two children with ASD. Biological parents, affected children, and unaffected siblings,

if available, were asked to participate. The research visit consisted of a diagnostic evaluation with affected children and a neuropsychological assessment with each of the participating family members. When possible, a blood sample and physical measurements were obtained in addition to behavioral measures from both parents, each affected child, and unaffected sibling(s). Recruitment occurred from a variety of sources, including newspaper articles, parent organizations, and a network of community service providers.

FSA Sample Description

Following the diagnostic evaluation of the probands, 311 families qualified as multiplex based on CPEA standards established by Catherine Lord among other experts in research diagnosis of ASD. The majority of families were composed of sibling pairs (n = 284 families), with substantially fewer families containing three (n = 25), four (n = 1), or five (n = 1) affected children. To be included in the study, families were required to contain at least two children who met the research diagnostic criteria specified below. The minimum age requirement for study participation was three years of age. Exclusionary criteria included the presence of a known genetic condition (e.g., neurofibromatosis and fragile X syndrome), history of serious head injury or neurological disease (e.g., encephalitis), significant sensory or motor impairment impacting measure completion, and language other than English as a primary language.

Current Multiplex Sample

In addition to general FSA study requirements, a number of specific conditions were put into place to address the research questions in this project. Firstly, only those families containing child(ren) *without a diagnosis* of ASD in addition to children with ASD were analyzed in order to assess the broader autism phenotype in ASD siblings. Specifically, we selected those families who at the time of study participation had at least one additional child in the family who did not

have (or subsequently receive) a clinical diagnosis of ASD and did not meet clinical cutoffs on diagnostic measures. At times, children were identified at screening as possibly having ASD, but following direct clinical assessment, did not receive a diagnosis. Additionally, we included only those families in which all children in the family were full biological siblings. This was verified by genotyping. Of the 311 total possible multiplex families, 97 met these criteria.

Further exclusionary criteria were applied in families that had more than one unaffected sibling. In these family constellations, only one unaffected sibling was occasionally assessed due to time constraints during a lengthy family session. When this occurred, the "least affected" sibling was selected to participate in terms of reported or observed ASD-related challenges. Since this procedure involved a systematic selection of subjects, those families with multiple unaffected children for which there were only data on one unaffected sibling were excluded from analyses (n = 7). Of the remaining 19 multiplex families containing multiple unaffected siblings, either all unaffected siblings were assessed (n = 11), sibling data was missing entirely (n = 6), or data were available on multiple unaffected siblings within the family (n = 2). These families were included in analyses because the siblings were not systematically selected for participation based on presenting behavior.

Lastly, an additional three families were excluded because the two affected children in the family were MZ twins. The presence of MZ twins concordant for ASD in a family does not necessarily suggest a genetic loading for ASD since identical twins share nearly all of their genetic material.

The twin and multiple unaffected sibling restrictions resulted in 87 families remaining for analysis. These families included 117 unaffected children (58 males and 59 females) and 184 affected children (149 males and 35 females) diagnosed with autistic disorder (n = 137),

Asperger's disorder (*n* = 23), or PDD-NOS (*n* = 24). Families had two (*n* = 79), three (*n* = 7), or five (*n* = 1) affected children and one (*n* = 68), two (*n* = 12), three (*n* = 5), four (*n* = 1), or six (*n* = 1) unaffected children. The most common family constellation was two affected children and one unaffected child (*n* = 62). Again, family data for the multiplex sample had already been collected and only those multiplex families who gave consent for secondary data analyses were included. Therefore, no additional procedures were necessary to recruit families to participate.

Simplex Families

Simplex families were ascertained from the SSC project that was privately funded by the Simons Foundation. The goal of the SSC was to examine the genetic risk associated with the single occurrence of ASD in the family. The University of Washington was one of 12 university-based centers that participated across the United States and Canada. Families at our site were generally seen at the University of Washington Autism Center. However, a team of clinicians traveled to neighboring states (Idaho and Oregon) in order to assess a limited number of families in their homes. Families were recruited if they reported having exactly one child in the family with diagnosed or suspected ASD. Four hundred fifty-three families were screened for study participation. Biological parents, the affected child, and an unaffected sibling, if available, were invited to participate. The research visit consisted of a diagnostic evaluation for the affected child and blood draws and physical measurements for parents, the affected child, and an unaffected sibling. Recruitment sources included referrals from clinical providers, general media advertising through the newspaper and radio, community talks, and website advertisements.

SSC Sample Description

As of June 30, 2011, the University of Washington SSC site evaluated 269 families that were determined by both the local team and the Simons Foundation to meet study criteria. The

majority of families contained at least one unaffected sibling (*n* = 227), with the remainder consisting of trio families in which the child with ASD did not have any full siblings. Inclusionary criteria for the SSC included having exactly one child in the family meeting research diagnostic criteria outlined below. Probands also were required to have a nonverbal mental age of at least 18 months. Only 14 families (out of 453 screened) were disqualified based on the requirements for nonverbal cognitive functioning. Therefore, this criterion likely did not affect the general severity level of probands in the simplex sample. The minimum age requirement for study participation for unaffected and affected children in the SSC was four years of age, with a maximum age of 17 years, 11 months for affected children and no upper age limit for siblings. There were no age restrictions for parents. Participating unaffected children were required to be full biological siblings and both biological parents were expected to participate.

Exclusionary criteria for the SSC regarding family history were stricter than the multiplex sample in order to prioritize children more likely to have a sporadic genetic event. Therefore, probands were excluded if they had a low birth weight and less than 36 weeks gestation, had a history of extensive pregnancy or birth complications, were positive for Fragile X Syndrome or Down syndrome, had sensory or motor difficulties that would preclude valid use of the diagnostic instruments, or had a history of severe nutritional or psychological deprivation.

Affected children were also carefully screened for a family history of ASD via parent interview. Families were excluded if biological grandparents, aunts/uncles, first cousins, parents, and/or siblings had a diagnosis of ASD (*n* = 13 families excluded for this criterion). Additionally, families with siblings and parents who were suspected to have a diagnosis of ASD or intellectual disability were excluded from study participation. Participating siblings must have

also received a composite score of 70 or above on the Vineland Adaptive Behavior Scales- 2nd Edition (Sparrow, Cicchetti, & Balla, 2005) to demonstrate adaptive abilities that were within two standard deviations of the norm. During the course of the study, new criteria were implemented to exclude families containing parents and siblings who demonstrated significant subclinical ASD-related traits. While these modified criteria could significantly sway study results, overall only four families out of 453 screened at University of Washington were excluded for the presence of subclinical ASD-related traits in immediate family members. Therefore, these modified criteria are unlikely to influence results. Furthermore, the majority of families invited back to participate in the current project were initially seen early in SSC data collection, during which time this criterion had not been established universally.

Current Simplex Sample

Specific criteria were implemented in the SSC sample to address the research questions in this project. Like the multiplex sample, only those families with at least one other participating unaffected sibling were recruited to participate in order to examine sibling presentation of the broader autism phenotype. Two hundred twenty-seven of the 269 families included in the larger sample met this criterion. As mentioned above, unaffected siblings and parents did not themselves participate in a behavioral assessment during the initial SSC visit. Therefore, in order to address the aims of this project, SSC families who consented to be contacted for future research studies were recontacted to participate in a second evaluation involving parents and siblings. Of the SSC families meeting these criteria who were contacted to participate in the second visit, 67% (n = 47) agreed to participate and 59% (n = 41) came in for a lab visit at the University of Washington Autism Center. 34% either declined to participate (largely because they were too busy at the time of contact) or did not return recruitment phone

calls. Affected children (35 males and 6 females) had diagnoses of autistic disorder (*n* = 27), Asperger's disorder (*n* = 9), or PDD-NOS (*n* = 5). There were no MZ twins in this subset of families.

We focused recruitment efforts on those families who had multiple unaffected siblings in the family. This was done for two reasons: 1) to better account for systematic differences in family size across simplex and multiplex families who, by definition, needed to have a minimum family size of three (two affected and one unaffected) to be included in these analyses, and 2) to theoretically increase the chance that ASD occurred in the proband as a result of a sporadic genetic mutation. The modal family constellation in the overall SSC sample consisted of one proband and exactly one unaffected sibling and we were not able to recruit a sample consisting entirely of families who had additional unaffected siblings. Overall, however, 73% (*n* = 30) of the simplex sample included multiple unaffected children while the remaining 27% (*n* = 11) had exactly one unaffected sibling. Families by definition had exactly one affected child and either one (*n* = 11), two (*n* = 21), three (*n* = 8), or five (*n* = 1) unaffected children. If there was more than one unaffected sibling, the closest in age to the proband was seen for evaluation for this study (20 males and 21 females).

Measures

Proband Measures

ASD Diagnosis

Diagnosis of the affected children in both the multiplex and simplex samples was derived from a combination of sources. In order to obtain parent report of child functioning as well as direct behavioral observation of child behavior, the ADI-R and Autism Diagnostic Observation Schedule (ADOS) were administered. The ADI-R (Lord et al., 1994) is a semi-structured

interview designed to elicit information from a child's caregiver about current and past social and communication skills as well as presence of restricted and repetitive behaviors and interests. The ADI-R takes approximately 3 hours to complete.

The ADOS (Lord et al., 2000) is a play-based assessment intended to directly assess ASD symptoms through a series of age appropriate activities designed to elicit social interactions. The ADOS takes approximately 45 minutes to complete. There are four modules that differ in terms of required language ability and age of the individual. Updated algorithms contain a subtotal for codes relating to social communication skills (ADOS Social Affect) and restricted/repetitive behaviors and interests (ADOS Restricted and Repetitive Behavior) as well as a total score summing these domains (ADOS Total). A calibrated severity score (CSS) can also be derived across Modules 1 – 3 standardizing severity of symptoms presented during the ADOS (Gotham et al., 2007).

Finally, a clinical diagnosis was given as defined in the DSM-IV (American Psychiatric Association, 1994) that was based on all available information obtained through the ADI-R, ADOS, cognitive testing, and any other experiences with the proband(s).

In both the multiplex and simplex samples, clinicians were trained to research reliability on the ADOS and ADI-R. Reliability checks were performed with local site supervisors who were reliable directly with the developers of the measures at the University of Michigan Autism and Communication Disorders Center (UMACC). In the simplex sample, quarterly reliability checks were also performed with UMACC consultants.

Children in both family types were considered to be "affected" if they: 1) met diagnostic cutoffs for ASD on the updated algorithms on the ADOS (Gotham et al., 2007), 2) met CPEA

ASD criteria on the ADI-R (see Schellenberg et al., 2006 for details), and 3) received a DSM-IV diagnosis of either autistic disorder, Asperger's disorder, or PDD-NOS.

In the multiplex sample, at least two children in the family met the above criteria while in the simplex sample, only one child in the family met the diagnostic criteria. In the multiplex sample, there were four families (n = 5 children total) who, in addition to having two children who met the specified diagnostic criteria, contained other children with ASD who met less stringent ADOS and ADI-R criteria. Specifically, four children with ASD diagnoses either met clinical cutoffs on the ADOS but not on the ADI-R (n = 2) or met on the ADI-R but not the ADOS (n = 2). One affected child in another family had a previous diagnosis of ASD, but could not participate in the lab visit. These five children were considered affected in analyses.

Because the updated ADOS algorithms were applied across the multiplex and simplex samples, children from the multiplex sample who were defined as "affected" in these analyses are slightly different from affected children in previous publications involving the FSA sample in which original ADOS algorithms were used (e.g., Schellenberg et al., 2006). A number of children who met original algorithm cutoffs no longer met new algorithm cutoffs and some children not included previously met new algorithm cutoffs.

Cognitive Testing

Cognitive level for the simplex sample was assessed via either the Differential Abilities Scale, 2nd Edition (DAS-II; Elliott, 2007) or the Mullen Scales of Early Learning (Mullen, 1995). The complete DAS-II School Age or Early Years Cores were given, depending on the individual's age and ability level. If a child received scores below the floor on more than half of the subtests within a domain on the DAS-II Early Years Core, then the Mullen was administered

in order to better capture their cognitive level. Therefore, the Mullen was generally used for children who were lower functioning.

In the multiplex sample, probands ages 3 – 4.5 years received the Mullen (Mullen, 1995), ages 4.5 – 7 years received the Wechsler Preschool and Primary Scale of Intelligence – Revised (WPPSI-R; Wechsler, 1989), ages 7 – 17 years received the Wechsler Intelligence Scale for Children – Third Edition (WISC-III; Wechsler, 1991), and ages 17+ received the Wechsler Adult Intelligence Scale– Third Edition (WAIS-III; Wechsler, 1997a). A short form of the Wechsler scales was administered consisting of the Vocabulary, Comprehension, Block Design and Object Assembly subtests. The short form yields an estimated full scale, verbal, and performance IQ as described by Sattler (Sattler, 1992), taking into account subtest reliability and validity. The Mullen, DAS-II, and Wechsler scales are normed, reliable, and valid and are used extensively in behavioral research studies.

Severity of the Affected Child

In order to address the fourth aim comparing the relationship between the symptom severity of the affected child(ren) and expression of the broader autism phenotype in family members across family types, an overall level of severity in families was required. This was challenging because families differed in terms of how many affected children participated (one, two, three, or five affected children). Additionally, affected sibling severity may not be correlated within families, so an average score may accurately reflect overall severity. Selecting one child to participate loses potentially important variability. Thus, a proportion score was instead created by summing all scores within a family and then dividing by the total possible score for that family (which varies depending on how many affected children are present) to

create a proportion of severity. The higher the proportion score, the greater the symptom severity within the family.

Severity proportions for ADOS Social Affect, ADOS Restricted and Repetitive Behavior, and ADOS Total Score were calculated separately. The total possible scores for each of these three composites are the same across ADOS Modules 1, 2, and 3 (20, 8, and 28, respectively for each composite). Therefore, scores across modules can be compared. These scores are from the updated ADOS algorithms (Gotham et al., 2007) and cannot be calculated for individuals who received ADOS Module 4. Therefore, families containing individuals receiving only Module 4 of the ADOS were excluded from Aim 4 analyses (N = 5 total, 2 multiplex and 3 simplex). Thus, families included in Aim 4 analyses were slightly different than families included in Aims 1 – 3.

Assessment of Family Members

In addition to a clinical evaluation of the affected child to confirm an ASD diagnosis, ASD parents and siblings were assessed in areas purported to be part of the broader autism phenotype. Social and communication skills (e.g., Bishop et al., 2006; Landa et al., 1992; Piven, Palmer, Landa et al., 1997), facial processing and memory (Dalton et al., 2007; Dawson et al., 2005), and phonological processing (Folstein et al., 1999; Schmidt et al., 2008) have all been shown to be impaired in ASD parents and siblings in previous research. Additionally, increased variability among verbal and nonverbal skills (Folstein et al., 1999; Pilowsky et al., 2003) and larger than normal head circumference (Lainhart et al., 2006; Miles et al., 2000) have been noted in unaffected family members of individuals with ASD. ASD parents and siblings in both the simplex and multiplex samples received the same test battery. ASD-related traits, cognitive

functioning, phonological short-term memory, face memory, object memory, head circumference, and height were assessed for all participating family members.

ASD-Related Traits

The Broader Phenotype Autism Symptom Scale (BPASS; Estes et al., 2004) was used to measure autism-related traits in family members. The BPASS has the benefit of assessing four domains of functioning (Social Interest, Expressiveness, Conversational Skills, and Range of Interests/Flexibility) using clinician ratings via both observation and interview. Composite scores are calculated by taking the average of individual scores within the domains. The BPASS can be used with individuals of a full range of ages, including parents, siblings, and affected children. Ratings for most trait domains range from impaired to non-impaired, and most also capture a level above the norm. Further information regarding BPASS composites as well as specific information for BPASS use in this study are provided below.

Social Interest.

The Social Interest domain of the BPASS is derived from interview questions assessing child- and adulthood social interest in peers and groups and assesses social motivation. Specific items include self-perception of social comfort in groups and preference for alone time versus time spent with others across settings.

Expressiveness.

The Expressiveness domain is based on nonverbal social communication observed during the BPASS interview and assesses social expressivity. Clinicians rate the use of appropriate and integrated eye gaze, social smiling, facial expressions, and prosody during the course of the interview.

Conversational Skills.

The Conversation domain is scored from clinical observations of conversation skills during the BPASS interview. Particular attention is paid to instances of excessive detail that impede conversation and decreased sensitivity to the listener by, for example, making comments out of context and/or without adequate background information.

Flexibility/Range of Interests.

The Flexibility/Restricted Interests domain pertains to parent- or self-report of flexibility and interests in both child- and adulthood. The breadth and type of interests are assessed as well as the intensity of these interests. Parents are also asked to describe how they and their children prefer to arrange their daily schedule and physical environment, with scores ranging from extremely flexible in routine and physical space to marked rigidity in these areas causing impairment in relationships or emotional distress if disrupted.

Applications of the BPASS.

The requirements set forth in the BPASS manual of establishing 80% scoring reliability with a reliable BPASS clinician were followed for both samples. The BPASS was administered by highly trained clinical examiners (doctoral level clinical psychology graduate students, Postdocs, and PhD level licensed psychologists) all of whom had attained research reliability on the ADOS and ADI-R. The potential for rater drift was addressed through regular clinician meetings focusing on BPASS scoring questions and watching recorded sessions. Given that the multiplex and simplex samples were collected independently from one another, additional study procedures were put into place to guard against rater drift. Specifically, 10% of BPASS administrations in both the multiplex and simplex samples were coded from videotape for reliability by a group of experienced BPASS clinicians. Percent agreements were greater than

80% in all tapes coded, which suggests that comparable BPASS coding procedures were maintained across both samples.

The proportion of family members with high scores (suggesting some degree of impairment) on the Social Interest and Range of Interests/Flexibility BPASS domains were analyzed in Aim 1. A high score was defined as receiving one of the two highest codes on any question within that domain. In the Social Interest domain of the BPASS, subjects were considered to have decreased social interest (and a "high score" in this area) if they were scored with a 4 or 5 on the question regarding sociability with peers (range of scores of 1 – 5) or a 3 or 4 on the question regarding sociability in groups (range of scores of 1 – 4). These codes suggest low to very low interest in interacting with others and substantial apprehension and rare initiation of social contact in groups. In the Range of Interests/Flexibility domain, subjects were considered to have restricted/repetitive behaviors (and a "high score" in this area) if they were scored with a 4 or 5 on the questions regarding range and type of interests, flexibility in physical environment, or flexibility in schedule and routine (range of scores on all three items of 1 – 5). These codes suggest unusual or impairing interests, a strong preference for predictability and routine that is bothersome to others, and limited adaptability and functionality in terms of preference for order in the physical environment.

For Aims 4 analyses, different BPASS composite scores were required to map onto the severity scores for the affected child described above. Items from the ADOS Social Affect composite take into account both verbal and nonverbal social communication skills (e.g., conversation skills, eye contact, facial expressions). These items are similar to items loading onto three domains of the BPASS: Social Interest (sociability with peers, sociability in groups), Expressiveness (eye gaze, social smiling, facial expressions), and Conversation Skills (detail in

conversation, sensitivity to listener). Scores from the eight items within the three domains were averaged to create a BPASS "Social Affect" composite similar to the ADOS Social Affect composite. The Restricted and Repetitive Behavior composite of the ADOS reflects many codes that are captured in the Range of Interests/Flexibility domain of the BPASS (e.g., interests, flexibility in physical environment, flexibility in schedule and routine). Lastly, an overall composite of the four BPASS domains was computed to be comparable to the ADOS Total score and ADOS calibrated severity score.

Cognitive Functioning

A variety of tests from the Wechsler Scales of Intelligence were administered to assess cognitive abilities in both the simplex and multiplex samples. In the simplex sample, family members were given the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). The WASI is an abbreviated measure of cognitive abilities, consisting of four subtests: Vocabulary, Block Design, Similarities, and Matrices. It yields an estimate of verbal, nonverbal, and full scale IQ and is appropriate for individuals aged 6 – 89 years of age. All participating siblings in the simplex sample were over six years of age and functioning in the average to above average IQ ranges. Therefore, the WASI could be used for all participating family members in the simplex sample. As reported in the normative data for the WASI (Wechsler, 1999), the WISC-III and the WASI are strongly correlated (FSIQ: r = .87, VIQ: r = .82, PIQ: r = .76) as are the WAIS-III and the WASI (FSIQ: r = .92, VIQ: r = .88, PIQ: r = .84).

In the multiplex sample, family members received the short form (described above in the affected child section) of the either the WPPSI-R (Wechsler, 1989), WISC-III (Wechsler, 1991), or the WAIS-III (Wechsler, 1997a), depending on their age. The short form of these measures also generates an estimate of verbal, nonverbal, and full scale IQ.

Several variables of interest were examined from the Wechsler scales. In addition to verbal, nonverbal, and full scale IQ composite scores, standardized scores from the Block Design subtest were examined as a potential proxy for central coherence. IQ discrepancies between verbal and nonverbal composites were also explored, given that significantly variability among composite scores has been noted both in children diagnosed with ASD (Happé, 1999; Lincoln et al., 1988) and ASD parents and siblings (Folstein et al., 1999; Pilowsky et al., 2003). An IQ discrepancy was identified when the difference between verbal and nonverbal IQ was significant at the .05 level as defined in the various Wechsler manuals. A significant discrepancy was considered to be 11.07 for all ages on the WPPSI-R, 11.30 for all ages on the WISC-III, 8.76 for all ages on the WAIS-III, 10.73 for children 6 – 16 years of age on the WASI, and 8.51 for individuals 17 – 89 years of age on the WASI. These numbers were rounded to the nearest whole number and used to compute an IQ discrepancy categorical variable in analyses.

Phonological Short-Term Memory

The Nonword Repetition subtest of the Comprehensive Test of Phonological Processing (CTOPP; Wagner, Torgesen, & Rashotte, 1999) was used to measure one aspect of phonological processing: phonological short-term memory. The Nonword Repetition subtest assesses an individual's ability to repeat nonsense words played on an audiocassette or CD player that range in length from 3 to 15 phonemes. The variable of interest for the CTOPP was the overall scaled score for the Nonword Repetition subtest. Scaled scores have a mean of 10 and a standard deviation of 2. Therefore, scores of 7 and below are considered to be below average. Normative data for the CTOPP are only available up to 24 years of age. Therefore, scores for adults were extrapolated upward from the oldest norm group.

Face Memory

Depending on participant's age, the immediate and delayed face memory tasks from either the Wechsler Memory Scale – Third Edition (WMS-III; Wechsler, 1997b) or Children's Memory Scale (CMS; Cohen, 1997) were administered to assess facial recognition. The WMS-III is a standardized measure for ages 16 and above, co-normed with the WAIS-III and provides scores for immediate and delayed memory for faces. The CMS is a similar assessment that is appropriate for children ages 5-16 years. Variables of interest were scaled scores for the immediate and delayed facial recognition subtests on the WMS-III and CMS. As with the CTOPP, scaled scores of 7 and below are considered to be below average.

It is conceivable that deficits observed in face memory could be a result of overall memory deficits, rather than challenges specific to faces. Therefore, a measure of object memory (Picture Recognition subtest of the Woodcock Johnson; Woodcock & Johnson, 1989) was also administrated to control for object memory. This measure generates an overall standardized score for object recognition. However, object memory was unrelated to face memory for both the simplex (r = .12 for immediate face memory, r = -.01 for delayed face memory) and multiplex (r = .03 for immediate face memory, r = .11 for delayed face memory) samples.

Head Circumference

Head circumference measurements were taken using a tape measure placed above the superior aspect of the supra orbital ridge and passing around the largest part of the occipital protuberance with measurements made to the nearest 1 mm by trained and reliable clinical staff. Bushby and colleagues (1992) reported significant effects of height on head circumference in adults. Therefore, height was also obtained from each subject. There was a moderate

relationship between height and head circumference in our sample as well (r = .34). *Z* scores for head circumference were computed using the normative samples reported by Roche et al. (1987). Because there are no generally accepted normative statistics for head circumference in adults, scores were extrapolated upward for parents from normative scores at 18 years of age in the Roche sample. *Z* scores for height were computed using the CDC norms published in 2000. Again, there are no widely used normative scores for height in adults and scores were extrapolated upward for parents from normative scores at 20 years of age in the CDC norms.

Missing Data

The amount of missing data was greater in the multiplex sample than the simplex sample. This is likely due to the fact that FSA families were often seen in their homes where multiple family members were often seen simultaneously by multiple clinicians. Therefore, it was difficult to ensure that all data was collected for all family members. Additionally, unlike the SSC, both biological parents were not required to participate. Therefore, there were some families who did not have any data available for fathers (n = 15) or mothers (n = 1). Other family members were missing particular measures. For example, the CMS/WMS-III, CTOPP, and Woodcock Johnson measures were added mid-way through FSA data collection. Therefore, there are considerable missing data for families who were seen in the earlier part of the study on these measures. Some of these parents were seen back at a later time and we were able to fill in missing data for a number of families in this manner.

The SSC, on the other hand, had strict requirements regarding acceptable levels of missing data. This requirement for data completeness was maintained in the follow-up evaluation of parents and siblings. Because of the significant discrepancy in missing data

between the multiplex and simplex sample, all analyses will report *n*'s and/or *df*'s for readers to determine how many family members had data for that particular variable.

Statistical Analyses

Data from mothers, fathers, and undiagnosed siblings in multiplex and simplex families were analyzed. We were only able to assess one unaffected sibling in the simplex sample despite there often times being multiple unaffected siblings in a household. In contrast, assessments were conducted on multiple unaffected siblings within 13 families in the multiplex sample. In order to better equate the two samples, we selected the sibling with the most available measures completed to participate in analyses for the 13 families with data for multiple unaffected siblings. When siblings completed an equal number of measures, the participating sibling was selected at random.

Aim 1. To obtain a multifaceted assessment of the broader autism phenotype in unaffected parents and siblings within simplex and multiplex families.

The primary aim was to assess and compare a number of different aspects of the broader autism phenotype in ASD parents and siblings within simplex and multiplex families. Outcome variables were social/communication skills and range of interests via the BPASS, cognitive abilities and variability in cognitive profiles via the Wechsler scales of intelligence, central coherence via the Block Design subtest of the Wechsler scales, phonological short-term memory via the CTOPP, and face memory via the WMS and CMS. There were no group differences on the object memory task between simplex and multiplex mothers, $t(72) = 0.63$, $p = .54$, fathers, $t(67) = 1.14$, $p = .26$, and siblings $t(64) = 1.76$, $p = .08$. Given that object memory was unrelated to face memory in our sample and did not differ significantly among groups, it was not entered as a covariate in face memory analyses.

Multivariate analyses of variance were run for each measure with simplex versus multiplex status included as a fixed factor. We ran separate models due to the varied amount of missing data across measures. Planned contrasts using independent samples *t* tests compared simplex mothers to multiplex mothers, simplex fathers to multiplex fathers, and simplex siblings to multiplex siblings.

As discussed, prior research suggests that broader autism phenotype traits are present in only a subset of ASD parents and siblings. Therefore, proportions of family members showing evidence for impairment was compared among simplex and multiplex families. Chi square analyses were used to compare the percentage of high scores on the BPASS Social Interest and Range of Interests/Flexibility domains, below average scores on the WMS-III/CMS face memory and CTOPP nonword repetition tasks, and significant variability in cognitive abilities on the Wechsler tests across mothers, fathers, and siblings within multiplex and simplex families.

Aim 2. To increase an understanding of head circumference in unaffected parents and siblings within simplex versus multiplex families

A secondary aim was to increase understanding of head circumference in relatives of individuals with ASD. Head circumference *z* scores were compared in mothers, fathers, and siblings in simplex versus multiplex families using multiple independent *t* tests. Although height was related to head circumference in our sample, it did not differ across mothers, $t(105) = 0.48$, $p = .64$, fathers, $t(89) = 1.00$, $p = .32$, and siblings, $t(80) = 0.59$, $p = .56$, in multiplex versus simplex samples. Therefore, it was unnecessary to include height in the model.

Aim 3. To assess the relation between head circumference and degree of expression of the broader autism phenotype in family members within simplex and multiplex families

One exploratory aim of the project was to examine the relationship between head circumference and degree of expression of the broader autism phenotype in ASD parents and siblings. We first examined overall correlations between head circumference and BPASS: Social Affect/BPASS: Range of Interests/Flexibility domains since this area has been very understudied in the literature. Multiple regressions were then run for each set of family members to examine this relationship within the desired comparison groups. BPASS: Social Affect and BPASS: Range of Interests/Flexibility scores were predicted by head circumference *Z* scores and simplex versus multiplex status. The two independent variables were entered simultaneously in the model.

Aim 4. To determine the relation between affected child characteristics and broader autism phenotype traits in family members within simplex and multiplex families

A final aim was to determine the relationship between affected child symptoms and presence of broader autism phenotype traits in mothers, fathers, and siblings of individuals with ASD in simplex and multiplex families. Separate multiple regressions for each contrast (i.e., mothers, fathers, and siblings) were run predicting BPASS: Social Affect, BPASS: Range of Interests/Flexibility, and BPASS: Overall Composite from simplex versus multiplex status as well as Proportion Severity scores for the affected child(ren) in the family in corresponding ADOS composites: ADOS: Social Affect, ADOS: Restricted and Repetitive Behaviors, and ADOS: Total score. Both independent variables were entered simultaneously into the model.

CHAPTER 3

RESULTS

Aim 1

BPASS

Mean scores, standard deviations, and number of family members with valid BPASS data are reported in Table 4. Results of the MANOVA for the four BPASS domains suggest significant differences across domains. Specifically, there was a significant main effect of simplex versus multiplex status on BPASS Social Interest, $F(1, 324) = 13.94$, $p < .001$, Expressiveness, $F(1, 324) = 28.31$, $p < .001$, Conversational Skills, $F(1, 324) = 11.43$, $p = .001$, and Range of Interests/Flexibility, $F(1, 324) = 3.96$, $p = .05$.

Planned contrasts using independent *t* tests suggest that differences exist in the BPASS Social Interest domain between simplex and multiplex mothers, $t(123) = -2.51$, $p = .01$, Cohen's $d = 0.48$, and fathers, $t(109) = -2.15$, $p = .03$, Cohen's $d = 0.42$, with a trend in differences observed in siblings, $t(96) = -1.81$, $p = .07$, Cohen's $d = 0.38$. All findings indicate decreased levels of social interest in multiplex family members compared to simplex family members. A similar pattern of results was observed in the BPASS Expressiveness domain. Simplex mothers were significantly more expressive in their use of nonverbal communication compared to multiplex mothers, $t(122) = -2.37$, $p = .02$, Cohen's $d = 0.47$, as were simplex fathers, $t(108) = -2.78$, $p = .006$, Cohen's $d = 0.57$, and siblings, $t(98) = -4.54$, $p < .001$, Cohen's $d = 0.98$. The BPASS Conversational Skills domain revealed differences between fathers, $t(108) = -2.28$, $p = .03$, Cohen's $d = 0.47$, and siblings, $t(108) = -2.04$, $p = .04$, Cohen's $d = 0.74$, but not mothers. The significant overall MANOVA result for the Range of Interests/Flexibility domain appeared to have been driven by the siblings given that simplex mothers and fathers did not differ

significantly on this domain. Again, multiplex siblings showed significantly greater rigidity and intense interests compared to simplex siblings, $t(96) = -2.30$, $p = .02$, Cohen's $d = 0.48$. Figures 3 – 6 depict the distribution of BPASS scores across domains and family members.

Table 4. Descriptive Statistics for BPASS Domains in Simplex and Multiplex Family Members

	Mother				Father				Unaffected Child			
	Simplex		Multiplex		Simplex		Multiplex		Simplex		Multiplex	
	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)
BPASS: Social Interest	41	2.17 (0.73)	84	2.52 (0.72)	40	2.50 (0.78)	71	2.82 (0.76)	41	1.88 (0.71)	57	2.18 (0.90)
BPASS: Expressiveness	41	1.22 (0.31)	83	1.38 (0.38)	40	1.38 (0.37)	70	1.61 (0.45)	40	1.16 (0.22)	60	1.52 (0.46)
BPASS: Conversational Skills	41	1.23 (0.41)	83	1.33 (0.42)	40	1.29 (0.44)	70	1.54 (0.60)	40	1.11 (0.31)	60	1.36 (0.72)
BPASS: Range of Interests/Flexibility	41	2.30 (0.61)	83	2.32 (0.56)	40	2.31 (0.59)	71	2.40 (0.56)	41	1.95 (0.45)	57	2.22 (0.63)

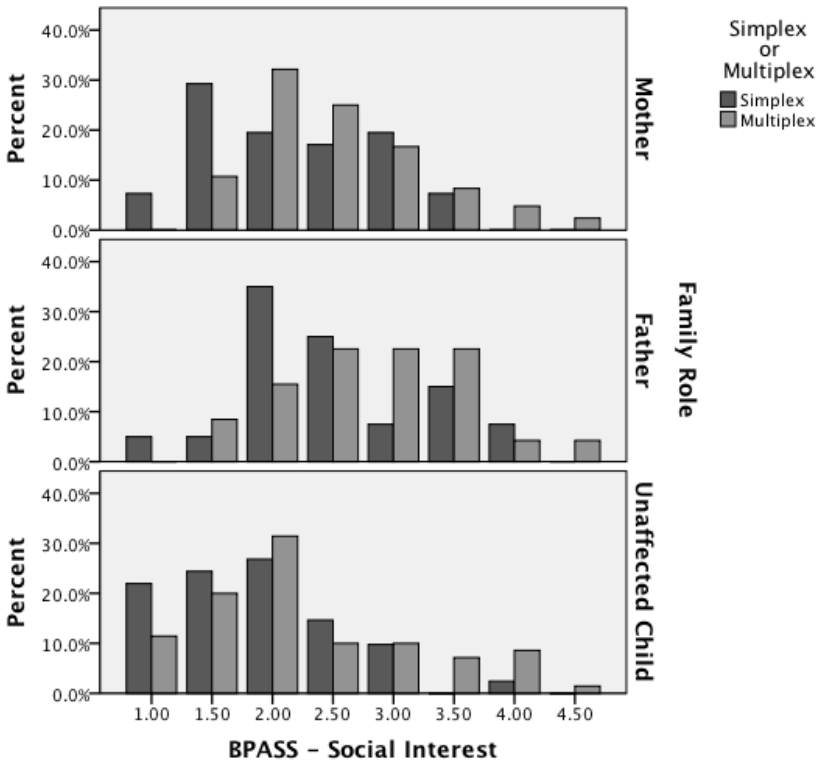


Figure 3. Distribution of BPASS: Social Interest domain scores for mothers, fathers, and unaffected siblings.

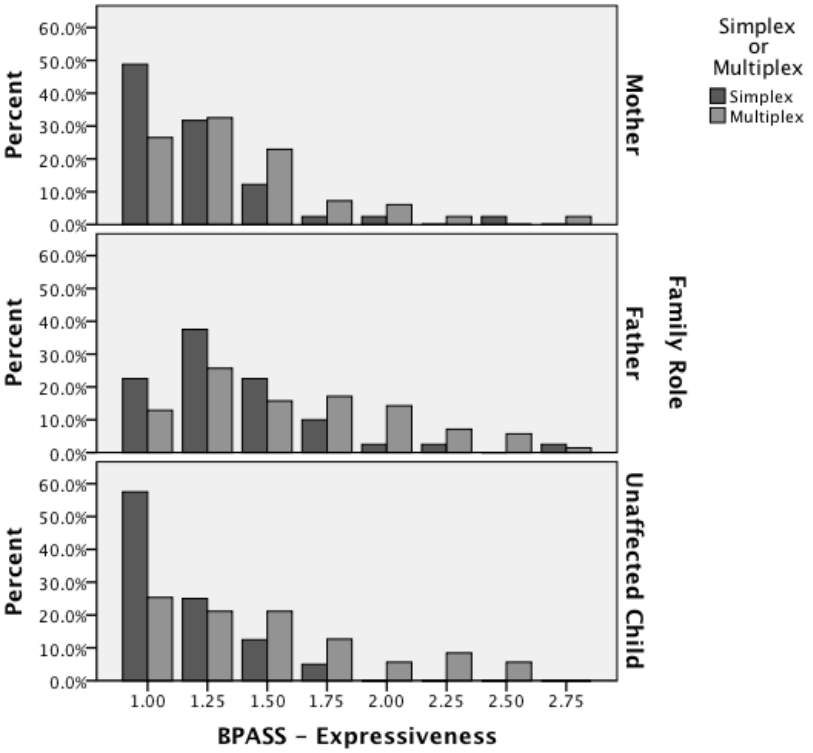


Figure 4. Distribution of BPASS: Expressiveness domain scores for mothers, fathers, and unaffected siblings.

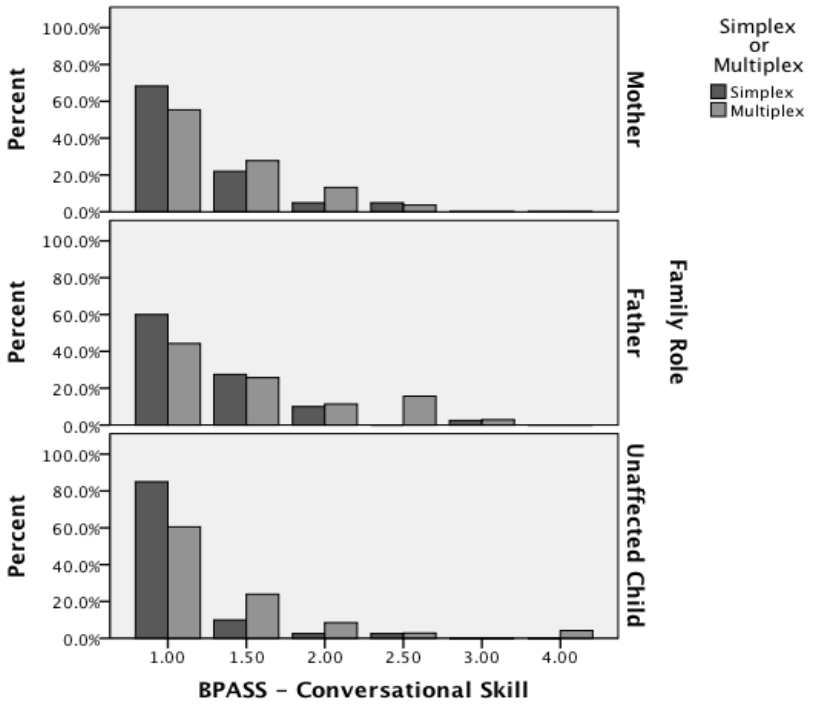


Figure 5. Distribution of BPASS: Conversational Skills domain scores for mothers, fathers, and unaffected siblings.

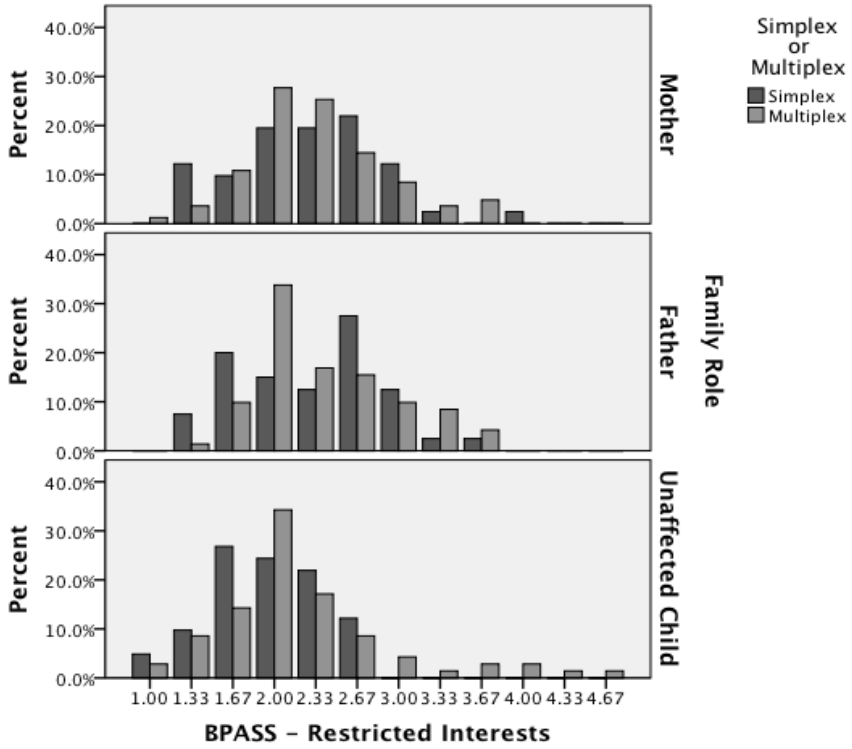


Figure 6. Distribution of BPASS: Range of Interests/Flexibility domain scores for mothers, fathers, and unaffected siblings.

The percentage of high scores (suggesting greater impairment) on the BPASS Social Interest and Restricted Interests domains were compared across simplex and multiplex families. Figure 7 shows proportions of high scores on these domains across family members. A chi square analysis indicated that a significantly greater percentage of multiplex family members were rated as having low to very low social interest compared to simplex family members, $\chi^2(1, N = 334) = 14.42$, $p < .001$. 66% of multiplex fathers received at least one high score on the BPASS: Social Interest domain compared to 33% of simplex fathers, $\chi^2(1, N = 111) = 11.70$, $p = .001$. 39% of multiplex siblings were rated highly compared to 22% of simplex siblings, $\chi^2(1, N = 98) = 3.06$, $p = .08$, which suggests a trend for multiplex siblings to show low levels of social interest compared to simplex siblings. Simplex mothers did not differ from multiplex mothers in the proportion of individuals showing low social interest (32% compared to 44%, respectively).

In the BPASS: Range of Interests/Flexibility domain, multiplex siblings more often had impairing repetitive behaviors compared to simplex siblings, 21% versus 5%, $\chi^2(1, N = 98) = 5.10$, $p = .02$. Mothers and fathers did not differ in their flexibility and range of interests.

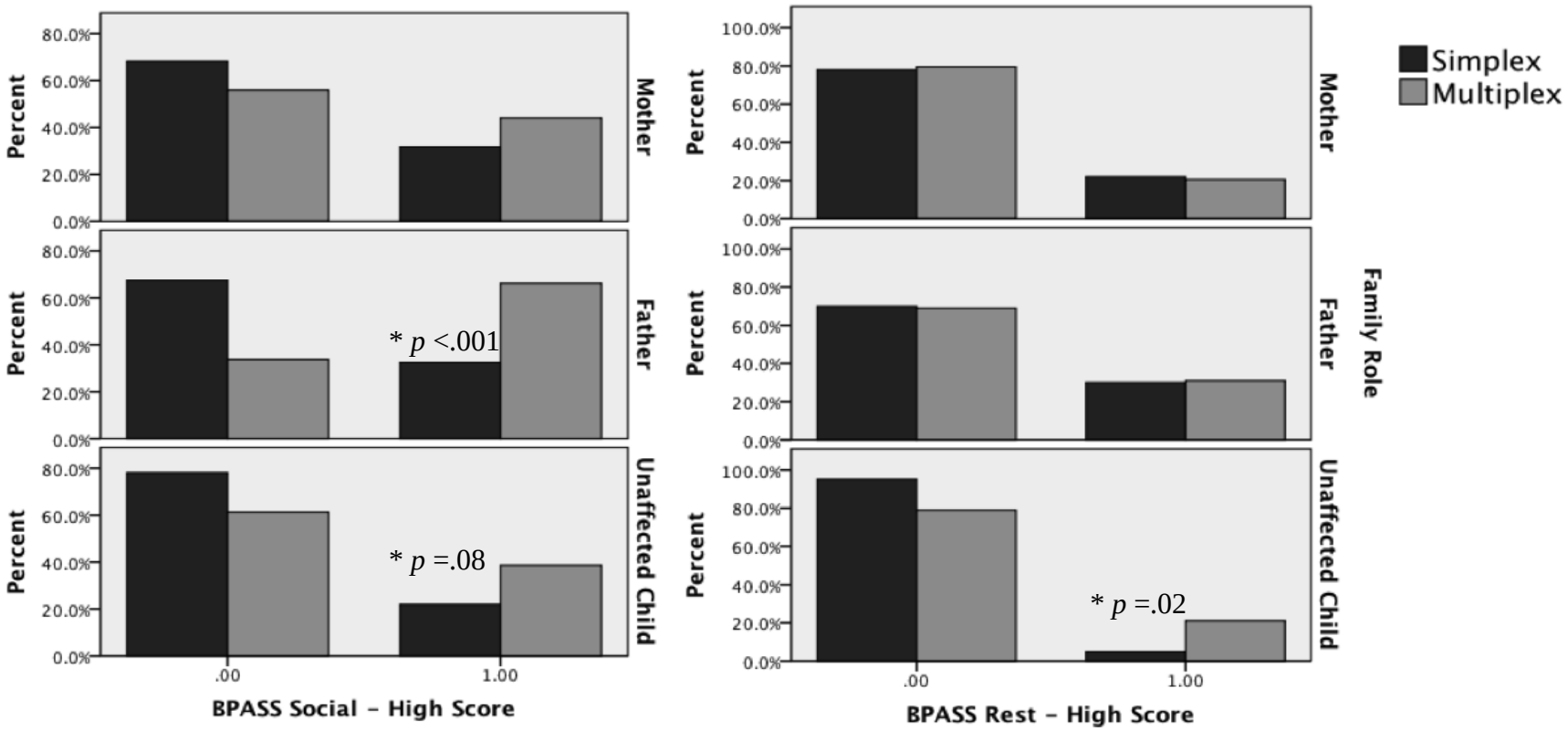


Figure 7. Bar graph showing percent of high scores on the BPASS: Social Interest and BPASS: Range of Interests/Flexibility domains for mothers, fathers, and unaffected siblings.

Cognitive Measures

Mean scores, standard deviations, and number of family members with valid data for the various cognitive measures are reported in Table 5. There is a significant amount of missing data in the multiplex sample for the three memory measures: Face Memory: Immediate, Face Memory: Delayed, and Phonological Short-Term Memory. Therefore, separate MANOVAs for the variables on the Wechsler scales and face memory tasks were run to maximize the available data. The nonword repetition task was analyzed using independent *t* tests since there is only one variable of interest from this measure.

IQ Measures

Data from 120 simplex family members and 210 multiplex family members were analyzed. Composite IQ scores differed significantly in simplex versus multiplex families. Significant differences were observed in full scale IQ, $F(1, 328) = 7.71$, $p = .006$, nonverbal IQ, $F(1, 328) = 3.96$, $p = .05$, and verbal IQ, $F(1, 328) = 9.01$, $p = .003$. However, scores on the Block Design subtest did not differ across groups.

Mean IQ scores were in the average to high average range in both samples. However, contrasts revealed significantly higher verbal IQs in simplex mothers, $t(121) = 2.03$, $p = .04$, Cohen's $d = 0.41$, and siblings, $t(104) = 2.89$, $p = .005$, Cohen's $d = 0.56$, compared to multiplex relatives. Nonverbal IQ was lower in multiplex fathers compared to simplex fathers, $t(109) = 2.29$, $p = .02$, Cohen's $d = 0.47$. Differences in full scale IQ scores between individual family members and across family types did not reach statistical significance. Figures 8 – 10 show the distributions of simplex and multiplex family members on IQ composites. There were no differences on the Block Design subtest of the Wechsler scales in any contrast.

Table 5. Descriptive Statistics for Neurocognitive Measures in Simplex and Multiplex Family Members

	Mother				Father				Unaffected Child			
	Simplex		Multiplex		Simplex		Multiplex		Simplex		Multiplex	
	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)
Full Scale IQ	40	115.08 (10.54)	86	110.20 (14.63)	39	117.23 (11.71)	72	112.49 (14.81)	41	113.02 (12.94)	66	106.68 (19.60)
Nonverbal IQ	41	113.07 (12.68)	86	109.35 (15.97)	40	119.53 (12.13)	71	112.65 (16.67)	41	111.10 (14.21)	66	109.53 (18.67)
Verbal IQ	40	113.83 (9.88)	83	108.98 (13.42)	39	111.49 (11.77)	68	110.00 (14.75)	41	111.93 (11.48)	65	102.11 (19.73)
Block Design T Score	41	56.68 (9.64)	86	55.83 (10.64)	40	60.78 (9.19)	71	59.56 (11.57)	41	58.32 (10.84)	61	57.05 (14.01)
Face Memory: Immediate	41	11.76 (3.12)	58	11.57 (3.15)	39	10.67 (2.79)	47	10.53 (2.43)	41	9.37 (2.91)	29	8.38 (3.51)
Face Memory: Delayed	41	11.59 (2.37)	55	11.91 (2.91)	39	11.13 (2.52)	44	10.30 (2.43)	41	9.37 (2.18)	26	7.81 (3.32)
Nonword Repetition	38	8.03 (1.95)	31	7.16 (2.61)	39	7.56 (2.00)	28	7.07 (2.24)	41	9.49 (2.20)	24	8.13 (2.19)

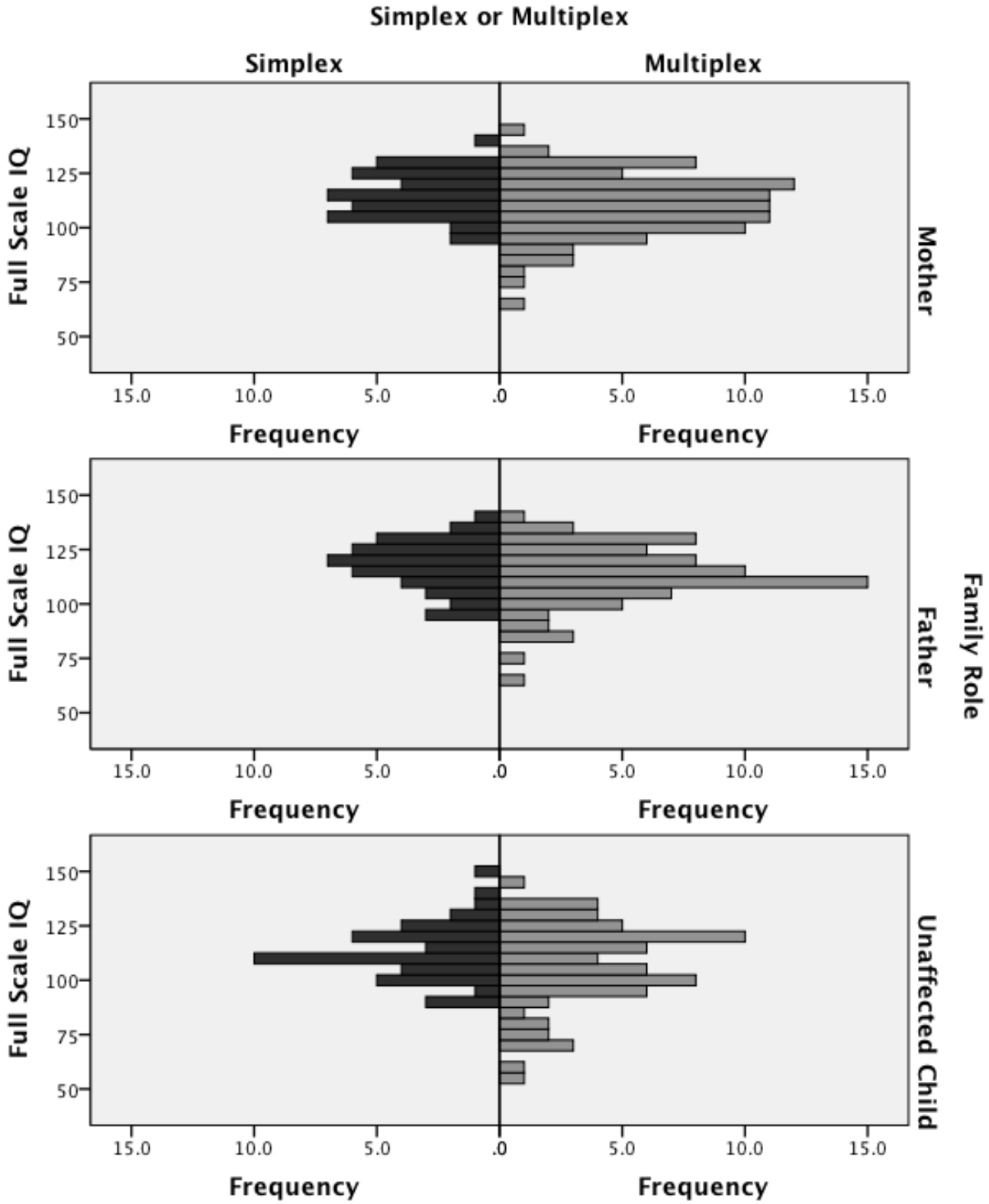


Figure 8. Frequency distributions of full scale IQs of mothers, fathers, and siblings in multiplex and simplex families.

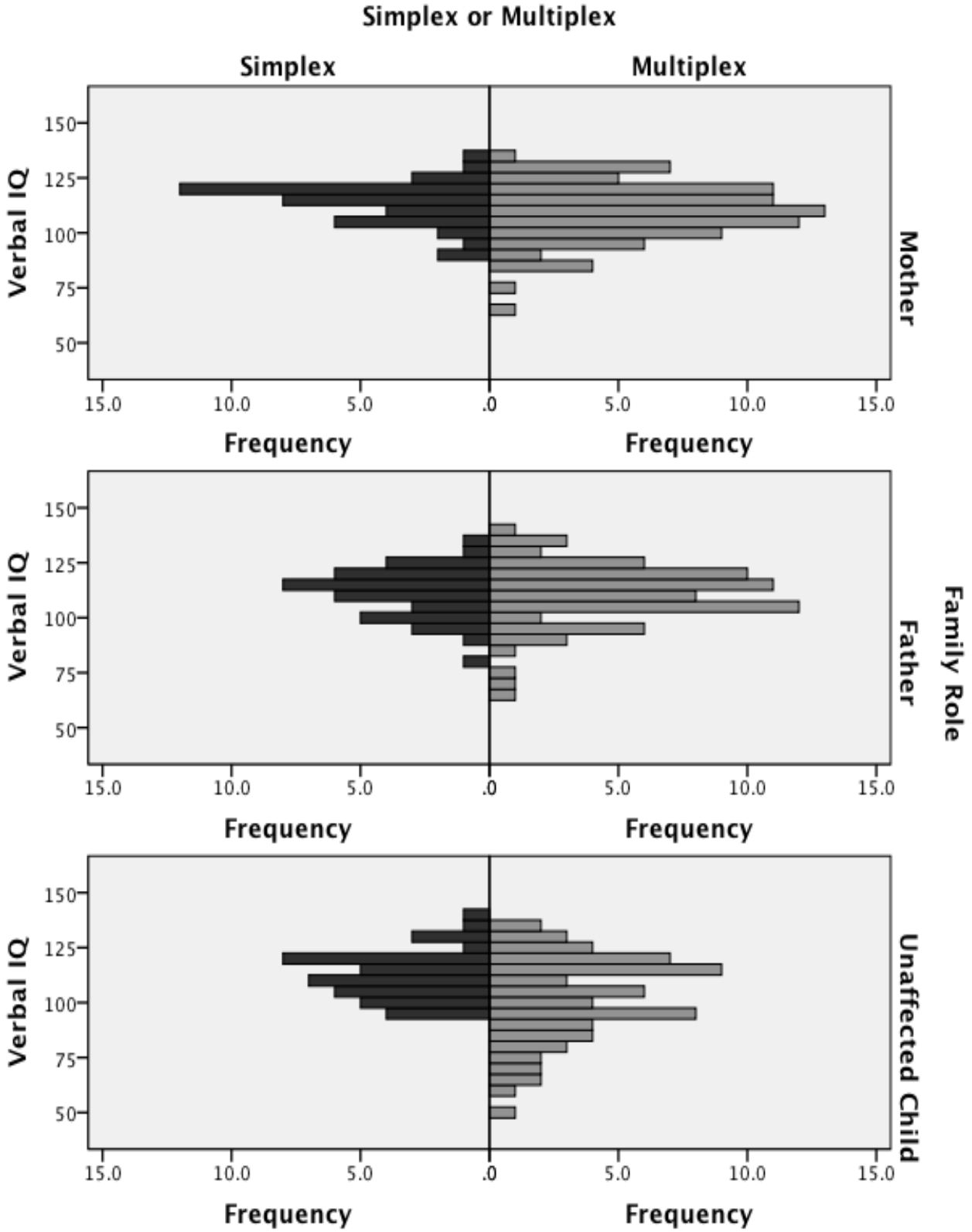


Figure 9. Frequency distributions of verbal IQs of mothers, fathers, and siblings in multiplex and simplex families.

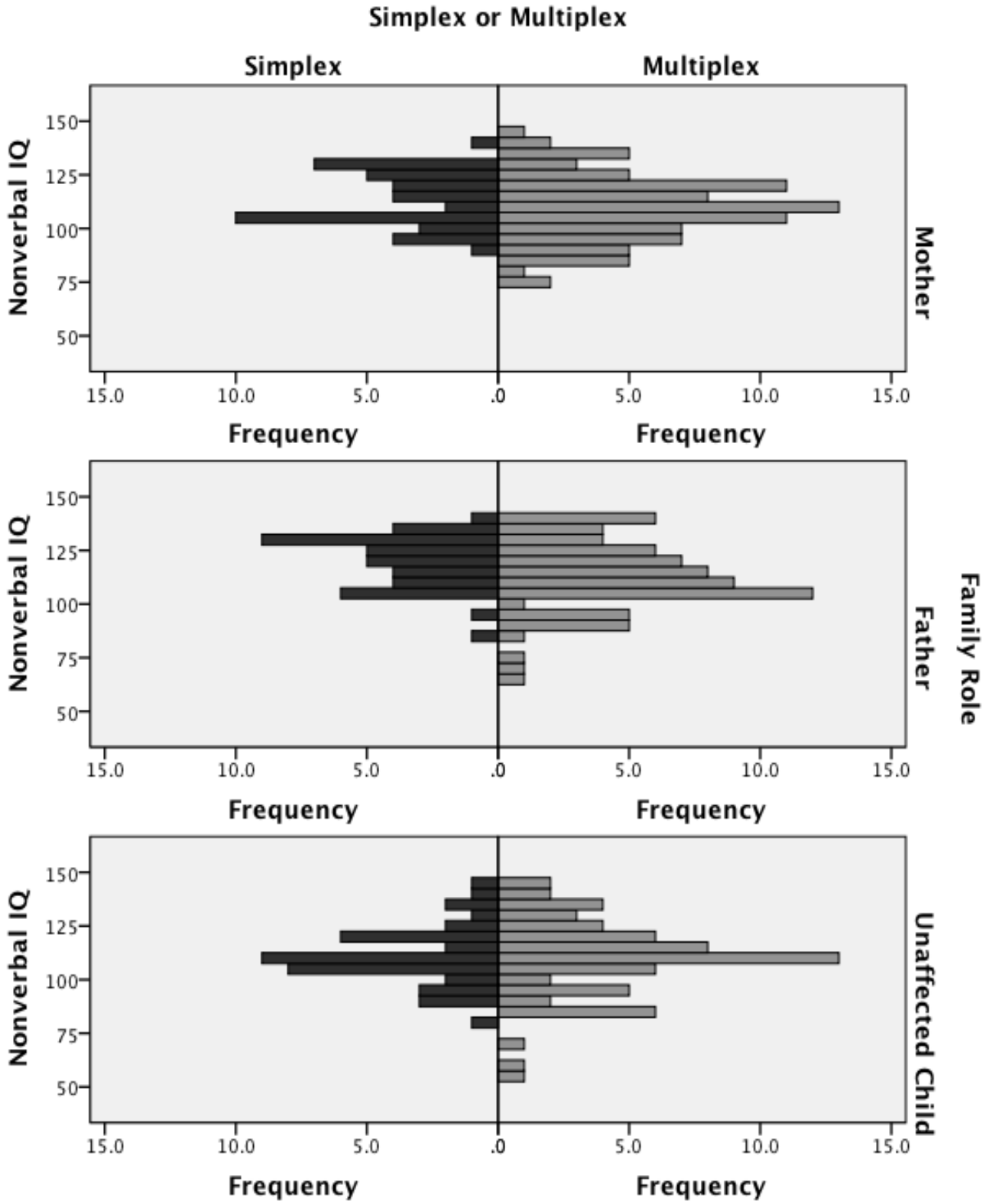


Figure 10. Frequency distributions of nonverbal IQs of mothers, fathers, and siblings in multiplex and simplex families.

Proportions of family members with significant variability in their cognitive profiles were also analyzed. 62% of multiplex family members versus 48% of simplex family members had a significant difference between their verbal and nonverbal IQ composites, $\chi^2(1, N = 330) = 5.74$, $p = .02$. This difference was mostly driven by multiplex siblings who more frequently had a significant difference in cognitive abilities than simplex siblings, 60% versus 34%, $\chi^2(1, N = 101) = 6.51$, $p = .01$. Figure 11 depicts the percentage of family members with significant IQ discrepancies. Table 6 shows whether the verbal or nonverbal IQ was higher across family types for family members who had significant IQ discrepancies.

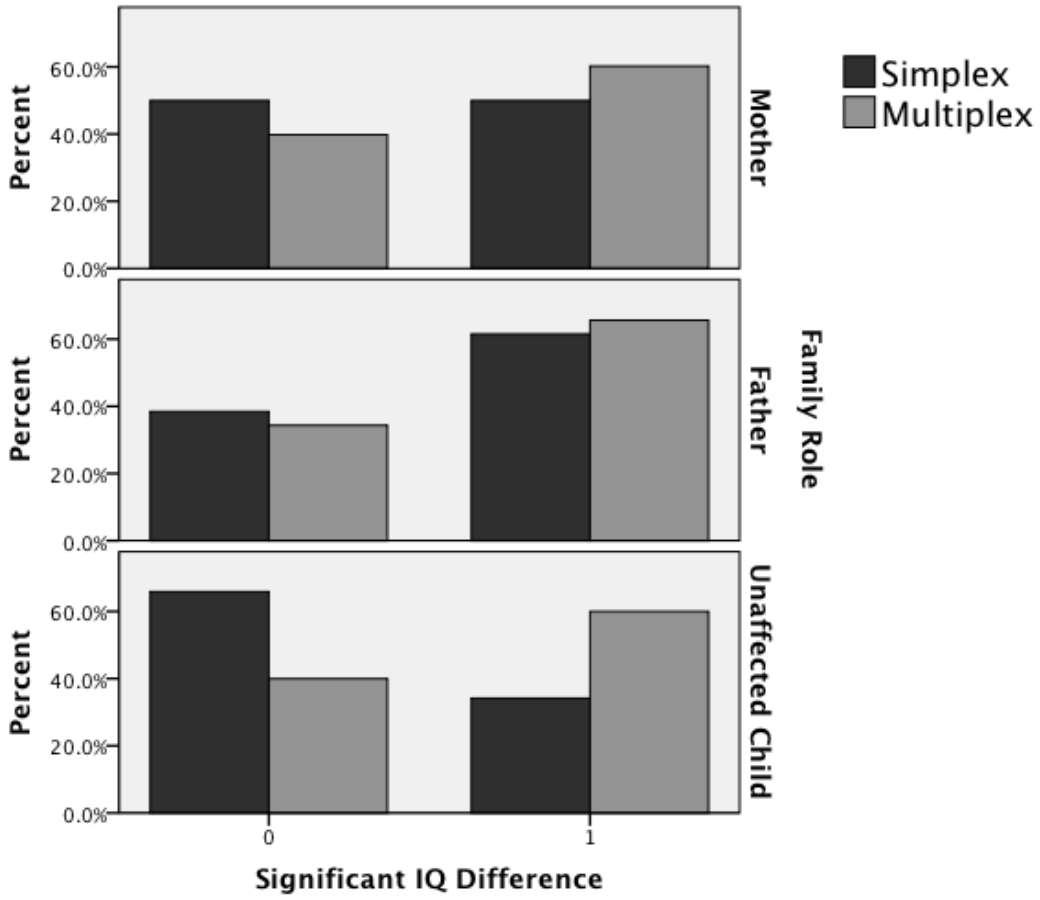


Figure 11. Bar graph showing percentage of mothers, fathers, and siblings in multiplex and simplex families with significant IQ discrepancies.

Table 6. Direction of Effect for IQ Discrepancies in Family Members.

	VIQ>NVIQ				NVIQ>VIQ			
	Simplex		Multiplex		Simplex		Multiplex	
	n	%	*n*	%	*n*	%	*n*	%
Mother	12	60%	22	44%	8	40%	28	56%
Father	4	17%	17	39%	20	83%	27	61%
Unaffected Child	8	57%	11	31%	6	43%	25	69%

Phonological Short-Term Memory

There were substantial missing data in the multiplex sample for the Nonword Repetition subtest of the CTOPP assessing phonological short-term memory. Data were available for 148 simplex family members and 83 multiplex family members. Overall, scores for both multiplex and simplex parents were in the below average range (mothers: $M = 7.64$, $SD = 2.29$, fathers: $M = 7.36$, $SD = 2.10$). However, there were no mean differences between simplex and multiplex parents. Multiplex siblings scored significantly lower on the Nonword Repetition subtest than simplex siblings, $t(63) = 2.41$, $p = .02$, Cohen's $d = 1.22$, suggesting greater impairment in phonological short-term memory. Additionally, a greater percentage of scores for multiplex siblings were in the below average range compared to simplex siblings (42% versus 12%), $\chi^2(1, N = 65) = 7.41$, $p = .006$. Proportions of low scores were comparable for mothers and fathers.

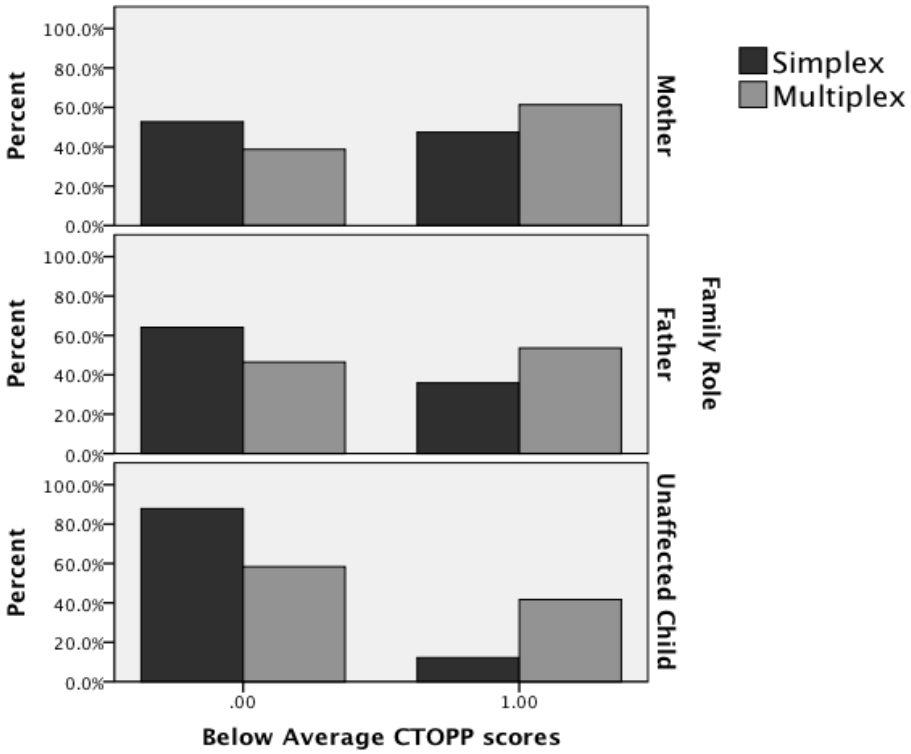
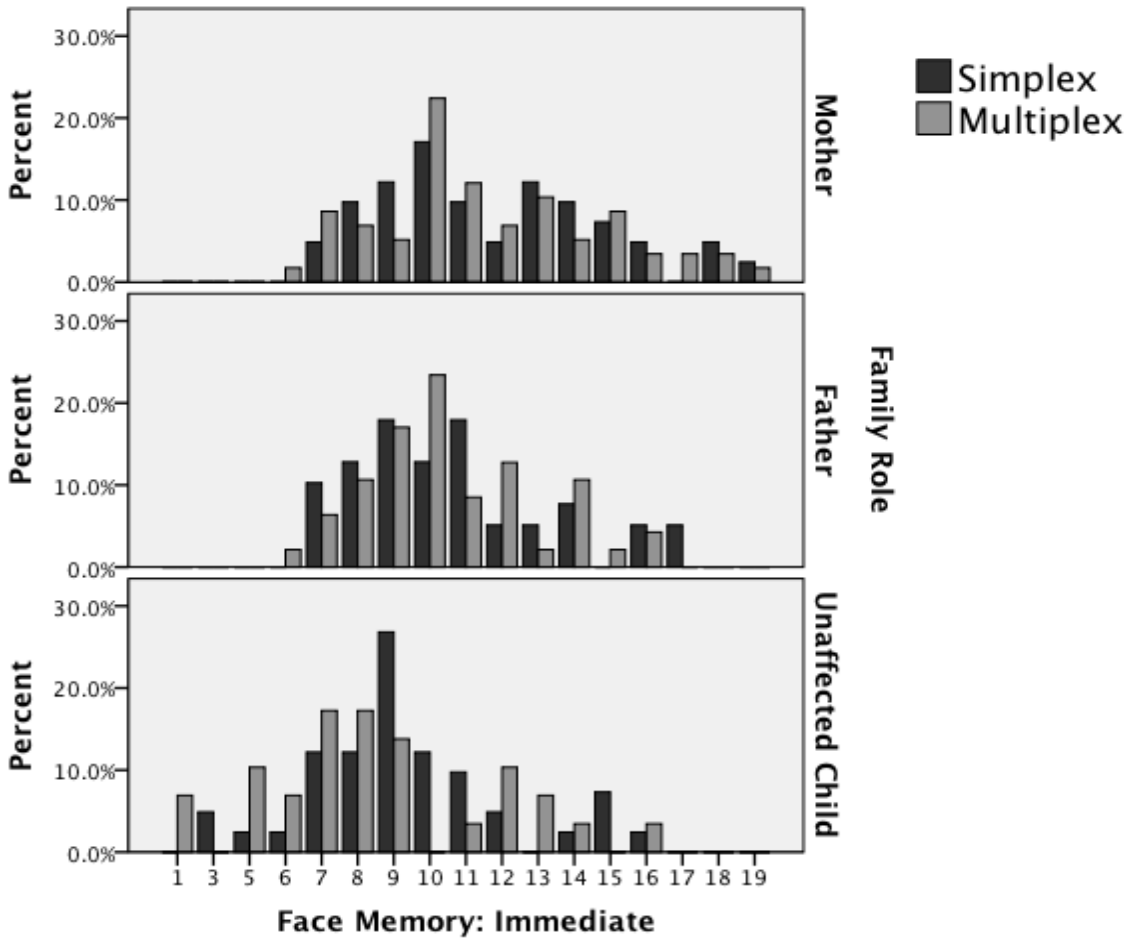
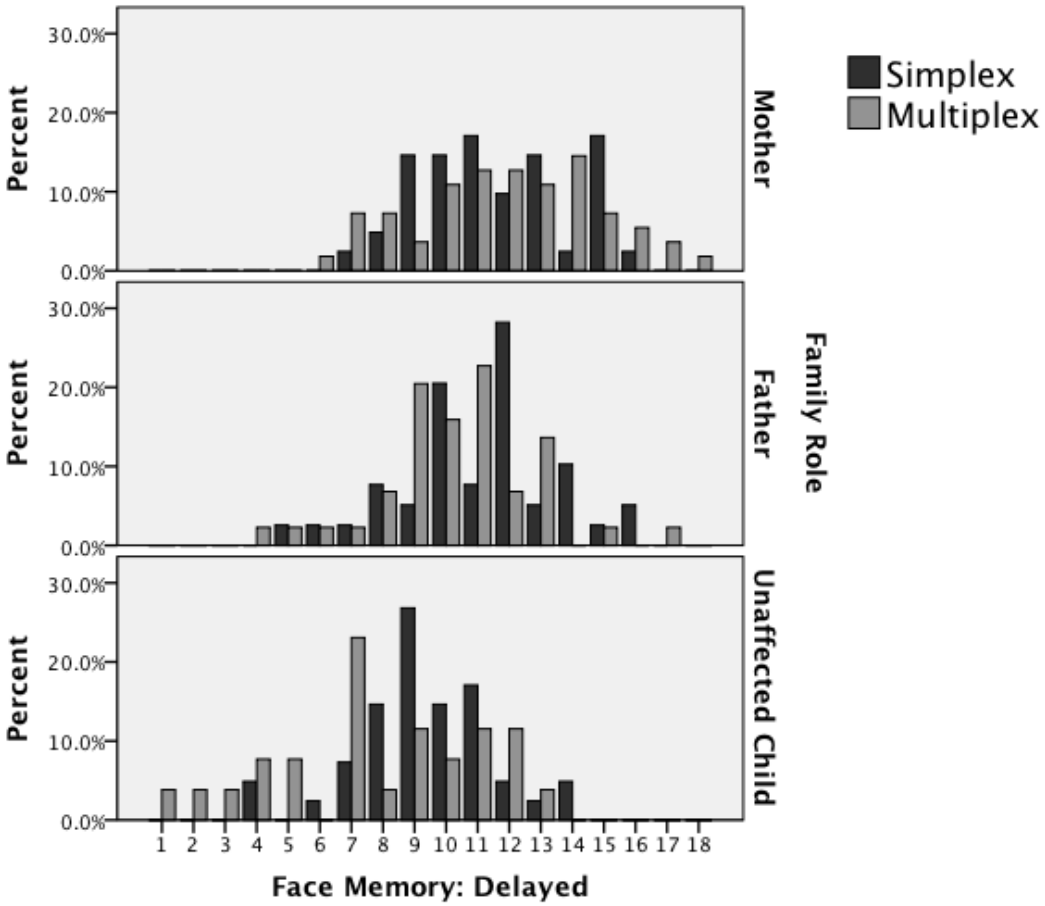


Figure 12. Proportion of below average CTOPP: Nonword Repetition scores for mothers, father, and siblings in multiplex versus simplex families.

Face Memory

As with the Nonword Repetition subtest of the CTOPP, there was a large amount of missing data in the multiplex sample for the face memory subtests of the WMS-III and CMS. Data were available for 121 simplex family members and 125 multiplex family members. There were no significant mean differences between groups. However, multiplex siblings were overrepresented in the lower range of scores on both the Face Memory: Immediate and Delayed subtests. Specifically, 22% of simplex siblings versus 41% of multiplex siblings, $\chi^2(1, N = 70) = 3.05$, $p = .08$, scored in the below average range on Face Memory: Immediate and 14% versus 50% respectively, $\chi^2(1, N = 67) = 9.80$, $p = .002$, scored in the below average range on Face Memory: Delayed. Figures 13 and 14 show the distribution of scores of these subtests.


Simplex
Multiplex
Family Role
Mother
Father
Unaffected Child
Percent
30.0%
20.0%
10.0%
0.0%
1 3 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Face Memory: Immediate



Figures 13 and 14. Distributions of Face Memory: Immediate and Delayed for mothers, fathers, and siblings in simplex and multiplex families.

Aim 2

Z scores for head circumference measurements were calculated based on age- and sex-based normative data (Roche et al., 1987). *Z* scores ranged from -3.42 to 6.42 and 40% of the entire sample had head circumference measurements at one standard deviation or greater than the norm. However, there were no mean differences in head circumference measurements between multiplex and simplex family members and both groups were equally likely to have measurements above one standard deviation (see Figure 15). Table 7 shows means, standard

deviations, and numbers of valid cases for the simplex and multiplex samples. Figure 16 shows distributions of head circumference scores.

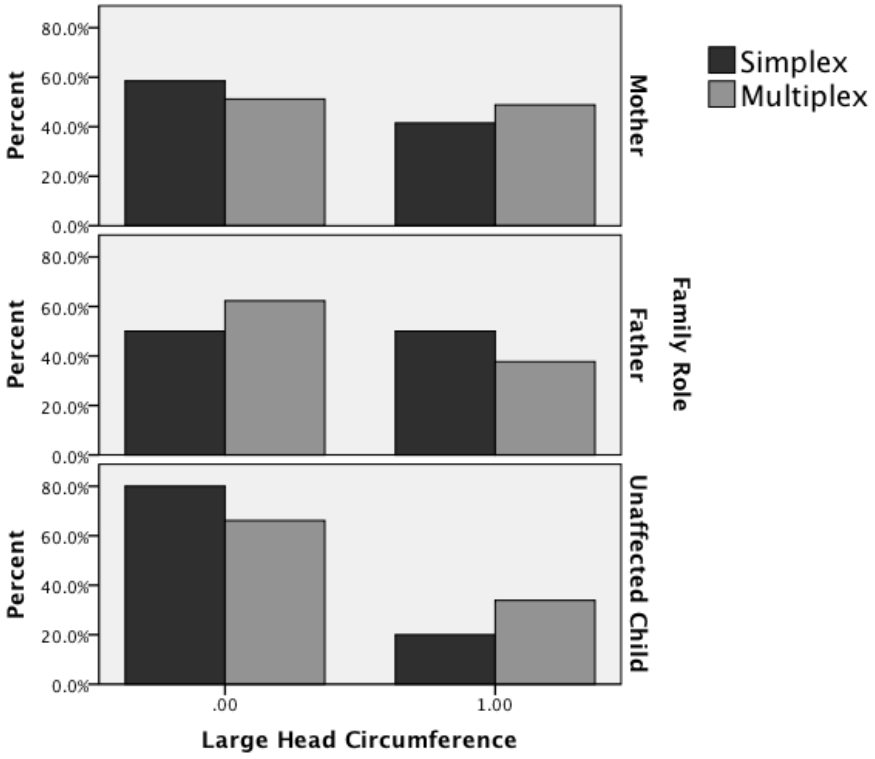


Figure 15. Proportions of multiplex and simplex family members with head circumferences at least one standard deviation above the norm.

Table 7. Head Circumference *Z* scores for Mothers, Fathers, and Siblings in Simplex and Multiplex Families

	Head Circumference *Z* scores			
	Simplex		Multiplex	
	Valid *N*	Mean (SD)	Valid *N*	Mean (SD)
Mothers	41	0.86 (1.38)	84	1.10 (1.54)
Fathers	40	1.07 (0.71)	69	0.77 (0.91)
Siblings	40	0.18 (1.15)	62	0.69 (1.39)

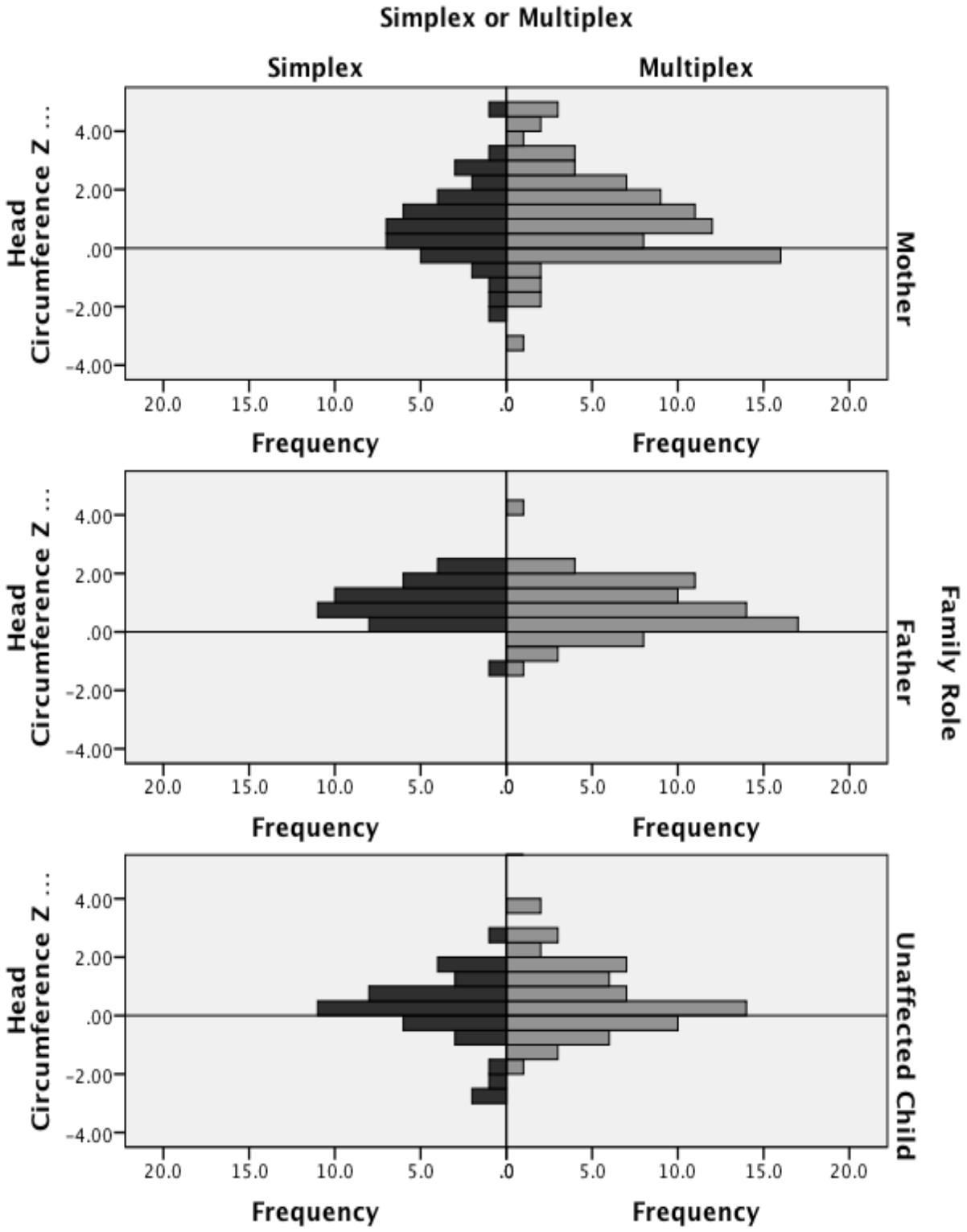


Figure 16. Frequency distributions of head circumference *Z* scores of mothers, fathers, and siblings in multiplex and simplex families.

Aim 3

To assess the relationship between head circumference and ASD-related symptoms, scores on the BPASS: Social Affect (the average of the three social and communication domains scores on the BPASS) and BPASS: Range of Interests/Flexibility domain were analyzed. Results of multiple regressions with simplex versus multiplex status and head circumference entered simultaneously are reported in Table 8.

A small positive relationship between head circumference *Z* score and BPASS: Social Affect domain was noted in family members across groups, $r(314) = .10$, $p = .08$, such that larger head circumference was related to higher Social Affect scores (suggesting decreased social interest and communication skills). However, a differential relationship in simplex and multiplex families was found only in siblings, $\beta = .22$, $t(85) = 2.15$, $p = .03$. Simplex versus multiplex status and head size explained a significant proportion of variance in BPASS: Social Affect, $R^2 = .18$, $F(2, 85) = 9.13$, $p < .001$. The positive relationship between sibling head size and social communication skills on the BPASS was stronger in simplex siblings compared to multiplex siblings. See Figure 17.

BPASS: Range of Interests/Flexibility domain score was unrelated to head circumference across the entire sample, $r(319) = .03$, $p = .57$. Regression models also did not suggest that multiplex and simplex family members had a differential relationship between head circumference and repetitive behaviors and interests, as measured by the BPASS. See Table 8.

Table 8. Regression Results for the Relationship Between Head Size and BPASS: Social Affect and Range of Interests/Flexibility.

	Mothers				Fathers				Unaffected Children			
	B	*SE B*	β	*N*	*B*	*SE B*	β	*N*	*B*	*SE B*	β	*N*
BPASS: Social Affect				122				106				88
Constant	1.54	0.06			1.76	0.09			1.37	0.07		
Head Circumference	0.01	0.02	.03		-0.03	0.05	-.06		0.07	0.03	.22^{+}	
Simplex v. Multiplex	0.20	0.07	.25*		0.24	0.09	.26*		0.29	0.09	.33*	
BPASS: Range of Interests/Flexibility				122				107				92
Constant	2.33	0.10			2.33	0.11			1.95	0.09		
Head Circumference	-0.03	0.04	-.08		-0.02	0.07	-.03		0.05	0.04	.12	
Simplex v. Multiplex	0.02	0.11	.02		0.06	0.16	.05		0.21	0.12	.19	

Note $^{+}p < .05$, $^{*}\ p < .01$

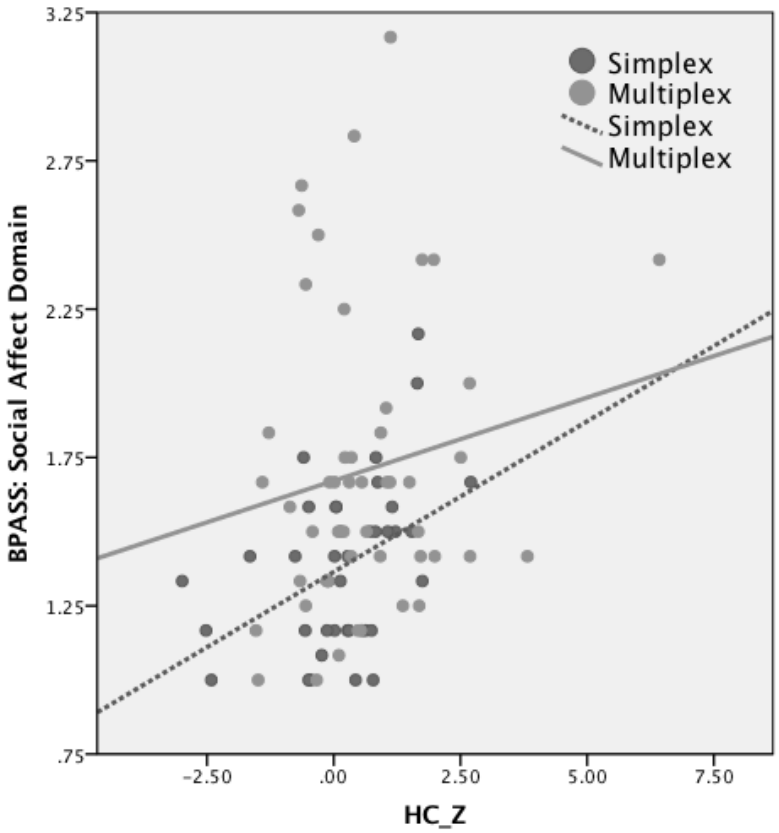


Figure 17. Scatter plot showing the relationship between head circumference and BPASS: Social Affect domain (average of three BPASS social and communication domains) in simplex and multiplex siblings.

Aim 4

The relationship between autism symptoms in the affected child(ren) and family member ASD-related traits was examined. Again, since few studies have addressed this research question, sample-wide correlations between affected child characteristics and whole family member traits were first calculated. As discussed in the Methods section, proportion severity scores were used for affected child characteristics and BPASS composites were used for family member traits. Proportion severity scores for affected children (ADOS: Total, ADOS: Social Affect, and ADOS: Restricted and Repetitive Behaviors) were unrelated to associated BPASS

domain scores (BPASS: Total, BPASS: Social Affect, BPASS: Range of Interests/Flexibility) in relatives, regardless of mother, father, or unaffected child status. Multiplex versus simplex status also did not reveal differential relationships between ASD-related traits in family members and autism symptoms in affected children. See Table 9.

Table 9. Regression Results for the Relationship Between Affected Child Symptoms and BPASS Scores.

	Mothers				Fathers				Unaffected Children			
	B	*SE B*	β	*N*	*B*	*SE B*	β	*N*	*B*	*SE B*	β	*N*
BPASS: Social Affect				119				105				87
Constant	1.70	0.12			1.78	0.15			1.33	0.16		
Proportion Severity Score: ADOS Social Affect	-0.29	0.18	-.15		-0.09	0.22	-.04		0.07	0.24	.04	
Simplex v. Multiplex	0.24	0.07	.30*		0.26	0.09	.28*		0.30	0.09	.34*	
BPASS: Range of Interests/Flexibility				119				106				93
Constant	2.25	0.15			2.24	0.16			1.95	0.16		
Proportion Severity Score: ADOS Restricted/Repetitive Total	0.14	0.26	.05		0.10	0.12	.08		-0.02	0.28	-.01	
Simplex v. Multiplex	0.02	0.12	.02		0.19	0.27	.07		0.28	0.13	.24	
BPASS: Overall Composite				119				105				87
Constant	1.70	0.12			1.78	0.15			1.47	0.14		
Proportion Severity Score: ADOS Total	-0.29	0.18	-.15		-0.09	0.22	-.04		0.09	0.24	.04	
Simplex v. Multiplex	0.24	0.07	.30**		0.26	0.09	.28*		0.30	0.09	.36**	

Note * $p < .01$, ** $p < .001$

CHAPTER 4

DISCUSSION

The main objective of this study was to conduct a comprehensive examination of the broader autism phenotype in family members from single-incidence (simplex) and multiple-incidence (multiplex) families. Areas of assessment included social communication, restricted interests and patterns of behavior, and cognitive abilities such as variability in IQ scores, central coherence, face memory, and phonological processing. These traits and skills were compared in mothers, fathers, and siblings within simplex and multiplex families. Physical attributes were also explored via a comparison of head circumference and behavioral correlates of head size among simplex and multiplex family members. A final goal of this study was to explore the relationship between characteristics of the affected child(ren) and family members in the two family types.

Recent genetic findings implicate potentially distinct genetic disease mechanisms in simplex and multiplex families such that sporadic genetic mutations are significantly more likely to occur in affected children in simplex families compared to multiplex families and controls (Marshall et al., 2008; Sanders et al., 2011; Sebat et al., 2007; Weiss et al., 2008). Understanding familial outcomes of these genetic events further elucidates autism etiology and increases understanding of the pathways between a genetic causal event and behavioral phenotype.

Aim 1

ASD-Related Traits

A comprehensive behavioral test battery was administered to unaffected family members within simplex and multiplex ASD families. The Broader Phenotype Autism Symptom Scale

(BPASS; Dawson et al., 2007) was used to assess social communication skills as well as presence of restricted interests and behaviors in undiagnosed relatives. The BPASS has the benefit of combining responses from a semi-structured clinical interview with behavioral observations from carefully trained clinicians who achieved research reliability on the measure. Adults who are cognitively capable are asked to report on their own social preferences, interests, and preferred organization of space and schedule. Parents provide responses about their children in these areas.

A recent study suggests that the BPASS differentiates parents of multiple children with ASD from parents of children with other developmental disabilities, parents of typically developing children, and parents who have just one child with ASD (Bernier et al., in press). However, this study reported on a small sample size that did not contain enough fathers within each group to explore sex differences in presentation of these BAP traits. Since sex differences in the BAP have been reported by a number of groups, it is an important research question. Additionally, undiagnosed siblings were not assessed and a careful family history to determine the presence of ASD in extended family members was not conducted in simplex ASD families. Results from the current study replicate and expand upon findings reported in this initial paper (Bernier et al., in press).

Overall, mothers and fathers from multiplex families were significantly more likely than mothers and fathers from simplex families to demonstrate decreased interest in interacting with peers (both in childhood and adulthood) as well as show low initiation of social interactions in group social situations. Additionally, 66% of multiplex fathers were rated as having low to very low social interest in at least one domain compared to only 33% of simplex fathers. Multiplex siblings showed a trend for decreased social interest as well and were significantly more likely to

be rated as having very low levels of social interest compared to simplex siblings (39% versus 22%, respectively).

Clinical observation of nonverbal social communication skills confirmed self- and parent-report of decreased social interest via the BPASS interview questions in multiplex versus simplex families. Mothers, fathers, and siblings from multiplex families less frequently used integrated eye gaze, social smiling, directed facial expressions, and typical prosody in their voices compared to the same family members in simplex families. Further, fathers and siblings from multiplex families offered excessive detail in conversation and failed to provide sufficient information in their verbal exchanges more often than fathers and siblings from simplex families. This suggests more impaired conversation skills in multiplex relatives compared to simplex relatives. Finally, siblings from multiplex families had, on average, more patterns of restricted behavior (e.g., preference for sameness in their schedule and physical space) and intense interests compared to simplex siblings and were more likely to be rated as having clinically impairing behaviors in these areas compared to simplex siblings (21% versus 5%, respectively). This pattern of increased repetitive behaviors and interests was not observed in mothers and fathers.

Findings on the BPASS of decreased social interest in multiplex parents and siblings compared to simplex relatives replicates previous studies using clinical interview and questionnaire-based methods via the SRS, which assesses social reciprocity traits in parents and siblings (Bernier et al., in press; Constantino, Zhang et al., 2010; Losh et al., 2008; Schwichtenberg et al., 2010; Virkud et al., 2009). Additionally, clinical observation of decreased expressiveness and impaired conversation skills in multiplex relatives, particularly males, replicates previous reports of comparative challenges in multiplex parents versus simplex parents using clinical interviews via the BPASS, MPAS-R, and FHI (Bernier et al., in press; Losh et al.,

2008; Szatmari et al., 2000). In general, differences in the BPASS were more likely to occur in fathers compared to mothers, supporting the role of sex differences in the differential broader phenotype presentation in simplex and multiplex families.

Cognitive Measures

An age-appropriate estimate of IQ was obtained for all family members via the Wechsler scales of intelligence (Wechsler, 1989, 1991, 1997a, 1999). Overall, simplex mothers and siblings had significantly higher verbal IQ scores than multiplex mothers and siblings and simplex fathers had higher nonverbal IQ scores than multiplex fathers. However, both groups as a whole had full scale, verbal, and nonverbal IQs in the average to high average ranges, replicating many prior reports of intact cognitive and intellectual abilities in relatives of individuals with ASD (e.g., Dawson et al., 2007; Fombonne et al., 1997b; Freeman et al., 1989; Gokcen et al., 2009; Lainhart et al., 2002; Szatmari et al., 1993).

Discrepancies between verbal and nonverbal IQ occurred in 57% of the sample. Nonverbal IQ was more often higher than verbal IQ and discrepancies were equally likely to occur in simplex and multiplex parents. However, multiplex siblings were significantly more likely to show a variable cognitive profile than simplex siblings (60% versus 34%, respectively), with nonverbal IQ more often being higher than verbal IQ.

The block design subtest of the Wechsler intelligence scales has been used in previous studies as a proxy for a cognitive processing style called central coherence. Weak central coherence involves a tendency to focus on details while sacrificing the gestalt/overall picture. Some groups have reported weak central coherence in individuals with ASD (Shah & Frith, 1983, 1993). We did not find a difference in standard scores on the block design subtest between simplex and multiplex relatives. Additionally, block design scores were generally in the average

to high average range, which was commensurate with other Wechsler subtest scores as well as overall IQ. These findings do not support the occurrence of weak central coherence in undiagnosed relatives of individuals with ASD and is consistent with a number of additional studies reporting intact central coherence in ASD relatives (Briskman et al., 2001; Losh et al., 2009; Nydén et al., 2011; Piven & Palmer, 1997; Scheeren & Stauder, 2008). Therefore, it is possible that central coherence is not part of the overall presentation the BAP in relatives.

Phonological processing, in particular, phonological short-term memory, was assessed using the nonword repetition subtest of the CTOPP (Wagner et al., 1999). Parents in both family types scored in the below average range as a whole, but scores did not differ across simplex and multiplex families. Multiplex siblings did, however, score significantly lower on this measure and were overrepresented in the below average range compared to simplex siblings. Specifically, 42% of multiplex siblings scored in the below average range compared to 12% of simplex siblings. A similar pattern was observed in the face memory measures. Despite a lack of mean difference between simplex and multiplex siblings on face memory, significantly more multiplex siblings had below average range facial recognition abilities than simplex siblings. No differences were observed in parents on face memory.

Aim 2

Head circumference was measured and compared in family members due to a consistent physical finding in ASD of larger brain volume and head size in individuals with ASD (Bailey et al., 1995; Courchesne et al., 2003; Dementieva et al., 2005; Deutsch & Joseph, 2003; Lainhart et al., 2006). Head circumference measurements were compared and *z* scores were generated from normative scores published in Roche et al. (1987) in order to contrast family members of varying ages.

Head circumference findings in the current study replicated previous reports (Fidler et al., 2000; Lainhart et al., 2006; Miles et al., 2000) of increased head size in ASD parents as 40% of the sample had head circumference measurements greater than one standard deviation above the norm. However, there were no differences in head size between multiplex and simplex parents and siblings and both groups were equally likely to have large head sizes. Thus, although head circumference does seem to be increased across family types, it may be not a specific phenotype of familial ASD as multiplex families were no more likely to have larger heads than simplex families.

Aim 3

The relationship between head circumference and social communication skills was assessed in the third aim of this project. Prior groups have reported inconsistent results in terms of the relationship between symptom severity and head size in children diagnosed with ASD (Dementieva et al., 2005; Fidler et al., 2000; Lainhart et al., 2006; Sacco et al., 2007). In the current study, we found a small positive relationship across the sample between head circumference and reported and observed social communication abilities on the BPASS. This partially replicates one prior study by Elder and colleagues (2008) reporting more negative social outcomes for infant siblings of older children with ASD who had faster rates of head circumference growth early in life. In the current study, the relationship was stronger in simplex than multiplex siblings.

Aim 4

In the final aim of the project, we examined the relationship between the symptom severity of the affected child(ren) in the family and BAP traits in family members. We predicted that the strength of the relationship would be more pronounced in family members within

multiplex families versus simplex families since genetic findings suggest that there may be more shared genetic variance and thus a higher genetic loading for traits among multiplex relatives than simplex relatives. Symptom severity of the affected children was assessed through three composite scores on the ADOS: Social Affect, Restricted Behavior, and Total Scores. Commensurate composite scores were used from the BPASS in family members. A single severity score for affected children was necessary to conduct analyses. Since multiplex families by definition had more than one affected child in the family, a single severity proportion score was calculated to represent overall symptom severity in families.

Results did not support a differential relationship between symptom severity of the affected child(ren) and BAP traits in family members in either multiplex or simplex families. Symptoms of the affected child and traits in family members were unrelated in both family types. This lack of relationship contradicts previous reports of a positive relationship between symptoms of the affected children and family member traits such that greater symptom severity in affected children predicted greater severity of BAP traits in ASD relatives (e.g., Pickles et al., 2000; Schwichtenberg et al., 2010).

Summary

In general, findings support a differential presentation of behavioral features of the broader autism phenotype in family members from multiplex families compared to simplex families. This was particularly relevant in social communication skills. Consistent with previous reports (Bernier et al., in press; Constantino, Zhang et al., 2010; Losh et al., 2008; Schwichtenberg et al., 2010; Virkud et al., 2009), multiplex family members showed decreased social interest, more impaired nonverbal communication abilities, and less flexible conversation

skills compared to simplex family members. Effects were moderate to large across skill areas and were most consistently observed in siblings, followed by fathers, and then mothers.

In the area of restricted and repetitive behaviors, multiplex siblings showed more impairing restricted patterns of behavior than simplex siblings. However, simplex parents did not differ from multiplex parents. A lack of difference between parents in these family types may be due to the fact that, despite an attempt to disentangle behaviors specific to child-rearing, the effects of having children with a disability who often benefit from increased structure overpowered previous preferences. These findings were similar to previous reports by Bernier and colleagues (in press) in which multiplex and simplex ASD parents and parents of children with non-ASD developmental disabilities showed similar preferences for increased structure in schedule and physical environment compared to parents of typically developing children.

Cognitive profiles seemed to present differently in parents compared to siblings. Specifically, differences between multiplex and simplex families in cognitive measures such as cognitive variability, face memory, and phonological processing were absent in parents, but were apparent in siblings. Multiplex siblings showed greater cognitive variability and were more likely to have impaired face memory and phonological processing skills compared to simplex siblings.

Different social communication traits are the most reliable phenotype observed in ASD parents and siblings compared to other control groups (e.g., Bishop et al., 2006; Landa et al., 1992; Piven, Palmer, Landa et al., 1997). This finding was observed in our study as well as differences were most consistently noted across family member types on the BPASS composites assessing social motivation, nonverbal communication, and conversational skills. This suggests that mild social communication challenges may be the most familial of ASD-related traits as

decreased abilities in these areas were more often observed in multiplex families than simplex families.

In general, siblings in multiplex families more consistently presented with decreased social motivation, lower observed communication skills, and impairing repetitive behavior patterns. In this way, undiagnosed children from multiplex families more closely modeled their siblings with ASD whose symptoms involve impairment in these same three domains: reciprocal social interactions, communication, and restricted and repetitive behaviors. Additionally, social and neuro-cognitive skills in ASD typically involve substantial variability in IQ with enhanced visuospatial abilities compared to verbal skills (Happé, 1999; Lincoln et al., 1988), challenges in phonological processing (Kjelgaard & Tager-Flusberg, 2001; Lindgren et al., 2009; Roberts et al., 2004; Whitehouse et al., 2008), and decreased recognition and attention to faces (e.g., Dawson et al., 2002; Hauck, Fein, Maltby, Waterhouse, & Feinstein, 1998). Multiplex siblings in the current study also showed greater IQ variability with higher nonverbal compared to verbal scores, below average face memory, and lower phonological processing skills compared to undiagnosed children in simplex families. Thus, multiplex siblings showed a number of similar elements of the cognitive phenotype in ASD in addition to milder levels of ASD behavioral symptoms compared to simplex siblings.

As in previous research, sex differences in the broader phenotype presentation were observed differently in multiplex versus simplex parents (Bolton et al., 1994; Pickles et al., 2000; Piven, Palmer, Jacobi et al., 1997; Schwichtenberg et al., 2010; Wheelwright et al., 2010). However, there were not enough siblings in the current study to allow for valid comparisons of the broader phenotype separately by sibling sex.

Despite reports that macrocephaly appears to be a familial trait in ASD (Lainhart et al., 2006; Miles et al., 2000), head circumference measurements did not differ between multiple-incidence and single-incidence families. However, the positive relationship between head circumference and increased BAP traits was more pronounced in simplex siblings compared to multiplex siblings. Given the variability in predictions of increased head circumference and ASD symptoms in affected individuals, this finding was not predicted and it is difficult to interpret it in isolation. Further work is necessary to replicate this relationship.

No relationship between the symptoms of the affected children and family member traits were found in the current study. Thus, increased symptom severity in affected children did not predict greater BAP traits in either simplex or multiplex families. This contradicts previous studies reporting a positive relationship between severity of BAP traits in ASD relatives and symptom severity in affected children (e.g., Pickles et al., 2000; Schwichtenberg et al., 2010). However, prior studies used the same measure to compare traits across family members (e.g., SRS and FHI) whereas the comparison in this study involved different measures (i.e., ADOS and BPASS). ADOS codes, and thus composite scores, are based on clinical observations only while BPASS scores combine clinical observation with direct interview questions. This difference may have affected results.

Etiological and Clinical Implications

The range of potential symptom profiles that can emerge from the varied deficits in social communication and behavior in ASD results in a complex behavioral presentation. The use of general, overarching diagnostic categories in complex psychiatric disorders such as ASD has often muddied genetic studies with these groups. Therefore, defining quantifiable components of the phenotype that are theoretically more closely tied to genetic vulnerability than a qualitative

diagnosis helps to isolate traits for genetic and neurobiological analysis (Berrettini, 2005; Gottesman & Gould, 2003; Gould & Gottesman, 2006). Investigations of component traits in biological relatives can also broaden understanding of the complex systems that underlie social communicative behavior and offer insight into how these systems go awry in ASD. An interactive model of genetic, neurobiological, environmental, and protective factors may explain why significant deficits are present in affected children while only mild challenges are measurable in relatives. In depth examinations of the familiality of component traits provide a more complete picture of ASD's etiology.

Examinations of BAP profiles in families provides an opportunity to gain insight into how phenotype profiles may be inherited within families and informs understanding of varying underlying genetic mechanisms in particular families. For instance, particular BAP profiles may be more suggestive of chromosomal variations impacting specific genomic regions. Specifying certain family types using behavioral measures may allow for the identification of differing genetic mechanisms. Examination of carefully defined phenotypes is crucial in providing insight into behavioral manifestations of genetic events and broadening understanding of the etiology of complex disorders such as ASD.

Results of this study lend support to the theory that families containing multiple children with ASD families carry a high loading for ASD-related traits. This was evidenced by the increased presence of mild social communication challenges in multiplex families compared to families containing just one child with ASD. Additionally, a significant greater proportion of family members in the multiplex group scored in the low range on measures, suggesting greater numbers of multiplex family members with impairment compared to simplex relatives.

This difference in behavioral presentation was more pronounced in siblings than parents such that multiplex siblings modeled their siblings with ASD in terms of behavioral and cognitive presentation more so than simplex siblings. Sibling presentation of the broader autism phenotype may be more representative of the complete range of underlying genetic influences in ASD. Parents by definition needed to have navigated at least one adult social relationship in order to have children. Additionally, families with both parents available to participate were prioritized in both the simplex and multiplex studies, which could have further served to inadvertently increase the level of parent social functioning. Finally, parents had to organize themselves and their family to participate in a relatively lengthy and complex family research study. These factors could have positively skewed the level of functioning of parents whereas siblings were not held to these same standards. Thus, they may be better representatives of the full range of functioning in ASD relatives. Despite these differences between parents and siblings, differences between multiplex and simplex parents were observed as well, particularly in fathers.

Findings are also consistent with the notion of distinct genetic mechanisms in single incidence versus multiplex incidence ASD. Recent genetic findings suggest that the development of ASD in simplex families may be more likely due to a *de novo* event not shared by other family members (Marshall et al., 2008; Sebat et al., 2007; State, 2011; Weiss et al., 2008). The lower level of BAP traits in both parents and siblings within simplex families support these genetic results in that ASD-related behavioral traits less often were shared in simplex compared to multiplex families. Thus, this study supports the theory that the types of genetic causes of ASD may indeed vary between single-incidence and multiple-incidence

families and that multiplex family members are more vulnerable to ASD symptoms given shared genetic variance.

Virkud and colleagues (2009) suggest that the increased presence of BAP traits in multiplex siblings versus multiplex parents suggests that the clinical presentation of ASD in a given child may result from interactive effects of multiple genetic loci in multiplex families as a result of particular combinations of alleles inherited from both parents independently. Perhaps subsets of those allelic combinations occurring in any one parent are inherited in siblings and are responsible for BAP manifestations in undiagnosed children.

In addition to offering insight into ASD etiology, consideration of BAP traits in family members also has important clinical implications in terms of treatment planning. An understanding and awareness of parent and family factors is crucial to the development of any intervention plan with a family seeking treatment for their child. Clinicians often individualize interventions for different children with the same diagnosis based on certain family characteristics and needs. Such family factors can include addressing specific treatment goals that parents have for their child, parent and child motivation for change, varying personality styles, skill level in implementing the intervention, and home and school environments. In ASD, awareness that BAP traits and certain cognitive features are present in some family members, particularly in families who have multiple children with ASD, should be part of these overall family considerations. Additionally, a careful family history prior to treatment onset may be helpful in determining the potential for broader phenotype expression in families who have multiple members with ASD and may be useful in predicting which families may benefit from additional attention. Thus, considerations of the BAP in clinical settings may be helpful in recommending and implementing the best and most appropriate treatments for a child.

The BAP has been used to help elucidate the genetic mechanisms in ASD and provide a clearer picture of the clinical phenotype of ASD. Although further work is certainly needed before the BAP can be fully incorporated into treatment planning, there have been significant advances in our understanding of and ability to assess the BAP. Clearly, these advances in our understanding have set the stage for future clinical and scientific pursuits.

Limitations

There were a number of important limitations to the current study that merit attention. The primary drawback of this project was that the simplex and multiplex samples were collected as part of separate research projects conducted over entirely nonoverlapping time periods. Although study protocols, measures, clinical raters, and inclusionary/exclusionary criteria were very similar across studies, it is nonetheless possible that cohort effects exist. However, there were differences on particular outcome variables in some groups and not others (e.g., in siblings but not mothers), so cohort effects likely do not explain the observed results completely.

A related concern was the large amount of missing data in the multiplex sample. The Family Study of Autism followed an in depth protocol with a primary focus on obtaining blood samples from each family member and behavioral measures for the participating affected children. Since there were multiple affected children within each family, at times the remaining family members did not receive a complete test battery. Additionally, a number of the neurocognitve measures (e.g., WMS-III, CMS, and CTOPP) were added to the protocol in the middle of data collection. Therefore, these measures are missing entirely from families seen in the first half of the study. Thus, a number of research questions, primarily those involving the neurocognitive measures, were likely underpowered. The varying amount of missing data also

affected the type of analyses possible. Separate MANOVAs were run for each measure, which increased the number of comparisons and may have inflated some test results.

Although there were sufficient numbers of participating fathers and mothers to allow separate analyses for parents, there was an inadequate number of participating siblings to conduct separate analyses for sex. Since the broader phenotype seems to present differently in males versus females, this would be a relevant research question. Results do suggest differences in multiplex versus simplex siblings, but it would be interesting to explore sex differences in siblings as well.

Additionally, it is possible that the findings of increased BAP traits in multiplex families could be due to increased parent stress related to the demands of raising multiple children with developmental disabilities. The BPASS is intended to assess and take into account current and past (i.e., from childhood and early adulthood) preferences in coding decisions. Nonetheless, it is possible that parents and clinicians struggled to disentangle current from earlier preferences.

Future Directions

Future studies should include measures of parenting-related stress and parental psychological distress to investigate and control for the possible influence of the potential stress involved in raising multiple children with ASD. In fact, although it would be challenging, an ideal comparison group would consist of families who had multiple children with non-ASD developmental disabilities.

Future research would also benefit from an examination of how the cognitive measures relate to social communication skills and restricted interests. For instance, an exploration of how face memory relates to level of social interest may provide additional insight into underlying social cognition contributions to symptom presentation. Losh and colleagues (2009) found that

only those parents with aloof personality styles demonstrated impairments on social cognitive measures. More in depth examinations of how this relationship might differ in simplex and multiplex families would add depth to an understanding of etiology.

This study originated from genetic findings of an increased prevalence of *de novo* genetic events in simplex versus multiplex families. However, the majority of simplex families do not have currently identifiable genetic events. Future research may benefit from focusing on those simplex families who have known *de novo* mutations to determine the presenting phenotype in the proband and family. This would likely serve to decrease variability in the simplex sample. Similarly, a careful genetic analyses of those family members with "high scores" on the BPASS, suggesting true challenges in social communication skills and restricted patterns of behavior that are impairing, may offer insight into how ASD-related traits are inherited within families.

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