DYRK1A haploinsufficiency as a subtype of ASD: Phenotypic presentation and the role of parental phenotype in accounting for variability in individuals with ASD and disruptive *DYRK1A* mutations

Rachel Kincade Earl

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Reading Committee:

Raphael Bernier, Chair

Janine Jones

Heather Mefford

Jeff Munson

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Abstract

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Rachel Kincade Earl

Chair of Supervisory Committee:

Raphael Bernier

Department of School Psychology

Background: DYRK1A is a gene recurrently disrupted in 0.1-0.5% of the ASD population. A growing number of case reports with *DYRK1A* haploinsufficiency exhibit common phenotypic features including microcephaly, intellectual disability, speech delay, and facial dysmorphisms.

Methods: Phenotypic information from previously published *DYRK1A* cases (n = 50) and participants in an ongoing study at the University of Washington (UW, n = 10) were compiled. Frequencies of recurrent phenotypic features in this population were compared to features observed in a large sample with idiopathic ASD from the Simons Simplex Collection (n = 1,981). UW *DYRK1A* cases were further characterized quantitatively and compared to a randomly subsampled set of idiopathic ASD cases

matched on age and gender (*n* = 10) and to cases with an ASD-associated disruptive mutation to *CHD8* (*n* = 12). Contribution of familial genetic background to clinical heterogeneity was assessed by comparing head circumference, IQ, and ASD-related symptoms of UW *DYRK1A* cases to their unaffected parents, and comparing *DYRK1A* to other ASD and ASD-associated groups.

Results: DYRK1A haploinsufficiency results in a common phenotypic profile including intellectual disability, speech and motor difficulties, microcephaly, feeding difficulties, and vision abnormalities. 89% of *DYRK1A* cases ascertained for ASD presented with a constellation of five or more of these symptoms. When compared quantitatively, *DYRK1A* cases presented with significantly lower IQ and adaptive functioning compared to idiopathic cases and significantly smaller head size compared to both idiopathic and *CHD8* cases. Phenotypic variability in parental head circumference, IQ, and ASD-related symptoms corresponded to observed variability in affected child phenotype.

Conclusions: Results confirm a core clinical phenotype for *DYRK1A* disruptions, with a combination of features that is distinct from idiopathic ASD. Cases with *DYRK1A* mutations are also distinguishable from disruptive mutations to *CHD8* by head size. Measurable, quantitative characterization of *DYRK1A* haploinsufficiency illuminates clinical variability, which may be, in part, due to familial genetic background.

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Chapter I: Introduction

Autism spectrum disorder (ASD) is characterized by tremendous heterogeneity in clinical presentation and level of functioning. Historical efforts to behaviorally subtype ASD has been largely unsuccessful due to lack of meaningful treatment implications by subtype and poor clinician reliability (King, Navot, Bernier, & Webb, 2014; Lord, Petkova, et al., 2012). Rates of ASD identification have increased rapidly in recent decades, as have disparities in diagnostic rates by ethnicity, socioeconomic status and region (Christensen et al., 2016). With these disparities come inequalities in age of diagnosis and access to early intervention (Mandell, Listerud, Levy, & Pinto-Martin, 2002; Mandell, Novak, & Zubritsky, 2005). Given the strong evidence to support the efficacy of early intervention for symptoms of autism, accurate diagnosis and tailored treatment planning are imperative (Ibanez, Stone, & Coonrod, 2014). Variability in the cognitive functioning, language ability, motor skills, and medical conditions of children with ASD provides strong evidence that treatment for the disorder is multi-faceted and cannot be uniformly applied.

Recent efforts have targeted the genetic etiology of ASD to better understand the heterogeneity of the disorder and to explore biologically-defined subtypes (Higdon et al., 2015; Jeste & Geschwind, 2014). Advances in genetic sequencing technology have allowed for the identification of increasingly smaller regions of the genome which are disrupted in individuals with ASD at higher rates than in the typical population (O'Roak et al., 2011). A series of chromosomal abnormalities, copy number variations (CNVs, i.e. deleted or duplicated regions within a chromosome), and single nucleotide variants

(SNVs, single gene mutations) have all been associated with ASD (Iossifov et al., 2014; Pinto et al., 2010; Sanders et al., 2015; Vorstman et al., 2006). Most recently, particular attention has been paid to the role of gene-disrupting SNVs and their role in ASD risk. *De novo* variants, those arising anew in the affected child, have emerged as key players in ASD risk, accounting for up to 30% of ASD cases (Iossifov et al., 2014). These discoveries have prompted a shift in ASD research; instead of using extensive phenotyping as the first step to reducing genetic heterogeneity, researchers have begun identifying genes of interest and exploring phenotype in specific gene cohorts (Stessman, Bernier, & Eichler, 2014). Specific disrupting gene mutations have emerged as high-confidence ASD risk genes. Known individuals with the same disrupted gene are observed to have similar medical, behavioral, and dysmorphic features, suggesting that these gene mutations might represent unique subtypes of ASD (Bernier et al., 2014; Helsmoortel et al., 2014; van Bon et al., 2016).

Methods for studying the impact of genetic events on a complex disorder like ASD and accounting for variability in comorbidities and level of functioning is an area of ongoing study. Researchers have begun to favor a dimensional framework for understanding ASD that better captures the overlap of cognitive, language, motor and medical complications (A. Moreno-De-Luca et al., 2013). In order to understand the impact of genetic mutation on the phenotype of a child with developmental disability, broader familial genetic background should be taken into consideration. The use of unaffected parental phenotypic background provides an important baseline with which to compare and affected child's phenotype and to estimate the disorder's impact. This

approach has been applied to other developmental disorders and to copy number variations associated with ASD, but has yet to be done for single gene mutations associated with ASD (Fraser & Sadovnick, 1976; Malich, Largo, Schinzel, Molinari, & Eiholzer, 2000; Moreno-De-Luca et al., 2015; Preece, 1996).

Dual-specificity tyrosine phosphorylation-regulated kinase 1A, or *DYRK1A*, is a gene recurrently disrupted in cases of ASD (Iossifov et al., 2014; O'Roak, Vives, Fu, et al., 2012). Individuals with disruptions to this gene have been reported to have similar features, including microcephalic head size, intellectual disability, speech delay, ASD, and facial dysmorphisms (Bronicki et al., 2015; Ji et al., 2015; van Bon, Coe, & deVries, 2015). Published cohorts of individuals affected with *DYRK1A* have compared small samples on categorical features only (i.e. presence and absence). To better understand the genetic impact of *DYRK1A* on phenotype and its uniqueness as a potential subtype of ASD, further study is needed which utilizes larger cohorts, compares individuals with *DYRK1A* to other ASD populations, approaches *DYRK1A* phenotypic features dimensionally, and accounts for familial phenotypic background.

Therefore, the aim of the proposed study is to examine a cohort of individuals with *DYRK1A* mutations, providing a summary of phenotype and comparing recurrent features to those of larger populations with ASD and cohorts with other disruptive, ASD-associated mutations. This study will examiner the effect size of *DYRK1A* by measuring the discrepancy between affected child and unaffected parents in the domains of cognitive, motor, behavior, and physiology. Effect sizes will be compared to large ASD-comparison samples and to cohorts with other disruptive mutations.

The dissertation proposal begins with a review of the literature regarding ASD diagnosis and prevalence, the genetic etiology of ASD, and dimensional methods utilizing familial background in developmentally disabled populations. Next, specific research aims and hypotheses are proposed, followed by proposal of methods, including participants, measures and analytic approaches.

Chapter II: Literature Review

Diagnosis, Prevalence, and Treatment of ASD

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by social communication impairments and the presence of repetitive behaviors and/or restricted interests. The disorder is defined based upon behavioral criteria and clinician judgment; there is no biological diagnostic indicator for ASD at present. The term ‘‘autism’’ was first used in the early 1900s and has since become a well-known disorder that affects 1 in 68 children in the United States, 1 in 160 worldwide (Christensen et al., 2016; Elsabbagh et al., 2012). In order to understand the current diagnostic criteria, process, and future of ASD, a review of its history, prevalence, and treatment implications is needed. It is important to note that ASD is characterized by tremendous variability in symptom presentation, which has fueled the ongoing pursuit of appropriate, therapeutically-applicable subtypes of ASD.

History of behaviorally-defined subtypes of ASD. The term “autism” was first used in 1910 by Eugene Bleuler to describe dissociation from reality as a feature of schizophrenia (Bernier & Gerdts, 2010; Bleuler, 1916). In 1938, Hans Asperger identified “autistic features” in children who did not meet criteria for schizophrenia. Asperger described symptoms in a series of the patients seen in his clinic, most of which had significant social and behavioral impairments despite average or advanced cognitive abilities (Asperger, 1944). Leo Kanner provided further evidence of autistic features in eleven unrelated children, publishing a series of case reports detailing early development, medical history, behaviors, and treatment history for each of these

patients (Kanner, 1943). These patients had varying language and cognitive abilities. Together, these two reports provided a set of common features, which included decreased social contact with others, repetitive behaviors and mannerisms, rigidity for "sameness," and unusual sensory interests.

When the first Diagnostic and Statistical Manual (DSM I) for psychiatric disorders was released by the American Psychiatric Association (APA) in 1952, autism was not included as a distinct diagnosis. Instead, the symptoms now understood to be autism were captured under "Schizophrenic Reaction, Childhood Type." This classification remained in the DSM-II (1968), with autism instead being included as a descriptor under Schizophrenic Reaction. It was not until 1980 and the release of the DSM-III that "Infantile Autism" was described as a unique disorder distinct from schizophrenia (APA, 1980). The term "infantile" was used to emphasize the early onset of autism symptoms before 30 months of age, in contrast to schizophrenia symptoms that emerge later in development. "Autistic disorder" was included in the revised third edition of the DSM (DSM-IIIR) and was organized into three symptom domains: communication, social interaction, and restricted or repetitive behaviors (APA, 1987).

As "Autistic Disorder" became used as a diagnosis, it was evident that symptom presentation was highly variable across children, particularly regarding language ability and cognitive functioning. With the release of the DSM-IV, a classification system for type and severity of symptom presentation was proposed, where the following diagnoses were introduced under the umbrella of Pervasive Developmental Disorders: Autistic Disorder, Asperger's Disorder, Rett Syndrome, Childhood Disintegrative

Disorder, and pervasive developmental disorder, not otherwise specified (PDD-NOS) (APA, 1994, 2000). The diagnoses of Autistic Disorder, Asperger's Disorder, and PDD-NOS were all considered to be types of autism spectrum disorders, while Rett Syndrome and Childhood Disintegrative Disorder represented pervasive developmental disorders outside the spectrum. What distinguished these three diagnoses within the autism spectrum were presence or absence of early language impairment and severity of behavioral symptoms.

Children with language impairment and more severe symptoms across social, communication, and behavioral domains met criteria for Autistic Disorder, while children with social and behavioral symptoms but no early language impairment could meet criteria for Asperger's Disorder. Cognitive impairments were co-occurring in approximately 30% of individuals with Autistic Disorder, while those with Asperger's disorder tended to be without cognitive impairments (Bernier & Gerdts, 2010). Those who had clinically significant symptoms but didn't meet full criteria for Autistic or Asperger's disorders might be given a diagnosis of PDD-NOS. The proposal of these three diagnoses offered diagnostic flexibility, but also sparked debate as to whether they truly represented distinct subtypes of autism (King et al., 2014; Volkmar, Reichow, & McPartland, 2012).

Over time, concerns mounted regarding the reliability and validity of these behaviorally defined subtypes of ASD (Lord, Petkova, et al., 2012; Sharma, Woolfson, & Hunter, 2012). Questions were raised regarding the stability of the criteria for children younger than three years of age (Lord, 1995). Lord and colleagues (2012) studied a

large sample of individuals with diagnoses of pervasive developmental disorders using gold-standard diagnostic measures, and found that a diagnosis of Asperger's Disorder was better accounted for by the evaluation site than specific symptom presentation. Furthermore, the three different types of autism spectrum disorders being used by clinicians did not have distinct treatment trajectories nor informed treatment recommendations in meaningful ways (King et al., 2014). Combined with growing genetic advances which have failed to find etiological differences between DSM-IV-defined subtypes, the DSM-5 removed existing subtypes in favor of a general continuum of Autism Spectrum Disorder (ASD) with varying levels of severity (King et al., 2014). This diagnostic reorganization was an attempt to preserve diagnostic flexibility while leaving room for a more dimensional understanding of ASD that could accommodate ongoing efforts to understand the biological etiology of the disorder (King et al., 2014; Stessman et al., 2014; Volkmar & McPartland, 2014).

Current diagnostic criteria for ASD. Social and communication domains, which were distinct in the DSM-IV, are collapsed in the DSM-5, creating two diagnostic domains: social communication and restricted and repetitive behaviors (APA, 2013).

Social communication domain. Social communication includes three types of impairments, which must each present to meet criteria in this domain. The first cluster is social-emotional reciprocity, which captures impairments related to reciprocal interaction, including back-and-forth play, conversations, recognition of social cues in others, and insight into the emotions of self and others. Second is nonverbal communication, which includes impairments in eye contact, directed facial expression,

and gestures. In an individual with language delay and without ASD, it would be anticipated that use of nonverbal communication would be used spontaneously and frequently to aid in communication. However, in children with ASD, regardless of language impairments, nonverbal communication is lacking. The last cluster of the social communication domain is interest in and maintenance of peer relationships. At younger ages, impairments in this area are often observed as a lack of interest in what other children are doing (e.g. less watching of other children's play or attempts to draw caregiver's attention to other children) and minimal cooperative play. In older children and adults, impairments in this area can also manifest as a disinterest in same-age peers as well as limited insight into the nature of social relationships and ways to make and keep friends. Sometimes, individuals with ASD can be motivated to make friendships, but have significant difficulty making appropriate overtures toward peers or engaging in activities with others that aren't related to their primary interests. When an individual has impairments in each of these three clusters, they meet criteria within the social communication domain.

Restricted and repetitive behaviors. The second domain, restricted and repetitive behaviors, requires the presence of two of four types of behavioral clusters. Each of these clusters may look very different depending on the individual, and examples are provided below but are by no means exhaustive. First is unusual sensory behaviors, including sensory-seeking and sensory aversions. Examples of sensory-seeking behaviors include touching objects to the mouth, rubbing another person's skin, pushing up against another person or object for high-pressure contact, and visually

examining objects like lights or ceiling fans. Sensory aversions may look like significant sensitivity to tags of clothes or seams on socks, strong aversions to everyday noises (e.g. hairdryers, blenders), or refusal to eat certain colored or textured foods. Both over- and under-sensitivity can occur for the same individual; those with ASD can have unusually dulled pain responses or notice of heat or cold.

Second in the restricted and repetitive behaviors domain are interests that unusual in subject matter and/or intensity. These interests are preoccupying and disrupt social rapport and family life in their intensity. Within this cluster, interests can unusual (e.g. street lights, toilets, brands of elevator manufacturers) or age-appropriate but unusually intense.

Difficulty with changes in routine is the third behavioral cluster, which is marked by significant distress at disrupted routines, either those created by the individual or by someone else. For example, a child with impairments in this area might throw tantrums when his caregiver takes a different route to school or if dinner is served fifteen minutes earlier or later than usual. Compulsive or ritualistic behaviors are also included in this cluster, such as requiring certain activities be completed in very specific ways.

Last in this behavioral domain is repetitive speech, motor movements, or play. Instances of repetitive speech include echoed speech (i.e. echolalia), such as repeating lines from television shows and movies with precise tone and cadence without broader context. Verbal rituals, in which a child has to say things in a certain way or order or requires that someone else says something a certain way. Complex motor mannerisms include repetitive hand movements (e.g. hand flapping or finger posturing) or whole

body movements (e.g. spinning, jumping and flapping one's arms) that are difficult for the child to disengage from. Repetitive play behaviors could include repeatedly lining up toys or interest in certain parts of a toy rather than playing with it as intended. For each of these behavioral clusters, the behaviors exhibited often cause significant distress if disrupted and cause clinical impairment to the individual's social interactions and to family functioning.

Meeting full criteria for impairments in both domains, with symptom onset prior to the age of 36 months, is required for a diagnosis of ASD. Additional specifiers were introduced with the DSM-5 to provide qualifiers of impairment that have attempted to explicitly inform treatment (APA, 2013; Volkmar & McPartland, 2014). First, a severity rating is given to specify current level of functioning and the level of support needed on a 3-point scale, with increasing numbers indicating increased severity of symptoms and need for support. These severity ratings support a more dimensional approach to understanding autism, but have yet to be fully studied for their reliability across clinicians or treatment utility. Both intellectual and language impairment are specified separately to inform current functioning. In a recent report released by the Center for Disease Control and Prevention (CDC), approximately a third of individuals with ASD also present with Intellectual Disability (ID), and significant heterogeneity exists in language abilities across ASD populations (Christensen et al., 2016). Specifiers noting whether there is a known etiological factor for the diagnosis, such as a genetic syndrome or medical condition, were also introduced. The introduction of a qualifier for genetic diagnosis was developed to provide room for ongoing discoveries regarding the

genetic etiology of ASD and to guide increasingly targeted treatment recommendations (Lord , Christina, & Grzadzinski, 2014; Volkmar & McPartland, 2014) The entire set of specifiers are intended to replace the multi-axial system that the DSM previously was based upon (Volkmar & McPartland, 2014).

The roll-out of the DSM-5 criteria also included an important caveat: anyone with a prior DSM-IV diagnosis could keep their diagnosis under the new criteria (APA, 2013). This helped to assuage fears regarding children and families losing important services. Researchers have argued, however, that future studies are needed to determine the impact of trying to proceed with the DSM-5 changes while still keeping a foothold in the DSM-IV (Volkmar & McPartland, 2014). An additional diagnosis was added to the DSM-5, called Social Communication Disorder, which captures impairments with verbal and nonverbal communication in the absence of restricted, repetitive behaviors. The prevalence of social communication disorder over time is an area in need of future study.

Evaluating the DSM-5 criteria for specificity and sensitivity. With the development of a new diagnostic structure, there were concerns that individuals might no longer qualify under the new criteria and their access to services would be jeopardized. Numerous studies have been conducted comparing the DSM 5 criteria to prior DSM-IV criteria to determine the DSM-5's specificity, the ability to correctly rule out ASD in those who didn't meet previously, and sensitivity, the ability to correctly diagnose those who previously met criteria for the disorder. In a study of 977 individuals, 657 of which has a prior diagnosis of an ASD and 276 who were diagnosed

with a non-ASD disorder, symptoms were mapped onto each diagnostic checklist. Specificity of DSM-5 criteria was determined to be high, with ASD accurately ruled out for 95% of participants. 60% of participant who had previously met criteria for ASD continued to meet under the DSM-5 criteria. Those who no longer met criteria tended to be participants with higher cognitive ability and those who has previously been diagnosed with Asperger's Disorder or PDD-NOS (McPartland, Reichow, & Volkmar, 2012). Other studies have shown varying rates of sensitivity, with many finding that over half of individuals with prior diagnoses meeting criteria under the DSM-5 (Huerta, Bishop, Duncan, Hus, & Lord, 2012; Worley & Matson, 2012). Worley and Matson (2012) found that the DSM-5 criteria required more severe levels of impairment compared to the DSM-IV, and identified this trend in both adults and toddlers. Frazier et al (2012) used national registry data and determined that 12% of studied individuals who had met DSM-IV criteria no longer met using DSM-5 criteria, many of which were females with average or above cognitive abilities.

However, a large study that used both parent report and observational diagnostic assessments reported a higher sensitivity rate of 91%, and found that sensitivity only decreased when one assessment method was used rather than two (Huerta et al., 2012). Mazefsky and colleagues (2013) echoed the importance of both parent report and clinician observation to accurately determine the sensitivity of DSM-5 criteria. In a study of 498 individuals who were considered high functioning and had met DSM-IV criteria for ASD, 93% continued to meet criteria when assessment prioritized both

developmental history and current behavioral observation (Mazefsky, McPartland, Gastgeb, & Minshew, 2013).

Comparative studies of DSM-IV and DSM-5 criteria highlight how variable symptom presentation of ASD can be, particularly regarding cognitive and language abilities. This line of research also emphasizes the impact that method of assessment has on whether or not a child may meet criteria for ASD. As Volkmar and McPartland (2014) point out, these studies have been conducted with research populations, which are historically susceptible to ascertainment bias and likely do not fully represent clinical populations. Indeed, beyond the concerns raised with changing diagnostic criteria lie larger systemic issues regarding the diagnostic process and access for families.

Diagnostic process. First concerns regarding ASD often accompany broader developmental delays brought to the attention of the child's primary care provider, teachers, or therapist (e.g. speech, occupational), who will then refer an individual for a diagnostic evaluation. The diagnostic evaluation is conducted by providers with specialized training, including licensed psychologists, psychiatrists, neurologists, and developmental pediatricians. Evaluation for ASD is generally composed of a parent interview to determine developmental history of ASD symptoms and direct observation of the client. Depending on the client's age and level of functioning, the direct observation may vary in its content and structure (Bernier & Gerdts, 2010). With younger children and those with limited language ability, the observation is oriented around play; observations of older individuals with developed language abilities are driven by conversations. In general, ASD is more likely to be diagnosed in early

childhood, due to increased awareness of symptoms and efforts to promote early screening and intervention. The median age for diagnosis is currently 40 months of age (Christensen et al., 2016).

Best practice for diagnosing ASD involves the use of well-validated, standardized diagnostic tools. For direct observation, the Autism Diagnostic Observation Schedule – 2nd Edition (ADOS-2) is considered the gold standard tool (Bernier & Gerdts, 2010). The ADOS-2 is a semi-structured play-based assessment that provides opportunities for the clinician to observe a client's social communication abilities and unusual behaviors (Lord et al., 2013). The gold standard for parent report is the Autism Diagnostic Interview – Revised (ADI-R), a comprehensive interview for developmental history and both past and current behaviors related to ASD (Lord, Rutter, & Le Couteur, 1994). Each of these assessments have separate coding schemes and a diagnostic algorithm with a cutoff to determine whether an individual does or does not meet criteria for ASD. The information gathered from these instruments are then applied to the DSM-5 criteria to determine whether a client has ASD or not.

Due to the variability in cognitive, language and adaptive abilities observed in cases of ASD, both cognitive and adaptive assessments are often used to determine whether an individual also meets criteria for a diagnosis of Intellectual Disability (Bernier & Gerdts, 2010; Lord et al., 2014; Volkmar & McPartland, 2014). Cognitive testing is a standardized method of assessment that generates scores which have been normed to a broader population of same-age peers. While intellectual impairment used to be strictly reliant of an individual's intelligence quotient (IQ) score, diagnostic assessment

of Intellectual Disability now places increased emphasis on adaptive functioning, which measures an individual's ability to function in their everyday environments, in the general areas of communication, social interaction, daily living skills, and motor skills (APA, 2013). Additional testing may be done as part of a diagnostic evaluation to determine the presence of other common psychiatric comorbidities, including ADHD, anxiety and mood disorders. Studies have shown that ADHD co-occurs in approximately 25-50% of individuals with ASD (Reiersen, 2011; van der Meer et al., 2014). Risk of comorbid anxiety and mood disorders are elevated in individuals with ASD, particularly those with higher cognitive abilities (Lugnegard, Hallerback, & Gillberg, 2011; Stewart, Barnard, Pearson, Hasan, & O'Brien, 2006; White, Schry, & Maddox, 2012).

Current challenges in diagnostic assessment. While clinical diagnosis of ASD remains categorical in its structure (i.e. ASD or no ASD, with or without cognitive and language impairments), dimensional approaches which quantify a child's degree of functioning and severity of impairment, are diagnostically meaningful for several reasons. In a clinical context, determination of scores that clarify an individual's cognitive, adaptive, and behavioral functioning is important for determining strengths and weaknesses that dictate goals for intervention. Secondly, dimensional assessment in a research context is essential to account for variations in ASD symptom presentation amongst individuals and clarify what ASD looks like across development. Particular emphasis is placed on standardized scores in research so that results can meaningfully translate across studies (Lord et al., 2014). What has proved challenging is how to

quantify the variable constellation of atypical behaviors that can be seen in ASD. Measures such as the Social Responsiveness Scale (2nd edition), the Social Communication Questionnaire, and the Childhood Autism Rating Scale (2nd edition) have been developed to quantify symptoms related to ASD, and are often used to determine severity of presentation in research settings (Constantino & Gruber, 2012; Lord et al., 2014; Rutter, Bailey, Lord , & Berument, 2003; Schopler, Van Bourgondien, Wellman, & Love, 2010). These measures also have clinical utility as screening measures in early childhood and a supplementary assessment of ASD symptoms in clinical and school evaluations (Ibanez et al., 2014). They are, however, most useful when used as a supplement with gold standard diagnostic measures (Lord et al., 2014).

Diagnostic assessment is limited in its utility for individuals that fall on the extremes of cognitive and language abilities (Lord et al., 2014). A portion of individuals with ASD have severely limited language abilities, and their abilities are below the baseline language abilities required of most cognitive assessments. Similar challenges have been identified for the ADOS-2 and ADI-R, and have been addressed with updated algorithms and calibrated scores (Bastiaansen et al, 2011; de Bildt et al, 2004) The SRS has the tendency to over-diagnose ASD in those with very low cognitive abilities and externalizing behavior problems (V. Hus, S. Bishop, K. Gotham, M. Huerta, & C. Lord, 2013a). Conversely, other measures, like the Social Communication Questionnaire, may miss individuals with ASD that have advanced cognitive and language abilities (Chesnut, Wei, Barnard-Brak, & Richman, 2016). Challenges have

also arisen in correctly identifying ASD in very young populations. For example, Lord and colleagues (1995) studied 30 toddlers referred for concerns of autism and found that while both the CARS and ADI-R were diagnostically reliable with clinician judgement of children at age 3, reliability diminished when assessing children age two or younger. Both measures tended to overdiagnose ASD in young children with severe developmental delay (Lord, 1995). Efforts have been made to make diagnostic measures more appropriate to young children through the development of updated algorithms (Kim & Lord, 2012; Kim, Thurm, Shumway, & Lord, 2013).

Ongoing efforts for improving diagnostic assessment are promising, yet highlight the limitations of behavioral assessment in its ability to profile a disorder as complex as ASD. Thus, many researchers of ASD have shifted their attention to the study of biomarkers and their utility as an additional tool for identifying ASD, such as genetic and neurological markers (Jeste, 2015; McPartland, 2016). Strong initiatives to understand the genetic underpinnings of ASD are in place to determine whether subtypes of ASD may exist along biological lines, as will be discussed in detail below (Stessman et al., 2014).

Diagnostic assessment as a guide for treatment. Domains assessed in a diagnostic evaluation depend on the presenting concerns of the individual. Evaluations are intended to support intervention tailored to a child's unique needs and be of use to community and school providers. Treatment for children and adolescents with ASD is provided in both private and school settings. Services are often provided by a child's school district under special education eligibility and an Individual Education Plan (IEP).

Treatment varies depending on individual needs; common treatments include speech therapy, occupational therapy, academic accommodations, social and behavioral support (Bernier & Gerdts, 2010). For most districts, a clinical diagnostic evaluation must be provided to the school to be used as part of a school evaluation to determine educational impact and school-based services. Others use standardized rating scales, such as the CARS-2 to determine autism symptoms that can inform eligibility; a formal diagnosis of ASD is never given as part of a school evaluation. It is important to note that not all individuals with a clinical diagnosis of ASD also receive special education under the eligibility category of autism, and vice versa (Pinborough-Zimmerman et al., 2012). The autism eligibility category within special education is shaped but not defined by DSM criteria; school providers determine eligibility based on a different set of standards under the Individuals with Disabilities Education Act (IDEA, 2004). Services provided under an educational eligibility of autism often include access to increased behavioral support at school than other eligibility categories.

Currently, behavioral intervention is the only treatment that has strong evidence to address symptoms of ASD (Bernier & Gerdts, 2010). Behavioral treatment is rooted in applied behavioral analysis (ABA), which operates on the fundamental principle of shaping behavior with contingencies of reinforcement. Dr. O. Ivar Lovaas first proposed the use of ABA as a treatment for autism symptoms (Lovaas, Berberich, Perloff, & Schaeffer, 1966). Initially, ABA was primarily discrete trial training, which involved long hours of trial-based learning in an isolated room. Over time, greater emphasis has been placed on embedding ABA therapy into a child's natural social environment, and there is

strong evidence to support the effectiveness of naturalistic behavioral intervention for teaching young children with ASD communication, social interaction, and daily living skills (Dawson et al., 2010; Goods, Ishijima, Chang, & Kasari, 2013; Ingersoll & Wainer, 2013; Mohammadzaheri, Koegel, Rezaee, & Rafiee, 2014; Schreibman et al., 2015). ABA services are generally provided in home, school, and clinical settings; the setting for service delivery varies depending on the region. It has long been recommended that intervention start as early as possible in a child's development to maximize the plasticity of the early developing brain (Dawson & Osterling, 1997). Much emphasis has been placed on researching the early signs of ASD to improve accuracy of diagnosis and targeted interventions at earlier ages (Lord, Luyster, Guthrie, & Pickles, 2012; Stenberg et al., 2014; Szatmari et al., 2016; Zwaigenbaum et al., 2015).

The remainder of therapies commonly recommended for children with ASD largely depend on their communication, motor, adaptive, and behavioral needs. For example, speech therapy goals can widely range from teaching augmentative communication devices for nonverbal individuals to teaching pragmatic social communication (e.g. reciprocal conversation, reading nonverbal cues) to those that are verbally fluent. Physical and occupational therapy similarly vary depending on an individual's fine motor and adaptive abilities. Given the frequency of psychiatric comorbidities, cognitive-behavioral therapy is often used to address internalizing behaviors in cognitively able children and adolescents with ASD. Additional medical complications may require medication management and frequent neurological appointments. Seizures occur in approximately a third of individuals with ASD and

ongoing gastrointestinal and sleep problems are also common (Bernier & Gerdts, 2010; Schieve et al., 2012).

With such varied presentation across individuals, it is currently difficult for providers to help families identify what treatments they should prioritize or to anticipate treatment needs over time. As diagnostic assessment becomes increasingly precise at younger ages, the need for targeted early intervention becomes paramount. However, despite tremendous gains in behavioral assessment and treatment over time, many children, particularly those from racial and ethnic minority groups and those in poverty, are not benefitting from these services at the ages researchers recommend. In order to maximize the effectiveness of early identification and treatment for ASD, it is imperative that issues related to culturally sensitive service delivery and systems of care are explored and addressed.

Demographic disparities in ASD diagnosis and treatment. Rates of ASD have seen dramatic increase in the past decades, yet the most recent epidemiological study conducted by the Center for Disease Control and Prevention (CDC) reported stable prevalence rates between 2010 and 2012. Increases in previous rates have been hypothesized to result from several factors, including increased societal awareness, changes in diagnostic criteria, changes in epidemiological study methodology, and financial incentives (Bernier & Gerdts, 2010; Fombonne, 2009). A Swedish study illustrates the important point of distinguishing prevalence rates of autism *symptoms* from prevalence rates of autism *diagnosis* (Lundstrom, Reichenberg, Anckarsater, Lichtenstein, & Gillberg, 2015). Lundstrom and colleagues (2015) conducted a large-

scale population study of twins in Sweden and found that the report of the autism symptom phenotype remained stable across ten years, while the prevalence of clinically diagnosed autism spectrum disorder steadily increased over time.

The most recent U.S. prevalence rates released by Christensen and colleagues (2016) reported on rates of ASD diagnosis of 8-year-old children across 11 state-wide networks, as reflected in educational and/or health records. This data collection was driven by the CDC's Autism and Developmental Disabilities Monitoring Network (ADDM). As has long been observed, ASD was observed to be a male-predominant disorder in this sample, with the ratio of males to females with ASD was 4.5:1. A higher percentage of females with ASD presented with intellectual disability compared to males. It is important to note that the 11 networks significantly differed from one another in prevalence rates, particularly the distribution of diagnoses given for individuals with and without comorbid intellectual disability. Seven states provided educational records, which showed that an average of 74% of children with ASD were also receiving special education services. These is also evidence that age of diagnosis differs between rural and urban environments, with children from rural settings receiving a diagnosis an average of 0.4 years later than those in urban settings (Mandell et al., 2005)

Comparisons in autism diagnostic rates were also made between different ethnic groups. Prevalence was highest in non-Hispanic White children (15.5 per 1,000), who were 20% more likely to have a diagnosis than non-Hispanic Black children (13.2 per 1,000), 40% more likely than Asian/Pacific Islander children (11.3 per 1,000), and 50% more likely than Hispanic children (10.1 per 1,000). Additionally, a significantly higher

percentage of non-Hispanic White children received diagnostic evaluations before 36 months of age compared to other ethnic groups. These discrepancies are consistent with previous reports, and suggest clear differences in rates of diagnosis and access to early evaluation amongst ethnicities (CDC, 2009, 2012, 2014). Rates of autism eligibility in special education have shown similar disparities over time, with Black and Latino children in the U.S. being consistently under-identified (Travers, Krezmien, Mulcahy, & Tincani, 2014).

In 2010, Durkin and colleagues conducted a cross-sectional analysis of the ADDM network data to study prevalence rates by socioeconomic status. Prevalence rates increased with increasing SES, as measured by percentage above the poverty line, percentage of parents with a Bachelors degree, and median household income (Durkin et al., 2010). This relationship was evident in both health and educational records, suggesting socioeconomic disparities in both clinical and school-based systems. In a survey of caregivers of children with ASD on service experiences, Mandell and colleagues (2005) found that children in poverty, on average, received a diagnosis 0.9 years later than children whose family income was over 100% above the poverty line. In a study of school districts across the state of Texas, and later nationwide, incidence of autism eligibility was directly related to amount of district revenue (Palmer, Blanchard, Jean, & Mandell, 2005). In other words, the more economically disadvantaged the school district, the lower their rates of students identified with ASD.

Given strong evidence for the benefits of earlier identification and intervention for children with ASD, these ethnic and socioeconomic disparities are alarming (Ibanez et

al., 2014; Zwaigenbaum et al., 2015). There is currently no conclusive evidence to support that the presentation of ASD symptoms differs by race, ethnicity, or socioeconomic status (Bertrand et al., 2001; Dyches, Wilder, Sudweeks, Obiakor, & Algozzine, 2004; Mandell & Novak, 2005). It is important to note, however, that most research exploring prevalence rates of ASD by racial and ethnic groups is reporting on children who have a pre-existing diagnosis, which limits findings due to ascertainment bias (Mandell et al., 2009). In other words, these epidemiological studies are rarely detecting individuals that show symptoms of ASD but have not been diagnosed, thus making it difficult to make clear conclusions about actual prevalence rates of ASD symptoms across diverse population groups. Additionally, evidence that diagnostic rates vary across states and countries limit the generalizability of findings across studies (Christensen et al., 2016; Elsabbagh et al., 2012). Despite methodological limitations, the strong evidence that diagnostic rates differ across groups suggest systemic issues regarding access to information about ASD and care for culturally diverse populations.

Accounting for ethnic and socioeconomic disparities in ASD identification. Research regarding the reasons for these disparities are ongoing. Particular attention has been paid to accounting for racial/ethnic discrepancies in age of diagnosis, which is found to be delayed in Black, Asian, and Hispanic children relative to White children (Christensen et al., 2016; Mandell et al., 2002; Mandell & Palmer, 2005). It is hypothesized that clinician bias plays a role. In a study of primary care providers, Begeer and colleagues (2009) found that providers were more likely to recommend further ASD evaluation for clinical vignettes of White children than vignettes of children

who from minority populations (Begeer, Bouk, Boussaid, Terwogt, & Koot, 2009). Biases amongst school staff have also been linked to disproportionate placement of minority populations in special education as a whole, particularly for the eligibility category of emotional disturbance (Serwatka, Deering, & Grant, 1995; Wiley, Brigham, Kauffman, & Bogan, 2013)

There is also evidence to suggest that clinicians may be less likely to give a diagnosis of ASD to non-White children. Mandell et al. (2002) studied specialty clinic records for 406 children in the Pennsylvania health system across 6 years, specifically tracking children's age at first appointment, at diagnosis, and the number of visits in between. They found that Black children participated in treatment for longer periods of time prior to receiving a diagnosis and were 2.6 times less likely to receive a diagnosis of ASD compared to White children (Mandell, Ittenbach, Levy, & Pinto-Martin, 2007; Mandell et al., 2002). When children who received a diagnosis other than ASD were compared, Black children were significantly more likely than White children to receive a diagnosis of conduct disorder compared to ADHD, the most common alternate diagnosis given to the sample as a whole. All minority groups had a significantly higher likelihood of being given an adjustment disorder diagnosis. There is evidence to suggest that providers in clinical and educational settings may bias their assessment toward conditions they feel that patients of different racial and ethnic groups are more likely to exhibit (Balsa & McGuire, 2001; Balsa, McGuire, & Meredith, 2005). Mandell et al (2007) hypothesize that this may be a driving factor in their findings.

This phenomenon has been the subject of much research in schools, where African American students are disproportionately placed in special education under the emotional disturbance (ED) category (Chinn & Hughes, 1987; Oswald, Coutinho, Best, & Singh, 1999; Wiley et al., 2013). While studies have yet to look at the potential over-representation of African American children with autism served under ED, it is possible that symptoms of autism in this population are being interpreted and treated as evidence to conduct problems, similar to Mandell's findings in 2007. More research is needed in this area to better understand the interplay of client culture and provider bias on diagnostic outcome.

The delay in first diagnosis for Latino families has also been associated with primary care provider referral. Researchers have found language barriers to be a key factor that negatively impacts provider's ability to effectively screen Latino children for ASD (Zuckerman et al., 2013). Additionally, providers consider Latino parents to be less knowledgeable about ASD. Other studies have found that indeed, Latino families (English and Spanish as primary language) have less access to community resources that provide information about early signs of ASD (Magana, Lopez, Aguinaga, & Morton, 2013). In general, Latino families access healthcare at lower rates than other ethnic groups due to language barriers, fear of exposing immigration status, and lack of culturally-sensitive care being provided (Alegria et al., 2007). Thus it follows that Latino families are also even less likely to access specialty services required to make a diagnosis of ASD (Zuckerman et al., 2013; Zuckerman et al., 2014).

Another important component to consider when exploring discrepancies in diagnostic rates of ASD is the impact of culture on beliefs related to developmental deviations, diagnosis, and intervention. As Mandell and Novak (2005) point out in their review of the role of culture on family treatment decisions, culture is a dynamic entity that is difficult to quantify. There are limitations to the extent to which cultural beliefs and values can be appropriately captured and analyzed in a research capacity, which impacts the robustness of the literature on culture and ASD to date. Efforts are being made to better understand the complex relationship of cultural perception on healthcare access and pursuit of intervention.

One primary cultural factor that should be considered is how parents perceive their child's behavior. It has been observed that in Asian/Pacific Islander and African American cultures, parents are less likely to see behavior as indicative of an underlying disorder (Lau et al., 2004). Parents from different cultural backgrounds can also describe symptoms in varying ways, and impact how a clinician conceptualizes a child's presentation (Mandell & Novak, 2005). In regards to a disorder like ASD that is characterized by early delays in social and communication abilities, parents from different cultural backgrounds may also prioritize different delays over others. In a study of Indian and American families of children with ASD, differences in what delays were flagged as concerning to Indian and American parents were noted (Daley, 2004). It is also important to consider that Western cultures are focused on pathology and labeling behavior that deviates from the norm, whereas some non-Western cultures place less emphasis and concern on abnormal behavior.

Family beliefs about the course of ASD may also impact the route a family takes to diagnosis and intervention. In a study of varied perceptions in need for treatment related to ASD by racial groups, Benevides et al. found that Hispanic and non-Hispanic Black families reported less need for prescription medication and mental health services that non-Hispanic White families. Hispanic and non-Hispanic Black caregivers placed stronger emphasis on speech, occupational and physical therapy than non-Hispanic White caregivers did (Benevides, Carretta, & Mandell, 2016). This highlights how important it is for providers to explore families' beliefs about the trajectory of the disorder. An ongoing, respectful dialogue is needed in order to disseminate information about ASD in a culturally sensitive way. Addressing issues related to clinician bias, accessibility of healthcare to underserved populations, and culturally responsive care will be a complex, multifaceted process that will take time and committed effort from providers and policy makers alike.

Considering a biology-driven approach to diagnosis and treatment. It is clear that behavioral assessment is limited in its ability to identify ASD in diverse populations and inform treatment for families. Specifically, accurate characterization of ASD becomes difficult with children who are severely impaired with low cognitive ability and medical complications. Access to information on ASD, timely referrals and diagnosis has been historically deficient for culturally and linguistically diverse families. Treatment continues to be highly variable across individuals, which makes it challenging for clinical and school providers to anticipate treatment needs over time. Given limited evidence for meaningful behavioral subtypes of ASD and the shift to a single diagnosis

with dimensional specifiers in the DSM-5, the clinical characterization of ASD has been primed to incorporate a more biologically driven understanding of the disorder (Lord et al., 2014; Volkmar & McPartland, 2014). It is hypothesized that genetic mutations result in biological disruptions and a shared constellation of cognitive, behavioral, and psychiatric complications that form distinct ASD subtypes (Jeste & Geschwind, 2014). The possibility of a genetic test to determine ASD risk and inform targeted intervention has great promise as a means to provide answers and support to families earlier. Collaboration between psychological and medical providers seems increasingly necessary to comprehensively treat the spectrum of ASD, and the use of biomarkers may help to reduce clinician subjectivity and improve the accuracy and efficiency with which the disorder is diagnosed.

It is important to acknowledge that behavioral assessment of ASD continues to have its place, and the flexibility and individualization of treatment in the field of psychology remains a strength of the current approach. Efforts to understand developmental trajectories of children with ASD will help to support families more fully over time, and may be better understood via genetic subtyping. And yet, genes are known to interact with the tremendous variation of a child's environment, interactions, and experiences to form behaviors. That variability is not likely to be fully accounted for with a shift to biological approaches. Nor should it be necessarily. Studies have shown that the outcome of individuals with ASD is highly correlated with the extent of parent and teacher expectations, suggesting that environment plays a very important role in supporting children with developmental challenges (Ivey, 2007; Kirby, 2016). Alongside

a clearer understanding of developmental trajectory, biologically defined subtypes of ASD related to cognitive impairments have the potential to limit caregiver expectation. Researchers and clinical providers must be careful to consider these factors as they proceed with understanding the genetic underpinnings of ASD and trying to biologically account for variability in developmental disorders.

The Genetic Etiology of ASD

Understanding of the genetic etiology of ASD is ongoing, and after forty years of study, remains complex and heterogeneous like the disorder's phenotypic presentation. The evolving landscape of genetics and ASD provides important insight into the multi-faceted nature of ASD, particularly the methodological and technological shifts that have revealed key pieces of the puzzle. A review of the trajectory of genetic research on ASD to date is detailed below, with particular focus on how this developing narrative begins to account for and parse apart the vast heterogeneity of ASD.

Scientific evidence for the biological heritability of ASD was first posed by Folstein & Rutter (1977), who demonstrated the utility of twin studies to inform the genetic nature of ASD. Looking at 21 same-sex twin pairs where one or both had ASD, 4 out of 11 twin pairs that shared 100% of their genetic makeup (monozygotic, MZ) were concordant for ASD (both twins met diagnostic criteria), while no dizygotic (DZ) twin pairs were concordant for ASD. Folstein & Rutter also observed that in MZ disconcordant twin pairs, the twin without ASD still presented with developmental deficits; in DZ disconcordant twin pairs, the twin without ASD was usually typically developing. This seminal work suggested that ASD was driven strongly by genetic

factors, and inspired additional studies of similar design in the decades following. A Nordic twin study found even starker differences in concordance between 11 MZ twin pairs and 10 DZ pairs; MZ twins had a 91% concordance rate versus 0% concordance in DZ twins (Steffenburg et al., 1989). Bailey (1995) studied a larger sample of 25 MZ twin pairs and 20 DZ pairs, and found that 60% of MZ twins were concordant for ASD and over 90% were concordant for broader cognitive and social difficulties. In contrast, DZ twins presented with 10% concordance for broader difficulties, 0% for full ASD. Rosenberg and colleagues (2009) screened 277 U.S. twin pairs for ASD and found similarly divergent concordance rates (88% MZ vs 31% DZ). Each of these studies served to highlight a strong genetic component for ASD. It should be acknowledged that twin studies of ASD do not negate the presence of a shared environmental component, which has been shown in some studies to be uniquely contributory to ASD risk (Hallmayer et al., 2011). However, the study of twins has been important for confirming a strong genetic liability for ASD.

The genetic influences evidenced in twin studies have been echoed in sibling studies. Studies have shown that families with a child with ASD are at elevated risk of having a second affected child. In a large study of 1,235 families with a child with ASD and another biological sibling, recurrence risk of strictly-defined ASD was found to be at 10%, with an additional 20% risk of broader communication deficits (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010). Recurrence risk for infant siblings meeting full criteria for ASD has been proposed to be as high as 20% (Ozonoff et al., 2011). Additional evidence of social and communicative impairments at sub-clinical levels in

parents and unaffected siblings of individuals with ASD supports a quantitative framework for autistic traits, in contrast to a dichotomous understanding of ASD (Ben-Yizhak et al., 2011; Constantino et al., 2006; Constantino & Todd, 2005). These sub-threshold traits of ASD found in family members is termed “broader ASD phenotype,” which is observed to be more prevalent in families with more than one individual affected with ASD (Gerdts, Bernier, Dawson, & Estes, 2013; Pickles et al., 2000). These families, termed “multiplex” families, have been recurrently contrasted to “simplex” families, who only have one affected child with ASD. It is important to note that the term “simplex” should be considered a temporary classification in the event that a sibling is born later who is also affected. Reduced broader ASD phenotype in simplex families compared to multiplex suggests that simplex cases of ASD have genetic mechanisms that are sporadically occurring in the affected child (hereafter referred to as the proband), while multiplex cases stem from inherited mechanisms (Gerdts et al., 2013). Distinguishing simplex from multiplex families suggests that more than one genetic mechanism results in ASD. In this way, the distinction between familial structures has become an important point of comparison in genetic studies of ASD, as will be referenced extensively below. The study of families of children with ASD forms the foundation for the genetic study of ASD, and remains an important methodological approach as genetic sequencing technology has evolved to provide more specific insight into the type of genetic variations that may be contributing to incidence of ASD and broader phenotype features.

Syndromic ASD. Thus far, researchers have been most successful at identifying forms of complex, syndromic ASD, which is characterized by clinically-recognizable dysmorphic features and additional features not required for an ASD diagnosis (Basu, Kollu, & Banerjee-Basu, 2009; Gurrieri, 2012). Cases of syndromic ASD often exhibit greater global impairment, worse prognosis, and lower recurrence in family members than non-syndromic, or "essential" ASD (Gurrieri, 2012). Throughout the past 25 years, forms of syndromic ASD have been growing as the precision of genetic sequencing technology increases, providing strong evidence of a heterogeneous etiology for ASD since each syndrome accounts for a very small percentage of all cases of ASD.

Monogenic disorders. The earliest identified forms of syndromic ASD were monogenic disorders that include symptoms of ASD as part of their phenotype. Fragile X Syndrome, which is predominantly characterized by cognitive impairment, was identified to be caused by genetic mutation of the fragile X mental retardation 1 gene (FMR1) on the X chromosome, with ASD occurring in more than 20% of cases, (Pieretti et al., 1991). Disruptive mutations to X-linked methyl-CpG-binding protein 2 (MeCP2) were discovered to be the cause of Rett Syndrome, which is marked by motor and speech regression early in life, as well as social communication deficits and repetitive, stereotyped mannerisms (Amir et al., 1999; Katz et al., 2016). Children with tuberous sclerosis complex, which results from mutations to TSC1 or TSC2 genes, present with cognitive and neurological deficits; 17-61% of which have ASD (Gillberg, Gillberg, & Ahlsen, 1994; Jeste, Sahin, Bolton, Ploubidis, & Humphrey, 2008; van Slegtenhorst et

al., 1997). Depending on the monogenic disorder, ASD is co-occurring in 18-80% of cases (Gurrieri, 2012). This variability suggests that perhaps the gene mutations causing these specific syndromes are related to ASD-specific genes, and elevate the risk of ASD, but don't necessarily cause it.

Chromosomal abnormalities. Several methods were important for the discovery of chromosomal abnormalities associated with particular syndromes that were associated with ASD. Karyotyping analysis, which looks at the appearance of chromosomes under a light microscope, identifies chromosomal abnormalities that were enriched in populations with ASD (Ziats & Rennert, 2016). Family linkage studies, which study families who have affected individuals across generations, identify genetic markers that are passed down in affected family members (Abrahams & Geschwind, 2008). Linkage studies have identified risk alleles that are passed within families and confer risk of specific ASD features (e.g. speech delay) in multiplex families (Chen, Kono, Geschwind, & Cantor, 2006; Schellenberg et al., 2006). Association studies look for correlations between incidence of disease and genetic variation, and have been important to identify candidate regions of the genome associated with ASD in larger cohorts of unrelated cases and controls (Lewis & Knight, 2012). For example, studies have identified variations on chromosome 7q35 that was significantly associated with clinical features, including age at first word (Alarcon et al., 2008; Arking et al., 2008). In a review of previous family linkage, association, and karyotyping studies, Vorstman and colleagues (2006) presented chromosomal regions that were recurrently disrupted, or rearranged, in ASD populations. These regions were found to be clustered together on

certain chromosomes rather than spread throughout the genome. The most recurrent anomalies occurred at chromosome 7, 15, and 22, specifically at regions 7q11, 15q11-13, and 22q11.2. These regions are also associated with other syndromes (e.g. 22q11.2 deletion syndrome termed DiGeorge Syndrome or Velo-cardio-facial Syndrome) that include features of ASD. Further discussion of more recently proposed single-gene syndromes associated with ASD will be provided below following discussion of recent advances in genetic sequencing technology.

Copy number variations in ASD. The application of array comparative genomic hybridization (array CGH) to screen chromosomal regions of interest in ASD allowed researchers to study microscopically visible chromosomal variations more closely. By studying these regions at a much higher resolution (Sebat et al., 2007; Vorstman et al., 2006), small rearrangements, duplications, or deletions of parts of chromosomes have been found. These copy number variations (CNVs), which are found in approximately 35% of the general population, have the potential to disrupt a varied number different genes (10s to 100s), depending on the chromosomal region. In a rare number of CNVs, these disruptions are capable of being pathogenic. The use of array technology to identify rare CNVs in ASD was first applied to known cases of syndromic ASD (M. L. Jacquemont et al., 2006). In 29 patients with syndromic ASD, 10 CNVs were identified to be possibly pathogenic, based upon previous evidence of their enrichment in ASD populations compared to controls, their size and the genes they contained. Of these 10, 7 arose anew in the affected child (termed *de novo*) and 3 were inherited from an unaffected parent; 7 CNVs were deleted regions of the genome and 3

were duplications. This study was the first to confirm the use of array comparative genomic hybridization (aCGH) to find more precise genetic variants in syndromic ASD compared to traditional karyotyping. Jacquemont and colleagues provided compelling evidence that copy number variations are an important component to the disorder's etiology.

De novo copy number variations. Particular attention quickly became directed at the discovery of *de novo* CNVs in a broader population of children with ASD with idiopathic (or non-syndromic) ASD. Children who meet criteria for ASD that is not tied to a known genetic cause or additional syndromal features are referred to as having "idiopathic" ASD (Sebat et al., 2007). In a seminal study, Sebat and colleagues (2007) performed a large-scale analysis of CNVs in individuals with idiopathic ASD and their families. 264 families were genetically sequenced, including 118 simplex families (with only one affected individual), 47 multiplex families (multiple affected siblings), and 99 control families with no history of ASD (Sebat et al., 2007). Spontaneous, large (mean size of 2.3 mega-base pairs (Mb)) *de novo* CNVs were significantly more frequent in individuals with ASD than unaffected individuals; more specifically, *de novo* CNVs were found at 10% frequency in simplex, sporadic cases of ASD, 3% in multiplex cases, and 1% in unaffected cases. These *de novo* CNVs were found in children with ASD and a wide range of cognitive abilities, suggesting that chromosomal abnormalities were not limited to cases of syndromic ASD and comorbid intellectual disability, as was previously thought (M. L. Jacquemont et al., 2006). This study by Sebat and colleagues

was the first to bring attention to the widespread impact of CNVs in cases of idiopathic ASD.

Increasingly larger samples of affected and unaffected families have been sequenced for CNV detection using aCGH and single nucleotide polymorphism (SNP) microarray, which provides the additional benefit of detecting specific inheritance patterns within families (Jeste & Geschwind, 2014; Szatmari et al., 2007). In a cohort of 427 families of children with ASD and 500 controls, *de novo* CNVs were identified through a genome-wide sequencing approach at rates similar to previous study, at 7% in simplex families and 2% in multiplex families (Marshall et al., 2008). This enrichment of *de novo* CNVs in simplex cases of ASD has been consistently echoed by subsequent studies (Pinto et al., 2010; Sanders et al., 2011). Marshall and colleagues then compared the CNV incidence and composition of the 27 ASD cases to 1152 matched controls, and did not find evidence of an identical CNV pattern in any of the controls. Thirteen CNVs were noted to be recurrent (intersecting the same region and/or overlapping the same gene(s)) in unrelated cases of ASD and absent in the large comparison sample, thus providing strong evidence of their potential importance in ASD. *SHANK3*, *NLGN4*, and *NRXN1* genes that had been identified in previous ASD cohorts, were noted to be disrupted in this ASD cohort, providing additional evidence for their role as specific candidate genes (De Jaco et al., 2005; Durand et al., 2007; Yangngam et al., 2014). Marshall et al. (2008) also brought the chromosomal region 16p11.2 to attention as a recurrent site for deletion and duplication CNVs, found in 1% of the sequenced ASD cohort.

In 2011, Sanders and colleagues published genetic findings from a cohort of 1,124 simplex families, 872 of which had two unaffected parents, an affected proband, and one unaffected sibling while 252 did not include an unaffected sibling. Once again, rare *de novo* CNVs were found to be more common in probands than unaffected siblings, and the CNV events of probands were both larger in size and contained more genes than CNVs identified in unaffected siblings. 16p11.2 was highlighted again as a region of interest for ASD with 11 recurrent *de novo* cases in probands, significantly more than would be expected in controls, based on the rates found in the study's large cohort of unaffected siblings. While 16p11.2 stood out as the most frequent recurrent CNV in this cohort, other regions were identified as recurrent and of interest to ASD: 1q21.1, 15q13.3, 16p13.2, and 16q23.3.

Sanders et al. (2011) performed rigorous analyses to account for possible ascertainment bias, which occurs when systematic factors impact recruitment of the studied sample, or ancestral clustering, which occurs when seemingly distinct families are in fact related by a common ancestor and possess shared genetic characteristics. After addressing the possibility of these confounds, the authors found that significant effects remained. Compelling evidence was built for phenotypic differences between deletion and duplication CNVs, exemplified by the 7q11.23 region at the Williams-Beuren Syndrome gene locus. Deletions to that region are characterized by intellectual disability and hyper-sociability while duplications have been recurrently identified in cases of ASD and social deficits (Levy et al., 2011; Sanders et al., 2011).

Inherited copy number variations. While estimated to have a smaller contribution to overall ASD risk, rare inherited CNVs have also been implicated in ASD (Pinto et al., 2010; Sanders et al., 2015). In a study of 1,275 cases of ASD, their parents and 1,981 controls, Pinto and colleagues (2010) identified 219 inherited CNVs enriched in ASD probands and not controls. Other studies including multiplex families have also identified inherited CNVs of interest to ASD (D. Moreno-De-Luca et al., 2013). Additionally, studies have reported *de novo* and inherited mutations occurring in the same CNV region in affected cohorts (Sanders et al., 2015). Although a large-scale study by Sanders et al (2011) failed to find significant inherited burden in simplex cohorts, it has since been argued that the study was designed to detect *de novo* events with far more accuracy than inherited mutations (Krumm et al., 2013).

Initially, it was hypothesized that inherited genetic mechanisms were exclusive to multiplex families. However, instances of broader ASD phenotype in simplex families and the presence of rare inherited mutations in simplex probands as mentioned above, suggest that inherited mutations are an important part of the genetic landscape of ASD (Davidson et al., 2014; Losh, Childress, Lam, & Piven, 2008). Zhao and colleagues (2007) articulated an important theory that supports the role of inherited mechanisms in simplex ASD. They propose that most cases of ASD arise from sporadic, *de novo* mutations occurring in the parental germ line of “low risk,” simplex families. When mutations are disruptive, they tend to have complete penetrance, leading to a clearly affected individual. However, when the *de novo* mutation occurs in a female, there is greater chance they will be asymptomatic, due to a higher mutation threshold. Thus

female carriers of the mutation may pass that mutation onto their offspring who, when male, are more likely to be impacted by the mutation and express the ASD phenotype. Zhao and colleagues proposed that this pattern of inheritance is the likely cause of “high risk,” multiplex families. This theory has been validated in subsequent studies supporting predominantly maternal transmission of rare inherited CNVs (Krumm et al., 2013; Krumm et al., 2015).

Large-scale studies of the role of CNVs in ASD over the past decade have highlighted several key findings. First, rare CNVs occur significantly more frequently in individuals with ASD than unaffected individuals, and *de novo* variants occurring more frequently than inherited variants in simplex cases of ASD (Sanders et al., 2011; Sanders et al., 2015). CNVs found in ASD cohorts are large in size and tend to encompass more gene-laden regions than variants in unaffected individuals (Levy et al., 2011; Pinto et al., 2010; Sanders et al., 2011). Additionally, deletions are seen more frequently in these *de novo* events than duplications, likely due to the more disruptive nature of deletions (Levy et al., 2011; Sanders et al., 2015). In alignment with Zhao and colleagues’ theory of inheritance, “higher risk” families appear to have more inherited duplications, which are less disrupting as a mutation and more likely to be carried by a seemingly unaffected (or broader ASD phenotype) parent (Levy et al., 2011; Zhao et al., 2007).

Given the larger proportion of males with ASD, studies of CNVs have compared mutation load between males and females. Sanders et al. (2011) did not find significant differences in frequency and size of CNVs in males and females, but found that males

seemed more vulnerable to reductions in IQ associated with rare CNVs than females. In contrast, Levy et al. (2011) found higher rates of *de novo* CNVs in females; interestingly, the CNVs in affected females contained significantly more genes that were larger and more likely impairing. A three-fold increase in disruptive CNVs was found in female probands compared to male in a study of 762 probands and their families (S. Jacquemont et al., 2014). This enrichment of large CNVs in females provides additional evidence that more harmful genetic variations are needed to cause ASD in females, or perhaps fewer target genes actually cause ASD in females (Sanders et al., 2015; Zhao et al., 2007).

Functional implications of CNVs. With a growing number of CNVs associated with ASD, efforts have been made to understand points of connectivity and functional similarity. Pinto and colleagues (2010) found that, in an ASD cohort of 996 and control cohort of 1,287, *de novo* CNVs found in the ASD cohort uniquely intersected genes that were involved in common cellular functions, including cellular propagation, motility, and signaling. Using previously sequenced dataset of 75 *de novo* CNV regions (Levy et al., 2011), Gilman and colleagues identified highly-connected clusters of genes within ASD-associated CNV regions. These clusters were then assessed for connectivity. The more highly-connected the genes, the more likely they were to be related to neurological development. In particular, genes in clusters were most often related to neural synapse structure and communication. Genes important to cellular adhesion, dendritic spine development and axon motility, were identified, including NRXN1 and NLGN4 genes which have been previously associated with ASD. In Gilman and colleagues' 2011

publication, cellular pathways such as the WNT neural signaling pathway (involved in dendritic spine development) are referenced as connective networks that may be disrupted in cases of ASD. The determination of functional groups of ASD-associated CNV regions have been important to determine potential groups of connected genetic events and to expose single genes of particular interest to the understanding of ASD.

Variable expressivity in ASD-associated CNVs. While great strides have been made in the association of rare CNVs with ASD, the observed genetic heterogeneity and phenotypic variability occurring within a single CNV has introduced new questions for researchers. Despite increasingly large-scale studies of ASD cohorts and increasing numbers of ASD-associated CNVs, recurrence rates have remained low, with the most recurrent CNV, 16p11.2 accounting for only 1% of cases, which has made widespread treatment options difficult to generate (Levy et al., 2011; Sanders et al., 2011). Levy and colleagues estimated 250-300 target genetic loci present in ASD-associated CNVs in 2011, and acknowledged that this was actually an underestimate. Rare CNVs occurring in cases of ASD tend to be large, thus containing multiple genes (Levy et al., 2011; Marshall et al., 2008; Pinto et al., 2010). Thus, looking at CNVs alone is not serving to significantly reduce the genetic heterogeneity of the disorder (Manolio et al., 2009).

Cases of syndromic ASD tend to arise from *de novo*, highly penetrant mutations, meaning the genetic variant results in a relatively consistent phenotype. The CNVs identified in disorders such as Angelman, Prader-Willi, and Williams-Beuren Syndromes are examples of highly penetrant CNVs (Girirajan & Eichler, 2010). These variants are

most commonly *de novo* and highly disruptive to phenotype in a rather consistent way (e.g. intellectual disability). However, as Girarajan and Eichler (2010) note, highly penetrant CNVs only account for a small portion of developmental disorders. They propose that many CNVs may instead be associated with a varied set of diagnoses.

This is observed in the phenotypes of 16p11.2 deletion and duplication CNVs. In 2010, Rosenfeld and colleagues found in a cohort of 16 16p11.2 deletions and 10 duplications where clinical information was available, 56% of those with a deletion met criteria for ASD and 12% of those with a duplication had ASD. In addition, individuals with the same 16p11.2 deletion or duplication presented with varied cognitive abilities, medical profiles, dysmorphology, and behavioral difficulties (Rosenfeld et al., 2010). In other words, not only did the phenotype of duplications and deletions differ from each other, but the phenotype amongst individuals with the same variant type differed.

Variability was echoed in later studies of larger cohorts with 16p11.2 CNVs using more substantial behavioral phenotyping efforts, and even smaller percentages of ASD were reported (Hanson et al., 2015; Hanson et al., 2010). So while rates of ASD and other psychiatric disorders are indeed higher in those with 16p11.2 CNVs compared to control populations, disruptions at this chromosomal region don't always result in ASD. Other CNVs that are enriched in ASD cohorts show similar variability, such as 1q21.1 deletions and duplications (Bernier et al., 2016; Mefford et al., 2008). Importantly, this variability highlights that these CNVs, along with being regions of interest for ASD, heighten risk for a broader constellation of psychiatric, developmental and medical disorders.

Incomplete penetrance has been hypothesized to result from various factors. First, variations in CNV size impact what genes are altered. Second, the presence of an additional variant next to the CNV or the requirement of an additional, functionally-connected genetic "hit" elsewhere in the genome may regulate whether a CNV results in the phenotype (i.e. a "dosage" effect) (Girirajan & Eichler, 2010). For example, in a study of probands with inherited 16p12.1 deletions, it was observed that carrier parents presented with learning disabilities and mood disorders, while probands presented with severe speech, motor, and cognitive impairments (Girirajan et al., 2010). In these cases, the presence of a second genetic hit was more frequent in probands and appeared to result in a more impaired phenotype.

In summary, large CNVs are well-established as important genetic components of ASD; the study of CNVs has uncovered recurrent chromosomal regions of interest for ASD. However, the observed variability in the penetrance and phenotype of ASD-associated CNVs highlights the role that single genes might play in parsing apart the genetic heterogeneity of ASD (Levy et al., 2011).

Disruptive single gene mutations. Through 2011, genome-wide sequencing efforts had identified numerous coding regions of the genome and had suggested single genetic variants that were associated with ASD, but larger cohorts were needed to look for recurrence rates of single genes that would be robust enough to draw meaningful conclusions. Proposing that a mutation is enriched in ASD populations requires identification of a sufficient number of people with the deleterious event and comparison to rates in typical populations. Further work is then required to prove pathogenicity,

which requires exploration of functional and animal model research regarding that gene mutation. The advent of exome sequencing provided a cost-effective way to sequence the protein-coding regions of the genome, or the "exome," in larger cohorts. This method was proposed in 2009 and began to be more widely adopted by researchers in 2011, which created a more streamlined, efficient, and accessible means of genetic sequencing (Ng et al., 2009).

De novo disruptive single nucleotide variants. In 2011, O'Roak and colleagues tested this technology on the exomes of 20 simplex families and confirmed that coding regions of probands were enriched with rare *de novo* (newly occurring) mutations to single genes that disrupted typical protein development (O'Roak et al., 2011). After sequencing 189 new simplex families, 126 protein-disrupting gene mutations were identified (O'Roak, Vives, Girirajan, et al., 2012). The contribution of rare *de novo* disruptive single nucleotide variants (SNVs) to ASD risk has been echoed in whole exome studies of simplex ASD cohorts (Iossifov et al., 2012; Neale et al., 2012; Sanders et al., 2015; Sanders et al., 2012). These disruptive *de novo* SNVs, or likely gene disrupting (LGD) point mutations as they will be subsequently referred to, tend to disrupt the DNA nucleotide sequence in a way that creates a stop codon, which halts the generation of the intended protein and therefore disrupts its intended function (Ronemus, Iossifov, Levy, & Wigler, 2014). *De novo* LGD mutations result from nonsense, frameshift, and splice-site mutations, all of which halt protein production. There is ongoing debate about the role that missense mutations play in ASD risk (Iossifov et al., 2014; Ronemus et al., 2014; Sanders et al., 2015). Missense mutations

alter nucleotide sequences to create different amino acids, which may or may not disrupt proteins. They occur more frequently in the general population and don't always have an observable effect. It is important to note that as many as 100 LGD genetic variants are present in every human's genome, and most do not cause disease (MacArthur et al., 2012). The LGD mutations identified in cases of ASD are indeed rare in the typical population, making their enrichment in ASD cohorts and absence in control populations suggestive of a strong ASD association.

Iossifov and colleagues (2014) estimated that while approximately 43% of LGD events contribute to ASD diagnoses, only 13% of missense mutations contribute to diagnoses. In genes with recurrent LGD mutations, a 90% contribution to ASD was estimated (Iossifov et al., 2014). In their large-scale whole exome sequencing study of 2,517 simplex families, Iossifov et al. found 353 *de novo* LGD mutations, 27 of which were recurrent in multiple probands. LGD mutations were observed to be particularly deleterious and enriched in individuals with comorbid intellectual disability. Similar to studies of large CNVs, *de novo* LGD mutations are enriched in females affected with ASD, further implicating the role of particularly disruptive or damaging mutations in female ASD. Overall, they estimated that both LGD mutations and CNVs in the coding regions of the genome together account for 30% of cases of simplex ASD. Sanders and colleagues (2015) analyzed CNVs and SNVs together and suggested that small CNVs are more likely to overlap high-effect, disruptive SNVs, while larger CNVs tend to contain multiple SNVs with more modest effects (Sanders et al., 2015). The ability to account for as much as 30% of simplex ASD by *de novo* genetic variation is considered

a significant milestone in the genetic study of ASD. However, several hundred implicated genetic loci still pose significant challenges to determining causal genes; attempts to account for this heterogeneity are ongoing and discussed further below.

Inherited single nucleotide variants. *De novo* genetic variants have been found to be predominantly deleterious, therefore providing a more straightforward mechanism incurring ASD risk compared to inherited events. Yet whole exome sequencing has made important contributions to the study of inherited genetic events as well. In a study of 16 families who were determined to have distant shared ancestry, many of which were multiplex families, whole exome sequencing revealed several rare inherited candidate genes involved in neuronal processes, *UBE3B*, *CLTCL1*, *NCKAP5L* and *ZNF18* (Chahrour et al., 2012). These gene mutations were found to be recessive, such that both unaffected parents possessed only one copy of the mutated gene while affected probands possessed two copies of the mutated gene. A similar recessive inheritance pattern for *AMT*, *PEX7*, and *SYNE1* in a study of three multiplex, consanguineous families (Yu et al., 2013). Challenges regarding small recurrence rates occur in the study of these inherited genetic mutations, moreso given smaller cohorts of multiplex families than simplex (Stein, Parikshak, & Geschwind, 2013). In 2015, Krumm and colleagues explored the role of inherited SNVs in simplex ASD, and found that inherited protein disrupting SNVs were enriched in those with ASD compared to controls. Additionally, these variants tended to impact genes that, when disrupted, had deleterious impacts on functioning. In alignment with Zhao and colleagues 2007 theory, these SNVs were more likely to be transmitted to a male proband by their mother

(Krumm et al., 2015) This study also analyzed the impact of inherited CNVs, as well as *de novo* CNVs and SNVs, and determined that all four types uniquely contributed to ASD risk.

Individual recurrent mutations and the exploration of phenotypic subtypes. The utility of identifying increasingly precise, recurrent mutations in large cohorts is to understand whether unique phenotypes emerge along genetic lines. In 2012, molecular inversion probe (MIP) sequencing introduced the lowest cost sequencing method to date which allowed researchers to finally target high-confidence risk genes individually in the size of cohorts that could detect more robust recurrence rates. 44 previously identified target genes were re-sequenced in a cohort of 2,494 probands with ASD (O'Roak, Vives, Fu, et al., 2012). The 44 genes that were targeted were likely gene disrupting, neurologically expressed mutations that had been associated with syndromic ASD and ASD-associated CNV regions. Six genes were found to have higher rates of *de novo* mutations than expected, *CHD8*, *GRIN2B*, *DYRK1A*, *PTEN*, *TBR1*, and *TBL1XR1*, accounting for approximately 1% of the sequenced cohort. Of these six genes, *chromodomain helicase binding protein 8* (*CHD8)* had the highest recurrence rate amongst *de novo* mutations (n = 8), with dual-specificity tyrosine phosphorylation-regulated kinase 1A (*DYRK1A*) next highest in recurrence (n = 3).

Efforts are ongoing to identify distinct phenotypic features of the most recurrent rare LGD mutations, in hopes that some of the phenotypic variability seen in ASD might be explained my distinct behavioral subtypes related to individual genetic variants. In an initial analysis of head circumference, O'Roak and colleagues (2012) found that

individuals with a *CHD8* mutation had significantly larger head sizes (i.e. macrocephaly) than probands without a *CHD8* mutation. Macrocephaly was also observed in individuals with a phosphatase and tensin homolog (*PTEN*) LGD mutation. In contrast, individuals with a disruptive mutation to *DYRK1A* displayed microcephalic head sizes. These findings suggested potential syndromic features to these specific genetic mutations, prompting an in-depth study of phenotype.

DYRK1A proposed to be syndromic form of ASD. There is growing literature suggesting that disruptive mutations to *DYRK1A* results in a distinct pattern of phenotypic traits with an elevated risk for ASD (Iossifov et al., 2014; O'Roak, Vives, Fu, et al., 2012; O'Roak, Vives, Girirajan, et al., 2012). *DYRK1A* was initially identified for it's role in intellectual disability, but has been found to be recurrent in as many as 0.5% of cases with ASD (O'Roak, Vives, Fu, et al., 2012; van Bon, Coe, & deVries, 2015). It is a gene in the Down syndrome critical region of chromosome 21 and is believed to play a causal role in intellectual disability when present in three forms in cases of Down syndrome (Redin et al., 2014; van Bon et al., 2016). Truncating mutations to *DYRK1A* in cases without Down Syndrome (two copies of the chromosome rather than three) result in loss of function to one copy of the gene, which creates a unique phenotype that is still characterized by intellectual disability. The gene appears to play a major role in brain development, specifically neurogenesis, neural plasticity, and cellular death (Bronicki et al., 2015).

In 2008, Moller and colleagues published phenotypic findings from two unrelated patients with truncating chromosomal rearrangements to *DYRK1A* and found shared

features of microcephaly and intellectual disability (ID). Severity differed between the two patients, with one presenting with a more significant seizure history and severe impairments (Moller et al., 2008). In 2011, Van Bon and colleagues reported on an additional patient with a deletion of *DYRK1A*, who presented with intellectual disability, growth retardation, febrile seizures and distinct facial dysmorphology consistent with Moller's patients. Autistic features (accompanied by anxiety) were noted in all three of these patients, although specific diagnoses were not included in the phenotypic classification. Van Bon et al. (2011) also summarized findings from animal models of *DYRK1A* mutations, which present with a phenotype consistent with humans. Drosophila (fruitfly) models of *DYRK1A* truncating mutations show microcephalic head size (Tejedor et al., 1995). In mice, mutations to *DYRK1A* result in reduced growth, developmental delays, motor and learning difficulties and behavioral deficits including anxiety (Fotaki et al., 2002; Fotaki, Martinez De Lagran, Estivill, Arbones, & Dierssen, 2004). Other reports of chromosomal rearrangements to *DYRK1A* showed recurring reports of ID and microcephaly (Fujita et al., 2010; Matsumoto et al., 1997; Oegema et al., 2010; Valetto et al., 2012; Yamamoto et al., 2011).

In 2012, disruptive *DYRK1A* SNVs and small insertion/deletions (indels) were identified as recurrent in cases of ASD, placing new emphasis on assessing for features of ASD in patients with *DYRK1A* mutations (O'Roak, Vives, Fu, et al., 2012; O'Roak, Vives, Girirajan, et al., 2012). Bronicki and colleagues (2015) published on 9 new patients with *DYRK1A* SNVs, one with a microdeletion and provided further evidence for a shared phenotype of intellectual disability, speech and motor delays, feeding

difficulties and microcephaly. A history of ASD diagnosis was documented for four of ten patients, although the presence of hand stereotypies, reduced eye contact, anxiety, and hyperactivity were noted in many of the remaining patients. Also in 2015, Ji et al. published on an additional 9 patients with *DYRK1A* SNV and indels and 5 with microdeletions, finding similar phenotypic features to Bronicki's cohort. ASD was found in the medical records of two participants, anxiety in four.

A 2016 study summarized the largest combined *DYRK1A* cohort to date (n = 15), and was one of the only studies to conduct clinical assessment for ASD in 8 of the patients (van Bon et al., 2016). Van Bon and colleagues found high rates of ASD (88% of those assessed for ASD) as well as recurrent dysmorphic features (e.g. prominent nose, deep-set eyes, and upslanted palpebral fissures at the eyes), intellectual disability, speech impairments, seizures, and hypertonia. Stereotypic behaviors were noted by history in 10 of 11 patients, and anxious behaviors were notes in 5 or 9 patients. Finally, Luco et al. (2016) published on two additional patients with *DYRK1A* SNVs, one noted to have behavioral issues but no diagnoses specified.

While Van Bon and colleagues (2016) were able to provide clinical assessment on 8 individuals with *DYRK1A*, the remaining were evaluated previously by researchers who had used medical records parent-reported diagnostic history only. This discrepancy is difficult to avoid, particularly in the early stages of phenotyping a gene event. However, it suggests the need for further comprehensive assessment. The presence of behavioral deficits and stereotypies in individuals without a formal ASD diagnosis suggest that dimensional assessment of ASD features might capture sub-clinical social

communication and behavior deficits. To date, the studies on *DYRK1A* provide strong evidence for its association with ASD, and suggest a complex phenotype that includes distinct dysmorphology as well as cognitive, neurological, and medical impairments. Further understanding of *DYRK1A*, its syndromic features, and its role as a subtype of ASD are needed.

Additional genes associated with ASD. *PTEN* was first identified for it's role in tumor suppression and its association with cancers when disrupted (Li et al., 1997). Small cohorts of individuals with ASD with *PTEN* were reported prior to 2012, suggesting that it may be a monogenic form of ASD (Butler et al., 2005; Herman et al., 2007; Varga, Pastore, Prior, Herman, & McBride, 2009). *PTEN* plays an important role in genetic pathways responsible for the regulation of cell growth and cellular signaling in social brain pathways; it has been proposed that most *PTEN* mutations associated with ASD are less severe than those impacting tumor-suppression and associated with cancers (Leslie & Longy, 2016; Zhou & Parada, 2012). In other words, ASD and cancers can co-occur in individuals with PTEN disruptions, but don't always. Individuals with *PTEN* and ASD have been observed to also present with macrocephalic head size, intellectual disability, and epilepsy (Zhou & Parada, 2012). Further study has shown that many individuals with *CHD8* present with dysmorphic facial features, such as prominent forehead, wide-set eyes, broad nose and pointed chin (Bernier et al., 2014; O'Roak, Vives, Fu, et al., 2012; Talkowski et al., 2012). In addition, individuals with a *CHD8* gene event frequently have chronic gastrointestinal issues and persistent difficulties falling

asleep. Importantly, Bernier et al (2014) found that 13 of 15 individuals identified with *CHD8* met criteria for ASD.

Another gene recurrently disrupted in cases of ASD is *ADNP*, which has been proposed to be another syndromic form of ASD. This gene disruption is estimated to account for 0.17% of ASD cases (Helsmoortel et al., 2014). *ADNP* is expressed during embryonic development and is believed to be involved in neural tube development and regulation of cellular death. In a study of 10 individuals with a *de novo* LGD *activity-dependent neuroprotective protein (ADNP)* gene mutation, patients were found to have similar facial features, including a prominent forehead, eversion of the eyelid, broad nasal bridge and thin upper lip. In addition to ASD, which was observed in 100% of cases, all patients presented with intellectual disability and most presented with gastrointestinal and sleep difficulties.

Additional genes have been recurrently implicated in ASD and additional comorbid disorders (Iossifov et al., 2014; Neale et al., 2012; Sanders et al., 2012). *GRIN2B* is involved in excitatory and inhibitory neurological signaling, and has been associated with intellectual disability, ASD, and schizophrenia (Dimassi et al., 2013; Tarabeux et al., 2011). *SCN2A* is a gene that regulates voltage-gated sodium channels and the generation of neural action potentials (Kamiya et al., 2004). Individuals with *SCN2A* mutations have presented with ASD, intellectual disability, ataxia, and most often, epilepsy (Celle, Cuoco, Porta, Gimelli, & Tassano, 2013; Tavassoli et al., 2014). Patients with a single functional copy of the *ARID1B* gene have been observed to present with ASD, intellectual disability, speech impairments, and corpus callosum

defects (Halgren et al., 2012). In some cases, Coffin-Siris Syndrome can also present, which is characterized by developmental delays, under-developed fingers and toes, excess hair, and dysmorphic facial features (Kosho, Miyake, & Carey, 2014).

These genes have been most frequently reported in ASD cohorts, and suggest the presence of unique subtypes of ASD. However, each gene only accounts for a fraction of the variability of ASD. At this point, small numbers of individuals with the same gene mutation remain a critical limitation; more robust samples are needed to understand these phenotypic patterns more fully. The existence of numerous other genes in ASD cohorts with even smaller rates of recurrence prompts the question: are these genes potentially linked by shared functions or pathways? Indeed, the discovery of genes like *ADNP* and *ARID1B*, which are involved in the same neuroprotective complex SWI/SNF, suggest points of interconnection and centralized mechanisms (Son & Crabtree, 2014). The discovery of viable connections between genes has the potential to group individuals with mutations in shared functional pathways for more robust analysis of phenotypic differences and has been the subject of much study recently.

Functional networks and pathways of genetic variants. With the discovery of several hundred ASD-associated gene loci prone to rare disruptive SNVs, O'Roak and colleagues (2012) found that 39% of the 126 truncating or severe missense mutations were part of interconnected protein network. ASD candidate genes were found to be the most connected within the network. Additionally, mutations to genes within this protein network were enriched in ASD cohorts only and were not found in unaffected family

members, strengthening evidence for this network's role in ASD risk. Members of this protein interaction network had established functioning in particular neurological processes, such as chromatin remodeling, cell signaling and neuronal development. In particular, many of the genes were part of the Wnt/β-catenin signaling pathway, an important pathway in neuronal communication previously proposed to be involved in ASD (De Ferrari & Moon, 2006). While this pathway may indeed play a role in ASD, it has since been established that it is not the only pathway involving *de novo* LGD mutations (Hormozdiari, Penn, Borenstein, & Eichler, 2015; Iossifov et al., 2014; Iossifov et al., 2012).

Iossifov and colleagues (2012) proposed different functional groups between identified LGD mutations. They found that many of the disruptive mutations identified through exome sequencing overlapped with Fragile X mental retardation protein (FMRP) targets, which were well-established to play a role in ASD in those with Fragile X Syndrome. Following the sequencing of a much larger cohort, Iossifov et al (2014) found that disruptive LGD mutations tended to belong to a series of four functional classes. They reconfirmed enrichment of FMRP targets, while also observing that ASD-associated LGD mutations overlapped with chromatin modifying and post-synaptic density regions, and many were expressed during embryonic development. These functional groups show promise and potential ways to cluster similar genes for analysis. However, many genes belong to more than one functional group, making comparison across groups limited to small sample sizes.

Clustering by timing and region of gene expression. Translational research efforts have proposed functional networks of genes based on where and when they are expressed in the brain during development. This approach has great promise, as it provides a foundation for clustering genes that are more likely to have overlapping phenotypes. Using data from four large-scale studies of *de novo* SNVs, Ben-David and Shifman (2013) found that ASD-associated genes from these studies were most commonly expressed in prenatal brain development compared post-natal development. They highlighted the role of these genes in chromatin regulation, which is vital to cellular transcription.

Willsey and colleagues (2013) developed coexpression networks based around nine high-confidence ASD risk genes based on prior research, including *CHD8*, *DYRK1A*, *SCN2A*, *GRIN2B*, *CUL3*, *ANK2*, *KATNAL2*, *POGZ*, and *TBR1*. By using a human brain transcriptome dataset, which provides data on gene expression across development, beginning post-conception into late adulthood. High-confidence ASD genes *TBR1*, *POGZ*, *SCN2A*, *DYRK1A, and CHD8* were found to be enriched in cortical glutamatergic projection neurons in the prefrontal cortex (Willsey et al., 2013). Specifically, gene expression was observed in the midfetal development, between 10-24 weeks post-conception. Importantly, these neurons are involved in forming early synaptic connections, which are likely to be especially vulnerable to genetic variations.

These findings are echoed by Parikshak and colleagues, which took a modular approach to grouping ASD risk genes based on function and expression similarities, and also found ASD genes to be enriched in stages of fetal cortical development. They

found that ASD risk genes were involved with glutamatergic neurons in the upper layers of the cortex, as well as excitatory and inhibitory cell processes. Additionally, ASD risk genes were linked by common transcriptional and translational processes of protein coding and production, specifically regulated by FMRP (Parikshak et al., 2013). They proposed clusters of ASD risk genes by cellular function that may be a promising means to comparing phenotypic differences between functional groups. However, Parikshak's functional modules do not include some of the most recurrent genes associated with ASD to date, such as *CHD8*, *DYRK1A* and *GRIN2B*.

Given the frequency of chromatin remodeling genes associated with ASD, a network has been proposed that centers around *CHD8* as a primary regulator of down-stream ASD genes (Cotney et al., 2015). Cotney and colleagues explored whether *CHD8* targeted other ASD risk genes within a regulatory network and whether the disruption of *CHD8* dysregulated other ASD risk genes. They found that *CHD8* targeted 47 ASD risk genes in the midfetal phase of brain development and in neural stem cells. In addition, when *CHD8* was knocked out, many ASD risk genes appeared to be more disrupted that other *CHD8* targets than are not ASD-associated. Functionally, the disruption for *CHD8* and subsequent ASD-risk genes decreased neuron reproduction and differentiation in the brain.

Recently, Hormozdiari and colleagues introduced a novel computational approach to identifying biological subnetworks of *de novo* LGD mutations. Two distinct modules were determined that are enriched, the first being significantly enriched for ASD risk genes identified in recurrent *de novo* SNVs (48 genes in the "best" module,

and 80 in the “extended” module). This module also contains genes that are linked to common signaling pathways and multiprotein complexes, including the WNT pathway and the SWI/SNF complex. Peak expression of the genes in Module 1 was identified to be between 8 and 16 weeks post-conception. A second module was identified as 24 genes enriched in probands, which were functionally linked as synaptic plasticity genes and were predominantly expressed postnatally rather than prenatally. Where genes were expressed in the brain was also studied, and the regions identified were consistent with those identified by Willsey et al in 2013. When looking for phenotypic differences between modules, Hormozdiari et al. found that for individuals with both ASD and ID had high overlap with the two initial modules presented. However, individuals with ASD without ID only overlapped with Module 1, suggesting that the genes within Module 2 are more explicitly linked to features of intellectual disability. The development of ID in those with Module 1 genes is proposed to be the result of disruptive, loss-of-function mutations, which appear to be linked to cognitive impairment than the less disruptive mutations that are found in higher functioning probands with ASD.

Each of these studies published in the last three years provide important insight on the potential functional similarities between ASD risk genes, and similar findings across studies is indeed encouraging. Findings thus far seem to suggest that genes diverge in whether they are expressed prenatally or postnatally, and by their roles in transcription, cellular signaling, and synaptic plasticity. It also appears that different groups of genes have different impacts on risk of cognitive impairment. What remains to be seen is whether these functional groupings are phenotypically meaningful in relation

to ASD symptoms specifically. Does severity of symptoms presentation, or the specific constellation of features look similar across these functional modules? Do they in turn look different from the features of another module? These questions are important ones to consider when exploring the utility of genetically-defined subtypes of ASD and highlights an area in need of further study.

Future directions to account for variability in ASD. Remarkable strides have been made in recent years to understand the key genetic factors believed to be impacting ASD risk. With increasingly precise technology, rare LGD mutations have taken the stage as critical genetic variants that are recurrent in populations with ASD and may result in a particular array of medical, cognitive, and behavioral features. Yet, even when comparing individuals with the same LGD mutation, variability in functioning and symptom presentation exists. For example, while a majority of individuals with a disruptive *DYRK1A* mutation have ASD, not all do (van Bon, Coe, Bernier, et al., 2015). Variations in cognitive and language impairments exist within all groups of specific LGD mutations. Accounting for this variability is crucial to understanding genetic impact and defining genetic subtypes of ASD.

Methods of accounting for familial background and dimensional assessment

While ASD diagnosis is based upon a set of categorical criteria, the overlap of ASD with cognitive, language, motor, and medical complications is well-established, as discussed in detail above. The presence and severity of these complications range across individuals and directly impact how ASD presents and implications for treatment. This diverse presentation is not unique to ASD; developmental disorders as a whole

have high rates of comorbidity. 30-50% of children with intellectual disability have an additional psychiatric disorder (Einfeld, Ellis, & Emerson, 2011). Individuals with cerebral palsy show elevated rates of intellectual disability, ASD, learning disorders, ADHD, and seizure disorders (Moreno-De-Luca, Ledbetter, & Martin, 2012; Odding, Roebroeck, & Stam, 2006). High rates of co-occurrence between these types of disorders suggest that, while they are categorically distinct diagnoses, they are bound by common biological mechanisms. Thus, in order to understand variability in ASD, researchers must situate the disorder in the context of a broader range of overlapping disorders and developmental deficits.

A dimensional framework for co-occurring disorders. Genetic research of ASD provides confirmation of the inter-connection of developmental disorders. Studies of 16p11.2 and 1q21.1 copy number variants, as discussed previously, reveal a dynamic range of co-occurring disorders that manifest differently across individuals including ASD, language disorders, intellectual disability, and motor deficits. Neurological findings have suggested that a common brain pathway for developmental disorders accounts for high rates of comorbidity (A.J. Capute & Accardo, 1991). In efforts to conceptualize the translation of neurological disruptions to clinical manifestations, Moreno-de-Luca and colleagues (2013) describe a “developmental brain dysfunction” framework, which builds off a term introduced by Capute and colleagues to describe the continuum of severity in developmental disorders (A.J. Capute & Accardo, 1991; A. J. Capute & Palmer, 1980; A. Moreno-De-Luca et al., 2013). This model proposes that categorical developmental disorders share common

risk factors and thus, frequently co-occur. Various etiological factors, including genetic disruptions and environmental trauma, impact the brain in ways that cause some form of cognitive, motor, behavioral, and neurophysiological manifestations. The precise constellation and severity of deficits is then dictated by an individual's larger genetic background, which includes the interaction of an expressed genetic variant with the combined genetic variety from an individual's parents.

Gene expressivity and penetrance. When considering variability in phenotype related to an individual's given genotype, one must consider variations due to both expressivity and penetrance. Expressivity captures the extent to which a given gene variant is expressed at the phenotypic level (i.e. observable features). Expressivity is impacted by the remaining genetic composition of an individual's genome, which may exacerbate or compensate for a gene mutation, thus affecting its phenotypic impact (Griffiths, Miller, Suzuki, Lewontin, & Gelbart, 2000). Penetrance, in contrast, refers to the proportion of people with the gene mutation that show signs of the genetic disorder, an all or nothing phenomenon (GHR, 2016). When a genetic variant is phenotypically expressed in some but not all affected individuals, it is referred to as reduced, or incomplete penetrance. Penetrance is also impacted by genetic factors, including family genetic background or sex of the affected individual (GHR, 2016; Zhao et al., 2007). Put another way, penetrance captures whether or not the variant is expressed and expressivity describes the level of expression (Griffiths et al., 2000).

Diverse presentations in developmental disorders have been attributed to both variable expressivity and incomplete penetrance, as discussed previously with 16p11.2

copy number variants, yet it is unclear how much each are playing a role in phenotypic variability. Moreno-De-Luca and colleagues assert that approaches to phenotype measurement influence the way expressivity and penetrance are understood. For example, if using dichotomous variables (i.e. the presence or absence of a disorder), then incomplete penetrance is a likely conclusion. If continuous variables are used, then all affected individuals may show some level of impairment, albeit sub-clinical in some. Thus the developmental brain dysfunction framework utilizes dimensional assessment in efforts to capture variable expressivity, relying more heavily on a quantitative measurement of symptoms than categorical diagnoses. Moreno-De-Luca et al. (2013) assert that this framework could account for variability within individuals with the same genetic mutation, whether copy number or single gene variant. They highlight the utility of comparing the phenotype of affected individuals to that of their parents in order to better account for genetic background. Comparison of parent and offspring has been a well-established method of confirming genetic heritability of phenotypic traits and medical risks. Understanding prior methods for capturing parent-offspring heritability is important prior to exploring its use in the study of ASD.

Parent-child comparisons as evidence of trait heritability. There is a long history of drawing comparisons between parents and their children on phenotypic traits and medical conditions. Most of these comparisons rely on correlations to demonstrate a relationship between generations. Preece (1996) found strong correlations between monozygotic twins and their family members on measures of height; 90% of the observed variance in stature was due to parental genes. Heritability of head

circumference in typically developing twins and their parents is estimated between 50-70% depending on the study (Clark, 1955; Weaver & Christian, 1980). Comparisons of obesity amongst family members has been well-documented, with parent body mass index (BMI) correlating significantly with the BMI of their children, independent of other environmental factors (Koomanaee et al., 2016; Xi et al., 2009). In addition to physiological traits, parents have been important subjects of study in research looking at addictive behaviors and medical risks. Studies have shown that smoking addiction is highly correlated in twins and is related to parental smoking behaviors (Brook, Whiteman, Czeisler, Shapiro, & Cohen, 1997; Griesler, Kandel, & Davies, 2002; Han, McGue, & Iacono, 1999; Vuolo & Staff, 2013). Determining familial heritability of various biological risk factors, such as cholesterol levels and insulin sensitivity, has been important to anticipate risk of disease across the lifespan. A study of 179 parents and their children found that blood pressure, cholesterol and insulin levels were significantly correlated across generations, even after controlling for BMI (Halvorsen et al., 2015). A familial connection in asthma risk and pulmonary conditions has also been found (Bouzigon et al., 2004).

Parental psychopathology is considered an important predictor of child psychiatric illness. Parents with ADHD are more likely to have children who meet criteria for the disorder, and mothers of children with ADHD show elevated anxiety and depressive symptoms, which are common comorbidities seen in their children (Xia, Shen, & Zhang, 2015). Studies have linked parental substance abuse and antisocial tendencies with disruptive disorders in their offspring (i.e. ADHD, oppositional defiant

disorder, conduct disorder) by a common heritable externalizing behavior characteristic (Bornovalova, Hicks, Iacono, & McGue, 2010; Hicks, Foster, Iacono, & McGue, 2013). Schizophrenia is thought to be hereditary, as children of parents with schizophrenia are at higher risk of developing the illness themselves (Gottesman, Laursen, Bertelsen, & Mortensen, 2010; Sanchez-Gistau et al., 2015). In most of these studies that compare parent and child physiology and psychopathology, researchers are careful to acknowledge that significant intra-familial correlations are likely attributable to a combination of genetics and shared environment.

Characteristics associated with developmental disorders such as ASD have also been found to be subject to heritability. Cognitive ability, as measured by an intelligence quotient (IQ) score, has been identified as a heritable trait amongst family members. Indeed, in studies of adopted twins raised apart, IQ remains highly correlated among twins and with birth parents (Devlin, Daniels, & Roeder, 1997). Studies show that 30-70% of the variance in cognitive ability is accounted for by parental cognition (Devlin et al., 1997; Furlow, Armijo-Prewitt, Gangestad, & Thornhill, 1997; Plomin, 1990). The heritability of social behavior has been demonstrated to be as high as 0.75, with correlations between parent and child at 0.50 (Constantino & Todd, 2005). As mentioned previously, there is strong evidence to support the existence of broader autism phenotype and its existence in parents of simplex families, suggesting that variations amongst parent phenotypes are important to take into consideration when evaluating proband phenotypic presentation.

Family member phenotypic contribution in genetically-defined developmental disorders. The abovementioned studies are looking at similarities between family members and children without the presence of a known disruptive mutation. It follows that if children and their family members are prone to displaying similar medical and behavioral conditions, larger genetic background will still play an important role in determining the characteristics of an individual with a disruptive gene variant. Comparison of unaffected family member phenotype to affected proband phenotype has been explored for a variety of genetic disorders thus far.

Fraser & Sadovnick (1976) studied 25 patients with Down Syndrome and compared their intellectual functioning to first-degree relatives. Down Syndrome occurs when there is an extra copy of the 21st chromosome, which results in a distinct physical profile and intellectual disability. This study found significant correlations between the IQ of patients with Down Syndrome, as measured by standardized cognitive assessment, and IQ of unaffected mothers and siblings (Fraser & Sadovnick, 1976). In a similar study of Klinefelter's Syndrome, a disorder seen in males and characterized by intellectual impairment and dysmorphology, the variability in intellectual functioning observed in probands was compared to that of unaffected siblings. In 24 proband-sibling pairs, they found that while proband IQ was significantly lower than sibling IQ, significant correlations remained between probands and siblings (Netley, 1987). Netley then used linear regression to estimate proband IQ based upon sibling IQ, concluding that there could be predictive value for families trying to understand their affected child's developmental trajectory.

In a study of individuals with Prader-Willi Syndrome (PWS) and their unaffected parents, Malich and colleagues (2000) compared parent and child presentation of both anthropomorphic and cognitive traits. PWS is a disorder that is caused by the absence of paternal contribution to chromosome 15q11-q13 and is characterized by short stature, obesity, microcephaly, and developmental delay. Much like genetically-defined developmental disorders, PWS has a variable presentation across individuals. Malich et al. found that proband height, BMI, and IQ were all positively correlated with the same traits in parents, particularly mothers.

Olszewski and colleagues (2014) studied the relationship between the cognitive ability of probands with 22q11.2 deletion (velo-cardio-facial) syndrome and their unaffected parents and siblings. They found that cognitive functioning of probands was significantly associated with their parents and sibling. In summary, these studies provide correlational evidence for the heritability of cognitive and physical traits in spite of a genetic variant that causes a developmental disorder. However, only Netley's 1987 study estimates the magnitude to which the gene is impacting phenotype, which has the potential to be predictive and inform treatment planning.

Quantifying parent-child effect sizes. Given evidence provided in the abovementioned studies, unaffected parental phenotype can serve as a good estimate of "expected" outcome in the absence of a disruptive genetic event. Actually measuring the discrepancy between "expected" and "observed" proband outcome could provide a quantitative measurement of a genetic variant's deleterious impact in a variety of phenotypic domains. Put in other words, Morrow (2015) describes this type of analysis

as measuring “the distance that a spontaneous genetic mutation leads the apple to roll away from the tree.” This approach has potential to inform variability in ASD symptoms, such as social behaviors, across affected probands with the same genetic variant. Two unrelated children with the same genetic mutation would be hypothesized to have different starting points or expected outcomes based on their family background. The impact of the gene mutation may be similar, but the observed outcomes would be different.

While families have been a critical part of genetic research of developmental disorders in the past, particularly ASD, most comparisons in phenotype across genetically-defined cohorts have been made using affected population means (Morrow, 2015). For example, Malich et al. (2000) measured proband discrepancy from population norms via Z-score and then compared Z scores of unaffected parents and controls. Researchers have proposed that measuring the extent to which a proband’s functioning deviates from the functioning of unaffected family members may be a better basis for measurement and account for variability seen in symptom presentation (Moreno-De-Luca et al., 2015; A. Moreno-De-Luca et al., 2013). Moreno-de-Luca and colleagues (2015) used this approach to quantify the impact of a *de novo* 16p11.2 CNV deletion, one of the most recurrent CNVs associated with ASD. Variant impact was calculated as the difference between proband and parental performance. On measures of cognition, social behavior and motor skills, 56 affected probands were compared to their unaffected parents and siblings. Relative to their parents, children with the deletion showed a 1.7 standard deviation (SD) decrease in cognitive ability, 2.2 SD decrease in

social behavior, and 1.3 SD decrease in motor performance. Similar to other studies of genetically-defined developmental disorders, significant correlations were found between unaffected parents and probands in all measured areas, indicating that the observed outcome of children with 16p11.2 deletions is still tied to familial traits. This is a promising means of quantifying genetic impact in developmental disorders that has yet to be done for LGD mutations associated with ASD.

Dimensional assessments. Measuring the discrepancy between parent and child phenotype, or effect size, relies on the assumption that phenotype varies along a continuum. To quantify genetic impact, dimensional assessment is required to capture a range of functioning. Current diagnostic criteria for developmental disorders (i.e. ASD, intellectual disability) remain descriptive and categorical at this time. Measures that quantify cognitive, behavioral, motor, and anthropomorphic qualities are required to effectively measure the extent to which a gene disrupts functioning across domains. To make meaningful comparisons across family members of varying ages, assessments with standardized norms that demonstrate strong reliability and validity are needed. The following measures are well-established dimensional assessments of phenotype related to developmental disorders.

Anthropomorphic traits, such as height, head circumference, and BMI are quantitatively measured and commonly converted to Z-scores to compare across individuals (Moreno-De-Luca et al., 2015). Motor dexterity has been previously measured by the Purdue Pegboard Test (PPT), which can be used to assess a wide age range and demonstrates satisfactory reliability and validity (Moreno-De-Luca et al.,

2015; Tiffin & Asher, 1948). The PPT measures both fine and gross motor abilities, in which participants are presented with a board with two rows of 25 holes down the center and four trays with small metal pegs. Participants are instructed to pick up and place as many pegs in the holes within 30 seconds, first with their dominant hand, then non-dominant hand, then both hands simultaneously. Standard scores are generated for each task, with a mean of 50 and standard deviation of 10.

Cognitive assessments are a naturally quantitative measurement tool, generally split into child and adult measures. A common cognitive assessment used in research on children with developmental disorders include the Differential Abilities Scales, DAS-II (Elliott, 2007). The DAS-II is normed for children ages 2 years, 6 months to 17 years, 11 months; it includes an Early Years battery (ages 2:6 – 6:11) and a School Age battery (ages 7:0 to 17:11). The Early Years lower level battery is composed of verbal and nonverbal domains, while the Early Years upper level and School Age batteries contains verbal, nonverbal and spatial domains. Scores on the core subtests for each battery yield standard scores for a General Conceptual Ability (GCA), Special Nonverbal Composite (SNC; EY upper level and SA), as well as verbal, nonverbal, and spatial domain scores. Standards scores have a mean of 100 and a standard deviation (SD) of 15. Individual subtests are measured in T scores, with a mean of 50 and a SD of 10. The DAS-II demonstrates excellent reliability, with high internal consistency reliability coefficients and test-retest stability coefficients. Criterion-related validity of the DAS-II is also satisfactory, with high correlations to other measures of intelligence and academic achievement (Sattler, 2008).

The Wechsler scales are common assessments for adult cognitive abilities, such as the Wechsler Adult Intelligence Scales, Fourth Edition (WAIS-IV) and an abbreviated cognitive screener, the Wechsler Abbreviated Scales of Intelligence, 2nd edition (Wechsler, Coalson, & Raiford, 2014; Wechsler & Hsiao-pin, 2011). The Wechsler Abbreviated Scales of Intelligence, 2nd edition (WASI-II) is considered a screening test that estimates overall intelligence of children and adults, ages 6-89, with satisfactory internal consistency and test-retest reliability and acceptable criterion-related validity (Sattler, 2008). The WASI-II has become a frequently used assessment tool for family members of probands with ASD, as it provides an assessment of both verbal and nonverbal cognitive abilities in a brief format. The WASI-II is not intended to be treated as a comprehensive cognitive assessment like the WAIS-IV, but rather as an IQ estimate (Axelrod, 2002; Sattler, 2008).

Importantly, these cognitive assessments are normed on typically developing populations rather than developmentally disabled populations. Where this poses a problem is when an individual is severely impaired in areas of cognition, language, or motor development, such that scores are not able to be generated. While this represents a small portion of individuals with ASD, those with *de novo* disruptive genetic mutations are often some of the most severely impacted individuals (Iossifov et al., 2014; Sanders et al., 2015). Thus, when studying rare disruptive genetic mutations associated with ASD, it is necessary to acknowledge the limitations of standardized cognitive assessment. It has been proposed that in situations where meaningful cognitive scores cannot be generated, that other measures may serve as a proxy for

verbal IQ, such as the Peabody Picture Vocabulary Test, 4th edition, PPVT-4 (Dunn & Dunn, 2007; Plesa Skwerer, Jordan, Brukilacchio, & Tager-Flusberg, 2016; Sykes et al., 2009). The PPVT-4 measures receptive language abilities in children and adults, and demonstrates good reliability and validity as a measure of language abilities for populations with and without special needs (Dunn & Dunn, 2007). This standardized measure generates standard scores with a mean of 100 and standard deviation of 15.

When quantifying social behaviors related to ASD, the Social Responsiveness Scale, 2nd edition (SRS-2) has become a commonly-used assessment (Constantino & Gruber, 2012). The SRS-2 is a 65-item questionnaire that takes approximately 15-20 minutes to complete, and measures behaviors related to reciprocal communication, social interaction, and repetitive, restricted behaviors. T scores are generated with a mean of 50 and standard deviation of 10. It is normed for both children and adults, making it a useful tool for measuring social behavior across family members of varying ages. The measure also demonstrates good reliability (Constantino & Gruber, 2012). SRS-2 scores have been demonstrated to be a continuously distributed measurement of severity of ASD-related behavioral impairments in an epidemiological sample of school-age children, which provides further evidence of it's utility as a dimensional assessment of ASD symptoms (Constantino, Przybeck, Friesen, & Todd, 2000). However, as discussed earlier, limitations to the SRS-2 have also been noted. Studies have found that the SRS-2 may not distinguish between ASD and non-ASD-related behavioral challenges for low functioning individuals and that the SRS-2 scores may be inflated for this population (Hus et al., 2013a).

Statistical analysis methods and current limitations. The utility of measuring effect sizes between parent and proband phenotype is maximized when studying a group of probands with the same genetic variant, as seen in the study of 16p11.2 (Moreno-De-Luca et al., 2015). Given strong evidence for the role that LGD mutations play in ASD risk, it follows that the use of a parent-child discrepancy approach for probands with high confidence ASD risk gene mutations could clarify genetic impact and clarify potential ASD subtypes. In their analysis of 16p11.2 CNVs, Moreno-De-Luca and colleagues (2015) hypothesized that since the affected region of the genome was kept constant across individuals, observed clinical variability should be explained by variable expressivity of genes in that CNV region and/or larger genetic background. With analysis of single gene mutations, the region of interest is made even more precise, and when there are not other disruptive mutations present to further impact phenotype, it can be hypothesized that parent genetic background plays a significant role in the observed phenotypic variability across individuals with the same single gene mutation.

To date, the ideal statistical analysis for parent-child phenotypic comparisons involves linear mixed modeling (Moreno-De-Luca et al., 2015). Linear mixed modeling (LMM) allows for within-family effects to be measured, which is important for obtaining accurate correlations between parent and probands. One limitation of this approach is that it relies on large sample sizes. However, an average discrepancy value, or delta between parent and proband, can still be useful without LMM and can be calculated as follows:

$$\Delta = \frac{proband\ mean - unaffected\ parent\ mean}{unaffected\ parent\ standard\ deviation}$$

Given how recently recurrent LGD mutations have been associated with ASD, sample sizes with the same single gene mutation are still small. Recent efforts to group recurrent genetic variants by biological function and pathway show promise as a means to generate groups large enough to statistically compare discrepancy values. However, there has yet to be a classification system that shows sufficient evidence that genes with a shared function or pathway would generate the same quantitative impact or same phenotype. For example, preliminary analyses compared parent-child effects sizes between probands with *CHD8* and *CHD8* target LGD mutations (termed "CHD8 target" group), as described by Cotney and colleagues (2015), and effect sizes for the *CHD8* target group were significantly larger for social behavior as measured by the SRS. However, while *CHD8* target genes are impacted by *CHD8*, many of gene mutations appear to have their own distinct phenotype, including unique cognitive, language, and neurobehavioral profiles. It is also not fully known how genes that result in loss of function or gain of function might impact phenotype differently. Thus at this time, use of family background to meaningfully quantify genetic impact and characterize phenotypic severity requires subjects that share the same gene mutation. Detection of increasingly large samples with the same *de novo* LGD mutation increases the need for comprehensive phenotyping efforts. Given promising evidence in the literature, the use of parent-child discrepancies should be considered as a useful sub-component of comprehensive phenotyping of single gene mutations, with future aims of more extensive statistical analysis.

Current Study

Statement of the problem. There is strong evidence that much of the variability observed in ASD is related to co-occurring behavioral, medical and neurological complications that impact a child's overall functioning and behavioral presentation. Study of the genetic causes of ASD has revealed a similarly diverse range of genetic mutations associated with the disorder. Disruptive SNVs have garnered much attention for their enrichment in ASD populations, particularly in those with comorbid intellectual impairments. Comprehensive phenotyping is necessary for each of these mutations in order to identify patterns in phenotypic profile that could inform providers' understanding of developmental course and treatment options. To date, many of these disruptive genetic variants appear to have distinct phenotypic features yet some level of variation in presentation exists between individuals. Phenotyping has also been limited to small sample sizes. Larger groups of affected individuals are needed to strengthen observed patterns in development and behavior.

DYRK1A is a gene recurrently disrupted in cases with ASD (O'Roak, Vives, Fu, et al., 2012). A growing body of literature suggests that disruptive mutations to *DYRK1A* result in a series of developmental challenges with an elevated risk for ASD (Iossifov et al., 2014; O'Roak, Vives, Fu, et al., 2012; O'Roak, Vives, Girirajan, et al., 2012). *DYRK1A*, a gene in the Down syndrome critical region of chromosome 21, was initially identified for it's role in intellectual disability (Redin et al., 2014; van Bon et al., 2016). Disruptive mutations to *DYRK1A* can result in loss of function to one copy of the gene, termed *DYRK1A* haploinsufficiency, which has been proposed to create a unique

phenotype. This emerging phenotype is primarily characterized by intellectual disability, ASD, febrile seizures, speech delays, motor deficits, and feeding difficulties. The gene appears to play a major role in brain development, specifically neurogenesis, neural plasticity, and cellular death (Bronicki et al., 2015). When a mutation disrupts *DYRK1A's* intended functioning, it is believed to cause reduced brain growth and visibly microcephaly, a predominant phenotypic feature. A variety of mutations to *DYRK1A* have been implicated in this emerging phenotype, including small disruptive SNVs (i.e. nonsense, frameshift, and splice-site mutations) and chromosomal rearrangements (i.e. translocations and microdeletions). Thus far, studies have found similar genotype-to-phenotype correlations, but sample sizes have been small (Ji et al., 2015; van Bon et al., 2016).

With approximately 0.5% recurrence in populations with ASD, there is evidence to suggest that *DYRK1A* may represent a unique subtype of ASD (O'Roak, Vives, Fu, et al., 2012). In previously published cases assessed for ASD, incidence of ASD remains variable, suggesting variable expressivity for the gene. While 87% of Van Bon et al's (2016) cohort (n = 8 assessed for ASD) met diagnostic criteria, 40% of Bronicki et al's (2015) cohort (n =10) had previous diagnoses of ASD. It is important to note, however, that there are inconsistencies in whether studies gather information about ASD by clinical assessment or medical record review. Reliance on medical records alone allows for the possibility that ASD features may be present, but not diagnosed. Additionally, a dichotomous diagnosis/no diagnosis approach has been taken to phenotyping *DYRK1A*, which does not capture sub-clinical or undiagnosed social communication

and behavior deficits. This is exemplified in Bronicki et al's publication on *DYRK1A*, where behavioral deficits and stereotypies in individuals are noted without an ASD diagnosis in an additional 50% (5/10 individuals) of the published cohort. Thus, a quantitative assessment approach may yield additional insight into *DYRK1A's* relationship with ASD symptoms. Additionally, patient ascertainment will significantly impact the prevalence of the ASD phenotype within *DYRK1A* cohorts.

The limitations of the existing literature indicate that additional quantitative characterization of autism symptoms and co-occurring impairments in individuals with mutations to *DYRK1A* is needed. Phenotyping efforts also have yet to situate individuals with *DYRK1A* in the context of larger familial genetic background, which may provide important information regarding genetic impact across domains of functioning. Sample sizes have remained too small to draw conclusions regarding the course of *DYRK1A*'s impact across development and have yet to compare phenotypic features of *DYRK1A* to a comparison group; report of a larger cohort and comparison to other developmentally disabled samples would allow for richer exploration of phenotypic profile.

Purpose of the study.

(See appendix table for summary table of aims and methods)

***Aim 1*:** To provide a summary of the genetic mechanism of *DYRK1A*, including a list of all known disruptive mutations associated with genetic changes, protein disruption, and resulting phenotype. To review linkage between genetic variation and phenotypic presentation through summary of known literature on functional models of *DYRK1A*.

***Aim 2*: (a)** To summarize gender, age and commonly observed phenotypic characteristics of known cases of *DYRK1A* disruptive SNVs (nonsense, splice-site, missense, and frameshift mutations) based upon information from 42 individuals, including cognitive, language, motor, behavioral and medical diagnoses across affected individuals. Additionally, to summarize characteristics of 19 individuals with chromosomal rearrangements to *DYRK1A* (translocations and microdeletions) and compare phenotypic features to those seen in the disruptive SNV cohort. Features considered common if observed in 75% or more of previously published cases. **(b)** To compare incidence of commonly observed phenotypic features in cases of *DYRK1A* to the incidence of those features in a large sample of individuals ascertained for ASD. **(c)** To examine quantitatively assessed domains of functioning in a subset of individuals with *DYRK1A* (n = 10) and compare IQ, adaptive functioning, social responsiveness, ASD-related features, head circumference, and developmental milestones observed in *DYRK1A* to the same phenotypic features in an age-matched subset of a larger sample of individuals with ASD and a cohort of individuals with another ASD-associated gene mutation, *CHD8*.

***Hypothesis*:** Common features will emerge across individuals with mutations to *DYRK1A*. Specifically, a majority of individuals with *DYRK1A* will exhibit intellectual disability, speech delays, motor deficits, ASD-related deficits (e.g. ASD diagnosis, stereotypic behaviors, anxious behaviors), feeding difficulties, vision abnormalities, and microcephaly as has been reported in smaller cohorts in previous literature (aim 2a). It is hypothesized that individuals with chromosomal rearrangements will show lower rates

of common features due to greater variability in the phenotype relative to the SNV group, but that similar phenotypic features will be present for both the SNV and chromosomal rearrangement cohorts, as has been found in previous literature (Ji et al., 2015; Luco et al., 2016) (aim 2a). Common features, including intellectual disability, microcephaly, motor deficits, feeding difficulties, and vision abnormalities, will occur at higher rates in the *DYRK1A* cohort than the larger ASD population (aim 2b). Specifically, those with *DYRK1A* will demonstrate greater impairment in the areas of IQ and adaptive functioning, as well as smaller head size, when compared to an age-matched subset of a large sample of children with ASD and the *CHD8* cohort (aim 2c).

***Aim 3*:** Given evidence of variability observed between individuals with the same genetic event, the third aim will situate observed phenotypic variability in probands in the context of their unaffected parents' phenotype, thus accounting for familial background and "expected" outcome in the absence of a mutation. **(a)** Regression analyses will be conducted to explore the impact of parental phenotype and gene event on child phenotype and group differences. The relationship between parent and affected child phenotype will be explored for other ASD-associated groups (e.g. idiopathic ASD, 16p11.2 copy number deletions, and *CHD8* SNVs) and compared to that of *DYRK1A*. **(b)** The impact of *DYRK1A* on IQ, ASD symptoms, and head circumference will be quantified by the discrepancy between affected proband and unaffected parent presentation, or "effect size" (defined in methods below). Effect size for *DYRK1A* will be compared to calculated effect sizes for idiopathic ASD, 16p11.2 deletions, and *CHD8* mutations.

***Hypothesis*:** The relationship between parental phenotype and child phenotype will differ for *DYRK1A* compared to other groups. It is hypothesized that given the scale of disruption observed in DYRK1A haploinsuffiency, parental contribution will be significant but weaker compared to groups with less disruptive genetic events (16p11.2) or no know genetic cause (e.g. idiopathic). Given the severity of symptoms presented by individuals with *DYRK1A*, it is hypothesized that parent-proband effect sizes (i.e. quantifiable genetic impact) for *DYRK1A* will be larger for full scale IQ and head circumference but not ASD symptoms when compared to effect sizes of other known genetic variants and the larger population of individuals with ASD.

Chapter III. Methods

Participants

***DYRK1A* sample.** Participants included 41 individuals with *de novo*, disruptive, pathogenic SNVs (nonsense, splice-site, frameshift, and missense mutations) at the *DYRK1A* gene. (Figure 1; see Additional files 1 and 2 for full variant information). The sample includes 10 individuals assessed as part of an ongoing study at the University of Washington (UW), including 6 new cases identified through clinical genetic testing, 3 previously published cases recruited from the Simons Simplex Collection (see below), and 1 previously published case recruited through clinical genetic testing. In addition to the 4 previously published cases studied at UW, 31 other previously published cases with disruptive SNVs were included in the sample. All subjects were identified via clinical exome sequencing, or exome or targeted sequencing of research cohorts ascertained for a diagnosis of ASD or ID.

Those seen at UW (UW-SNV group; $n = 9$ *de novo* and $n = 1$ non-maternal; see Table 1 for variant information ("The Genotype-Tissue Expression (GTEx) project,")) completed standardized behavioral measures and medical evaluations by clinicians naïve to gene group membership as part of a study evaluating individuals ages four and older with ASD-associated, disruptive mutations. Biological parents of the participants were also characterized.

Thirty-two previously published cases of *DYRK1A* disruptive SNVs (Pub-SNV group) included 31 *de novo* cases and 1 non-maternal case (Bronicki et al., 2015; Iglesias et al., 2014; Ji et al., 2015; Luco et al., 2016; Okamoto et al., 2015; Ruaud et

al., 2015; van Bon et al., 2016) with available medical history, physical features, and diagnoses.

Additionally, the phenotype of 19 previously published cases of *de novo DYRK1A* chromosomal rearrangements (Pub-CHR group), including microdeletions and translocations, was described and compared to those with disruptive SNVs (Bartsch et al., 1997; Courcet et al., 2012; Fujita et al., 2010; Ji et al., 2015; Matsumoto et al., 1997; Oegema et al., 2010; Valetto et al., 2012; van Bon et al., 2011; Yamamoto et al., 2011). See Table 2 for participant characteristics for the 61 total *DYRK1A* sample participants.

Comparison samples. Secondary data from an idiopathic subset of the Simons Simplex Collection (SSC), a large sample ascertained for ASD, were used as a comparison cohort for Aims 2b, 2c, and 3a. The SSC was a cohort of 2,446 simplex families, including a single proband with ASD age 4-18, unaffected biological parents, and any unaffected siblings (Fischbach & Lord, 2010). As part of the SSC, probands were assessed on measures of neurocognitive functioning, social communication behaviors, motor skills, physical features (e.g. head circumference), and medical history (measures described below). Probands were included in the idiopathic subset (n = 1,981) if they had no known disruptive SNVs or deleterious CNVs, as determined by sequencing efforts by Sanders and colleagues in 2015 (Sanders et al., 2015). In order to account for high rates of ID seen in the *DYRK1A* haploinsufficiency, a subset of the idiopathic group with full-scale IQ below 70 (n = 487) served as an additional comparison group. Additionally, a randomly selected age- and gender-matched subset

of this sample was used for comparison to the subset of *DYRK1A* cases assessed quantitatively at the UW (n = 10) in Aim 2c.

Twelve individuals with disruptive SNVs at a different high-confidence ASD risk gene, *CHD8* (chromodomain helicase-DNA-binding protein 8), participating in the same UW characterization study and assessed by clinicians naïve to implicated gene disruption, served as a comparison cohort matched on ascertainment approach. This sample served as a comparison group in Aims 2c and 3b.

Additionally, participants from the Genetic Linkage Study of Autism (GEN) study will be used as a comparison group for Aim 3a. IQ for parents and probands was collected in this study of 250 multiplex families. Lastly, 56 probands with *de novo* 16p11.2 copy number deletions and their unaffected parents, a cohort characterized in the Simons Variation in Individuals Project (SVIP), will be utilized to explore the relationship of parent phenotype on child phenotype as it relates to different ASD-associated gene mutations in aim 3a.

Measures

Categorical assessment of diagnostic history and developmental characteristics. Psychiatric and medical history, developmental milestones, and physiological characteristics were gathered from Pub-SNV and Pub-CHR cases. In additional to published data, supplemental case reports were reviewed when available, detailing medical history and developmental trajectory.

For UW-SNV study participants, a structured caregiver interview, adapted from the SSC, was administered to gather information about developmental, psychiatric and

medical history. When caregiver-endorsed diagnoses required additional clarification, medical records were reviewed for confirmation. Current psychiatric diagnoses were confirmed by a licensed clinical psychologist following characterization using all available information. A physical and dysmorphology exam was conducted by a licensed medical geneticist.

Quantitative assessment of *DYRK1A* UW-SNV (*n* = 10) and *CHD8* (*n* = 12).

Head circumference. Orbital frontal head circumference was measured and standardized values calculated using a normative population reference (Roche, Mukherjee, Guo, & Moore, 1987).

Cognitive functioning. Full-scale IQ was assessed in probands and unaffected parents. Probands ages 4 years, 0 months to 17 years, 11 months were administered the Differential Abilities Scales, 2nd Edition (Elliott, 2007). Probands 18 and older, as well as unaffected parents, were administered the Wechsler Abbreviated Scales of Intelligence (Wechsler & Hsiao-pin, 2011). For all assessments, IQ scores were generated using deviation (standard; mean = 100, SD = 15) or ratio scores (mental age equivalent/chronological age x 100). Ratio scores were derived using age equivalence values if standard scores were not possible to calculate due to subject's level of functioning.

Adaptive functioning. Caregivers were administered the Vineland Adaptive Behavior Scales, 2nd edition (VABS-2) to measure adaptive functioning across communication, daily living skills, and social domains (Sparrow, Balla, Ciccheti, & Doll, 2005).

ASD-specific assessment. Research-reliable clinicians administered the appropriate module of the Autism Diagnostic Observation Schedule, 2nd Edition (ADOS-2; (Lord , Rutter, DiLavore, & Risi, 1999; Lord et al., 2013) and Autism Diagnostic Interview-Revised (ADI-R; (Lord et al., 1994). ADOS calibrated severity scores and items regarding age of first words and age of first steps from the ADI were used in analyses. Total T score from the Social Responsiveness Scale (SRS-2; (Constantino & Gruber, 2012) was used to quantify ASD associated symptoms in all UW-SNV family members.

Analytic approach

Aim 1. In accordance with the first aim, genetic data from 61 participants was compiled and presented in relation to clinical phenotype. Specifically, by reviewing genetic reports of Pub-SNV, UW-SNV, and Pub-CHR cases, a list of specific variants and resulting genetic changes was summarized and compared across participants.

Aim 2a and 2b. Fisher's exact tests were used to compare frequencies of features commonly found for *DYRK1A* across disruptive SNV (Pub-SNV and UW-SNV) and chromosomal rearrangement (Pub-CHR) groups. These features include intellectual disability, speech delay (first words after 24 months of age), motor deficits (e.g. delayed walking, poor coordination, abnormal gait), ASD-related deficits (e.g. ASD diagnosis, stereotypic behaviors, anxious behaviors), feeding difficulties, seizures, vision abnormalities, and microcephaly. The frequency of most common features (present in 75% or more cases) was compared across *DYRK1A* and idiopathic ASD (full idiopathic and IQ < 70) groups. Only characteristics specifically noted in case reports

were included in analyses; if a phenotypic characteristic was not reported, it was treated as missing for that individual, thus total frequencies reflect those who reported on the presence or absence of a given characteristic.

Aim 2c. UW-SNV *DYRK1A* participants were compared to (1) a randomly subsampled age and gender-matched subset of the SSC idiopathic sample and (2) a cohort with disruptive *CHD8* mutations on quantitatively assessed domains of functioning, including head circumference, IQ, adaptive functioning, ASD severity (ADOS calibrated severity score), age of first words (ADI-R), and age of first independent steps (ADI-R). Independent sample *t*-tests were used to compare *DYRK1A*, idiopathic, and *CHD8* groups, using Bonferroni adjustment for multiple comparisons ($p < 0.002$).

Aim 3. Regression analyses were conducted to explore the relationship of parental phenotype and gene event on child phenotype and potential differences between groups, using the following model:

$$ProbandIQ = ParentIQ + Group + ParentIQ*Group$$

Separate regression analyses were run for maternal and paternal contributions to proband phenotypic features: head circumference, ASD symptoms, and IQ. DYRK1A contribution was analyzed with the following comparison groups in separate regression analyses: idiopathic ASD, 16p11.2 microdeletion, and *CHD8* samples.

Nonparametric Wilcoxon Signed Rank tests were used to compare parental and proband phenotype for UW-SNV participants across head circumference, IQ score, and ASD symptoms (SRS). Gene "effect size," as measured by the difference between parental and proband phenotype, was calculated as follows:

$$\text{Effect size } = \frac{proband\ mean - unaffected\ biparental\ mean}{unaffected\ biparental\ standard\ deviation}$$

When both maternal and paternal data were available, biparental means were calculated as the average of maternal and paternal scores. If only one parent's data was available, that parent's score was used instead of a biparental mean.

Chapter IV. Results

Aim 1: Genetic mechanism of DYRK1A

Dual-specificity tyrosine phosphorylation-regulated kinase 1A, or *DYRK1A*, is a highly conserved gene in the Down Syndrome critical region of chromosome 21 (Iossifov et al., 2014; O'Roak, Vives, Fu, et al., 2012), and appears to play a major role in brain development, specifically neurogenesis, neural plasticity, and cellular death (Bronicki et al., 2015). Animal models have resulted in a recurrent phenotype. In *Drosophila* models, truncating mutations to *DYRK1A* (*Drosophila* ortholog termed the Minibrain *(Mnb)* gene) result in microcephaly, including intact but smaller brain structures (Tejedor et al., 1995). *Dyrk1A*-null mice models (-/-) result in mid-gestational death, supporting the gene's key role in early neurological development (Fotaki et al., 2002). Mice heterozygous for *Dyrk1A* (+/-) present with reduced growth, developmental delays, motor and learning difficulties, and atypical behaviors, including anxiety (Fotaki et al., 2002; Fotaki et al., 2004).

Gene variant information was compiled for published cases with SNV or chromosomal rearrangements to *DYRK1A*, as well as the 6 new cases seen at UW. Figure 1 presents all known variants on *DYRK1A* isoform NM101395.2, selected for its high expression in human tissues (see Supplementary Table 1 for comprehensive variant data). Disruptive SNVs include splice-site, nonsense, missense, and frameshift mutations, all *de novo* (or confirmed non-maternal in 2 cases). The majority of SNVs affected the kinase region of the *DYRK1A* protein, confirming its importance within cellular processes and the impact of disruptions to this region. While some cases whose

mutations fell outside the kinase region (e.g., p.Ile48Lysfs*2, p.Ala498Profs*61) presented with milder cognitive impairments, this was not a consistent pattern across all cases. Chromosomal rearrangements included copy number variants, mosaic variants, translocations, and inversions. While chromosomal rearrangements to *DYRK1A* often impacted other closely situated genes on the 21st chromosome (up to 35 genes), cases were not found to be phenotypically distinct from those with single disruptions to *DYRK1A* (see Aim 2a for phenotypic analyses). In six cases, secondary hits to *KCNJ6* were noted, which is believed to impact potassium channel function and has been associated with epilepsy. Four of these cases were reported to have intractable seizures (two cases had no report of seizure activity, one of which presented with variant mosaicism).

Detailed gene variant information is presented for 10 UW-SNV cases in Table 1. Of these ten cases, two were found to have secondary genetic events of unknown significance. Patient 7 had an interstitial duplication of the short arm of chromosome 11, with duplications of the following genes: *DEPDC7*, *TCP11L1*, *NCRNA00294*, *CSTF3*, *LOC338739*, *HIPK3*, *C11orf41*. Patient 10 was found to have maternally inherited variants at *KCNQ2* and *TPP1* genes (unknown clinical significance, mother healthy and developed typically).

Aim 2a: Clinical Phenotype of *DYRK1A*

There were no significant differences between disruptive SNV (Pub-SNV and UW-SNV) and chromosomal rearrangement (Pub-CHR) groups on frequency of phenotypic characteristics (Table 3). Language delay was noted for 60/60 (100%); 21

individuals were nonverbal at the time of their evaluation. Intellectual Disability and/or Global Developmental Delay (depending on age) were reported in 59/60 (98%) cases. Presence of motor difficulties, including delayed walking, abnormal gait, and poor coordination were noted for 51/52 (98%). A common abnormal gait was observed across UW-SNV participants, specifically a lilting gait with a forward lean to the upper body, arms bent and held tight against the body, and hands splayed. Feeding difficulties in infancy, including poor suck, were observed in 50/53 (94%) of those with reports of feeding abilities in early development. Microcephaly, defined as head circumference two or more standard deviations below the mean for age, either primary (present throughout development) or acquired at a later age, was reported in 57/60 cases (95%). Vision abnormalities were identified in 34/42 (81%) cases, including impairments such as strabismus, astigmatism, optic nerve dysfunction, and corneal clouding. Febrile and non-febrile seizures were reported in 42/58 cases (72%).

Diagnoses of ASD were reported in 18/42 cases (43%), suggesting elevated risk well above the general population percentage of 1.5% (Christensen et al., 2016). The frequency increased to 42/60 cases (69%) when broadening the criteria to include ASD-related behaviors without a formal diagnosis, such as stereotypic behaviors (e.g., complex motor mannerisms, repetitive and self-stimulatory behaviors), limited eye contact (reported in those without known severe vision impairments), inappropriate laughter, and limited social engagement. Anxious behaviors were reported in 12/43 cases (28%) and hyperactivity was reported in 14/42 cases (33%). Seven of the ten

UW-SNV cases were confirmed to have ASD; three who did not meet diagnostic criteria presented with notable stereotypies and socially anxious behaviors.

Co-occurrence of the seven most common phenotypic features (reported in 75% or more of cases) was evaluated: microcephaly, intellectual impairment, speech delay, motor difficulties, feeding difficulties, vision abnormalities, and ASD. 53% of the total *DYRK1A* sample (32/60) possessed six or more features. 70% (42/60) presented with six or more features when the ASD category was broadened to include other behavioral difficulties, including stereotypic, anxious, and hyperactive behaviors.

Facial dysmorphisms were reported in 49/50 (98%) previously published cases (excluding UW-SNV cases who were previously published, $n = 4$). Similar dysmorphic facial features were observed in eight UW-SNV cases who participated in a standardized medical exam (4 new cases, 4 previously published), including deep-set eyes with a hooded appearance, slightly upslanting palpebral fissures, bitemporal narrowing, large forehead and brow with high anterior hairline, tubular-shaped nose, prominent nasal bridge, retrognathic jaw, and small chin (Figure 2a). Additionally, prominent, low-set, or malformed ears were also reported across cases. 4/8 UW-SNV cases presented with thick, overfolded ear helices (Figure 2b). Foot anomalies were also noted across patients, including toe syndactyly (webbing of the toes), arachnodactyly, crooked toes, and proximal placement of the first toe (Figure 2c). Observed commonalities in facial, ear, and foot characteristics in UW-SNV cases were consistent with reports of previously published cases. In the larger sample, spine or

chest abnormalities, including pectus excavatum and scoliosis, were reported in 13/25 cases with documented skeletal observations.

Aim 2b: Phenotypic comparisons of *DYRK1A* to idiopathic ASD

Rates of microcephaly, intellectual disability, speech delay, motor difficulties, vision impairments, and feeding difficulties were significantly higher in the total *DYRK1A* group (Pub-SNV, UW-SNV, and Pub-CHR combined) relative both to the full idiopathic SSC comparison cohort and the subset with IQ below 70 (Table 4; Figure 3).

The likelihood of five or more of these six features occurred in combination was significantly higher in the DYRK1A group (79%) than in the full idiopathic and IQ < 70 groups (0.3% and 1%, respectively). When frequencies were evaluated for the subset of individuals with *DYRK1A* ascertained for an ASD diagnosis (n = 18), the percentages and group differences remain consistent (Figure 3). Notably, co-occurrence of five or more features increased to 89% in those with *DYRK1A* mutations ascertained for ASD.

Aim 2c: Quantitative phenotype of *DYRK1A*

In order to better understand the quantitative phenotype of *DYRK1A* and its distinction from other groups with ASD, the *DYRK1A* cases seen at UW (n = 10) were compared to an age and gender-matched subset of a sample of individuals with idiopathic ASD (n = 10) and a sample with mutations to another high-confidence ASD risk gene, *CHD8* (n = 12). Independent-samples t-tests were conducted to separately compare *DYRK1A,* idiopathic, and *CHD8* groups.

DYRK1A versus idiopathic ASD. Independent samples *t*-tests revealed significant differences between groups on measures of head circumference, cognitive

ability, and adaptive functioning (see Figure 4). On head circumference (reported in *Z*-scores), *DYRK1A* cases ($M = -3.62$, $SD = 2.17$) had significantly smaller head sizes than idiopathic cases ($M = 1.21$, $SD = 0.39$), $t(18) = 6.94$, $p = 0.001$, $d = 3.10$. *DYRK1A* cases presented with significantly lower full-scale IQ scores ($M = 45.30$, $SD = 18.14$) compared to idiopathic cases ($M = 81.20$, $SD = 26.12$), $t(18) = 3.57$, $p = 0.002$, $d = 1.60$. On adaptive functioning, *DYRK1A* cases had significantly lower scores ($M = 54.80$, $SD = 9.04$) than idiopathic cases ($M = 70.50$, $SD = 9.40$), $t(18) = 3.81$, $p = 0.001$, $d = 1.70$. There was no difference between *DYRK1A* and idiopathic groups on autism symptom severity, $t(18) = 1.24$, $p > 0.002$, age of first words, $t(15) = -3.576$, $p > 0.002$, or age of first unaided steps, $t(17) = -3.154$, $p > 0.002$. Importantly, three of ten *DYRK1A* cases were not given an ASD diagnosis, but still presented with notable stereotypies and socially anxious behaviors.

DYRK1A versus CHD8. No significant differences were found between idiopathic and *CHD8* groups. Independent samples t-tests revealed significant differences between *DYRK1A* and *CHD8* groups on head circumference only (see Figure 4). *DYRK1A* cases ($M = -3.62$, $SD = 2.17$) had significantly smaller head sizes than idiopathic cases ($M = 1.76$, $SD = 1.62$), $t(19) = 6.49$, $p < 0.001$, $d = 2.84$. There was no evidence of differences between *DYRK1A* and *CHD8* groups on IQ, $t(19) = 1.52$, $p > 0.002$, adaptive functioning, $t(20) = 1.59$, $p > 0.004$, and autism symptom severity, $t(19) = 1.66$, $p > 0.002$. On measures of developmental milestones, groups did not differ is age of first words, $t(17) = -1.58$, $p > 0.002$, or age of first steps, $t(20) = -0.56$, $p > 0.002$.

Aim 3a: Exploration of remaining genetic background

A series of multiple linear regressions with sequential predictor entry were used to explore parental contribution to proband head circumference, cognitive ability, and ASD symptoms. Separate regressions were conducted to explore the extent maternal and paternal phenotype accounted for variance in proband phenotype. Additionally, contribution of *DYRK1A* group status was compared to the following ASD-associated groups: idiopathic ASD (simplex for head circumference and ASD symptoms, multiplex for IQ), 16p11.2 deletion, and *CHD8* SNV. Results are organized by phenotypic characteristic, followed by groups comparisons.

Head circumference

Idiopathic-DYRK1A Maternal head circumference. In the first block, results showed that the main effects of proband height accounted for significant variance in proband head circumference, $R^2 = 0.12$ (Adjusted $R^2 = 0.12$), $F(1,1901) = 248.42$, $p < 0.001$. The addition of group (Idiopathic ASD vs. DYRK1A) and maternal head circumference (controlling for maternal height) in the second block revealed main effects of group and maternal head circumference, which accounted for significant variance in proband head circumference, $R^2_{change} = 0.15$, $F_{change}(2,1899) = 188.19$, $p < 0.001$, $R^2_{total} = 0.26$, Adjusted $R^2 = 0.26$. In the third block, the addition of an interaction term (group-by-maternal head circumference) accounted for an additional 0.2% of the variance in proband head circumference, which was significant, $R^2_{change} = 0.002$, $F_{change}(1,1898) = 4.91$, $p < 0.05$, $R^2_{total} = 0.26$ and $R^2_{adjusted} = 0.26$. Table 6 and Figure 5 provide correlations and regression analyses.

Results from the final block, with all predictors entered in the model, showed that the average proband head circumference z score was 0.57 (*SE* = 0.03), holding all other variables constant, $t(1898) = 20.13$, $p < 0.001$. Proband height uniquely predicted proband head circumference, holding all else constant, ($b = 0.37$, $SE = 0.02$, $t(1898) = 15.87$, $p < 0.001$, $sr^2 = 0.01$). Specifically, for every standard deviation increase in proband height, head circumference was predicted to increase by 0.37 SD. Group uniquely predicted proband head circumference, with DYRK1A subjects showing 4.92 SD smaller head circumference than idiopathic subjects, holding all else constant, ($b = -4.92$, $SE = 0.41$, $t(1898) = -11.90$, $p < 0.001$, $sr^2 = 0.06$). Maternal head circumference also uniquely predicted proband head circumference, holding all else constant, ($b = 0.41$, $SE = 0.03$, $t(1898) = 15.40$, $p < 0.001$, $sr^2 = 0.09$). For every standard deviation increase in maternal head circumference, proband head circumference was predicted to increase by 0.41 SD.

There was also a significant interaction between group and maternal head circumference, ($b = 0.80$, $SE = 0.36$, $t(1898) = 2.22$, $p = 0.03$, $sr^2 = 0.002$). To understand the nature of the interaction, predicted values were plotted for each group by three levels of maternal head circumference (low = -1 *SD*, mean, and high = +1 *SD*). As illustrated in Figure 6a, the interaction was ordinal, indicating that the effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose mothers had relatively smaller head circumference (*DYRK1A* cases whose maternal head circumference was one standard deviation below average had a predicted decrease in head size of 5.72 *SD* compared to idiopathic cases). In contrast, the effect of a *DYRK1A*

mutation on head circumference was smaller for cases with mothers with relatively large head size (when maternal head circumference one standard deviation above average, *DYRK1A* cases had a predicted decrease of 4.11 *SD* compared to idiopathic cases).

Idiopathic-DYRK1A Paternal head circumference. In the first block, results showed that the main effects of proband height accounted for significant variance in proband head circumference, $R^2 = 0.12$ (Adjusted $R^2 = 0.12$), $F(1,1875) = 258.72$, $p < 0.001$. The addition of group (Idiopathic ASD vs. DYRK1A) and paternal head circumference (controlling for paternal height) in the second block revealed main effects of group and paternal head circumference, which accounted for an additional 11% of the variance in proband head circumference, $R^2_{change} = 0.11$, $F_{change}(2,1873) = 137.82$, $p < 0.001$, $R^2_{total} = 0.23$ and $R^2_{adjusted} = 0.23$. In the third block, the addition of an interaction term (group-by-paternal head circumference) accounted for an additional 0.4% of the variance in proband head circumference, which was significant, $R^2_{change} = 0.004$, $F_{change}(1,1872) = 8.66$, $p < 0.01$, $R^2_{total} = 0.24$ and $R^2_{adjusted} = 0.24$. Table 7 and Figure 5 provide correlations and regression analyses.

Results from the final block, with all predictors entered in the model, showed that the average proband head circumference z score was 0.56 ($SE = 0.03$), holding all other variables constant, $t(1872) = 19.71$, $p < 0.001$. Proband height uniquely predicted proband head circumference, holding all else constant, ($b = 0.38$, $SE = 0.02$, $t(1872) = 16.08$, $p < 0.001$, $sr^2 = 0.11$). Specifically, for every standard deviation increase in proband height, head circumference was predicted to increase by 0.38 SD. Group uniquely predicted proband head circumference, with DYRK1A subjects showing 4.05

SD smaller head circumference than idiopathic subjects, holding all else constant, (b = -4.05, SE = 0.48, t(1872) = -8.45, $p < 0.001$, sr^2 = 0.03). Paternal head circumference also uniquely predicted proband head circumference, holding all else constant, (b = 0.39, SE = 0.03, t(1872) = 14.25, $p < 0.001$, sr^2 = 0.08). Specifically, for every standard deviation increase in paternal head circumference, proband head circumference was predicted to increase by 0.39 SD.

Lastly, there was also a significant interaction between group and paternal head circumference, (b = 2.58, SE = 0.88, t(1872) = 2.94, p = 0.003, sr^2 = 0.003). To understand the nature of the interaction, predicted values were plotted for each group by three levels of paternal head circumference (low = -1 *SD*, mean, and high = +1 *SD*). As illustrated in Figure 6b, the interaction was ordinal, indicating that the effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose fathers had relatively smaller head circumference (*DYRK1A* cases whose paternal head circumference was one standard deviation below average had a predicted decrease in head size of 6.63 *SD* compared to idiopathic cases). In contrast, the effect of a *DYRK1A* mutation on head circumference was smaller for cases with fathers with relatively large head size (when paternal head circumference was one standard deviation above average, *DYRK1A* cases had a predicted decrease of 1.47 *SD* compared to idiopathic cases).

16p11.2-DYRK1A Maternal head circumference. In the first block, results showed that the main effects of proband height accounted for significant variance in proband head circumference, R^2 = 0.16 (Adjusted R^2 = 0.15), F(1, 72) = 13.98, $p <$

0.001. The addition of group (16p11.2 vs. DYRK1A) and maternal head circumference (controlling for maternal height) in the second block revealed main effects of group and maternal head circumference, which accounted for an additional 60.5% of variance in proband head circumference, $R^2_{change} = 0.605$, $F_{change}(2,70) = 91.15$, $p < 0.001$, $R^2_{total} = 0.77$ and $R^2_{adjusted} = 0.76$. In the third block, the addition of an interaction term (group-by-maternal head circumference) accounted for an additional 2% of the variance in proband head circumference, which was significant, $R^2_{change} = 0.02$, $F_{change}(1,69) = 7.14$, $p = 0.01$, $R^2_{total} = 0.79$ and $R^2_{adjusted} = 0.78$. Table 8 and Figure 5 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that the average proband head circumference z score was 1.46 ($SE = 0.14$), holding all other variables constant, $t(69) = 10.80$, $p < 0.001$. Proband height uniquely predicted proband head circumference, holding all else constant, ($b = 0.57$, $SE = 0.08$, $t(69) = 6.88$, $p < 0.001$, $sr^2 = 0.14$). Specifically, for every standard deviation increase in proband height, head circumference was predicted to increase by 0.57 SD. Group uniquely predicted proband head circumference, with DYRK1A subjects showing 6.02 SD smaller head circumference than 16p11.2 subjects, holding all else constant, ($b = -6.02$, $SE = 0.42$, $t(69) = -6.02$, $p < 0.001$, $sr^2 = 0.62$). Maternal head circumference did not uniquely predict proband head circumference, $p > 0.05$.

Finally, there was a significant interaction between group and maternal head circumference, ($b = 1.00$, $SE = 0.38$, $t(69) = 2.67$, $p = 0.01$, $sr^2 = 0.02$). To understand the nature of the interaction, predicted values were plotted for each group by three

levels of maternal head circumference (low = -1 *SD*, mean, and high = +1 *SD*). As illustrated in Figure 7a, the interaction was ordinal, indicating that the effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose mothers had relatively smaller head circumference (*DYRK1A* cases whose paternal head circumference was one standard deviation below average had a predicted decrease in head size of 7.02 *SD* compared to 16p11.2 cases). In contrast, the effect of a *DYRK1A* mutation on head circumference was smaller when mothers with relatively large head size (when maternal head circumference was one standard deviation above average, *DYRK1A* cases had a predicted decrease of 5.02 *SD* compared to 16p11.2 cases).

16p11.2-DYRK1A Paternal head circumference. In the first block, results showed that the main effects of proband height accounted for significant variance in proband head circumference, $R^2 = 0.23$ (Adjusted $R^2 = 0.22$), $F(1, 61) = 18.43$, $p < 0.001$. The addition of group (16p11.2 vs. DYRK1A) and maternal head circumference (controlling for maternal height) in the second block revealed main effects of group and paternal head circumference, which accounted for an additional 51% variance in proband head circumference, $R^2_{change} = 0.51$, $F(2,59) = 57.96$, $p < 0.001$, $R^2_{total} = 0.74$ and $R^2_{adjusted} = 0.73$. In the third block, the addition of an interaction term (group-by-paternal head circumference) accounted for an additional 5% of the variance in proband head circumference, which was significant, $R^2_{change} = 0.05$, $F_{change}(1,58) = 12.09$, $p = 0.001$, $R^2_{total} = 0.79$ and $R^2_{adjusted} = 0.77$. Table 9 and Figure 5 provide correlations and regression analyses.

Results from the final block, with all predictors entered in the model, showed that the average proband head circumference z score was 1.49 (*SE* = 0.14), holding all other variables constant, $t(58) = 11.02$, $p < 0.001$. Proband height uniquely predicted proband head circumference, holding all else constant, ($b = 0.53$, $SE = 0.09$, $t(58) = 6.17$, $p < 0.001$, $sr^2 = 0.14$). Specifically, for every standard deviation increase in proband height, head circumference was predicted to increase by 0.53 SD. Group uniquely predicted proband head circumference, with DYRK1A subjects showing 5.28 SD smaller head circumference than 16p11.2 subjects, holding all else constant, ($b = -5.28$, $SE = 0.45$, $t(58) = -11.79$, $p < 0.001$, $sr^2 = 0.51$). Paternal head circumference also uniquely predicted proband head circumference, holding all else constant, ($b = 0.42$, $SE = 0.14$, $t(58) = 3.03$, $p = 0.004$, $sr^2 = 0.03$). Specifically, for every standard deviation increase in paternal head circumference, proband head circumference was predicted to increase by 0.42 SD.

There was also a significant interaction between group and paternal head circumference, ($b = 1.97$, $SE = 0.57$, $t(58) = 3.48$, $p = 0.001$, $sr^2 = 0.05$). To understand the nature of the interaction, predicted values were plotted for each group by three levels of paternal head circumference (low = -1 *SD*, mean, and high = +1 *SD*). As illustrated in Figure 7b, the interaction was ordinal, indicating that the effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose fathers had relatively smaller head circumference (*DYRK1A* cases whose paternal head circumference was one standard deviation below average had a predicted decrease in head size of 7.25 *SD* compared to 16p11.2 cases). In contrast, the effect of a *DYRK1A*

mutation on head circumference was smaller for cases with fathers with relatively large head size (when paternal head circumference was one standard deviation above average, *DYRK1A* cases had a predicted decrease of 3.30 *SD* compared to 16p11.2 cases).

CHD8-DYRK1A Maternal head circumference. In the first block, results showed that the main effects of proband height accounted for significant variance in proband head circumference, $R^2 = 0.67$ (Adjusted $R^2 = 0.64$), $F(1,13) = 26.09$, $p < 0.001$. The addition of group (*CHD8* vs. *DYRK1A*) and maternal head circumference (controlling for maternal height) in the second block revealed main effects of group and maternal head circumference, which accounted for an additional 25% of variance in proband head circumference, $R^2_{change} = 0.25$, $F_{change}(2,11) = 15.96$, $p = 0.001$, $R^2_{total} = 0.92$ and $R^2_{adjusted} = 0.89$. In the third block, the addition of an interaction term (group-by-maternal head circumference) did not account for significant variation in the outcome, $R^2_{change} = 0.01$, $F_{change}(1,10) = 0.69$, $p = 0.43$. Table 10 and Figure 5 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that the average proband head circumference z score was 0.14 ($SE = 0.83$), holding all other variables constant, $t(10) = 0.16$, $p > 0.05$. Proband height uniquely predicted proband head circumference, holding all else constant, ($b = 0.75$, $SE = 0.27$, $t(10) = 2.79$, $p = 0.02$, $sr^2 = 0.06$). Specifically, for every standard deviation increase in proband height, head circumference was predicted to increase by 0.75 SD. Group uniquely predicted proband head circumference, with *DYRK1A* subjects showing 4.03 SD

smaller head circumference than *CHD8* subjects, holding all else constant, (b = -4.03, SE = 0.99, t(10) = -4.06, p = 0.002, sr^2 = 0.13). Maternal head circumference and the interaction terms of group by maternal head circumference did not uniquely predict proband head circumference, $p > 0.05$.

CHD8-DYRK1A Paternal head circumference. In the first block, results showed that the main effects of proband height accounted for significant variance in proband head circumference, R^2 = 0.65 (Adjusted R^2 = 0.62), F(1,11) = 20.38, p = 0.001. The addition of group (*CHD8* vs. *DYRK1A*) and maternal head circumference (controlling for maternal height) in the second block revealed main effects of group and maternal head circumference, which accounted for an additional 18% of variance in proband head circumference, R^2_{change} = 0.18, F_{change}(2,9) = 4.58, p = 0.04, R^2_{total} = 0.83 and $R^2_{adjusted}$ = 0.77. In the third block, the addition of an interaction term (group-by-paternal head circumference) accounted for an additional 9% of the variance in proband head circumference, which was significant, R^2_{change} = 0.09, F_{change}(1,8) = 9.35, p = 0.02, R^2_{total} = 0.92 and $R^2_{adjusted}$ = 0.88. Table 11 and Figure 5 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that the average proband head circumference z score was -0.13 (SE = 0.65), holding all other variables constant, t(8) = -0.191, $p > 0.05$. Proband height uniquely predicted proband head circumference, holding all else constant, (b = 0.83, SE = 0.24, t(8) = 3.50, p = 0.01, sr^2 = 0.12). Specifically, for every standard deviation increase in proband height, head circumference was predicted to increase by 0.83 SD. Group uniquely

predicted proband head circumference, with *DYRK1A* subjects showing 2.30 SD smaller head circumference than *CHD8* subjects, holding all else constant, (b = -2.30, SE = 0.96, $t(8)$ = -2.39, p = 0.04, sr^2 = 0.06). Paternal head circumference did not uniquely predict proband head circumference, $p > 0.05$.

However, there was a significant interaction between group and paternal head circumference, (b = 2.55, SE = 0.83, $t(8)$ = 3.06, p = 0.02, sr^2 = 0.09). To understand the nature of the interaction, predicted values were plotted for each group by three levels of paternal head circumference (low = -1 *SD*, mean, and high = +1 *SD*). As illustrated in Figure 8, the interaction was ordinal, indicating that the effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose fathers had relatively smaller head circumference (*DYRK1A* cases whose paternal head circumference was one standard deviation below average had a predicted decrease in head size of 4.85 *SD* compared to *CHD8* cases). In contrast, the effect of a *DYRK1A* mutation on head circumference was smaller for cases with fathers with relatively large head size (when paternal head circumference was one standard deviation above average, *DYRK1A* cases had a predicted decrease of 0.36 *SD* compared to *CHD8* cases).

ASD Symptoms

Idiopathic-DYRK1A Maternal ASD symptoms. In the first block, results showed that the main effects of proband full-scale IQ accounted for significant variance in proband ASD symptoms, as measured by Social Responsiveness Scale (SRS) total raw score, R^2 = 0.06 (Adjusted R^2 = 0.06), $F(1,1846)$ = 115.40, $p < 0.001$. The addition of group (Idiopathic ASD vs. DYRK1A) and maternal ASD symptoms in the second

block revealed main effects of both predictors, which accounted for an additional 0.8% of the variance in proband ASD symptoms, $R^2_{change} = 0.008$, $F_{change}(2,1844) = 8.11$, $p < 0.001$, $R^2_{total} = 0.07$ and $R^2_{adjusted} = 0.07$). In the third block, the addition of an interaction term (group-by-maternal ASD symptoms) did not account for significant variation in the outcome, $R^2_{change} = 0.001$, $F_{change}(1,1843) = 2.16$, $p = 0.14$. Table 12 and Figure 9 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that average proband ASD symptoms was 97.37 points on the SRS ($SE = 0.61$), holding all other variables constant, $t(1843) = 160.62$, $p < 0.001$. Proband IQ uniquely predicted ASD symptoms, holding all else constant, ($b = -6.63$, $SE = 0.61$, $t(1843) = -10.91$, $p < 0.001$, $sr^2 = 0.06$). Specifically, for every standard deviation decrease in proband IQ, ASD symptoms were predicted to increase by 6.63 points (increased severity of symptoms). Group did not uniquely predict proband ASD symptoms, p = 0.36. Maternal head circumference uniquely predicted the outcome, holding all else constant, ($b = 2.31$, $SE = 0.61$, $t(1843) = 3.78$, $p < 0.001$, $sr^2 = 0.007$). Specifically, for every standard deviation increase in maternal ASD symptoms, proband ASD symptoms were predicted to increase by 2.32 points. The interaction of group and maternal ASD symptoms did not uniquely predict the outcome, $p = 0.14$.

Idiopathic-DYRK1A Paternal ASD symptoms. In the first block, results showed that the main effects of proband full-scale IQ accounted for significant variance in proband ASD symptoms, $R^2 = 0.06$ (Adjusted $R^2 = 0.06$), $F(1,1836) = 113.67$, $p < 0.001$. The addition of group (Idiopathic ASD vs. DYRK1A) and paternal ASD symptoms in the

second block revealed main effects of both predictors, which accounted for an additional 2% of the variance in proband ASD symptoms, $R^2_{change} = 0.02$, $F_{change}(2,1834) = 14.78$, $p < 0.001$, $R^2_{total} = 0.07$ and $R^2_{adjusted} = 0.07$). In the third block, the addition of an interaction term (group-by-paternal ASD symptoms) did not account for significant variation in the outcome, $R^2_{change} = 0.000$, $F_{change}(1,1833) = 0.83$, $p = 0.36$. Table 13 and Figure 9 provide correlations and regression analyses.

Results from the final block, with all predictors entered in the model, showed that average proband ASD symptoms was 97.45 points ($SE = 6.01$), holding all other variables constant, $t(1833) = 160.83$, $p < 0.001$. Proband IQ uniquely predicted proband ASD symptoms, holding all else constant, ($b = -6.72$, $SE = 0.61$, $t(1833) = -11.05$, $p < 0.001$, $sr^2 = 0.06$). Specifically, for every standard deviation decrease in proband IQ, ASD symptoms were predicted to increase by 6.72 points. Group did not uniquely predict proband ASD symptoms, p = 0.31. Paternal ASD symptoms did uniquely predict proband ASD symptoms, holding all else constant, ($b = 3.29$, $SE = 0.61$, $t(1833) = 5.42$, $p < 0.001$, $sr^2 = 0.02$). Specifically, for every standard deviation increase in paternal symptoms, proband symptoms were predicted to increase by 3.29 points. The interaction of group and paternal ASD symptoms did not uniquely predict the outcome, $p = 0.36$.

16p11.2-DYRK1A Maternal ASD symptoms. In the first block, results showed that the main effects of proband full-scale IQ accounted for significant variance in proband ASD symptoms, as measured by SRS total raw score, $R^2 = 0.12$ (Adjusted $R^2 = 0.10$), $F(1,61) = 7.94$, $p = 0.007$. The addition of group (16p11.2 vs. DYRK1A) and

maternal ASD symptoms in the second block did not account for significant variation in the outcome, $R^2_{change} = 0.07$, $F_{change}(1,59) = 2.49$, $p = 0.09$. In the third block, the addition of an interaction term (group-by-maternal ASD symptoms) did not account for significant variation in the outcome, $R^2_{change} = 0.003$, $F_{change}(1,58) = 0.24$, $p = 0.62$. Table 14 and Figure 9 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that average proband ASD symptoms was 82.33 points ($SE = 4.91$), holding all other variables constant, $t(58) = 16.77$, $p < 0.001$. Proband IQ uniquely predicted ASD symptoms, holding all else constant, ($b = -16.26$, $SE = 6.26$, $t(58) = -2.60$, $p = 0.01$, $sr^2 = 0.09$). Specifically, for every standard deviation decrease in proband IQ, ASD symptoms were predicted to increase by 16.77 points (increased severity of symptoms). Neither group nor maternal ASD symptoms uniquely predicted proband ASD symptoms, $p > 0.05$. The interaction of group and maternal ASD symptoms did not uniquely predict the outcome, $p = 0.62$.

16p11.2-DYRK1A Paternal ASD symptoms. In the first block, results showed that the main effects of proband full-scale IQ accounted for significant variance in proband ASD symptoms, as measured by SRS total raw score, $R^2 = 0.12$ (Adjusted $R^2 = 0.12$), $F(1,56) = 7.79$, $p = 0.01$. The addition of group (16p11.2 vs. DYRK1A) and paternal ASD symptoms in the second block did not account for significant variation in the outcome, $R^2_{change} = 0.05$, $F_{change}(1,54) = 1.75$, $p = 0.18$. In the third block, the addition of an interaction term (group-by-paternal ASD symptoms) did not account for

significant variation in the outcome, $R^2_{change} = 0.01$, $F_{change}(1,53) = 0.47$, $p = 0.50$. Table 15 and Figure 9 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that average proband ASD symptoms was 82.33 points ($SE = 5.23$), holding all other variables constant, $t(53) = 15.75$, $p < 0.001$. Proband IQ did not uniquely predicted ASD symptoms, holding all else constant, ($b = -12.95$, $SE = 6.44$, $t(53) = -1.97$, $p = 0.05$, $sr^2 = 0.06$). Specifically, for every standard deviation decrease in proband IQ, ASD symptoms were predicted to increase by 12.95 points (increased severity of symptoms). Neither group nor paternal ASD symptoms uniquely predicted proband ASD symptoms, $p > 0.05$, although paternal ASD symptoms trended toward significant contribution, $p = 0.059$. The interaction of group and maternal ASD symptoms did not uniquely predict the outcome, $p = 0.498$.

CHD8-DYRK1A Maternal ASD symptoms. In the first block, results showed that the main effect of proband IQ did not account for significant variance of proband ASD symptoms, $R^2 = 0.15$ (Adjusted $R^2 = 0.10$), $F(1,15) = 2.73$, $p = 0.12$. In the second block, the addition of group and maternal ASD symptoms did not account for significant variance in proband ASD symptoms, $R^2_{change} = 0.31$, $F_{change}(2,13) = 3.78$, $p = 0.051$, $p = 0.55$, $R^2 = 0.47$, Adjusted $R^2 = 0.34$. The addition of an interaction term (group-by-maternal ASD symptoms) also did not account for significant variation in the outcome, $R^2_{change} = 0.001$, $F_{change}(1,12) = 0.03$, $p = 0.87$. Given weak correlations between variables and the lack of significance of the either block, it was not surprising that neither group, maternal ASD symptoms, nor the interaction term uniquely predicted

proband ASD symptoms. Table 16 and Figure 9 provide correlations and regression analyses.

CHD8-DYRK1A Paternal ASD symptoms. In the first block, results showed that the main effect of proband IQ did not account for significant variance of proband ASD symptoms, $R^2 = 0.10$ (Adjusted $R^2 = 0.03$), $F(1,13) = 1.46$, $p = 0.25$. In the second block, the addition of group and paternal ASD symptoms did not account for significant variance in proband ASD symptoms, $R^2_{change} = 0.09$, $F_{change}(2,11) = 0.64$, $p = 0.55$, $R^2 = 0.20$, Adjusted $R^2 = -0.03$. The addition of an interaction term (group-by-paternal ASD symptoms) also did not account for significant variation in the outcome, $R^2_{change} = 0.05$, $F_{change}(1,10) = 0.62$, $p = 0.45$. Again, given weak correlations between variables and the lack of significance of the either block, it was not surprising that neither group, maternal ASD symptoms, nor the interaction term uniquely predicted proband ASD symptoms. Table 17 and Figure 9 provide correlations and regression analyses.

Full-Scale IQ

Multiplex ASD-DYRK1A, Maternal IQ. In the first block, results showed that the main effects of group and maternal IQ accounted for significant variance in proband IQ, $R^2 = 0.14$ (Adjusted $R^2 = 0.14$), $F(2,269) = 22.13$, $p < 0.001$. The addition of an interaction term (group-by-maternal IQ) did not account for significant variation in the outcome, $R^2_{change} = 0.01$, $F_{change}(1,268) = 2.52$, $p = 0.11$. Table 18 and Figure 10 provide correlations and regression analyses.

Results from the final block, with all predictors entered in the model, showed that the average proband IQ was 82.33 points ($SE = 1.52$), holding all other variables

constant, $t(268) = 54.19$, $p < 0.001$. Group status uniquely predicted proband IQ, holding all else constant, with DYRK1A cases performing 40.74 points lower than multiplex cases ($b = -40.74$, $SE = 10.35$, $t(268) = -3.94$, $p < 0.001$, $sr^2 = 0.05$). Maternal IQ also uniquely predicted proband IQ, holding all else constant, ($b = 8.16$, $SE = 1.53$, $t(268) = 5.34$, $p < 0.001$, $sr^2 = 0.09$). Specifically, for every standard deviation increase in maternal IQ, proband IQ was predicted to increase by 8.16 points. The interaction of group and maternal IQ did not uniquely predict the outcome, $p = 0.11$.

Multiplex ASD-DYRK1A, Paternal IQ. In the first block, results showed that the main effects of group and paternal IQ accounted for significant variance in proband IQ, $R^2 = 0.10$ (Adjusted $R^2 = 0.09$), $F(2,215) = 11.38$, $p < 0.001$. The addition of an interaction term (group-by-paternal IQ) did not account for significant variation in the outcome, $R^2_{change} = 0.000$, $F_{change}(1,214) = 0.09$, $p = 0.77$. Table 19 and Figure 10 provide correlations and regression analyses.

Results from the final block, with all predictors entered in the model, showed that the average proband IQ was 82.66 points ($SE = 1.72$), holding all other variables constant, $t(214) = 48.05$, $p < 0.001$. Group status uniquely predicted proband IQ, holding all else constant, with DYRK1A cases performing 30.01 points lower than multiplex cases ($b = -30.01$, $SE = 11.20$, $t(214) = 3.66$, $p < 0.01$, $sr^2 = 0.03$). Paternal IQ also uniquely predicted proband IQ, holding all else constant, ($b = 6.30$, $SE = 1.72$, $t(214) = 3.66$, $p < 0.001$, $sr^2 = 0.06$). Specifically, for every standard deviation increase in paternal IQ, proband IQ was predicted to increase by 6.30 points. The interaction of group and paternal IQ did not uniquely predict the outcome, $p = 0.77$.

16p11.2-DYRK1A Maternal IQ. In the first block, results showed that the main effects of group and maternal IQ accounted for significant variance in proband IQ, R^2 = 0.36 (Adjusted R^2 = 0.34), $F(1,75) = 20.97$, $p < 0.001$. In the second block, the addition of an interaction term (group-by-maternal IQ) did not account for significant variation in the outcome, $R^2_{change} = 0.02$, $F_{change}(1,74) = 2.27$, $p = 0.14$. Table 20 and Figure 10 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that average proband IQ was 84.58 points (SE = 1.94), holding all other variables constant, $t(74) = 43.50$, $p < 0.001$. Group uniquely predicted proband IQ, holding all else constant, (b = -41.76, SE = 6.52, $t(74)$ = -6.41, $p < 0.001$, $sr^2 = 0.34$). Specifically, the *DYRK1A* group performed 41.76 points lower than 16p11.2 cases. Neither maternal IQ nor the group-maternal IQ interaction term uniquely predicted proband ASD symptoms, $p >$ 0.05.

16p11.2-DYRK1A Paternal IQ. In the first block, results showed that the main effects of group and paternal IQ accounted for significant variance in proband IQ, R^2 = 0.33 (Adjusted R^2 = 0.31), $F(1,61) = 15.14$, $p < 0.001$. In the second block, the addition of an interaction term (group-by-paternal IQ) did not account for significant variation in the outcome, $R^2_{change} = 0.00$, $F_{change}(1,60) = 0.47$, $p = 0.90$. Table 21 and Figure 10 provide correlations and regression analyses.

Results from the final block, with all predictor entered in the model, showed that average proband IQ was 85.05 points (SE = 2.05), holding all other variables constant, $t(60) = 41.49$, $p < 0.001$. Once again, group status uniquely predicted proband IQ,

holding all else constant, (b = -32.52, SE = 7.08, $t(60)$ = -4.59, $p < 0.001$, sr^2 = 0.24). Specifically, the *DYRK1A* group performed 32.52 points lower than 16p11.2 cases. Neither paternal IQ nor the group-paternal IQ interaction term uniquely predicted proband ASD symptoms, $p > 0.05$.

CHD8-DYRK1A Maternal IQ. In the first block, results showed that the main effects of group and maternal IQ did not account for significant variance in proband IQ, R^2 = 0.35 (Adjusted R^2 = 0.25), $F(2,13) = 3.53$, $p = 0.06$. The addition of an interaction term (group-by-maternal IQ) also did not account for significant variation in the outcome, $R^2_{change} = 0.14$, $F_{change}(1,12) = 3.26$, $p = 0.10$. Given the lack of significance of the either block, it was not surprising that neither group, maternal IQ, or the interaction term uniquely predicted proband IQ. Table 22 and Figure 10 provide correlations and regression analyses.

CHD8-DYRK1A Paternal IQ. In the first block, results showed that the main effects of group and paternal IQ did not account for significant variance in proband IQ, R^2 = 0.15 (Adjusted R^2 = -0.02), $F(2,10) = 0.88$, $p = 0.45$. The addition of an interaction term (group-by-maternal IQ) also did not account for significant variation in the outcome, $R^2_{change} = 0.14$, $F_{change}(1,9) = 0.16$, $p = 0.16$. Given the lack of significance of the either block, it was not surprising that neither group, paternal IQ, or the interaction term uniquely predicted proband IQ. Table 23 and Figure 10 provide correlations and regression analyses.

Aim 3b: Gene effect size for *DYRK1A*

When comparing parental and proband head circumference *Z*-scores, *DYRK1A* haploinsufficiency accounted for a 2.93 SD decrease in head size for probands. Wilcoxon Signed Rank tests showed that both mothers and fathers exhibited significantly larger head size, controlling for age and gender, compared to their affected child, $Z = -2.67$, $p = 0.008$ and $Z = -2.20$, $p = 0.028$, respectively. When comparing social responsiveness of parents and probands, *DYRK1A* accounted for a 5.51 SD increase in SRS total T score. Wilcoxon Signed Rank tests showed that both mothers and fathers display significantly lower SRS scores compared to their affected child, $Z = -3.62$, $p < 0.001$ and $Z = -3.41$, $p = 0.001$, respectively. On measures of full-scale IQ, *DYRK1A* accounted for a 6.09 SD decrease in IQ for probands compared to biparental IQ. Wilcoxon Signed Rank tests showed that both mothers and fathers display significantly higher IQ compared to their affected child, $Z = -2.67$, $p = 0.008$ and $Z = -2.20$, $p = 0.028$, respectively.

Figure 11 presents parental and proband data for (a) head circumference, (b) ASD symptoms, and (c) IQ. Probands with smaller head sizes relative to other *DYRK1A* cases correspond to parents who also have smaller head size and vice versa (see Figure 11a). There are similar patterns in cognition, though perhaps more pronounced for fathers, such that fathers with higher IQ have children (i.e., probands) with higher IQ relative to others with *DYRK1A* cases (Figure 11c). For ASD symptoms, higher parental scores (i.e., greater social impairment) correspond to probands with greater social impairment (Figure 11b). Lastly, it is also of interest to note the apparent wider range of

IQ variability for fathers (*SD* = 14.99) relative to mothers (*SD* = 9.42) and the wider range of head circumference variability for mothers (*SD* = 1.81) relative to fathers (*SD* = 0.52). Although these observations remain exploratory due to small sample sizes, findings suggest parental phenotype may account for some of the phenotypic variability observed within *DYRK1A* cases.

Parent-proband effect sizes: gene group comparisons

Parent-proband relationships for *DYRK1A* phenotype were then compared to several comparison groups to situate *DYRK1A*'s genetic impact within the context of other ASD (or ASD-associated) groups: Idiopathic ASD, *CHD8*, and 16p11.2 deletion (See Figures 6-8). See Table 24 for mean and standard deviations of probands and unaffected parents by group on measures of head circumference, ASD symptoms, and IQ. The *DYRK1A* group had a larger effect size (-2.93 SD) for head circumference than idiopathic, *CHD8*, and 16p11.2 deletion groups (-0.25, 0.09, and -0.03 SD, respectively). On a measure of ASD symptoms, all groups showed positive effect sizes, indicating probands across groups displayed more severe ASD symptoms than their parents. The *DYRK1A* group demonstrated the largest effect size (5.51 SD), followed by *CHD8* (4.45 SD), Idiopathic (3.88 SD), and 16p11.2 (2.68 SD) groups. On IQ, negative effect sizes were observed for all groups (i.e. probands showed lower IQ scores compared to parents). *DYRK1A*'s effect size for IQ (-6.09 SD) was smaller than *CHD8* (-7.32 SD), but larger than Idiopathic and 16p11.2 groups (-2.50 and -2.60 SD, respectively). See Table 25 for effect sizes by group and Figures 12-14 for parent-proband comparisons across group by phenotypic feature.

Chapter V. Discussion

Clinical phenotype of *DYRK1A*

This study of the *DYRK1A* haploinsufficiency phenotype, compiling previously published and newly identified cases, confirms a phenotype characterized by microcephaly, intellectual disability, speech delay, motor difficulties, feeding difficulties, and vision abnormalities. 78% of cases presented with 5 or more symptoms in combination, which suggests that this particular constellation of features may be indicative of *DYRK1A* haploinsufficiency. A common facial gestalt included deep-set eyes with a hooded appearance, slightly upslanted palpebral fissures, tubular-shaped nose with pronounced broad tip, high nasal bridge, large forehead and brow with high anterior hairline, retrognathic jaw, and small chin. Dysmorphic feet, including proximal placement of the first toe, syndactyly of the second and third toe, and unusually long and/or crooked toes, and protruding and post-rotated ears with overfolded, thick helices were also commonly observed. Those with *de novo* disruptive mutations and chromosomal rearrangements did not differ in clinical features.

ASD risk in *DYRK1A* haploinsufficiency. 43% of all case reports reviewed indicated an ASD diagnosis. Among 15 cases who received gold-standard ASD assessment, rates increased to 73%. Additionally, features common to ASD, such as stereotypic and anxious behaviors, were noted in many cases where reference to ASD diagnosis was absent. This suggests rates of ASD in *DYRK1A* cohorts may be higher than reported in our total sample.

There are several reasons for the potential underestimated prevalence rate of ASD among *DYRK1A* cases. First, most previously published cases relied on medical records, which varied greatly in the detail provided and comprehensiveness of prior evaluations; as such, it is unknown whether ASD was evaluated and confirmed absent or not evaluated at all. Second, it can be difficult to tease apart symptoms of ASD from those of intellectual disability and speech impairments without specialized training and experience with differential diagnosis within developmental disabilities. Additionally, with the many complications that arise from cognitive, language, and motor impairments, along with medical complications to vision, growth, and seizures, ASD may not be the most pressing concern for families regarding their child's development. As *DYRK1A* haploinsufficiency continues to be explored within ASD risk, these factors need to be considered when determining rates in this population.

***DYRK1A* haploinsufficiency as a distinct ASD subtype**

In an effort to situate the *DYRK1A* phenotype in the context of ASD, we found the *DYRK1A* group exhibited significantly higher incidence of key features compared to those with idiopathic ASD: intellectual disability, speech delay, motor difficulties, vision abnormalities, feeding difficulties, and microcephaly. Frequency of these features also significantly differed between the *DYRK1A* group and the comparison group with both idiopathic ASD and an IQ below 70. This suggests that the combination of features observed in *DYRK1A* haploinsufficiency is distinct from that observed in those with intellectual disability and ASD alone. This is consistent with prior evidence that disruptive mutations result in significantly more impairing comorbidities than in idiopathic

ASD (Iossifov et al., 2014; Sanders et al., 2015). Notably, when those with *DYRK1A* who were originally ascertained for an ASD diagnosis are compared to the idiopathic groups (also ascertained for ASD), the profile remains the same. These findings suggest the phenotype commonly exhibited in individuals with *DYRK1A* disruptions and ASD is indeed distinct from idiopathic ASD. The co-occurrence of five or more of these phenotypic features in *DYRK1A* cases (78%) was significantly higher than co-occurrence in idiopathic cases (0.3%), suggesting the combination of these core phenotypic features may be clinically suggestive of *DYRK1A* haploinsufficiency in an individual presenting with concerns of ASD.

Prior publications of *DYRK1A* cases have relied on categorical data to describe clinical phenotype. Our exploration of a quantitative phenotype provided further evidence of differences in head size, cognitive functioning, adaptive ability between *DYRK1A* and idiopathic cases, as well as differences in head size between *DYRK1A* and *CHD8* cases. Interestingly, *DYRK1A* and *CHD8* cases did not differ quantitatively in other phenotypic domains. This is perhaps due to the wide variability in symptoms severity observed in *CHD8* cases. It is also possible that further phenotypic differences exist which have not been detected by current diagnostic tools given the level of resolution inherent in clinical assessment. Markers relying on quantitative, brain-based measures may reveal gene-specific profiles. For instance, recent work highlights divergent information processing systems for children with 16p11.2 CNVs (Hudac, Kresse, et al.) and children with early-emerging disruptive mutations (Hudac, Stessman, et al.). Considering the intellectual disability associated with *DYRK1A*, a passive,

noninvasive neuroimaging approach may help illuminate neuroendophenotypes that links the behavioral phenotype to the underlying neural mechanisms.

Familial contribution to phenotypic variability in *DYRK1A* haploinsufficiency

Exploring quantitative phenotype illuminated phenotypic heterogeneity between individuals. While *DYRK1A* mutations significantly impact development, the severity of impairment varied between individuals, which may be, in part, due to contributions of the family background. A series of sequential entry multiple linear regressions were conducted to explore the relationship between parental (maternal and paternal separately) and proband phenotype for *DYRK1A* cases in the context of other ASD-associated groups: idiopathic cases (simplex and multiplex), 16p11.2 deletion (CNV), and *CHD8* SNV. Regressions focused on phenotypic variables of particular interest in *DYRK1A* haploinsufficiency: head circumference, ASD symptoms, and IQ. It is important to note that these regression analyses are indeed exploratory, as the three comparison groups differed widely in sample size from eachother and from the *DYRK1A* group (n = 10).

Head Circumference. Variation in proband head circumference was explored in relationship to a series of predictors: proband height, group status (*DYRK1A* vs. (1) Idiopathic, (2) 16p11.2, (3) *CHD8*), maternal or paternal head circumference, and an interaction of group and parental head circumference. Proband height was moderately to highly correlated to proband head circumference across all groups and accounted for significant variation in each model (Figure 6). This suggests that the relationship between height and head size in the typically developing population is consistent across

ASD populations, including those with *DYRK1A* mutations, a finding that is consistent with prior studies of typically developing and ASD populations (Bale, C.I., Parry, & Bale, 1991; Lainhart et al., 2006)

The inclusion of group and parental head circumference (maternal and paternal in separate analyses) accounted for significant variation (ranging widely from 11-60%) across Idiopathic-*DYRK1A*, 16p11.2-*DYRK1A*, and *CHD8-DYRK1A* models. Group was a significant predictor across models, with the *DYRK1A* group presenting with smaller head sizes than all comparison samples. This is consistent with the phenotypic analyses conducted above. Maternal and paternal head circumference were each a significant predictor in Idiopathic-*DYRK1A* models, accounting for similar variation in head circumference (0.39-0.41 SD change in proband head circumference per 1 SD change in parental head circumference). Interestingly, paternal but not maternal head circumference was a unique predictor in 16p11.2-*DYRK1A* models, following a stronger correlation between paternal and proband head circumference than maternal and proband head circumference. Paternal contribution was strikingly similar for 16p11.2 and idiopathic models. The significant contribution of parental head circumference to variation in proband head circumference is consistent with studies of other ASD populations (Lainhart et al., 2006). Neither maternal nor paternal head circumference uniquely predicted proband head circumference in *CHD8-DYRK1A* models; parental and proband head circumferences were moderately correlated, but parental predictors likely didn't reach significance due to small sample sizes.

Interactions of group and parental head circumference were observed in all three comparison group analyses. Idiopathic-*DYRK1A* and 16p11.2-*DYRK1A* analyses yielded interactions for both group-maternal and group-paternal head circumference, with larger coefficients observed for group-paternal interactions. In *CHD8-DYRK1A* analyses, a significant interaction between group and paternal head circumference was observed. Across each interaction, the same pattern emerged. Parental head circumference more heavily influenced that of *DYRK1A* probands than probands in idiopathic, 16p11.2, and *CHD8* comparison groups. Interactions also suggest that differences in head circumference between *DYRK1A* and comparison groups were more pronounced in cases whose parents had smaller head sizes than those whose parents had larger head sizes. In other words, relatively smaller parental head sizes (even low average) were related to more severely microcephalic *DYRK1A* cases than cases whose parental head sizes were larger (high average). These findings are contrary to our initial hypotheses, in which we anticipated that parental phenotype would be less influential in the *DYRK1A* group than in the idiopathic and 16p11.2 comparison groups, as *DYRK1A* SNVs are considered more deleterious than 16p11.2 CNVs and idiopathic ASD. *CHD8* and *DYRK1A* groups were hypothesized to have similar parental contribution given their disruptive SNV statuses.

Our contrary findings are indeed surprising. Given the small sample size, correlations appear to be driven by a few participants and their parents with relatively small and large head sizes. There is a wider range in proband head circumference in the *DYRK1A* group than other gene comparison groups (4 SD range vs. 2 SD range,

respectively) that may be driving stronger correlation slopes between parent and proband head size in the *DYRK1A* group compared to idiopathic, 16p11.2, and *CHD8* groups. Future studies with a larger *DYRK1A* group will be important to better understand whether the interaction observed in this study is more than a spurious finding. However, it is also possible that *DYRK1A*'s impact on physiological features, like head circumference, is more heavily moderated by other genes (and thus parental background) than genes such as a *CHD8* or 16p11.2. Many genes have been identified as influential to head circumference, opening the possibility that secondary genetic events could also be at play. At this time, this finding should be interpreted with caution and it will be important to determine whether this pattern persists with larger samples in the future. Overall, we see promising evidence that parental head circumference is indeed contributory to variations observed in *DYRK1A* proband head circumference.

ASD Symptoms. Variation in proband ASD symptoms was analyzed in the context of the following predictors: proband full-scale IQ, group status, maternal or paternal ASD symptoms, and an interaction of group and parental ASD symptoms. Ranging from weakly to moderately correlated, proband IQ accounted for significant variation in proband ASD symptoms across Idiopathic-*DYRK1A* and 16p11.2-*DYRK1A* analyses (6-12%). While IQ accounted for 15% of variation of proband IQ in *CHD8-DYRK1A* analyses, the contribution was not significant, perhaps due to small sample sizes. Overall, proband IQ and ASD symptoms were negatively correlated, and regressions indicated that as IQ decreased, ASD symptoms increased. This is consistent with prior literature, indicating that intellectually impaired individuals with ASD

tend to also have more severe ASD symptoms (Constantino et al., 2000; Constantino et al., 2010; Hus et al., 2013).

While controlling for proband IQ, the addition of group status and parental ASD symptoms into the models accounted for minimal variation in proband ASD symptoms across idiopathic, 16p11.2, and *CHD8* analyses. Although statistically significant, the addition of group and parental ASD symptoms in Idiopathic-*DYRK1A* models accounted for negligible variance (0.8% maternal and 2% paternal) in proband ASD symptoms. These predictors did not contribute significant variation to 16p11.2 or *CHD8* analyses. ASD symptom severity within the *DYRK1A* group was similar to Idiopathic, 16p11.2, and *CHD8* groups. For idiopathic-*DYRK1A* analyses, maternal and paternal ASD symptoms were unique predictors and had similar effects on proband ASD symptoms (2-3 point increase in proband ASD symptoms per 1 SD increase in parental ASD symptoms). The significance of these predictors and their contribution to the overall model is likely due to the large sample size of the Idiopathic group. A 2-3 point increase on the SRS represents minimal change in clinical severity. There were no significant interactions between group and parental ASD symptoms in any of the models, suggesting that there is not differential contribution of parental ASD phenotype by group.

Overall, parental variation in ASD symptoms appeared to have minimal contribution to proband ASD symptom severity. This finding is not surprising for *DYRK1A* and *CHD8* groups, both of which have a disruptive SNV that appears to be driving their ASD risk amidst a typically developing family. Both the Idiopathic group (no

known genetic cause for ASD) and 16p11.2 group (a gene event that ranges in phenotypic expression) were anticipated to have higher correlations between parental and proband ASD symptoms than were observed. This could be due in part to selection bias in our samples, as it may require a high level of parental functioning to participate in research. Prior studies of the Social Responsiveness Scale as a measure of ASD symptoms severity have found a variety of phenotypic factors to be related to severity scores, thus it is likely that other variables, such as comorbid psychopathology, are driving the variation we see in ASD symptom severity (Constantino et al., 2000; V. Hus, S. Bishop, K. Gotham, M. Huerta, & C. Lord, 2013b).

Full-scale IQ. Variation in proband IQ was explored with the following predictors: group status and parental IQ, and the interaction of group and parental IQ. Parental IQ (both maternal and paternal) were weakly correlated across idiopathic-*DYRK1A*, 16p11.2-*DYRK1A*, and *CHD8-DYRK1A* analyses. However, inclusion of group and parental IQ in Idiopathic-*DYRK1A* and 16p11.2-*DYRK1A* models accounted for significant variation (10-14% and 33-36%, respectively). These predictors were not significant contributors within the *CHD8-DYRK1A* models, again potentially due to sample size constraints. Though not significant, it is interesting that while 35% of the variation in proband IQ for the *CHD8-DYRK1A* sample was accounted for by group and maternal IQ, only 15% was accounted for by group and paternal IQ, suggesting that a mother's IQ could be a stronger predictor of proband IQ than a father's IQ. Larger sample sizes would be needed in the future to understand maternal-paternal differences. In Idiopathic-*DYRK1A* models, group status was a unique predictor,

indicating *DYRK1A* cases presented with lower IQ than idiopathic cases. This finding is consistent with earlier findings that intellectual impairment is a distinguishing factor for the *DYRK1A* phenotype.

Both maternal and paternal IQ were unique predictors to proband IQ in multiplex-*DYRK1A* analyses (a 6-8 point increase in proband IQ per 1 SD increase in parental IQ). However, weak correlations between parental and proband IQ suggest that the large sample size of the multiplex group may be driving significance of these findings. Parental IQ and interaction terms were not uniquely predictive in 16p11.2-*DYRK1A* or *CHD8*-*DYRK1A* models. Overall, findings suggest parental IQ is likely not a primary driver in cognitive variation in these various ASD groups. However, with greater sample sizes, the ability to evaluate correlations within-families through multi-level linear modeling may illuminate significant relationships between parental and proband phenotype and is an important consideration for future study (Moreno-De-Luca et al., 2015).

Overall, findings suggest that parental phenotype is indeed a contributor to variability in proband head circumference but less so for ASD symptoms and IQ. Physiological characteristics are highly correlated between parents and children in typically developing populations, ranging from 0.5 to 0.7, which may explain why parental contribution is most evident for head circumference in ASD samples, including the *DYRK1A* group (Clark, 1955; Weaver & Christian, 1980). Our findings suggest that, even in the presence of a *de novo*, disruptive *DYRK1A* mutation, parental phenotype may still impact their affected child's presentation. Of course, secondary genetic events,

embryonic or early developmental influences, and treatment must also be considered as potential factors contributing to the variability.

Parent-proband effect size of *DYRK1A* versus other ASD-associated mutations

While regression analyses informed the general relationships between parental and proband phenotypic features across ASD groups, further analysis was conducted to quantify the effect of a *DYRK1A* mutation relative to other ASD-associated mutations on clinical phenotype.

It was hypothesized that, given the severity of the *DYRK1A* haploinsufficiency phenotype, parent-proband effect sizes (as a quantifiable measure of genetic impact) for the *DYRK1A* group would be larger than those of idiopathic, 16p11.2, and *CHD8* groups on measures of head circumference and IQ, but not ASD symptoms. The *DYRK1A* group did indeed present with the largest effect size for head circumference (-2.93 SD), indicating an average -2.93 SD impact of *DYRK1A* on head circumference, taking parental head circumference into account. This is consistent with the recurrent phenotype of microcephaly in cases with *DYRK1A* haploinsufficiency. Comparison group effect sizes for head circumference were all small (less than 0.5 SD). *CHD8* effect size for head circumference (0.09 SD) was smaller than anticipated given the group's trend toward macrocephaly. This small effect size may be due to the fact that mean parental head circumference for the *CHD8* group were larger than other parental groups and remarkably similar to *CHD8* proband mean head circumference *Z* score (Mean Proband HC = 1.76 SD, Mean Biparental HC = 1.68 SD). The negative effect size of the

idiopathic group was also unexpected, but likely driven by a few idiopathic proband outliers with severe microcephaly (see Figure 12).

When comparing effect sizes for ASD symptoms, the *DYRK1A* group displayed the largest effect size (5.52 SD), indicating a 5.52 SD increase in ASD symptom severity for probands compared to their unaffected parents. This finding is contrary to our hypotheses and is likely due in part to unaffected parents in the DYRK1A group having the lower ASD symptom scores compared to other groups as well as one *DYRK1A* case with a very high ASD symptom score (see Figure 11b). The 16p11.2 group had the smallest effect size, which is consistent with reports of highly variable ASD symptomology amongst 16p11.2 cases, perhaps due in part to the incomplete penetrance and variable expressivity of the CNV(Hanson et al., 2015; Hanson et al., 2010). Other influential factors could include measurement errors and ascertainment factors.

It was anticipated that, given the significant intellectual impairment that is common in *DYRK1A* haploinsufficiency, *DYRK1A* effect size for IQ would be larger than comparison groups. Instead, *DYRK1A* parent-proband effect size for IQ (-6.09 SD) was smaller than the CHD8 group (-7.32 SD). This is likely due to tighter variation in parental IQ for the *CHD8* group compared to the *DYRK1A* group. While all groups displayed effect sizes suggesting lower IQ in probands compared to unaffected parents, *DYRK1A* and *CHD8* groups had far larger effect sizes, highlighting the intellectual impairment in the phenotype of both disruptive SNVs. Overall, these effect sizes provide quantifiable estimates of genetic impact across three phenotypic domains, which improve

understanding of the varied impact different genetic events have on proband phenotype in the context of their remaining genetic background.

Limitations and Future Directions

Our findings must be considered in the context of limitations of this study. First, information available for previously published cases varied widely. While some case reports provided detailed record of psychiatric history, others only included medical history, which also varied in its extensiveness. Full assessment history was unknown for previously published cases, raising questions whether phenotypic features left out of a case report were previously ruled out or were not assessed. These variations highlight the importance of consistency in phenotypic assessment across future *DYRK1A* studies to ensure comprehensive and accurate phenotyping efforts. In the future, more detailed assessment of common phenotypic characteristics of *DYRK1A* haploinsufficiency (e.g. vision impairments, feeding difficulties, motor impairments) will increase clinical utility and accuracy with which those with *DYRK1A* mutations might be clinically distinguishable from other patients with developmental disabilities.

Second, the *DYRK1A* subset who participated in the same quantitative assessment battery was small. With a larger sample, it will be useful to compare phenotype across different types of genetic variants, secondary genetic events, prenatal environmental influences. Additionally, it is important to note that head circumference measurements used in this study were taken at the time of study participation and do not reflect head size or growth in early development. Given the literature suggesting the rate of postnatal head growth in infancy to be important in developmental outcomes, it is

important to acknowledge that impact of small variations in microcephaly on development may be better captured by longitudinal tracking of head growth early in development (Gross, Oehler, & Eckerman, 1983). Sample size within this study limits the generalizability of group comparisons and regression analyses. Sample size discrepancies between large comparison samples and the *DYRK1A* sample made interpretation across models difficult. With a larger *DYRK1A* sample, multi-level modeling would likely be a more effective way to exploring parental-proband relationships, as it would allow for variation to be measured within families. Analyses of parental contribution to proband variation remain exploratory due our study's limitations, and further study is indeed needed to better understand the quantitative phenotype of *DYRK1A* and familial contribution to variability between affected individuals.

Also, while comparison of *DYRK1A* cases to idiopathic ASD provides important confirmation of a distinct combination of comorbidities, it is important to acknowledge that individuals in the idiopathic group may, with future advances in our understanding of the genetics of ASD, no longer be identified as idiopathic. The idiopathic group analyzed in this study likely represents a population with fewer syndromic features than populations with ASD and other genetic events. Furthermore, the use of secondary data for large ASD comparison samples prevented the use of a consistent idiopathic group in regression analyses. Due to strong evidence for divergent genetic mechanisms for simplex and multiplex autism, findings from these two comparison groups (simplex sample for head circumference and ASD symptoms and multiplex sample for IQ) must be interpreted separately. Further study of parental contribution for simplex and

multiplex probands is needed to better understand differences between these two populations. The formation of similarly sized and matched ASD-associated comparison groups will be important when studying increasingly larger samples with *DYRK1A* haploinsufficiency. Continued study of ASD-associated mutations, including *DYRK1A*, will allow for improved understanding of distinct subtypes of ASD and inform future approaches to personalized treatment.

Implications

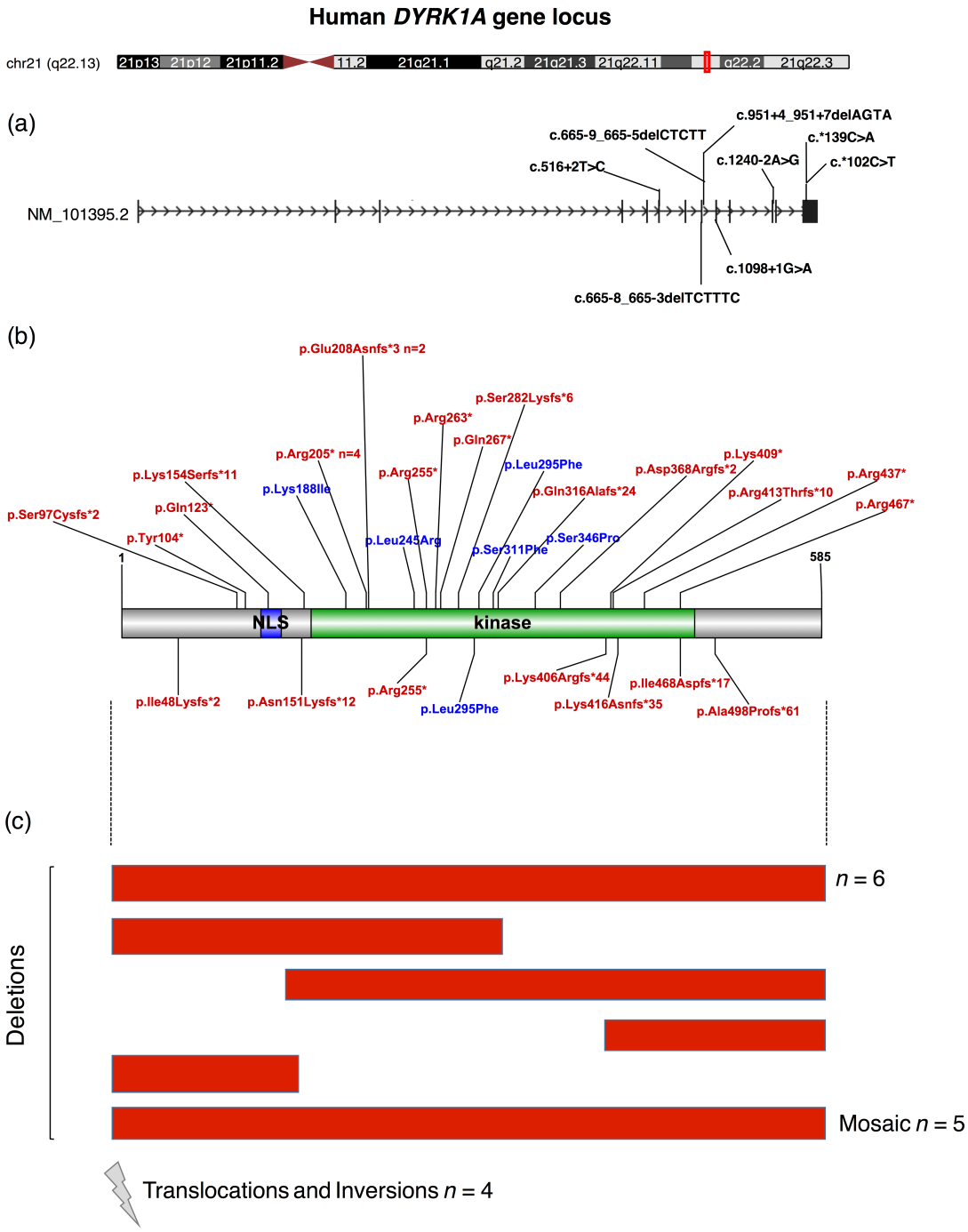
Further study of *DYRK1A* haploinsufficiency holds important utility for understanding ASD more broadly and developing modes of intervention for the disorder. First, determination of genetically-defined subtypes of ASD, alongside increasing availability of whole exome sequencing, may allow for earlier identification of mutations like *DYRK1A* and increase understanding of an affected child's developmental trajectory and risks, including ASD. Identifying specific genes or gene pathways that confer ASD risk is an important step to parsing the clinical heterogeneity of the disorder and conceptualizing symptoms in the broader context of associated comorbidities and varied levels of functioning. Understanding the constellation of phenotypic impairments associated with *DYRK1A* mutation will inform specific domains to target in behavioral intervention, such as speech, motor, and social-communication development.

Second, understanding the relationship between disruptive mutations to *DYRK1A* and phenotypic impairments is the first step to identifying specific cellular mechanisms associated with specific symptoms. Just like with previously identified single gene disorders, translational research can expose potential pathways related to *DYRK1A* that

might be susceptible to pharmacological intervention (Ziats & Rennert, 2016). For example, given *DYRK1A*'s known role in brain development, the observed brain size reductions that occurs when the gene is disrupted may be eventually counteracted through intervention that promotes brain growth during early development. Numerous studies indicate that many ASD-associated genes, including *DYRK1A*, appear to be functionally connected, suggesting that the identification of targeted interventions for *DYRK1A* may inform similar interventions for other individuals with ASD (Hormozdiari et al., 2015; Iossifov et al., 2014; O'Roak, Vives, Girirajan, et al., 2012).

In summary, *DYRK1A* haploinsufficiency results in a distinct clinical phenotype, which includes microcephaly, intellectual impairment, the presence of vision and motor difficulties, language delays, and ASD risk. The *DYRK1A* profile is distinct from that observed in idiopathic ASD, suggesting a potential subtype of ASD. Despite a consistent profile, quantitative assessment highlights heterogeneity in the severity of impairments. Parental phenotype, reflecting genetic background, is a likely contributor to that variability among individuals. These findings serve to strengthen both genetic and phenotypic understanding of a strongly ASD-associated mutation and promote the utility of a genetics-first approach to understanding variations in ASD.

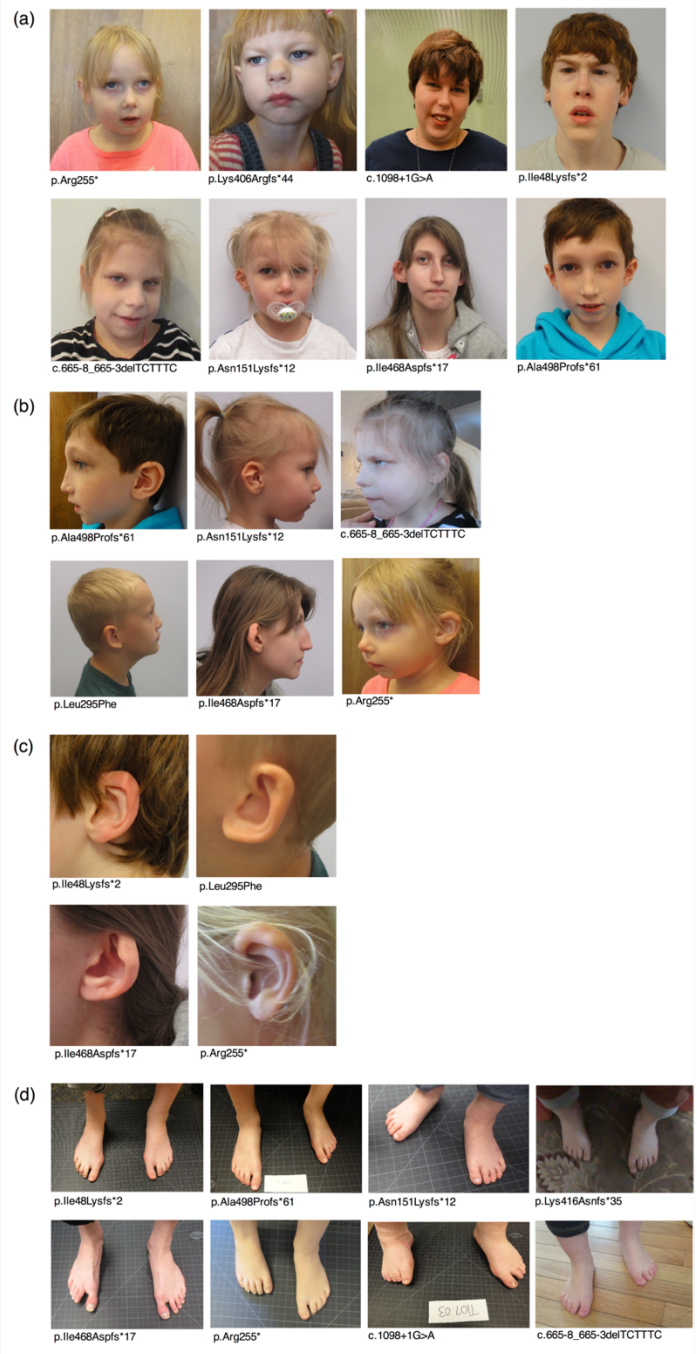
Figure 1. Summary of *DYRK1A* gene variants



Schematic depicting the locations of disruptive variants (truncating, missense, and splice site mutations), copy number variations, and chromosomal rearrangements affecting *DYRK1A*. The ideogram of human chromosome 21 and isoform NM101395.2 coding sequence was obtained from the UCSC genome browser (Kent et al., 2002). (a) NM101395.2 coding sequence with 8 reported splice site mutations (presented in HGVS cDNA notation). Mutations below the sequence are UW-SNV participants, above are Pub-SNV mutation cases. (b) The *DYRK1A*

protein (NP_567824.1) with truncating (red) and missense (blue) mutations (presented in HGVS notation). Mutations below the protein are UW-SNV cases, above are Pub-SNV mutation cases. (c) Copy number deletions and chromosomal rearrangements, including 6 deletions of entire gene, four partial deletions, 5 mosaic deletions, and 4 translocations/inversions (lightning bolt).

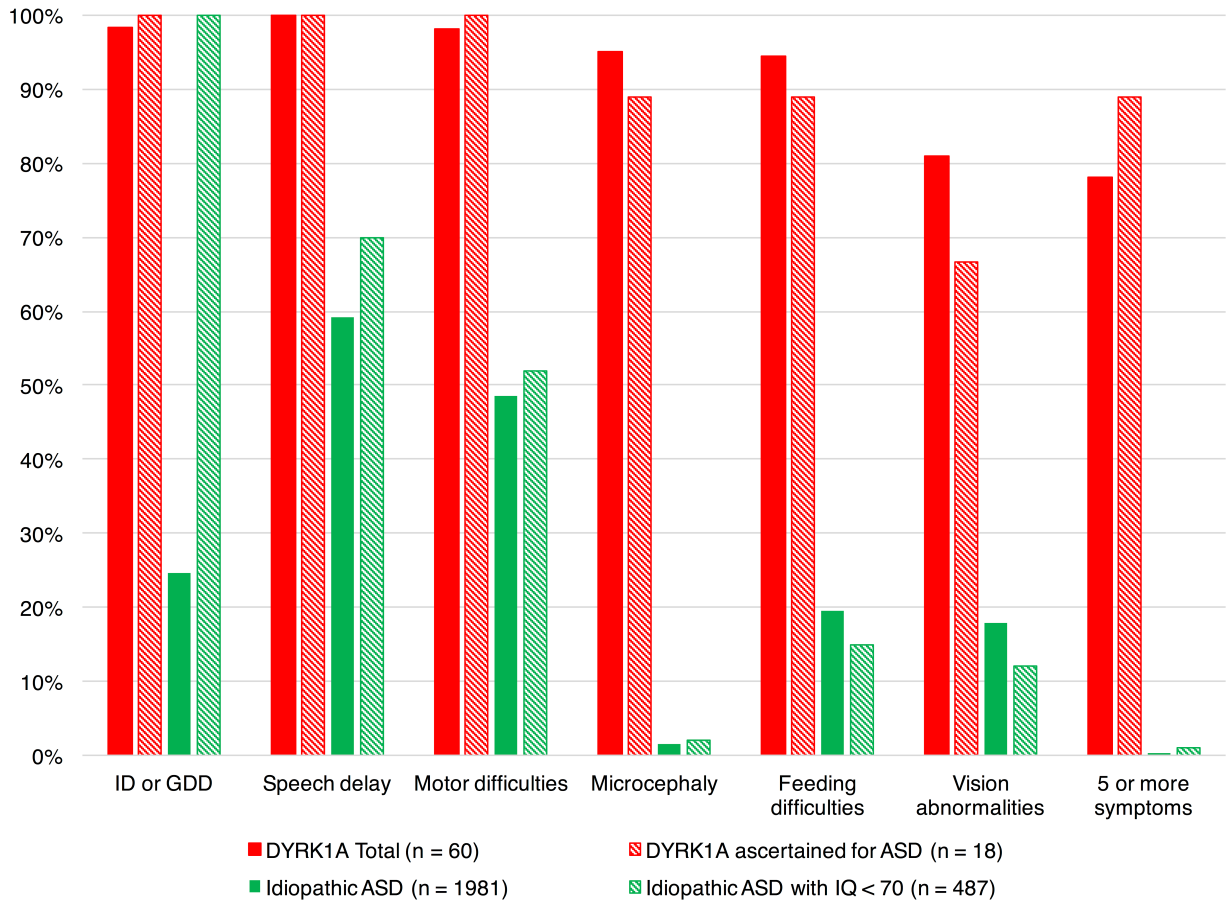
Figure 2. Common dysmorphic features in UW-SNV patients with *DYRK1A* haploinsufficiency



(a) Facial features of eight UW-SNV patients with *DYRK1A* haploinsufficiency. Note common features across patients, including prominent brow with high anterior hairline, slightly upslanted palpebral fissures, retrognathic jaw, deepset eyes with a hooded appearance, bitemporal narrowing, high nasal bridge with tubular-shaped, broad-tipped nose, and protruding ears. (b) Profiles of six UW-SNV patients. Note prominent brows with high anterior hairlines as well as low-set, posteriorly rotated ears in a subset of patients. (c) Ear abnormalities in four UW-SNV patients, including post-rotated and protruding ears with protruding thick and over-folded helices

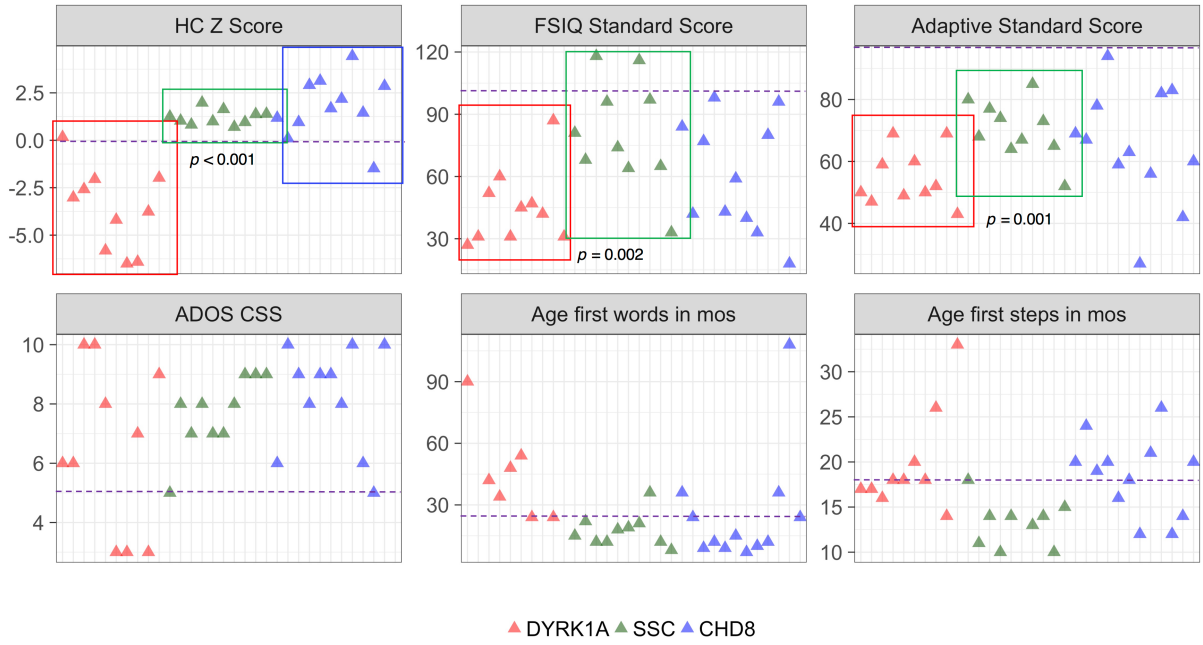
(i.e. outer fold of the ear). (d) Foot abnormalities in eight UW-SNV patients. Common features include proximal placement of the first toe, crooked toes, and syndactyly of the second and third toes. Frameshift, nonsense, and missense cases identified by HGVS protein notation; cases with splice site variants identified by HGVS cDNA notation.

Figure 3. Phenotypic features in total *DYRK1A* sample, *DYRK1A* sample ascertained for ASD, and Idiopathic ASD samples



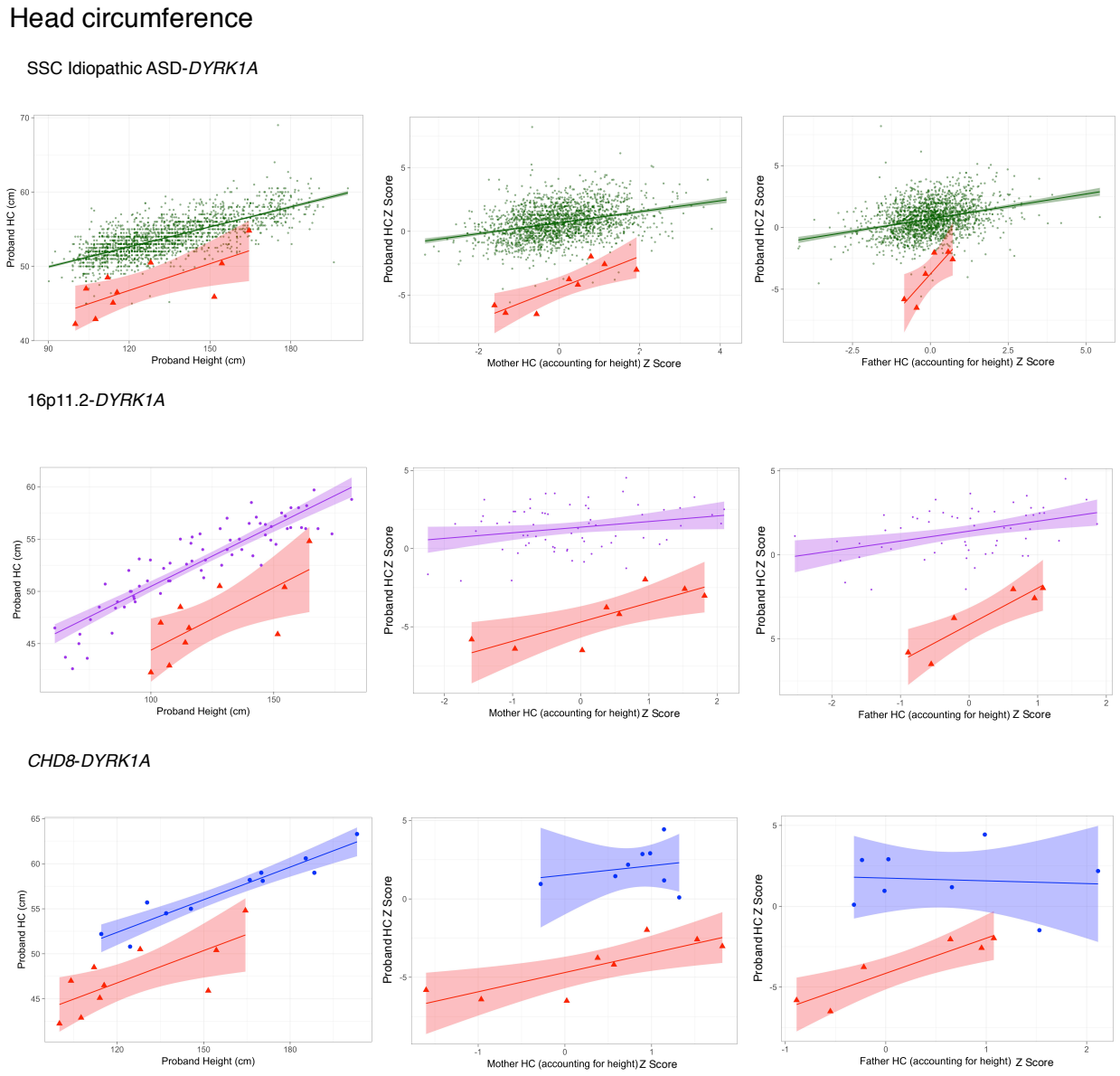
Bar graph presented frequencies of core phenotypic features observed in 75% or more of *DYRK1A* patients. Total *DYRK1A* sample (Pub-SNV, UW-SNV, Pub-CHR) and *DYRK1A* sample ascertained for ASD were compared to frequencies of features in Idiopathic ASD samples (total and IQ < 70) using Fisher's exact tests ($p < 0.001$).

Figure 4. Quantitative phenotype of *DYRK1A*, Idiopathic, and *CHD8* samples



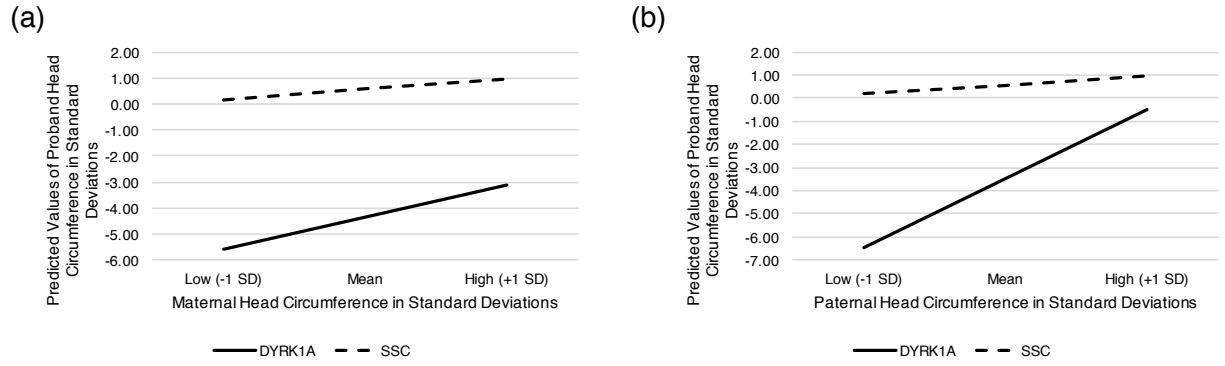
Scatterplots of core phenotypic features in UW-SNV *DYRK1A* sample, (*n* = 10), Idiopathic subset matched for age and gender (randomly sample, *n* = 10), and *CHD8* sample (*n* = 12). Dotted lines designate conservative averages for typical population. HC = head circumference, FSIQ = full-scale IQ, ADOS CSS = calibrated severity score. Independent sample *t* tests comparing *DYRK1A*, Idiopathic, and *CHD8* groups, *p* value adjusted for multiple comparisons.

Figure 5. Correlations of proband and parent head circumference by gene group



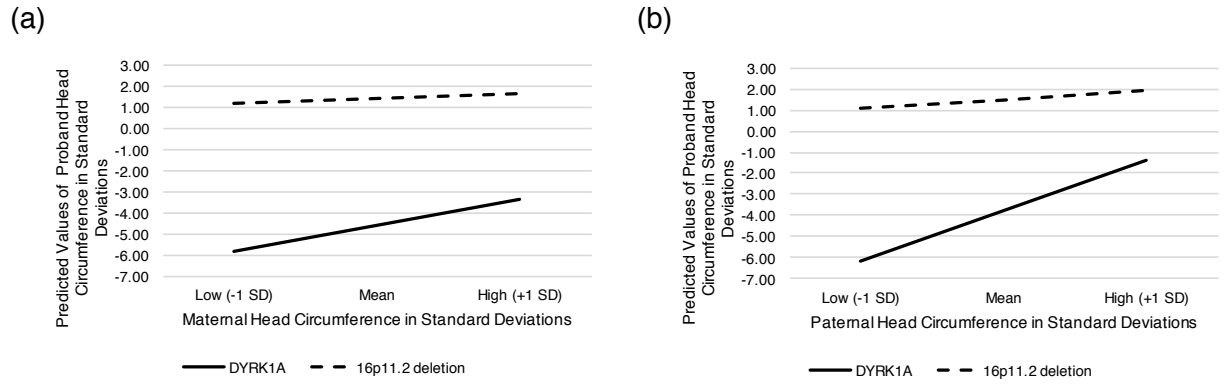
Correlations between proband head circumference and proband height, maternal head circumference, and paternal head circumference for *DYRK1A* (UW-SNV, presented in red) and the following comparison groups (from top to bottom): SSC simplex idiopathic ASD (green), 16p11.2 deletion (purple), and *CHD8* SNV (blue). Parental head circumference variables are standardized and control for parental height.

Figure 6. Interaction of Group and Parental Head Circumference (*DYRK1A* vs. SSC simplex idiopathic ASD) on proband head circumference, (a) maternal head circumference, (b) paternal head circumference.



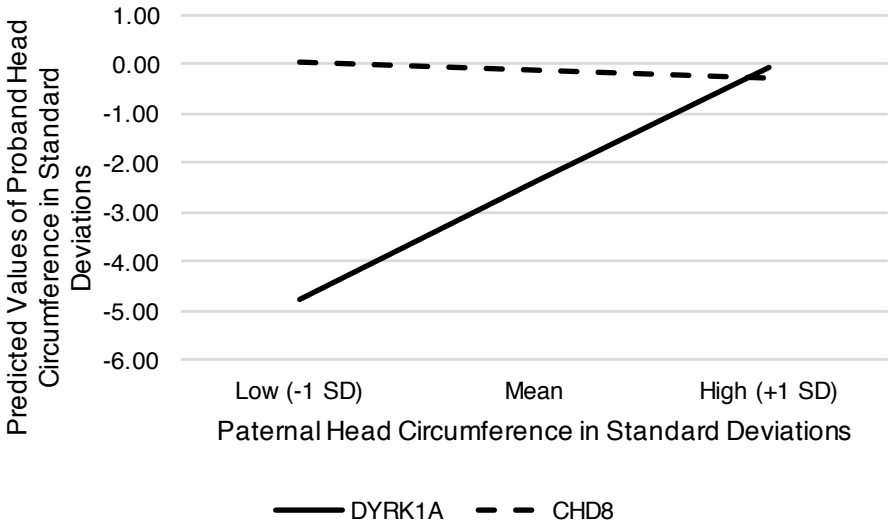
(a) There is a significant ordinal interaction between group and maternal head circumference. The effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose mothers had relatively smaller head circumference (-1 SD), while the effect of a *DYRK1A* mutation on head circumference was smaller for cases with mothers with relatively large head size (+1 SD). (b) A similar ordinal interaction was observed between group and paternal head circumference. The effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose fathers had relatively smaller head circumference (-1 SD), while the effect of a *DYRK1A* mutation on head circumference was smaller for cases with fathers with relatively large head size (+1 SD). In both models, the effect of parental head circumference was larger for the DYRK1A group compared to the SSC group.

Figure 7. Interaction of Group and Parental Head Circumference (*DYRK1A* vs. 16p11.2 deletion) on proband head circumference, (a) maternal head circumference, (b) paternal head circumference.



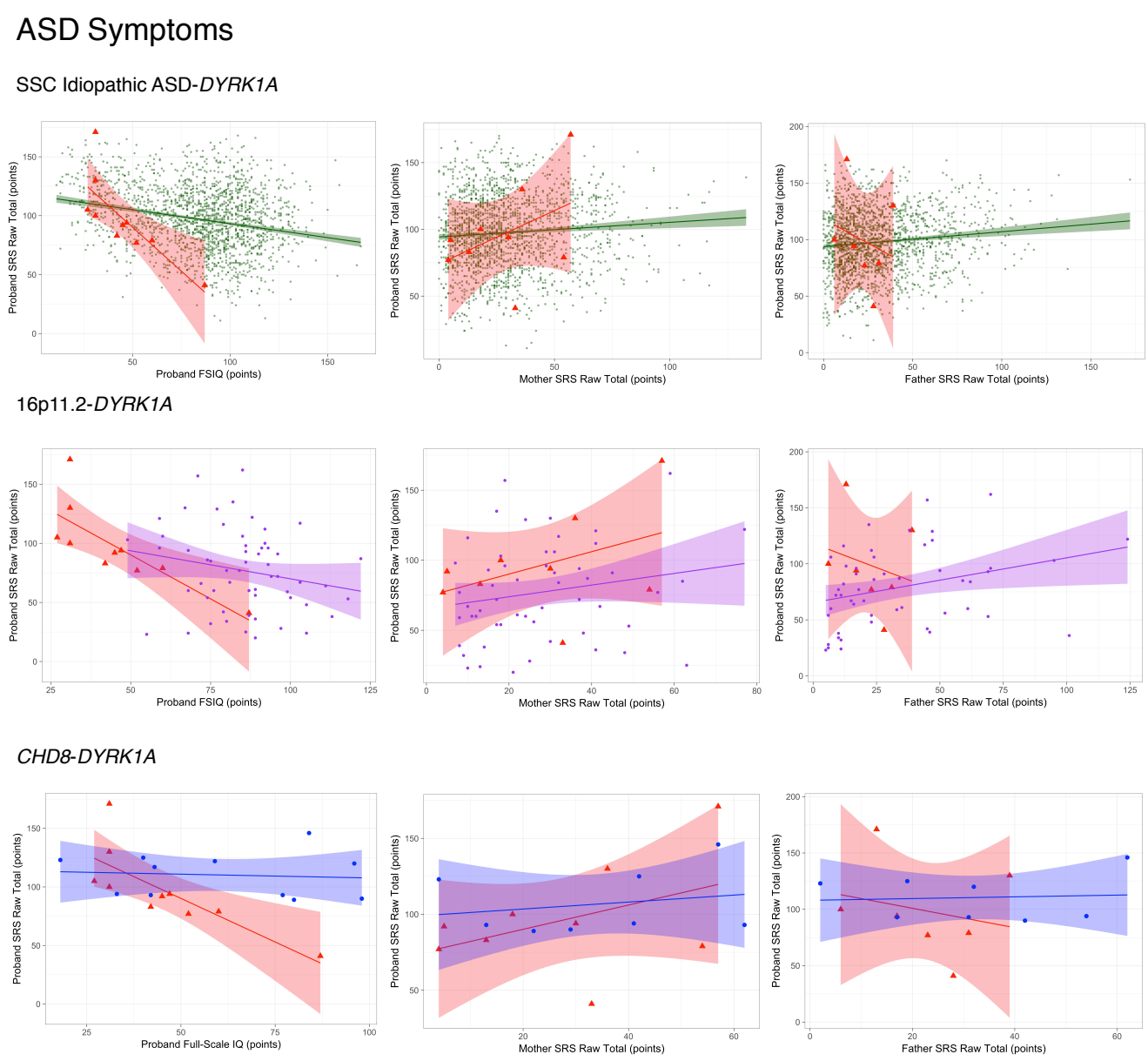
(a) There is a significant ordinal interaction between group and maternal head circumference. The effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose mothers had relatively smaller head circumference (-1 SD), while the effect of a *DYRK1A* mutation on head circumference was smaller for cases with mothers with relatively large head size (+1 SD). (b) A similar ordinal interaction was observed between group and paternal head circumference. The effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose fathers had relatively smaller head circumference (-1 SD), while the effect of a *DYRK1A* mutation on head circumference was smaller for cases with fathers with relatively large head size (+1 SD). In both models, the effect of parental head circumference was larger for the DYRK1A group compared to the 16p11.2 group.

Figure 8. Interaction of Group and Paternal Head Circumference (*DYRK1A* vs. *CHD8*) on proband head circumference



A significant ordinal interaction was observed between group and paternal head circumference. The effect of having a *DYRK1A* mutation on head circumference was greater for individuals whose fathers had relatively smaller head circumference (-1 SD), while the effect of a *DYRK1A* mutation on head circumference was smaller for cases with fathers with relatively large head size (+1 SD). The effect of paternal head circumference was larger for the DYRK1A group compared to the *CHD8* group.

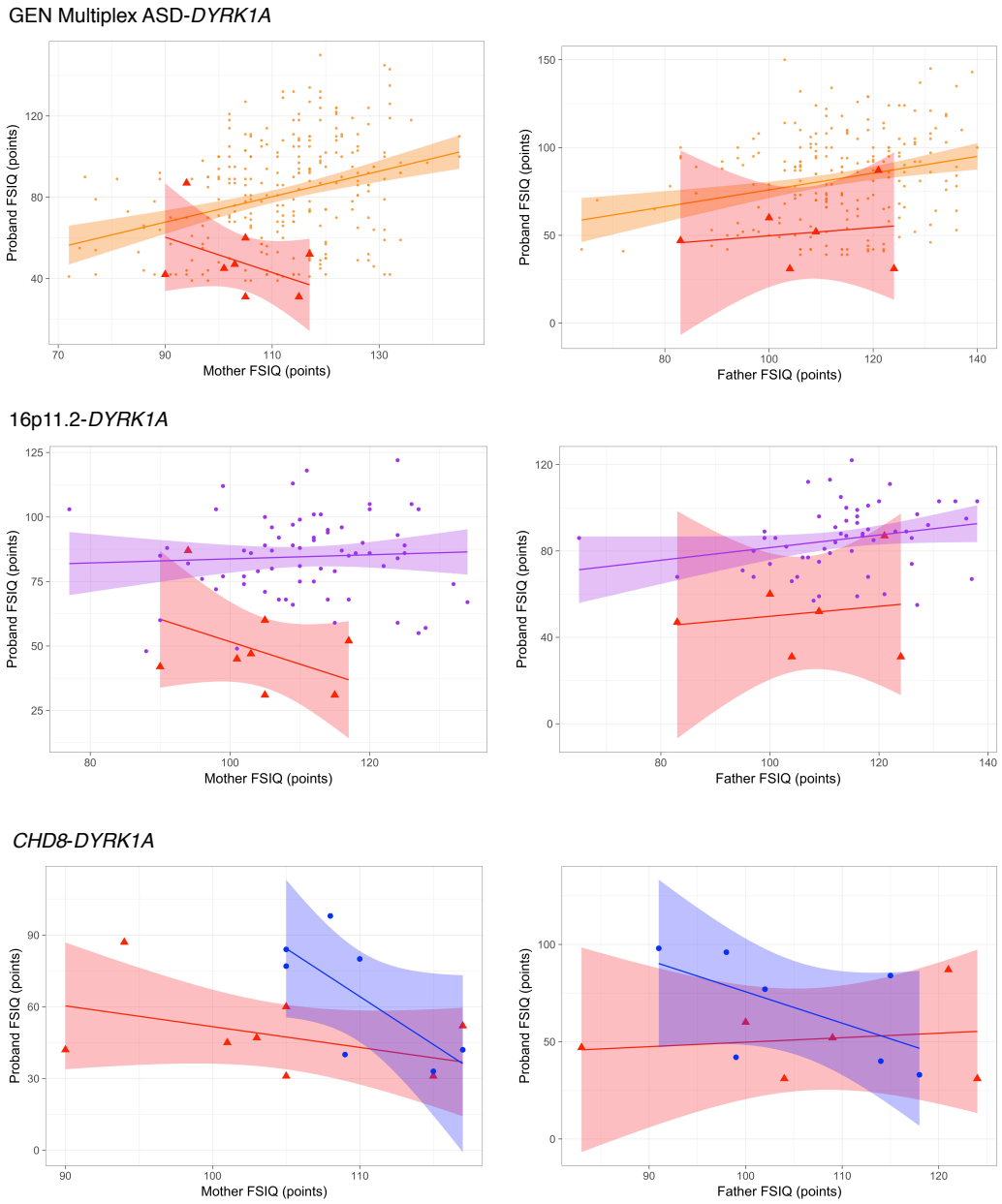
Figure 9. Correlations of proband and parent ASD symptoms by gene group



Correlations between proband ASD symptoms (SRS Raw Total Score) and proband IQ, maternal ASD symptoms, and paternal ASD symptoms for DYRK1A (UW-SNV, presented in red) and the following comparison groups (from top to bottom): SSC simplex idiopathic ASD (green), 16p11.2 deletion (purple), and *CHD8* SNV (blue).

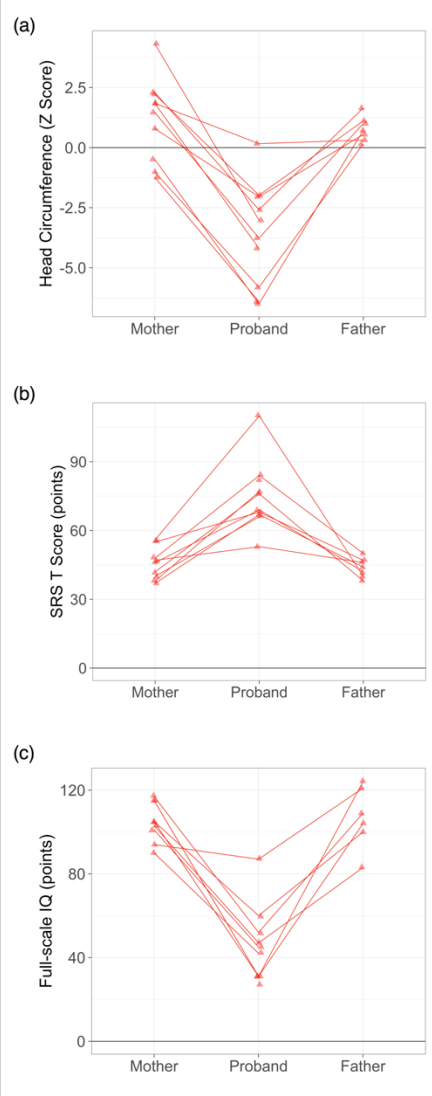
Figure 10. Correlations of proband and parent IQ by gene group

Full Scale IQ



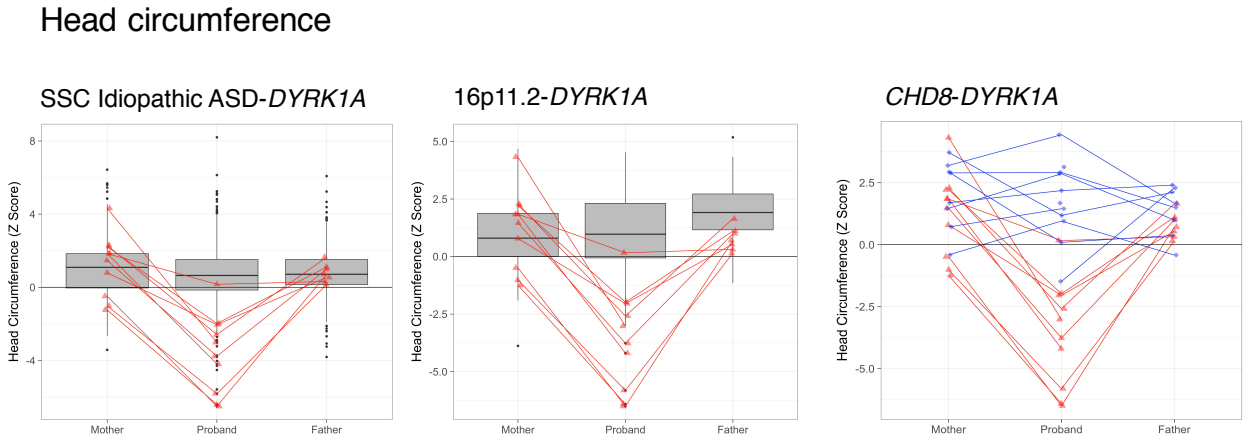
Correlations between proband IQ, maternal IQ, and paternal IQ for DYRK1A (UW-SNV, presented in red) and the following comparison groups (from top to bottom): SSC simplex idiopathic ASD (orange), 16p11.2 deletion (purple), and *CHD8* SNV (blue).

Figure 11. Contribution of familial genetic background to DYRK1A cases on measures of head circumference, ASD symptoms, and IQ



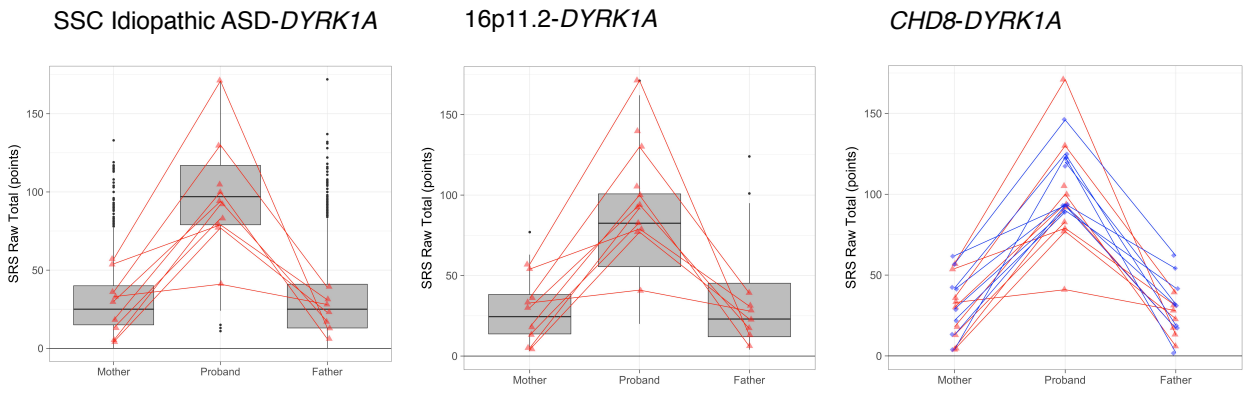
UW-SNV cases are presented with their unaffected mothers and fathers on three phenotypic measures: (a) head circumference (Z score, SD), (b) ASD symptoms (Social Responsiveness Scale T score), and (c) IQ (Full-scale standard score). Affected children presented with significantly more severe phenotypes compared to both unaffected mothers and fathers using Wilcoxon rank sum tests ($p < 0.001$). Variability in parental phenotype corresponds to proband variation. Probands with smaller head sizes relative to other UW-SNV cases correspond to parents who also have smaller head size and vice versa. There are similar patterns in cognition, perhaps more pronounced for fathers, such that fathers with higher IQ have probands with higher IQ relative to other *DYRK1A* cases. Related to social responsiveness, higher parental scores (i.e., greater social impairment) correspond to probands with greater social impairment. Also note the apparent wider range of IQ variability for fathers ($SD = 14.99$) relative to mothers ($SD = 9.42$) and the wider range of head circumference variability for mothers ($SD = 1.81$) relative to fathers ($SD = 0.52$).

Figure 12. Contribution of familial genetic background to head circumference for *DYRK1A*, idiopathic, 16p11.2, and *CHD8* groups



Head circumference Z scores for UW-SNV *DYRK1A* cases are presented in red with their unaffected mothers and fathers, alongside the following gene comparison groups (from left to right): SSC simplex ASD, 16p11.2 deletions, and *CHD8* SNV. Variability in parental phenotype corresponds to proband variation across groups, including *DYRK1A* cases. Unaffected parents of *DYRK1A* cases present with head sizes predominantly in the average range, yet parents with relatively smaller head sizes correspond to more severely microcephalic probands and parents with relatively larger head sizes correspond to probands with relatively larger head sizes. When compared to each gene group, *DYRK1A* cases exhibit clear microcephaly compared to slight macrocephaly in idiopathic and 16p11.2 cases and more pronounced macrocephaly in *CHD8* cases.

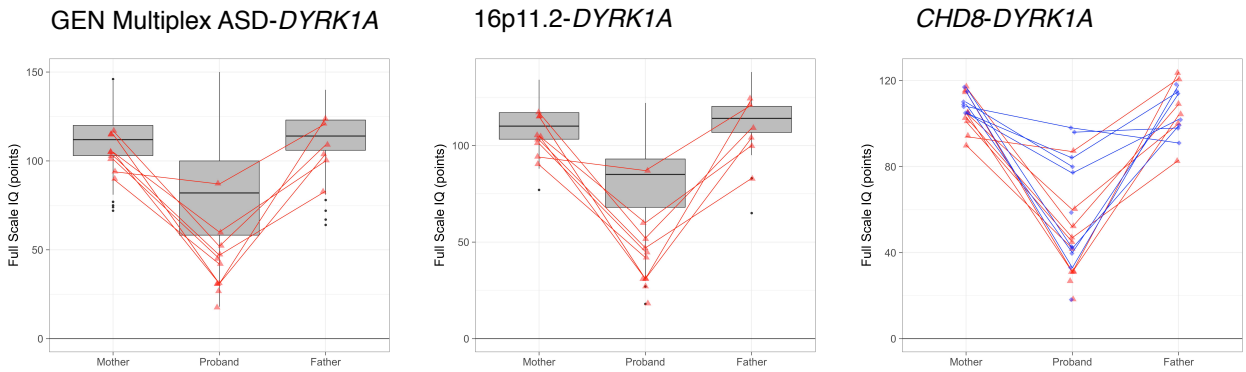
Figure 13 Title: Contribution of familial genetic background to ASD symptoms for *DYRK1A*, idiopathic, 16p11.2, and *CHD8* groups



ASD symptoms (SRS Raw Total Score) for UW-SNV *DYRK1A* cases are presented in red with their unaffected mothers and fathers, alongside the following gene comparison groups (from left

to right): SSC simplex ASD, 16p11.2 deletions, and *CHD8* SNVs. Variability in parental phenotype corresponds to proband variation, although less pronounced than for head circumference. When compared to each gene group, *DYRK1A* cases and their parents display similar patterns of ASD symptoms compared to idiopathic, 16p11.2, and *CHD8* groups. Severity of ASD symptoms look similar for probands across groups.

Figure 14. Contribution of familial genetic background to IQ for *DYRK1A*, idiopathic, 16p11.2, and *CHD8* groups



IQ (Full-scale IQ standard score) for UW-SNV *DYRK1A* cases are presented in red with their unaffected mothers and fathers, alongside the following gene comparison groups (from left to right): SSC multiplex ASD, 16p11.2 deletions, and *CHD8* SNVs. Variability in parental phenotype appears to correspond to proband variation in IQ, although this is less pronounced than for head circumference. Parental IQ fell predominantly in the average range across gene groups, including *DYRK1A*. Interestingly, more than half of the parents of *DYRK1A* cases displayed IQs in the lower average range 1 SD below that of idiopathic and 16p11.2 groups. When compared to each gene group, *DYRK1A* cases consistently lower IQ compared to multiplex and 16p11.2 cases. *CHD8* cases present with a wider range in IQ compared to *DYRK1A* cases.

Tables

TABLE 1. *DYRK1A* variant information for UW-SNV mutation patients

Patient	Position	Type of mutation	cDNA	Protein	Inheritance
1	21:38865466	splice site	c.1098+1G>A	-	*De novo*
2	21:38845116	frameshift	c.143_144delTA	p.Ile48Lysfs*2	*De novo*
3	21:38877833	frameshift	c.1491delC	p.Ala498Profs*61	*De novo*
4	21:38868533	frameshift	c.1217_1220delAGAA	p.Lys406Argfs*44	*De novo*
5	21:38862575	nonsense	c.763C>T	p.Arg255*	*De novo*
6	21:38877746	frameshift	c.1401delAinsGG	p.Ile468Aspfs*17	*De novo*
7	21:38853064	frameshift	c.452dupA	p.Asn151Lysfs*12	*Not maternal*
8	21:38862695	missense	c.883C>T	p.Leu295Phe	*De novo*
9	21:38862463	splice site	c.665-8_665-3delTCTTTC	-	*De novo*
10	21:38877590	frameshift	c.1248delA	p.Lys416Asnfs*35	*De novo*

Variant information for UW-SNV patients using NCBI reference sequence for *DYRK1A* isoform NM_101395.2, GRCh37 (hg19) build version (Ensembl id: ENST00000338785). This isoform was selected because it was the highest expressing isoform in human tissues in the GTEx database [https://gtexportal.org/home/gene/DYRK1A]) ("The Genotype-Tissue Expression (GTEx) project,"). Patients 1-3 were first identified through the Simons Simplex Collection, patients 4-10 underwent clinical genetic testing prior to research participation. cDNA and protein (NP_567824.1) annotation follows HGVS guidelines.

TABLE 2. Demographics

	***DYRK1A* Sample**			**SSC Idiopathic Sample**		***CHD8* Sample**
	disruptive SNVs		*CHR*	*No disruptive SNVs or deleterious CNVs*		*disruptive SNVs*
	Pub-SNV	UW-SNV	Pub-CHR	Total Sample	IQ < 70	
Total N (male)	31(22)	10(4)	19(9)	1,981 (1,705)	487 (407)	12(9)
Mean Age in months (SD)	124.12 (128.85)	108.40 (69.12)	102.22 (88.06)	107.66 (42.34)	114.00 (44.00)	148.08 (64.56)

SNV = single nucleotide variant, Pub-SNV = published disruptive SNV cases, UW-SNV = UW study cases with disruptive SNVs, Pub-CHR = published chromosomal rearrangement, CNV = copy number variant. Note that there are four individuals in the UW-SNV group that were previously published, three ascertained from the Simons Simplex Collection and one previously published in another report by Ji and colleagues (2015). DYRK1A sample significantly differed from Idiopathic ASD samples (total and IQ < 70) in gender ratio, χ^2 (1, n = 2041) = 66.88, $p < 0.001$ and χ^2 (1, n = 547) = 36.25, $p < 0.001$, respectively. Samples did not significantly differ in age, $p > 0.05$. No significant differences in age or gender for *DYRK1A* and *CHD8* samples.

TABLE 3. Phenotypic characteristics of *DYRK1A*

	Pub-SNV & UW-SNV (*n* = 41)			Pub-CHR (*n* = 19)			Total (*n* = 60)			
Phenotypic characteristic	**N**	**Total**	**%**	**N**	**Total**	**%**	**N**	**Total**	**%**	**Sig (Fisher's exact tests)**
Intellectual disability or Global Developmental Delay	40	41	98%	19	19	100%	59	60	98%	NS
Speech delay	41	41	100%	19	19	100%	60	60	100%	NS
Motor difficulties	37	37	100%	14	15	93%	51	52	98%	NS
Microcephaly	38	41	93%	19	19	100%	57	60	95%	NS
Feeding difficulties	36	39	92%	14	14	100%	50	53	94%	NS
Vision abnormalities	26	33	79%	8	9	89%	34	42	81%	NS
Seizures	26	39	67%	16	19	84%	42	58	72%	NS
ASD Diagnosis	16	35	46%	2	7	29%	18	42	43%	NS
Stereotyped behaviors	22	35	63%	4	9	44%	26	44	59%	NS
Anxious behaviors	11	35	31%	1	8	13%	12	43	28%	NS
Hyperactive behaviors	10	34	29%	4	8	50%	14	42	33%	NS
Behavioral differences	35	41	85%	7	19	37%	42	60	70%	NS
6+ symptoms including ASD	25	41	61%	7	19	37%	32	60	53%	NS
6+ symptoms including broader behavioral difficulties	32	41	78%	10	19	53%	42	60	70%	NS

Totals reflect those with complete data. Groups did not significantly differ in gender ratio (Fisher's exact test) or age (Independent sample *t* test), $p > 0.05$. Fisher's exact tests used for group comparisons, Sig = significance, NS = not significant.

TABLE 4. Phenotypic comparisons of *DYRK1A* to Idiopathic ASD

	Total *DYRK1A* Sample (*n* = 60)		*DYRK1A* ascertained for ASD (*n* = 18)		SSC Idiopathic ascertained for ASD (*n* = 1,981)		Sig (Total DYRK1A vs. Idio Total)	SSC Idiopathic ASD with IQ < 70 (*n* = 487)		Sig (Total DYRK1A vs. Idio IQ < 70)
Phenotypic characteristic	**N/Total**	**%**	**N/Total**	**%**	**N/Total**	**%**		**N**	**%**	
Intellectual Disability or Global Developmental Delay	59/60	98%	18/18	100%	487/1974	25%	**p<0.001**	487/487	100%	**--**
Speech delay	60/60	100%	18/18	100%	1173/1981	59%	**p<0.001**	343/487	70%	**p<0.001**
Motor difficulties	51/52	98%	18/18	100%	963/1981	49%	**p<0.001**	253/487	52%	**p<0.001**
Microcephaly	57/60	95%	16/18	89%	31/1958	2%	**p<0.001**	10/485	2%	**p<0.001**
Feeding difficulties	50/53	94%	16/18	89%	386/1981	19%	**p<0.001**	112/487	15%	**p<0.001**
Vision abnormalities	34/42	81%	16/18	67%	355/1981	18%	**p<0.001**	60/487	12%	**p<0.001**
5+ symptoms	47/60	78%	16/18	89%	6/1981	0.30%	**--**	5/487	1%	**--**

Totals reflect those with complete data. Fisher's exact tests used to compare Total *DYRK1A* sample to both idiopathic samples on each phenotypic characteristic; all group differences significant, $p < 0.001$. Sig = significance.

TABLE 5. Quantitative phenotype and group differences between *DYRK1A*, Idiopathic, and *CHD8* groups

	***DYRK1A* (UW-SNV)**			**SSC Idiopathic subset**			***CHD8***			***t* statistics *DYRK1A* vs SSC**		***t* statistics *DYRK1A* vs *CHD8***		***t* statistics *CHD8* vs** SSC	
Phenotypic characteristic	***Mean***	***SD***	***N***	***Mean***	***SD***	***N***	***Mean***	***SD***	***N***	***t***	***d***	***t***	***d***	***t***	***d***
Head circumference Z score	-3.62	2.17	10	1.21	0.39	10	1.76	2.9	11	6.94*	3.10	6.49**	2.84	NS	--
Full-scale IQ	45.30	18.14	10	81.20	26.12	10	60.91	27.39	11	3.57*	1.60	NS	--	NS	--
Overall Adaptive functioning	54.80	9.04	10	70.50	9.40	10	65.00	18.49	12	3.81*	1.70	NS	--	NS	--
Autism Severity (ADOS CSS)	6.50	2.80	10	7.70	1.25	10	8.18	1.78	11	NS	--	NS	--	NS	--
Age walked unaided	19.70	5.64	10	13.22	2.59	10	18.50	4.38	12	NS	--	NS	--	NS	--
Age of first single words	45.14	22.82	7	17.50	7.92	10	25.17	28.01	12	NS	--	NS	--	NS	--

Group comparisons using independent sample t tests between (1) *DYRK1A* and SSC groups, (2) *DYRK1A* and *CHD8* groups, and (3) *CHD8* and SSC groups. * = significant differences between *DYRK1A* and Idiopathic groups, $p < 0.002$; ** = significant differences between *DYRK1A* and *CHD8* groups, $p < 0.001$. Independent sample *t* and Cohen's *d* values provided when significant, *p* value adjusted for multiple comparisons, NS = not significant.

TABLE 6. Idiopathic Simplex ASD vs. DYRK1A Head Circumference, Maternal Contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Proband HC, Maternal HC contribution

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband Head Circumference	0.68	(1.35)	--				
Block 1 Predictors							
2. Std Proband Height	0.38	(1.14)	.34 ***	--			
Block 2 Predictors							
3. Group	0.00	(0.07)	-.24 ***	-.04 *	--		
4. Std Maternal Head circumference (accounting for maternal height)	0.00	(1.00)	.33 ***	.06 **	.01	--	
Block 3 Predictors							
5. Group*Mother Head Circumference	0.00	(0.07)	.04 *	.00	.11 ***	.07 *	--

Note. N=1903. Std = Standardized Z score, HC = Head circumference, Ht = Height

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband Head Circumference, Maternal contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.12 ***	0.12 ***	0.12			0.15 ***	0.26 ***	0.26			0.00 *	0.26 *	0.26		
Coefficients															
Intercept				0.53 ***					0.57 ***					0.57 ***	
Std Proband Ht				0.40 ***	0.12				0.37 ***	0.10				0.37 ***	0.10
Group									-4.81 ***	0.05				-4.92 ***	0.05
Std Maternal HC									0.41 ***	0.09				0.41 ***	0.09
Group*Maternal HC														0.80 *	0.00

Note. N=1903. Block 1 *F*-change test *df* = 1, 1901; Block 2 *df* = 2, 1899; Block 3 *df* = 1, 1898. Std = standardized by Z score, Ht = Height, HC = Head circumference, Maternal height controlled for in Maternal head circumference predictor

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 7. Idiopathic Simplex ASD vs. DYRK1A Head Circumference, Paternal Contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Proband HC, Paternal HC contribution

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband HC	0.69	(1.34)	--				
Block 1 Predictors							
2. Std Proband Ht	0.38	(1.14)	.35 ***	--			
Block 2 Predictors							
3. Group	0.00	(0.06)	-.19 ***	-.06 **	--		
4. Std Paternal HC (accounting for maternal height)	0.00	(1.00)	.31 ***	.05 *	.00	--	
Block 3 Predictors							
5. Group*Paternal HC	0.00	(0.03)	.07 **	-.01	.00	.03	--

Note. N=1877. Std = Standardized Z score, HC = Head circumference, Ht = Height

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband Head Circumference, Paternal Contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.12 ***	0.12 ***	0.12			0.11 ***	0.23 ***	0.23			0.00 **	-0.24 **	0.24		
Coefficients															
Intercept				0.54 ***					0.56 ***					0.56 ***	
Std Proband Ht				0.41 ***	0.06				0.38 ***	0.10				0.38 ***	0.11
Group									-4.05 ***	0.03				-4.05 ***	0.03
Std Paternal HC									0.39 ***	0.08				0.39 ***	0.08
Group*Paternal HC														2.58 **	0.00

Note. N=1877. Block 1 *F*-change test *df* = 1, 1875; Block 2 *df* = 2, 1873; Block 3 *df* = 1, 1872. Std = standardized by Z score, Ht = Height, HC = Head circumference, Paternal height controlled for in Paternal head circumference predictor

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 8. 16p11.2 deletion vs. DYRK1A Head Circumference, Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Proband HC, Maternal HC contribution

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband Head Circumference	0.71	(2.29)	--				
Block 1 Predictors							
2. Std Proband Height	-0.19	(1.53)	.40 ***	--			
Block 2 Predictors							
3. Group	0.11	(0.31)	-.76 ***	-0.01	--		
4. Std Maternal Head circumference (accounting for maternal height)	-0.09	(1.00)	.09	.12	.15	--	
Block 3 Predictors							
5. Group*Std Maternal Head Circumference	0.04	(0.38)	-.03	-.01	.28 **	.39 ***	--

Note. N=74. Std = standardized, HC = Head circumference

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband HC- Maternal HC contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.16 ***	0.16 ***	0.15			0.61 ***	0.77 ***	0.76			0.02 **	0.79 **	0.78		
Coefficients															
Intercept				0.83 **					1.48 ***					1.46 ***	
Std Proband Ht				0.60 ***	0.16				0.56 ***	0.14				0.57 ***	0.14
Group									-5.75 ***	0.60				-6.02 ***	0.62
Std Maternal HC									0.37 **	0.03				0.23	0.01
Group*Std Maternal HC														1.00 **	0.02

Note. N=74. Block 1 *F*-change test *df* = 1, 72; Block 2 *df* = 2, 70; Block 3 *df* = 1, 69. Ht = Height, HC = Head circumference, Maternal height controlled for in Maternal head circumference predictor

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 9. 16p11.2 deletion vs. DYRK1A Head Circumference, Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Proband HC, Paternal HC contribution

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband Head Circumference	0.88	(2.12)	--				
Block 1 Predictors							
2. Std Proband Height	-0.23	(1.55)	.48 ***	--			
Block 2 Predictors							
3. Group	0.10	(0.30)	-.72 ***	-.11	--		
4. Std Paternal Head circumference (accounting for paternal height)	-0.05	(0.97)	.26 *	.17	.07	--	
Block 3 Predictors							
5. Group*Std Paternal Head Circumference	0.02	(0.24)	.09	-.09	.21 *	.25 *	--

Note. N=63. Std = standardized, HC = Head circumference

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband HC- Paternal HC contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.23 ***	0.23 ***	0.22			0.51 ***	0.74 ***	0.73			0.05 **	0.79 **	0.77		
Coefficients															
Intercept				1.03 ***					1.50 ***					1.49 ***	
Std Proband Ht				0.66 ***	0.23				0.49 ***	0.13				0.53 ***	0.14
Group									-4.99 ***	0.48				-5.28 ***	0.51
Std Paternal HC									0.55 ***	0.06				0.42 **	0.03
Group*Std Paternal HC														1.97 **	0.04

Note. N=63. Block 1 *F*-change test *df* = 1, 61; Block 2 *df* = 2, 59; Block 3 *df* = 1, 58. Ht = Height, HC = Head circumference, Paternal height controlled for in Paternal head circumference predictor

TABLE 10. CHD8 vs. DYRK1A Head Circumference, Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Proband HC, Maternal HC contribution

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband Head Circumference	-1.36	(3.60)	--				
Block 1 Predictors							
2. Std Proband Height	1.01	(1.83)	.82 ***	--			
Block 2 Predictors							
3. Group	0.53	(0.52)	-.90 ***	-.75 **	--		
4. Std Maternal Head circumference (accounting for maternal height)	-0.02	(1.00)	.40	.07	-.22	--	
Block 3 Predictors							
5. Group*Std Maternal Head Circumference	-0.11	(0.93)	.33	-.01	-.12	.95 ***	--

Note. N=15. Std = standardized, HC = Head circumference

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband HC- Maternal HC contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.67 ***	0.67 ***	0.64			0.25 **	0.92 **	0.89			0.01	0.92	0.89		
Coefficients															
Intercept				-2.98 ***					-0.03					0.14	
Std Proband Ht				1.61 ***	0.67				0.75 **	0.06				0.75 *	0.06
Group									-3.88 **	0.13				-4.03 **	0.13
Std Maternal HC									0.93 *	0.06				0.09	0.00
Group*Std Maternal HC														0.93	0.01

Note. N=15. Block 1 *F*-change test *df* = 1, 13; Block 2 *df* = 2, 11; Block 3 *df* = 1, 10. Ht = Height, HC = Head circumference, Maternal height controlled for in Maternal head circumference predictor

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 11. CHD8 vs. DYRK1A Head Circumference, Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Proband HC, Paternal HC contribution

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband Head Circumference	-0.90	(3.36)	--				
Block 1 Predictors							
2. Std Proband Height	0.75	(2.04)	.81 ***	--			
Block 2 Predictors							
3. Group	0.46	(0.52)	-.83 ***	-.71 **	--		
4. Std Paternal Head circumference (accounting for paternal height)	-0.14	(0.83)	.32	.04	-.19	--	
Block 3 Predictors							
5. Group*Std Paternal Head Circumference	-0.14	(0.56)	.56 *	.12	-.29	.67 **	--

Note. N=13. Std = standardized, HC = Head circumference

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband HC- Paternal HC contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.65 **	0.65 **	0.62			0.18 *	0.83 *	0.77			0.09 *	0.92 *	0.88		
Coefficients															
Intercept				-1.19 *					-0.06					-0.13	
Standardized Proband Ht				1.33 **	0.65				0.80 *	0.11				0.83 **	0.12
Group									-2.83	0.09				-2.30 *	0.06
Standardized Paternal HC									0.91	0.05				-0.18	0.00
Group*Std Paternal HC														2.55 *	0.09

Note. N=13. Block 1 *F*-change test *df* = 1, 11; Block 2 *df* = 2, 9; Block 3 *df* = 1, 8. Ht = Height, HC = Head circumference, Paternal height controlled for in Paternal head circumference predictor

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 12. Idiopathic Simplex ASD vs. DYRK1A ASD Symptoms Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Maternal ASD Symptoms

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband SRS Raw Total	97.35	(26.90)	--				
Block 1 Predictors							
2. Std Proband IQ	0.00	(1.00)	-.24 ***	--			
Block 2 Predictors							
3. Group	0.00	(0.07)	.00	-.09 ***	--		
4. Std Maternal SRS Raw Total	0.01	(0.99)	.08 ***	.02	-.01	--	
Block 3 Predictors							
5. Group*Std Maternal SRS Raw Total	0.00	(0.06)	.04	.01	-.10 ***	.06 **	--

Note. N=1848. Std = standardized, SRS = Social responsiveness scale

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband ASD symptoms - Maternal ASD symptom contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.06 ***	0.06 ***	0.06			0.01 ***	0.07 ***	0.07			0.00	0.07	0.07		
Coefficients															
Intercept				97.35 ***					97.37 ***					97.37 ***	
Std Proband IQ				-6.63 ***	0.06				-6.63 ***	0.06				-6.63 ***	0.06
Group									-9.33	0.00				-8.07	0.00
Std Maternal SRS Raw Total									2.37 ***	0.01				2.31 ***	0.01
Group*Std Maternal SRS														14.20	0.00

Note. N=1848. Block 1 *F*-change test *df* = 1, 1846; Block 2 *df* = 2, 1844; Block 3 *df* = 1, 1843. Std = Standardized Z Score, SRS = Social Responsiveness Scale

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 13. Idiopathic Simplex ASD vs. DYRK1A ASD Symptoms Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Paternal ASD Symptoms

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband SRS Raw Total	97.46	(26.91)	--				
Block 1 Predictors							
2. Std Proband IQ	0.00	(1.00)	-.24 ***	--			
Block 2 Predictors							
3. Group	0.00	(0.06)	.00	-.08 ***	--		
4. Std Maternal SRS Raw Total	0.01	(1.00)	.11 ***	.06 **	-.02	--	
Block 3 Predictors							
5. Group*Std Maternal SRS Raw Total	0.00	(0.03)	-.02	.06 **	-.58 ***	.04	--

Note. N=1838. Std = standardized, SRS = Social responsiveness scale

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband ASD symptoms - Paternal ASD symptom contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.06 ***	0.06 ***	0.06			0.02 ***	0.07 ***	0.07			0.00	0.07	0.07		
Coefficients															
Intercept				97.46 ***					97.45 ***					97.45 ***	
Std Proband IQ				-6.50 ***	0.06				-6.72 ***	0.06				-6.72 ***	0.06
Group									-5.89	0.00				-12.18	0.00
Std Paternal SRS Raw Total									3.27 ***	0.01				3.29 ***	0.01
Group*Std Paternal SRS														-19.32	0.00

Note. N=58. Block 1 *F*-change test *df* = 1, 1836; Block 2 *df* = 2, 1834; Block 3 *df* = 1, 1833. Std = Standardized Z Score, SRS = Social Responsiveness Scale

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 14. 16p11.2 deletion vs. DYRK1A ASD Symptoms, Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Maternal ASD Symptoms

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband SRS Raw Total	79.91	(35.42)	--				
Block 1 Predictors							
2. Std Proband IQ	0.09	(0.88)	-.34 **	--			
Block 2 Predictors							
3. Group	0.14	(0.35)	.19	-.64 ***	--		
4. Std Maternal SRS Raw Total	0.00	(0.99)	.23 *	.08	.00	--	
Block 3 Predictors							
5. Group*Std Maternal SRS Raw Total	0.00	(0.42)	.16	.02	.00	.42 ***	--

Note. N=63. Std = standardized, SRS = Social responsiveness scale

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband ASD symptoms - Maternal ASD symptom contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.12 **	0.12 **	0.10			0.07	0.18	0.14			0.00	0.19	0.13		
Coefficients															
Intercept				81.08 ***					82.37 ***					82.33 ***	
Std Proband IQ				-13.66 **	0.11				-16.36 *	0.10				-16.26 *	0.09
Group									-7.10	0.00				-6.91	0.00
Std Maternal SRS Raw Total									9.34 *	0.07				8.37	0.04
Group*Std Maternal SRS														5.48	0.00

Note. N=63. Block 1 *F*-change test *df* = 1, 61; Block 2 *df* = 2, 59; Block 3 *df* = 1, 58. Std = Standardized, SRS = Social Responsiveness Scale

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 15. 16p11.2 deletion vs. DYRK1A ASD Symptoms, Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Paternal ASD Symptoms

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband SRS Raw Total	81.26	(35.88)	--				
Block 1 Predictors							
2. Std Proband IQ	0.14	(0.87)	-.35 **	--			
Block 2 Predictors							
3. Group	0.12	(0.33)	.18	-.60 ***	--		
4. Std Paternal SRS Raw Total	0.04	(1.04)	.24 *	-.03	-.14	--	
Block 3 Predictors							
5. Group*Std Paternal SRS Raw Total	-0.04	(0.19)	-.18	.46 ***	-.62 ***	.20	--

Note. N=58. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband ASD symptoms - Paternal ASD symptom contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.12 **	0.12 **	0.11			0.05	0.18	0.13			0.01	0.18	0.12		
Coefficients															
Intercept				83.28 ***					82.62 ***					82.33 ***	
Std Proband IQ				-14.47 ***	0.12				-13.70 *	0.07				-12.95	0.06
Group									2.04	0.00				-4.07	0.00
Std Paternal SRS Raw Total									8.08	0.05				8.58	0.06
Group*Std Paternal SRS														-21.39	0.01

Note. N=58. Block 1 *F*-change test *df* = 1, 56; Block 2 *df* = 2, 54; Block 3 *df* = 1, 53. Std = Standardized, SRS = Social Responsiveness Scale

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 16. CHD8 vs. DYRK1A ASD Symptoms, Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Maternal ASD Symptoms

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband SRS Raw Total	101.18	(29.98)	--				
Block 1 Predictors							
2. Std Proband IQ	-1.05	(1.03)	-.39	--			
Block 2 Predictors							
3. Group	0.53	(0.51)	-.18	-.25	--		
4. Std Maternal SRS Raw Total	0.16	(1.16)	.36	.31	-.16	--	
Block 3 Predictors							
5. Group*Std Maternal SRS Raw Total	0.00	(0.82)	.37	.02	.00	.71 *	--

Note. N=17. Std = standardized, SRS = Social responsiveness scale

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband ASD symptoms - Maternal ASD symptom contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.15	0.15	0.10			0.31	0.47	0.34			0.00	0.47	0.29		
Coefficients															
Intercept				89.12 ***					88.03 ***					88.63 ***	
Std Proband IQ				-11.45	0.15				-17.82 *	0.32				-17.52 *	0.29
Group									-14.63	0.06				-14.83	0.06
Std Maternal SRS Raw Total									13.03 *	0.23				12.00	0.09
Group*Std Maternal SRS														1.88	0.00

Note. N=17. Block 1 *F*-change test *df* = 1,15; Block 2 *df* = 2,13; Block 3 *df* = 1,12. Std = Standardized, SRS = Social Responsiveness Scale

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 17. CHD8 vs. DYRK1A ASD Symptoms, Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Paternal ASD Symptoms

Measure	*M*	*(SD)*	1.	2.	3.	4.	5.
Outcomes							
1. Std Proband SRS Raw Total	105.07	(31.56)	--				
Block 1 Predictors							
2. Std Proband IQ	-0.95	(1.15)	-.32	--			
Block 2 Predictors							
3. Group	0.47	(0.52)	-.19	-.24	--		
4. Std Paternal SRS Raw Total	-0.14	(0.66)	.00	.52 *	-.31	--	
Block 3 Predictors							
5. Group*Std Paternal SRS Raw Total	-0.16	(0.35)	-.07	.28	-.53 *	.54 *	--

Note. N=15. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband ASD symptoms - Paternal ASD symptom contribution

	Block 1					Block 2					Block 3				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.10	0.10	0.03			0.09	0.20	-0.03			0.05	0.24	-0.06		
Coefficients															
Intercept				96.75 ***					101.29 ***					100.76 ***	
Std Proband IQ				-8.73	0.10				-12.68	0.15				-13.05	0.16
Group									-15.54	0.06				-22.60	0.10
Std Maternal SRS Raw Total									7.54	0.02				13.55	0.05
Group*Std Maternal SRS														-26.29	0.05

Note. N=15. Block 1 *F*-change test *df* = 1,13; Block 2 *df* = 2,11; Block 3 *df* = 1,10. Std = Standardized, SRS = Social Responsiveness Scale

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 18. Multiplex ASD vs. DYRK1A IQ Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Maternal IQ

Measure	*M*	*(SD)*	1.	2.	3.	4.
Outcomes						
1. Proband IQ	81.25	(26.57)	--			
Block 1 Predictors						
2. Group	0.03	(0.18)	-.24 ***	--		
3. Std Maternal IQ	-0.01	(0.99)	.31 ***	-.09	--	
Block 2 Predictors						
4. Group*Std Maternal IQ	-0.02	(0.15)	.10	-.59 ***	.15 **	--

Note. N = 272. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband IQ - Maternal IQ contribution

	Block 1					Block 2				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.14 ***	0.14 ***	0.14			0.01	0.15	0.14		
Coefficients										
Intercept				82.33 ***					82.33 ***	
Group				-31.11 ***	0.04				-40.74 ***	0.05
Std Maternal IQ				7.86 ***	0.09				8.16 ***	0.09
Group*Std Maternal IQ									-19.76	0.01

Note. N=272. Block 1 F-change test df = 2, 269; Block 2 df = 1, 268. Std = Standardized

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 19. Multiplex ASD vs. DYRK1A IQ Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Paternal IQ

Measure	*M*	*(SD)*	1.	2.	3.	4.
Outcomes						
1. Proband IQ	82.06	(26.14)	--			
Block 1 Predictors						
2. Group	0.03	(0.16)	-.20 **	--		
3. Std Paternal IQ	0.03	(1.00)	.25 ***	-.07	--	
Block 2 Predictors						
4. Group*Std Paternal IQ	-0.01	(0.18)	.09	-.38 ***	.18 **	--

Note. N = 218. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband IQ - Paternal IQ contribution

	Block 1					Block 2				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.10 ***	0.10 ***	0.09			0.00	0.10	0.08		
Coefficients										
Intercept				82.66 ***					82.66 ***	
Group				-28.76 **	0.03				-30.01 **	0.03
Std Paternal IQ				6.21 ***	0.06				6.30 ***	0.06
Group*Std Paternal IQ									-3.12	0.00

Note. N=218. Block 1 *F*-change test *df* =2, 215; Block 2 *df* =1, 214. Std = Standardized

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 20. 16p11.2 deletion vs. DYRK1A IQ, Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Maternal IQ

Measure	*M*	*(SD)*	1.	2.	3.	4.
Outcomes						
1. Proband IQ	80.33	(20.03)	--			
Block 1 Predictors						
2. Group	0.12	(0.32)	-.60 ***	--		
3. Std Maternal IQ	0.00	(1.04)	.11	-.17	--	
Block 2 Predictors						
4. Group*Std Maternal IQ	-0.06	(0.32)	.17	-.48 ***	.32 **	--

Note. N=78. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband IQ - Maternal IQ contribution

	Block 1					Block 2				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.36 ***	0.36 ***	0.34			0.02	0.38	0.35		
Coefficients										
Intercept				84.63 ***					84.58 ***	
Group				-37.26 ***	0.35				-41.76 ***	0.34
Std Maternal IQ				0.09	0.00				0.86	0.00
Group*Std Maternal IQ									-10.27	0.02

Note. N=63. Block 1 *F*-change test *df* = 2, 75; Block 2 *df* = 1, 74. Std = Standardized

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 21. 16p11.2 deletion vs. DYRK1A IQ, Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Paternal IQ

Measure	*M*	*(SD)*	1.	2.	3.	4.
Outcomes						
1. Proband IQ	82.25	(18.54)	--			
Block 1 Predictors						
2. Group	0.09	(0.29)	-.54 ***	--		
3. Std Paternal IQ	0.06	(1.01)	.28 *	-.15	--	
Block 2 Predictors						
4. Group*Std Paternal IQ	-0.04	(0.35)	.23 *	-.34 **	.36 **	--

Note. N=64. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband IQ - Paternal IQ contribution

	Block 1					Block 2				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.33 ***	0.33 ***	0.31			0.00	0.33	0.30		
Coefficients										
Intercept				85.06 ***					85.05 ***	
Group				-32.25 ***	0.26				-32.52 ***	0.24
Std Paternal IQ				3.69	0.04				3.77	0.04
Group*Std Paternal IQ									-0.79	0.00

Note. N=64. Block 1 *F*-change test *df* =21,61; Block 2 *df* =1,60. Std = Standardized

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 22. CHD8 vs. DYRK1A IQ, Maternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Maternal IQ

Measure	*M*	*(SD)*	1.	2.	3.	4.
Outcomes						
1. Proband IQ	55.00	(22.80)	--			
Block 1 Predictors						
2. Group	1.44	(0.51)	.39	--		
3. Std Maternal IQ	-0.34	(0.59)	-.30	.32	--	
Block 2 Predictors						
4. Group*Std Maternal IQ	-0.39	(0.69)	-.46 *	.17	.95 ***	--

Note. N= 16. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband IQ - Maternal IQ contribution

	Block 1					Block 2				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.35	0.35	0.25			0.14	0.49	0.36		
Coefficients										
Intercept				14.32					16.85	
Group				24.09 *	0.26				16.26	0.10
Std Maternal IQ				-18.05	0.20				30.37	0.05
Group*Std Maternal IQ									-41.97	0.14

Note. N=16. Block 1 *F*-change test *df* = 2,13; Block 2 *df* = 1,12. Std = Standardized

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 23. CHD8 vs. DYRK1A IQ, Paternal contribution, (A) Correlations, (B) Regression analysis

A. Correlation Table for Linear Regression using Sequential Predictor Entry - Paternal IQ

Measure	*M*	*(SD)*	1.	2.	3.	4.
Outcomes						
1. Proband IQ	59.85	(25.33)	--			
Block 1 Predictors						
2. Group	1.54	(0.52)	.32	--		
3. Std Paternal IQ	-0.47	(0.88)	-.23	-.07	--	
Block 2 Predictors						
4. Group*Std Paternal IQ	-0.76	(1.32)	-.41	-.25	.93 ***	--

Note. N=13. Std = standardized

* $p < .05$, ** $p < .01$, *** $p < .001$.

B. Multiple Linear Regression with Sequential Predictor Entry for Proband IQ - Paternal IQ contribution

	Block 1					Block 2				
	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2	R^2_{change}	R^2_{total}	R^2_{adj}	*b*	sr^2
Model Fit	0.15	0.15	-0.02			0.18	0.33	0.11		
Coefficients										
Intercept				33.17					49.82	
Group				15.13	0.10				2.82	0.00
Std Paternal IQ				-6.04	0.04				28.57	0.11
Group*Std Paternal IQ									-25.39	0.18

Note. N=13. Block 1 *F*-change test *df* =2,10; Block 2 *df* =1,9. Std = Standardized

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 24. Phenotype descriptives of probands and unaffected parents

	Head Circumference (Z score)				ASD Symptoms (SRS Raw Total)				Full-Scale IQ			
	Proband		Biparental Average		Proband		Biparental Average		Proband		Biparental Average	
Group	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
DYRK1A	-3.62	2.17	1.13	1.62	97.20	34.53	23.61	13.34	45.30	27.39	105.11	9.82
Idiopathic ASD	0.67	1.32	0.92	0.98	97.55	26.78	29.92	17.44	82.12	26.06	111.86	11.92
CHD8	1.76	1.62	1.68	1.03	110.18	19.13	32.39	17.50	60.91	18.14	107.13	6.31
16p11.2 del	1.34	1.43	1.37	1.14	77.00	34.80	29.22	17.84	85.10	6.00	111.68	10.23

DYRK1A group includes UW-SNV (n = 10), Idiopathic ASD includes participants from the Simons Simplex Collection (SSC, n = 1,981), CHD8 group (n = 12), 16p11.2 deletion (n = 56).

TABLE 25. Parent-child effect sizes as measure of quantifiable gene status impact

	Effect sizes (SD) by phenotypic features		
Group	**Head Circumference**	**ASD symptoms**	**Full-Scale IQ**
DYRK1A	-2.93	5.52	-6.09
Idiopathic ASD	-0.25	3.88	-2.50
CHD8	0.09	4.45	-7.32
16p11.2 deletion	-0.03	2.68	-2.60

Effect size = proband mean – biparental mean/ biparental standard deviation. DYRK1A group includes UW-SNV (n = 10), Idiopathic ASD includes participants from the Simons Simplex Collection (SSC, n = 1,981), CHD8 group (n = 12), 16p11.2 deletion (n = 56).

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Appendix

Aim 1: To summarize the genetic mechanism of *DYRK1A*, including functional models of *DYRK1A*. Summarize known disruptive mutations associated with genetic changes, protein disruption and the resulting phenotype of DYRK1A.

Literature review of functional model research on DYRK1A and genetic record review of known cases with *DYRK1A* mutations (published cases and TIGER study participants, n = 61).

Aim 2a: To summarize gender, age and phenotypic characteristics of known cases of *DYRK1A* LGD mutations (SNVs, splice-site, and frameshift mutations). Additionally, to summarize characteristics of 19 individuals with chromosomal rearrangements to *DYRK1A* (translocations and microdeletions) and compare phenotypic features to those seen in the LGD mutation cohort.

Groups	Variables	Statistical Analysis
1. *DYRK1A* LGD group (published and TIGER participants, n = 42) 2. *DYRK1A* Chromosomal rearrangement group (published, n = 19)	Frequency of: 1. Intellectual disability or Developmental Delay 2. Speech delay (first words after 24 months of age) 3. Motor deficits (delayed walking, poor coordination, abnormal gait) 4. ASD-related deficits (e.g. ASD diagnosis, stereotypic behaviors, anxious behaviors) 5. Feeding difficulties 6. Vision abnormalities 7. Microcephalic head size (<-2 SD) 8. Presentation of all 6 or more symptoms	Fisher's exact tests

Aim 2b: To compare incidence of commonly observed phenotypic features in cases of *DYRK1A* (included if present in 75% or more of cases) to the incidence of those features in a large sample of individuals ascertained for ASD.

Groups	Variables	Statistical Analysis
1. *DYRK1A* combined group (n = 61) 2. SSC idiopathic group (n = 1,981)	Frequency of: 1. Intellectual disability or Developmental Delay 2. Microcephalic head size (<-2 SD)	Fisher's exact tests

	3. Speech delay (first words after 24 months of age) 4. Motor deficits (delayed walking, poor coordination, abnormal gait) 5. Feeding difficulties 6. Vision abnormalities 7. Presentation of all 5 or more symptoms	
Aim 2c: To examine dimensionally assessed domains of functioning in a subset of individuals with *DYRK1A* (n = 10) and compare common phenotypic features observed in *DYRK1A* to the same phenotypic domains observed in a larger sample of individuals with ASD and a cohort of individuals with another disruptive genetic event associated with ASD, *CHD8*.		
Groups	Variables	Statistical Analysis
1. *DYRK1A* UW-SNV group (n = 10) 2. SSC group (age-matched subset) 3. *CHD8* SNV group (n = 12)	1. IQ (full-scale, DAS-II) 2. Adaptive functioning (VABS Composite Score) 3. ADOS calibrated severity score 4. Age of first words 5. Age of first independent steps 6. Head circumference	Independent-sample t-tests (*DYRK1A* vs. SSC, *DYRK1A* vs. *CHD8*, SSC vs. *CHD8*)
Aim 3a: To situate phenotype in the context of familial background and calculate the discrepancy between affected child (proband) and unaffected parent. To explore the relationship of parental phenotype and gene status with child phenotype.		
Groups	Variables	Statistical Analysis
1. *DYRK1A* UW-SNV group (n = 10) 2. SSC idiopathic group (n = 1,981)	1. Social responsiveness (SRS Total Score) 2. Head circumference	Regression analysis to explore potential relationships between groups (e.g. ProbandSRS = ParentSRS + Group+ ParentSRS*Group)

1. *DYRK1A* UW-SNV group (n = 10) 2. GEN group (n = 250)	1. IQ (full-scale, DAS-II and Wechsler scales)	Regression analysis to explore potential relationships between groups (e.g. ProbandIQ = ParentIQ + Group+ ParentIQ*Group)
1. *DYRK1A* UW-SNV group (n = 10) 2. *CHD8* SNV group (n = 12)	1. IQ (full-scale, DAS-II and Wechsler scales) 2. Social responsiveness (SRS Total Score) 3. Head circumference	Regression analysis to explore potential relationships between groups (e.g. ProbandIQ = ParentIQ + Group+ ParentIQ*Group)
1. *DYRK1A* UW-SNV group (n = 10) 2. 16p11.2 CNV group (n = 56)	1. IQ (full-scale, DAS-II and Wechsler scales) 2. Social responsiveness (SRS Total Score) 3. Head circumference	Regression analysis to explore potential relationships between groups (e.g. ProbandIQ = ParentIQ + Group+ ParentIQ*Group)
Aim 3b: An effect size for core phenotypic features will be calculated for subset of individuals with DYRK1A (n = 10) and compared to a large sample of children with ASD. Additionally, to calculate effect sizes of other ASD-associated mutations, *CHD8* and 16p11.2 deletions and compare to *DYRK1A*.		
Groups	Variables	Statistical Analysis
1. *DYRK1A* UW-SNV group (n = 10) 2. SSC Idiopathic group (n = 1,981) 3. 16p11.2 CNV group (n = 56) 4. *CHD8* SNV group (n = 12)	1. IQ (full-scale, DAS-II and Wechsler scales) 2. Social responsiveness (SRS Total Score) 3. Head circumference	Calculate parent-proband effect size $\Delta = \frac{proband\ mean - unaffected\ parent\ mean}{unaffected\ parent\ standard\ deviation}$