Developmental Predictors of Cognitive and Adaptive Outcomes in Genetic Subtypes of Autism Spectrum Disorder

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Approximately one-fourth of autism spectrum disorder (ASD) cases are associated with a disruptive genetic variant. Many of these ASD genotypes have been described previously, and are characterized by unique constellations of medical, psychiatric, developmental, and behavioral features. Development of precision medicine care for affected individuals has been challenging due to the phenotypic heterogeneity that exists even within each genetic subtype. In the present study, we identify developmental milestones that predict cognitive and adaptive outcomes for five of the most common ASD genotypes. Sixty-five youth with a known pathogenic variant involving *ADNP, CHD8, DYRK1A, GRIN2B*, or *SCN2A genes* participated in cognitive and adaptive testing. Exploratory linear regressions were used to identify developmental milestones that predicted cognitive and adaptive outcomes within each gene group. We hypothesized that the earliest and most predictive milestones would vary across gene groups, but would be consistent across outcomes within each genetic subtype. Within the *ADNP* group, age of walking predicted cognitive outcomes, while age of first words predicted adaptive behaviors. Age of phrases predicted adaptive functioning in the *CHD8* group, but cognitive outcomes were not clearly associated with early developmental milestones. Verbal milestones were the strongest predictors of cognitive and adaptive outcomes for individuals with mutations to *DYRK1A, GRIN2B*, or *SCN2A*. These trends inform decisions about treatment planning and long-term expectations for affected individuals, and they add to the growing body of research linking molecular genetic function to brain development and phenotypic outcomes. ***Autism Res*** *2020, 13: 1659–1669.*

Lay Summary: Researchers have found many genetic causes of autism including mutations to *ADNP*, *CHD8*, *DYRK1A*, *GRIN2B*, and *SCN2A* genes. We found that each genetic cause had different early developmental milestones that explained the overall functioning of the children when they were older. Depending on the genetic cause, the age that a child first starts walking and/or talking may help to better understand and support a child's development who has a mutation to one of the above genes.

Keywords: subtypes of ASD; genetics; genetic/genomic syndromes; intellectual disability; developmental psychology

Introduction

The etiology of autism spectrum disorder (ASD) is highly heterogeneous; in the majority of affected individuals, ASD arises from multiple, additive, and interactive effects of common genetic variants [Bai et al., 2019]. However, it has been estimated that in up to 25% of cases, the etiology may be attributed to a *de novo* copy number variation or single-nucleotide variant [Iossifov et al., 2014]. Although not all individuals with mutations to these genes develop the full ASD phenotype, most show at least some symptoms of atypical development, including delayed developmental milestones, atypical social communication, motor stereotypies, craniofacial abnormalities, seizures, and intellectual disability (ID) [Arnett et al., 2018; Ben-Shalom et al., 2017; Bernier et al., 2014]. As awareness of genetic subtypes of ASD has expanded, families of individuals with disruptions to the same gene have connected in person and *via* social media to provide social support, raise research funds, and share information about developmental trajectories and treatment options. Simultaneously, large- and small-scale research efforts, such as Simons Searchlight (https://www.simonssearchlight.org/) and our own The Investigation of Genetic Exome Research (TIGER) Study at the University of Washington, are amassing comprehensive phenotypic information about genetic disruptions associated with ASD. In the current study, we present the first report

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on very early predictors of cognitive and adaptive outcomes in five of the most common ASD-linked gene disruptions.

Genetic subtypes of ASD are associated with unique behavioral, medical, and cognitive phenotypes [Arnett, Trinh, & Bernier, 2019]. Mutations in *CHD8* are among the most commonly identified, and include relatively high rates of ASD, macrocephaly, and gastrointestinal slowing [Sugathan et al., 2014]. Cognitive and adaptive outcomes among individuals with a *CHD8* mutation are highly variable, with some individuals showing minimal impairment and others meeting criteria for ID [Beighley et al., 2020]. In contrast, individuals with a disruption to *ADNP* show a more consistent presentation that includes ID, ASD symptoms, minimal language development, hypotonia, and early tooth eruption [Gozes et al., 2017; Van Dijck et al., 2019]. Among individuals with *ADNP* syndrome, verbal intelligence is strongly correlated with social skills development, suggesting *ADNP* expression is broadly critical to neurodevelopment [Arnett et al., 2018]. Mutations in *SCN2A* contribute to distinct phenotypes according to their function; loss-of-function mutations are associated with ASD and/or ID as well as significant motor and verbal delays, while gain-of-function missense mutations predict either infantile epileptic encephalopathy with severe developmental delay or benign infantile seizures with minimal long-term neuropsychiatric consequences [Sanders et al., 2018]. Individuals with mutations in *GRIN2B* or *DYRK1A* present with ID in nearly all reported cases [Earl et al., 2017; Platzer & Lemke, 2018] and ASD in a significant minority (~25 and ~40%, respectively) [Platzer et al., 2017; Earl et al., 2017]. Mutations to *GRIN2B* are also associated with malformations of cortical development, which may reflect disrupted neuronal migration, while mutations in *DYRK1A* typically result in microcephaly, speech delay, and motor difficulties as well as a characteristic dysmorphology [Earl et al., 2017; Platzer & Lemke, 2018]. Thus, although cognitive and adaptive delays are common among individuals with mutations to ASD-associated genes, the profiles, correlates, and severity of adaptive and cognitive deficits vary widely across specific genetic subtypes.

A plethora of longitudinal studies have reported early predictors of cognitive and adaptive outcomes among children with ASD. Toth, Munson, Meltzoff, and Dawson [2006] found that play skills and delayed imitation in preschoolers with ASD predicted adaptive communication at school age. Gillespie-Lynch et al. [2012] reported that language ability in early childhood predicted adaptive functioning in young adults with ASD. Among children with ASD and language delay, fine motor skills appear to be the best predictor of later language development [Bal et al., 2020]. For parents and clinicians, knowledge about how early development predicts later functioning promotes proactive, targeted interventions, as well as opportunities for psychoeducation and social emotional support [Bryson, Rogers, & Fombonne, 2003; Warren et al., 2011]. Unfortunately, it is unclear to what extent longitudinal studies of the general ASD population apply to children with ASD-linked genetic mutations. Compared to children with ASD without a known cause, children with *de novo* genetic mutations have greater delays in motor development, stronger social abilities, and less discrepancy between verbal and nonverbal intelligence [Bishop et al., 2017]. However, given the variability in phenotypic outcomes across genotypes within the *de novo* mutation population, it is clinically relevant to identify predictors of developmental outcomes within genetic subtypes of ASD. We expect that the exploratory analyses conducted in this study will provide preliminary evidence on which to base future work, including development of guidelines for clinicians and families seeking to support and better manage affected individuals across the lifespan.

Methods

Procedures

One hundred seventy individuals aged 2–51 years old with a known disruptive mutation in a gene that has been previously associated with ASD [O'Roak et al., 2012, 2014] have participated in the ongoing TIGER Study at the University of Washington. This represents one of the largest collections of autism patients with different genetic etiologies subjected to standardized phenotyping. Enrollment exclusion criteria included diagnosis of a common syndromic disorder associated with ASD (e.g., Fragile X). For in person visits, data collection included standardized testing and took place in the laboratory or in the participant's home and via telephone interview with clinicians naïve to genetic diagnosis; remote participation consisted of telephone interview and sharing of medical records. Parents participated in diagnostic interviews with research-reliable clinicians, including the Autism Diagnostic Interview Revised (ADI-R) [Lord, Rutter, & Le Couteur, 1994], the Vineland Adaptive Behavior Scales, Second or Third Edition [Sparrow, Cicchetti, & Balla, 2005; Sparrow, Cicchetti, & Saulnier, 2016] and a family and medical history. Licensed psychologists determined psychiatric diagnoses (e.g., ASD, ID) using criteria from the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) following completion of data collection [American Psychological Association, 2013]. Parents provided written consent. Participants over age 13 provided written consent if developmentally appropriate, and younger participants and those unable to consent provided verbal or written assent, as appropriate. All procedures were

approved by and in compliance with the University of Washington Institutional Review Board.

Participants

Participants for the current study were selected from five gene groups for which full data were available on at least nine individuals between the ages of 5–21 years. The age range was selected to maximize the possibility that language and motor milestones would have been obtained by the low age cutoff and that parents would be accurate reporters of milestones at the high cutoff. Full data were defined as all predictor variables and at least one set of outcome variables (i.e., cognitive or adaptive). Cognitive variables and ASD or ID diagnosis were not obtained for individuals who participated remotely. One participant with a *DYRK1A* mutation did not complete the adaptive behavior interview due to difficulties with scheduling. One other participant with a *DYRK1A* mutation was interviewed with Version 3 of the Vineland Adaptive Behavior Scales; this individual's adaptive behavior outcomes were not included in the current analyses due to poor reliability across the second and third versions [Farmer, Adedipe, Bal, Chlebowski, & Thurm, 2020]. The final sample included the following gene groups: *ADNP* (n = 13), *CHD8* (n = 14), *DYRK1A* (n = 19), *GRIN2B* (n = 9), and *SCN2A* (n = 10). Participant demographics are described in Table 1.

Genotyping

For all participants, presence of a disruptive variant was confirmed through review of the clinical genetic testing lab report or through targeted or exome sequencing conducted as part of a previous study [Stessman et al., 2017]. Gene and variant information for participants are listed in Table S1. Most genetic mutations were found to be *de novo* (n = 53); however, two were inherited and ten were of unknown inheritance because one or both parents did not complete genetic testing. All inherited variants and variants of unknown inheritance were confirmed as disruptive by at least one publicly available pathogenic scoring metric (see Table S1 and footnote). The phenotypes of the inherited cases are elaborated in the Discussion section.

Behavioral Measures

Milestone attainment. Caregivers reported age of independent walking, age of first meaningful single words, and age of first meaningful phrases (defined as phrases of at least two words containing a verb) during the ADI-R, which was administered to primary caregivers by research reliable clinicians. Individuals who had not yet attained a milestone by the time of research participation (see Table 1) were assigned a value equivalent to one standard deviation above their age, to clearly place the milestone in the delayed range without introducing non-uniformity in the data. For these calculations, standard deviations

Table 1. Participant Demographics

	ADNP	*CHD8*	*DYRK1A*	*GRIN2B*	*SCN2A*
n	13	14	19	9	10
	115	145	143	114	143
Age at testing in months	(63–206)	(65–260)	(60–262)	(63–186)	(61–253)
Female (*n*/%)	7/53%	5/36%	6/32%	5/56%)	6/60%
Remote participation (*n*/%)	1/8%	1/7%	0/0%	2/22%	2/20%
ID diagnosis (*n*/%)	12/100%	7/54%	16/84%	7/100%	7/88%
ASD diagnosis (*n*/%)	7/58%	13/100%	16/84%	4/57%	5/63%
	36.33	53.38	42.16	40.43	18.13
Nonverbal ratio IQ	(20–49)	(13–100)	(12–115)	(16–56)	(7–39)
	36.08	56.18	38.47	41.00	21.38
Verbal ratio IQ	(17–51)	(15–100)	(4–120)	(16–56)	(6–72)
	30.69	61.21	52.06	55.44	39.20
Vineland adaptive behavior composite	(20–48)	(24–83)	(20–69)	(29–68)	(25–62)
Spoke first words prior to evaluation (*n*/%)	10/77%	14/100%	12/63%	7/78%	4/40%
	46.20	24.57	44.27	39.86	28/75
Age first words	(22–78)	(7–108)	(11–120)	(27–60)	(12–54)
Walked independently prior to evaluation (*n*/%)	13/100%	14/100%	17/94%	7/78%	8/80%
	30.69	17.43	22.94	23.29	17.50
Age walking	(20–48)	(12–26)	(12–54)	(16–33)	(12–30)
Spoke in phrases prior to evaluation (*n*/%)	6/46%	12/86%	9/47%	6/67%	2/20%
	71.50	46.25	57.67	50.00	26.50
Age first phrases	(44–108)	(12–150)	(36–120)	(30–72)	(23–30)

Note. Milestone age means and standard deviations derive from individuals who achieved the milestone prior to the evaluation. Values in parentheses are ranges.

derived from the individual's own gene group. Because of the small sample sizes that result from the rarity of these genetic events, we acknowledge that our analyses are underpowered. Thus, we report *P* values in the results but will focus on effect sizes (i.e., regression coefficients) and consistency of results across the multiple outcomes in our interpretation.

Outcome variables. The Differential Abilities Scale, Second Edition (DAS-II) [Beran, & Elliot, 2007] was administered to generate verbal and nonverbal ratio IQ scores for participants ages 5–17 years. The Wechsler Abbreviated Scales of Intelligence, Second Edition (WASI-II) [Wechsler, 2011] was administered for three participants who were at least 18 years old and for whom accurate age equivalent scores could be generated. Participants who earned a raw score of zero on all subtests in the age-appropriate verbal or nonverbal domain of the WASI-II, DAS-II School Age battery, or DAS-II Upper Early Years battery were administered the next lowest verbal or nonverbal battery. Twenty-one probands were administered a verbal or nonverbal DAS-II battery below their chronological age. The Mullen Scales of Early Learning [Mullen, 1995] was administered to eight participants who were unable to complete DAS-II items and whose mental age was clearly below 4 years as confirmed by adaptive behavior and clinician judgment.

Verbal and nonverbal ratio IQs were calculated by dividing mental age (i.e., normative group-referenced age equivalent score) by chronological age. When participants earned scores below the floor of the test, the age equivalent was estimated as the lowest age equivalent available for that subtest minus 3 months. Four participants were administered the DAS-II and were not able to complete the test items due to functioning at a level below the floor of the test before the Mullen Scales were available as part of the research protocol. In these cases, floor age equivalent scores minus 3 months were assigned to calculate a conservative estimate of their ratio IQ. To measure adaptive functioning, the Vineland Adaptive Behavior Scales, Second Edition caregiver interview was administered to a primary caregiver. The Vineland adaptive behavior composite score, which reflects functioning across communication, daily living and social domains, was used in the current analyses. Ratio IQ scores and adaptive skills are reported using standard scores with a mean of 100 and standard deviation of 15.

Analytic Plan

Analyses were done in IBM SPSS Statistics 19. Hierarchical linear regressions were conducted within each gene group. Separate regressions were run for each of the dependent variables: verbal ratio IQ, nonverbal ratio IQ, and adaptive functioning. Age of attainment of single words, walking, and phrases were entered one at a time in that order, which we expected would reflect chronological order of typical development. Our goal was to identify the earliest and strongest predictors of later cognitive and adaptive functioning within each gene group. Thus, in the results we report significant predictors that emerge at each stage of the regression models as well as the final model results. Lastly, the child's age at the time of the ADI-R interview was added as a fourth independent variable to control for telescoping bias in parents' recollection of milestone attainment [Hus, Taylor, & Lord, 2011].

We hypothesized that younger age of early milestone attainment would predict stronger cognitive and adaptive outcomes, and that the most predictive milestone(s) would be consistent across outcomes within each gene group. There is no precedent for these exploratory analyses in the literature, thus we did not have specific hypotheses about which milestones would be most predictive of outcomes in each gene group.

Results

Results are summarized in Table 2. Detailed model results are reported in Table S2. Regression coefficients reported in the text are unstandardized.

Table 2. Summary of Developmental Predictors of Cognitive and Adaptive Outcomes by Gene Group

	ADNP	*CHD8*	*DYRK1A*	*GRIN2B*	*SCN2A*
Nonverbal cognition	Walking	(Phrases)	Words Phrases	Words Phrases	All
Verbal cognition	Walking	(Phrases)	Words Phrases	Words	All
Adaptive functioning	Words	Phrases	Words Phrases	Words	Words

Note. Earliest and/or strongest milestone predictors of each outcome are listed by gene group. All = no single milestone was significantly predictive of outcomes, but the milestones together explained a significant proportion of variance in the outcome. Parentheses around results in the *CHD8* column indicate this result was driven by outliers.

Activity Dependent Neuroprotector Homeobox Gene (ADNP)

Nonverbal cognition. In the *ADNP* group, age of walking explained a significant amount of variance in nonverbal ratio IQ over age of first words (ΔR^2 = 0.561, P = 0.006), while the other indicators did not independently explain significant variance in nonverbal cognition (ΔR^2 range = 0.039–0.099). Examination of regression coefficients indicated age of walking had a strong linear association with nonverbal ratio IQ (β = −0.774, SE = 0.188, P = 0.006) that was reduced but still approached significance when all predictors were included in the model (β = −0.623, SE = 0.238, P = 0.058).

Verbal cognition. Age of first walking explained significant variance in verbal ratio IQ (R^2 = 0.784, ΔR^2 = 0.663, P = 0.001), without additional variance contributed by the remaining predictors (ΔR^2 range = 0.005–0.121; final R^2 = 0.834). Age of walking remained significantly associated with verbal ratio IQ after all predictors were included in the model (β = −0.626, SE = 0.195, P = 0.025).

Adaptive functioning. Age of first words explained variance in adaptive functioning outcomes in the *ADNP* group (R^2 = 0.469, P = 0.010). Age of walking, phrases and age at ADI-R interview did not explain additional variance on their own (ΔR^2 range = 0.002–0.073; final R^2 = 0.579). Age of first single words was linearly associated with adaptive functioning (β = −0.685, SE = 0.051, P = 0.010). This effect remained statistically significant when all predictors were in the model (β = −0.626, SE = 0.055, P = 0.031).

Chromodomain Helicase DNA Binding Protein 8 Gene (CHD8)

Nonverbal cognition. Among individuals with a mutation to *CHD8*, age of first phrases explained significant variance in nonverbal ratio IQ over and above ages of walking and first words (ΔR^2 = 0.545, P = 0.008) and had a strong linear association with nonverbal ratio IQ over and above the other milestone ages (β = −0.902, SE = 0.071, P = 0.008). However, Figure 1 suggests this effect may be driven by the two individuals who had not attained phrase speech by the time of the evaluation. Thus, analyses were rerun without these two individuals (Table S3). Results with the reduced dataset were similar, with age of phrases explaining significant variance in nonverbal outcomes (ΔR^2 = 0.561, P = 0.001) and maintaining a linear association with nonverbal outcomes once all predictors were included in the final model (β = −1.710, SE = 0.466, P = 0.060).

Verbal cognition. With the full sample of *CHD8* individuals, ages of milestone attainment and age at the ADI-R interview only explained a modest amount of variance in verbal cognition among individuals with a *CHD8* mutation (R^2 = 0.416) and none of the developmental milestones had a strong linear association with verbal ratio IQ (β range = −0.362 to 0.004). When outliers were removed, age of first phrases independently explained variance in verbal cognition (ΔR^2 = 0.554, P = 0.014) and the combination of predictors explained a majority of variance (R^2 = 0.740). In the final model with this restricted sample, only age of phrases was significantly associated with verbal ratio IQ (β = −2.429, SE = 0.648, P = 0.036).

Adaptive functioning. Age of first phrases explained significant variance in adaptive functioning within the full *CHD8* group, over and above that explained by earlier milestones (R^2 = 0.848, ΔR^2 = 0.622, $P < 0.001$). None of the other independent variables contributed significant variance (ΔR^2 range = 0.027–0.200). The regression coefficient for age of first phrases remained statistically significant when all indicators were included in the model (β = −0.691, SE = 0.043, P = 0.011). Results were similar with the restricted sample of *CHD8* individuals who had attained phrase speech by the time of the evaluation.

Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A Gene (DYRK1A)

Nonverbal cognition. For individuals with a mutation to *DYRK1A*, age of first words explained a modest amount of variance in nonverbal ratio IQ (R^2 = 0.246, P = 0.031), with the remaining predictors not independently explaining additional variance (ΔR^2 range = 0.002–0.167; final R^2 = 0.431). When age of first words was the only predictor in the regression model, this milestone was linearly related to nonverbal ratio IQ (β = −0.496, SE = 0.073, P = 0.031), but this effect was no longer significant once age of walking was added, suggesting shared predictive variance across single word and walking milestones. In the final model, the regression coefficient for age of first phrases approached significance over and above the other predictors (β = −0.680, SE = 0.110, P = 0.054).

Verbal cognition. Age of first words explained a modest amount of variance in verbal ratio IQ among individuals with a *DYRK1A* mutation (R^2 = 0.263, P = 0.025), and age of first phrases partially explained additional variance over and above words and walking (ΔR^2 = 0.165, P = 0.055). Age of walking and age of ADI-R interview did not explain significant variance on their own (ΔR^2 = 0.000 and 0.017, respectively). The final model explained less than half the variance in verbal cognition (R^2 = 0.444). Examination of the regression coefficients revealed that age of first words was linearly associated with verbal ratio IQ (β = −0.513, SE = 0.081, P = 0.025), but this effect was

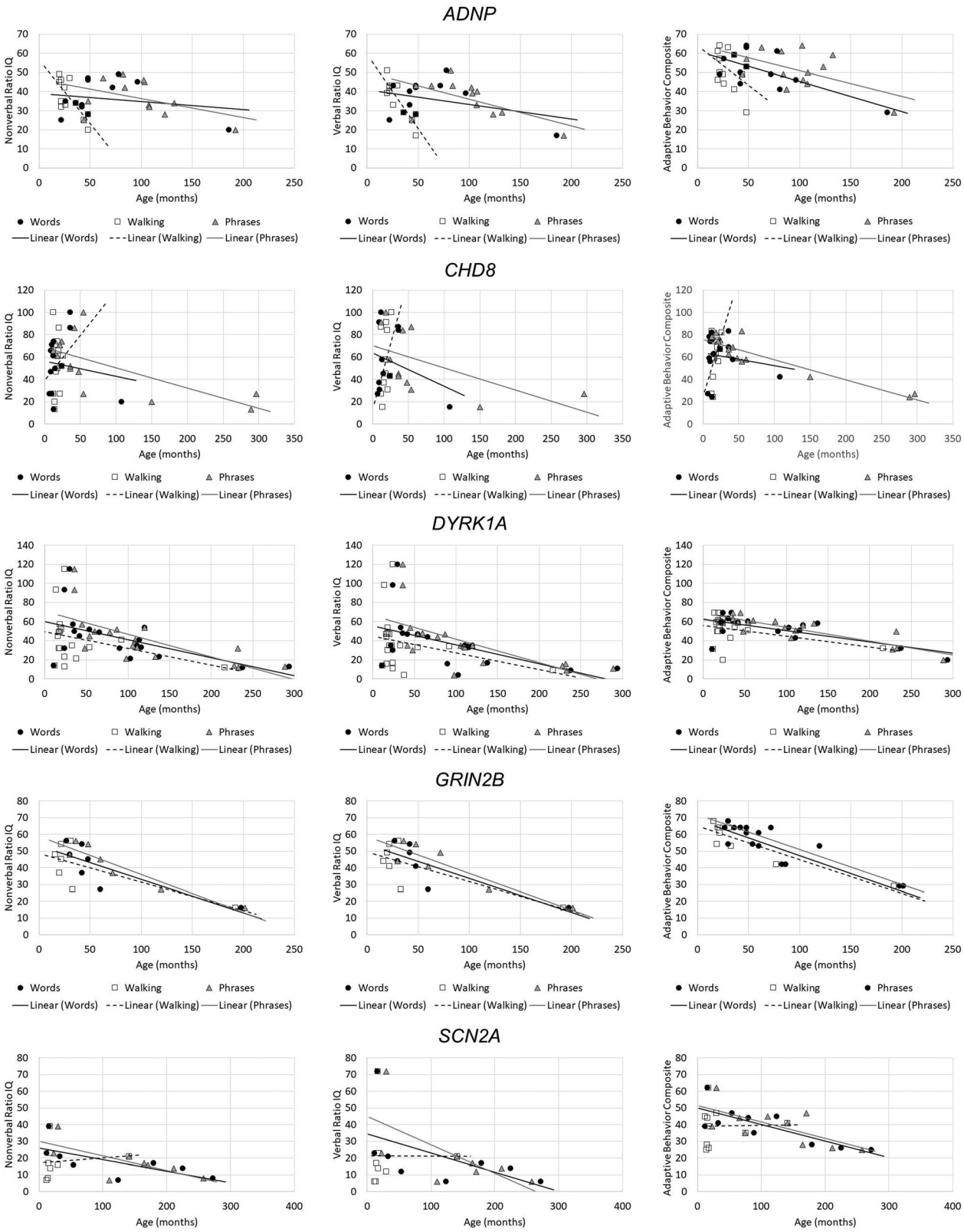

Figure 1. Linear associations between ages of attainment of developmental milestones and cognitive and adaptive outcomes for each gene group.

reduced when the other milestone ages were added to the model. With all predictors included, the regression coefficient for age of phrases approached significance (β = −0.677, SE = 0.120, P = 0.053).

Adaptive functioning. Variance in adaptive functioning in the *DYRK1A* group was explained by age of first single words (R^2 = 0.468, P = 0.002). Age of first phrases explained additional variance over and above age of words and walking (ΔR^2 = 0.287, P = 0.002). In all, a large amount of variance in adaptive functioning was explained by milestone ages in this group (R^2 = 0.756) with nonsignificant contribution by ADI-R interview age (ΔR^2 = 0.014, P = 0.413). Regression coefficients indicated a linear association between age of first single words and adaptive outcomes (β = −0.684, SE = 0.032, P = 0.002) that was reduced following addition of age of first phrases (β = −0.088, SE = 0.038, P = 0.698). In the final model, age of first phrases was strongly related to adaptive outcomes in this group, over and above the other indicators (β = −0.698, SE = 0.037, P = 0.008).

Glutamate Ionotropic Receptor NMDA Type Subunit 2B Gene (GRIN2B)

Nonverbal cognition. Among individuals with a mutation to *GRIN2B*, age of first words explained a moderate amount of variance in nonverbal ratio IQ (R^2 = 0.673, P = 0.024). Although the remaining predictors did not independently explain additional variance (ΔR^2 range = 0.052–0.140), a large amount of variance in nonverbal ratio IQ was explained by all predictors together (R^2 = 0.989). Age of single words was linearly associated with nonverbal ratio IQ (β = −821, SE = 0.062, P = 0.024), but this effect was reduced when age of walking was added to the model, suggesting shared variance across these milestones. In the final model, age of first phrases showed a strong linear association with nonverbal ratio IQ, over and above the other milestones and interview age (β = −1.252, SE = 0.061, P = 0.038).

Verbal cognition. Age of first words explained a majority of variance in verbal ratio IQ in the *GRIN2B* group (R^2 = 0.696, P = 0.020), while the remaining variables did not independently explain additional variance (ΔR^2 range = 0.005–0.098; final R^2 = 0.859). Single word attainment was linearly associated with verbal outcomes (β = −0.834, SE = 0.060, P = 0.020), but this was no longer statistically significant when age of walking was added to the model (β = −2.609, SE = 0.318, P = 0.117) and the effect was further reduced with the addition of phrases as a predictor. None of the regression coefficients were statistically significant in the final model. Altogether, these results indicate the three developmental milestones shared predictive variance for later verbal cognitive outcomes in this group.

Adaptive functioning. Age of first words explained a large amount of variance in adaptive outcomes (R^2 = 0.814, P = 0.001), with little additional variance explained by the other predictors (ΔR^2 range = 0.000–0.038; final R^2 = 0.864). Age of first words was linearly associated with adaptive outcomes in this group (β = −0.902, SE = 0.039, P = 0.001), but the effect was significantly reduced when age of walking was added to the model and further reduced with the addition of all independent variables (β = 1.00, SE = 0.380, P = 0.567), indicating shared predictive variance across all milestones.

Sodium Voltage-Gated Channel Alpha Subunit 2 Gene (SCN2A)

Nonverbal cognition. For the *SCN2A* group, variance in nonverbal ratio IQ was partially explained by age of first words (R^2 = 0.465, P = 0.063). With all predictors in the model, a large amount of variance in nonverbal cognition was explained (final R^2 = 0.802), although no single predictor explained significant variance on its own (ΔR^2 range = 0.016–0.273). Likewise, the regression coefficient for age of first words approached significance (β = −0.682, SE = 0.030, P = 0.063), but was reduced with the addition of other predictors to the model (β = −0.307, SE = 0.065, P = 0.669), indicating shared predictive variance across the milestones.

Verbal cognition. Altogether, ages of milestone attainment explained a minority of variance in verbal ratio IQ among individuals with a mutation to *SCN2A* (R^2 = 0.427). However, no milestone explained a significant amount of variance on its own (ΔR^2 range = 0.050–0.294). Regression coefficients for the independent variables were not statistically significant at any stage of the model (P > 0.195).

Adaptive functioning. Variance in adaptive functioning in the *SCN2A* group was largely explained by age of first words (ΔR^2 = 0.621, P = 0.007). Independent variables added later in the model did not explain significant variance on their own (ΔR^2 range = 0.002–0.074). Age of first words was linearly associated with adaptive outcomes (β = −0.788, SE = 0.027, P = 0.007) but the effect was reduced once age of first phrases was added (β = −1.064, SE = 0.060, P = 0.067), indicating shared predictive variance across the verbal milestones. With age of ADI-R interview added, none of the independent variables were linearly associated with adaptive outcomes at a statistically significant level, which may further suggest influence of telescoping bias. Visual inspection of the

scatterplots indicates that there is significant heterogeneity in cognitive and adaptive outcomes for children with a mutation to *SCN2A* who show on-time attainment of developmental milestones.

Discussion

In this study, we present evidence that age of attainment of developmental milestones may be used to predict later cognitive and adaptive functional outcomes among children with mutations in five genes associated with ASD and ID. As expected, the developmental milestones that were most predictive of later outcomes varied across genetic subtypes, but were largely consistent within each gene group. This is the first study to provide clinical markers of long-term outcomes in children with ASD-linked genetic mutations.

There was considerable heterogeneity in developmental milestones and outcomes within each gene group, likely driven by individual variability in specific genetic variants and additional genetic factors. Although individuals in the *ADNP* group all met criteria for ID, they had clinically significant variability in nonverbal and verbal cognitive outcomes. Visual inspection of Figure 1 suggests that children with an *ADNP* mutation who walk by 3 years are likely to develop conceptual abilities equivalent to those of a typical preschool or early elementary school aged child. Children with an *ADNP* mutation who do not meet those milestones may have severe difficulty engaging in both verbal and nonverbal problem-solving tasks, likely requiring close supervision and needing more support with personal care even after extensive teaching. In addition, they may have very limited speech as well as difficulty attaining basic understanding of concepts related to time, money, and math. Interestingly, the *ADNP* group was the only group for whom age of walking was a statistically significant predictor. This may be due to consistent, severe impairment in language and oral motor skills among individuals with an *ADNP* mutation. On the other hand, although motor delay is a key feature of mutations in *ADNP*, affecting 95% of *ADNP* patients [Van Dijck et al., 2019], all individuals in our sample did eventually walk independently by about 4 years of age. Thus, motor coordination and strength may be a sensitive measure of individual differences in the impact of *ADNP* dysfunction on neurodevelopment.

Among individuals in our study with a *CHD8* mutation, verbal and nonverbal ratio IQ ranged from 13 to 100, and age of first phrases ranged from 12 months to never obtained (n = 2). Visual inspection of Figure 1 shows that individuals with a *CHD8* mutation who attained verbal and motor milestones by age 5 years had highly variable nonverbal and verbal ratio IQ scores (range = 27–100). Thus, even without including the individuals who had not obtained phrase speech by the time of the evaluation, associations between phrase speech and cognitive outcomes were largely driven by one individual who developed phrase speech after 10 years of age. This finding is therefore less applicable to individuals with *CHD8* who meet milestones broadly on time (i.e., before age 4.5 years). On the other hand, the association between age of phrase speech and adaptive skills was more clear and consistent across the full sample of individuals with a *CHD8* variant. Given that *CHD8* is expressed most strongly during the early prenatal and mid-prenatal period [Bernier et al., 2014], this finding highlights an association between postnatal adaptive skill acquisition and early fetal brain development. A potential implication is that for individuals with *CHD8* variants, phrase speech may not serve as a key treatment target, but may, like walking in *ADNP*, be an important clue as to the level of impact that the disrupted gene variant has had on neurodevelopment more broadly.

In the *DYRK1A* group, there appeared to be a bimodal distribution of the age of development of phrase speech. Among this sample, 50% developed phrase speech by 80 months, while the other half had not developed phrase speech by the time of the evaluation. The ages of those who had not yet developed phrase speech ranged from 60 to 262 months, with three of those individuals younger than 80 months at the time of the assessment; thus, it is possible some of these children have gone on to develop phrase speech since participating in the research evaluation. The two individuals who developed phrase speech before 40 months had a nonverbal ratio IQ in the average range, while the rest of the sample had nonverbal ratio IQ scores in the intellectually impaired range. Additional data points would be necessary to determine whether 40 months is a reliable cutoff point to predict average cognition among children with a *DYRK1A* mutation. Interestingly, only one of these high functioning *DYRK1A* cases was inherited; the other inherited case was diagnosed with Severe ID and had not yet developed phrase speech at the time of the evaluation. Yet, both inherited cases met criteria for ASD, suggesting distinct gene–gene and gene x environment interactions drive cognitive vs. social skill development among individuals with *DYRK1A* mutations.

Single words and phrases consistently and strongly predicted *GRIN2B* cognitive and adaptive outcomes. Notably, although all individuals with a *GRIN2B* mutation showed nonverbal and verbal cognitive abilities in the impaired range (ratio IQs < 60), adaptive skills were relatively preserved. More than half of the participants with a *GRIN2B* mutation had either adaptive communication or adaptive social skill scores greater than 60, consistent with relatively lower rates of ASD diagnosis in this group (57%). *GRIN2B* is expressed prenatally [Endele et al., 2010], which is consistent with high rates of ID

and broad cognitive deficits. However, the relative strength observed in adaptive skills in this group may suggest that adaptive functioning is also sensitive to postnatal brain development and thus is a good candidate focus of intervention for children with *GRIN2B* and other prenatally expressed genetic disruptions.

Results of the regression analyses and visual inspection of the scatterplots in Figure 1 show that outcomes for the *SCN2A* group were generally predicted by the combination of verbal milestones, rather than a single predictor. Interestingly, while verbal and nonverbal ratio IQs in this group were generally below 45, one individual had a verbal ratio IQ of 72 and a nonverbal ratio IQ of 39. This split between verbal and nonverbal reasoning is quite unusual and suggests a target for further investigation, in the context of the individual's specific genetic variant. *SCN2A* encodes for a neuronal voltage-gated sodium channel, with disruptive variants of *SCN2A* often categorized as either "gain of function" or "loss of function" [Ben-Shalom et al., 2017]. Whereas the former category is associated with early onset seizures, the latter is more closely associated with ASD. Despite this often binary phenotypic outcome, visual inspection of Figure 1 suggests consistent linear associations between development of verbal milestones and nonverbal and adaptive outcomes among individuals with an *SCN2A* variant.

Prior research indicates that parents accurately report age of first independent walking on the ADI-R, but accuracy of reported age and first words decreases over time [Hus et al., 2011]. We saw little indication of telescoping bias by reporters on the ADI-R in the current study. Nonetheless, prospective and real-time data collection methods would improve accuracy of results and specificity for clinical guidelines. Social networks of families who have children with an ASD-linked gene have been critical to the research process [Gozes et al., 2017], and present a novel medium through which to collect milestone data in real time. Phone- and internet-based apps like Groopit (groopit.co) have already been leveraged by some family groups to collect and share timely data in a standardized way. Future real-time and prospective data collection efforts should aim to include additional early developmental data, such as timing and level of mastery of fine, gross and oral motor, nonverbal and verbal communication, and multiple adaptive functioning milestones.

The genetics first recruitment approach may be limited by the fact that individuals with greater impairment are more likely to be referred for clinical or research genetic testing. Thus, while our results are likely to be representative of the cases who present to clinicians seeking prognosis and treatment guidelines, we may not be capturing variance across the full spectrum of phenotypic presentations associated with these genetic events. However, we believe this work remains important, given that individuals with significant impairment secondary to genetic disruptions are most in need of clinical research like the current study. Another limitation of our study was that we did not track developmental regression that may have occurred after the evaluation. In the future, prospective samples will allow for modeling developmental trajectories and identification of covariates, like seizures, that may predict nonlinear development. In the current study, seizures are most common among individuals with variants to *SCN2A*; however, those individuals tend to have infant-onset seizures, rather than later-onset that would be expected to disrupt a linear trajectory. Finally, given the rarity of these genetic events, statistical power in this study was limited. However, even with small sample sizes, we achieved statistical significance and observed consistency in predictors and effect sizes across multiple outcomes. Thus, we predict that these results will only be strengthened with additional data points.

For some families and providers, the data presented in this study will provide treatment guidelines, including behavioral intervention targets, special education services, proactive acquisition of department of disability funds, and long-term care planning. Data collected over hundreds of genotypes associated with ASD and related disorders will provide the most effective management tools for customized behavioral therapies in the future. For other families, the uncertainty around what to expect for their child over the long-term may constitute a significant emotional and practical burden. For those individuals, we hope the trends reported here will simply provide clarity and ease of mind.

Conclusions

Early language and motor milestones are strong and consistent predictors of cognitive and adaptive outcomes among individuals with ASD-linked gene disruptions. Associations between early milestones and later outcomes were specific to distinct genetic subtypes, and in a few cases, to either cognitive or adaptive outcomes. These trends have potential to inform decisions about treatment planning and long-term expectations for families and providers of affected individuals. Additionally, the specificity of these associations adds to the growing body of research linking molecular genetic function to brain development and phenotypic outcomes.

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Author contributions

A.A. conceptualized the paper, ran analyses and drafted the majority of the manuscript. J.S.B. contributed to conceptualization, organized the phenotypic data, and drafted sections of the introduction and methods. E.C.K-N. contributed to conceptualization, organized genetic data, and drafted sections of the introduction and methods. K.H. and T.W. analyzed and checked accuracy of the genetic data and reviewed the manuscript. R.A.B. and E.E.E. provided conceptual input, reviewed the manuscript, and drafted sections of the discussion.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Gene and Variant Information for Participants
Table S2 Regression Model Results
Table S3 Regression Model Results of CHD8 Participants Excluding Outliers