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ARTICLE

Developmental Trajectories as Autism Phenotypes

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Numerous studies of Autism Spectrum Disorder have attempted to link behavioral phenotypes to genetic findings. Reliance on cross-sectional behavioral data in samples that span wide age ranges may have limited this endeavor because ASD behaviors are not static within individuals across development. This study uses quantitative methods to describe specific aspects of changes in autism-related and more general behaviors in order to yield trajectories that could be used in place of single time-point data as behavioral phenotypes in neurobiological studies of both Autism Spectrum Disorders and overlapping conditions. Building on previous analyses, we examined trajectories of parent-reported social-communication deficits, social adaptive functioning, and two types of repetitive behaviors, repetitive sensory motor (RSM) behaviors and insistence on sameness (IS) behaviors, in a relatively large sample of participants referred for possible autism at age 2 years and followed into young adulthood (n = 85). A strength of this sample was the diverse range of outcomes, including young adults with intellectual disability and persistent autism related difficulties, those with IQs in the borderline or average range who continued to experience functional impairment related to Autism Spectrum Disorders, and a small group of young adults (n = 8) with IQs in the average range who were judged to be functioning socially and adaptively at age-appropriate levels at age 19 years, despite a previous childhood diagnosis of autism. © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

There have been multiple attempts in recent years to link behavioral phenotypes to molecular genetics within Autism Spectrum Disorders (ASD) [e.g. Brune et al., 2006; Hu and Steinberg, 2009; Bernier et al., 2014; Merikangas et al., 2014]. In behavioral genetics studies, primarily with typical populations, there have been consistent findings of associations between various parent-reported behaviors generally related to ASD and concordance between identical twins, even those who score at the extremes [Reiersen et al., 2008]. In studies of selected populations with different genetic patterns, a few associations have been found, for example, between 16p11.2 and a shift downward from a normal distribution of IQ [Hanson et al., 2014], as well as between general social communication-behavioral deficits and *CHD8* mutations [Bernier et al., 2014] and between several ASD traits and fragile X [Abbeduto et al., 2014].

One of the most striking and puzzling aspects of ASD is the heterogeneity in outcomes, not just in terms of overall independence, but also in terms of changes over time in behaviors that define ASD [Richler et al., 2010; Fountain et al., 2012; Troyb et al., 2014]. Furthermore, the nature of behavioral heterogeneity in ASD, including the range of "expected" behaviors varies across development. So, whereas a certain level of social motivation or communicative competency might be incompatible with a diagnosis of ASD in a very young child and/or one with low cognitive abilities, these skills might be reasonably expected in an older and/or cognitively

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higher functioning individual. Thus, because ASD-related symptoms and behaviors change over time, it is potentially quite problematic to try to link cross-sectional behavioral data to genetics, since findings of relationships become dependent on the specific point in time at which the behavior was measured. This may help explain why attempts to increase genetic homogeneity via reduced phenotypic heterogeneity have proven less fruitful than had originally been hoped [see Chaste et al., 2014]. However, as we have developed a better understanding of how to characterize at least some of these behavioral clusters that define ASD [Lecavalier, 2006; Gotham et al., 2012; Bishop et al., 2013], and as longitudinal data become more available, the possibility of using trajectories of change as behavioral phenotypes becomes an option. This approach has proven fruitful in other areas of medicine, such as breast cancer and Alzheimer's disease, where different patterns of disease onset have been linked to specific genetic findings [Cruchaga et al., 2012; Bettens et al., 2013; Ahsan et al., 2014]. Even within the area of pervasive developmental disorders, it could be argued that observations of discrepancies in the developmental courses of children with Rett syndrome compared to "idiopathic autism" led to more targeted investigations and ultimately discovery of the genetic cause of Rett [Amir et al., 1999; Cuddapah et al., 2014].

The purpose of this paper is to use quantitative methods to describe specific aspects of changes in ASD-related and more general behaviors in a relatively large sample of participants referred for possible autism at age 2 years and followed into young adulthood. Unlike our previous analyses of this sample, this paper describes the trajectories in various domains that led to different outcomes (as opposed to focusing on the outcomes and predicting them) and includes additional dimensions than discussed in previous papers in order to provide information for other researchers about dimensions to study. The hope is that these trajectories could be used as behavioral phenotypes in neurobiological studies of both ASD and other overlapping conditions.

A substantial literature now exists that attempts to define how various repetitive and sensory-related interests and behaviors (referred to as RRBs) cluster in individuals with ASD [Bishop et al., 2006; Szatmari et al., 2006; Lam et al., 2008; Richler et al., 2010]. Many of these analyses are based on either the Autism Diagnostic Interview-Revised [ADI-R; Rutter et al., 2003] or the Autism Diagnostic Observation Schedule [ADOS; Lord et al., 2000], but a recent paper [Bishop et al., 2013] showed good convergence between the ADOS, ADI-R and a more scalable, parent questionnaire, the Repetitive Behavior Scale-Revised [RBSR; Bodfish et al., 1999]. This is particularly encouraging because the instruments rely on different methods of reporting and yet show good agreement for four areas within RRBs: Repetitive Sensory Motor Behaviors (RSM), Insistence on Sameness (IS), Self-injury and Circumscribed Interests. Figure 1 depicts these relationships.

In the present paper, the focus is on two areas in RRBs that begin in early development, often continue into adulthood, and that have emerged as subcategories in factor analyses of RRBs in multiple samples using different measures: RSM and IS. Previous analyses of these areas [Richler et al., 2010] from ages 2 to 9 in the same sample reported here had shown three classes of trajectories within ASD for RSM: one group which started with many behaviors even at age 2 and increased steadily from 2 to 9 (25%); the largest group (50%), which started moderately high and decreased gradually from 2 to 9, and another group that started low and remained low throughout childhood (25%). Within IS, three trajectory groups were identified: a smaller group (13%) with consistently mild symptoms, an increasing group (71%), which showed relatively modest increases between the ages of 2 and 5 years, and a moderate group with a steady course (16%). In this paper, we look at trajectories of the same clusters into young adulthood.

Identifying measures of social-communicative behavior in ASD that predict outcomes has been more difficult than for RRBs. Parent reports of social communicative behaviors are differentially related to a child's language level, IQ and more general behavior problems for various instruments [Charman et al., 2007; Hus et al., 2013; Hus and Lord, 2014] and so, in analyses, these factors must be taken into account. Different ADOS trajectories in Social Affect were identified in the present sample up to age 12; with nearly 80% of the sample remaining at the same level of severity from age 2 onward into adolescence [Gotham et al., 2012]. In the present

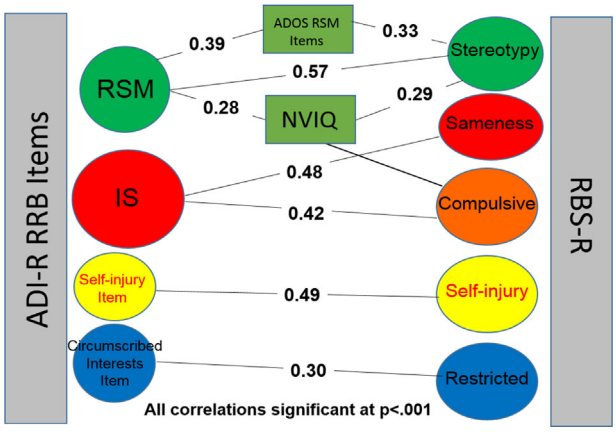

Figure 1. Relationships among ADI-R, RBS-R and ADOS items.

paper, we look both at a Social Communication Deficits trajectory from the ADI-R from 2 to 19 years, and also at changes in the social skills domain of the Vineland Adaptive Behavior Scales as a measure of social adaptation [Klin et al., 2007]. We used several independent measures of outcome at age 19, based on directly assessed intelligence and interviews (ADOS), self and parent-reported use of support services, parent reports of current symptoms (ADI-R), as well as clinician judgment to create trajectory groups with markedly different functioning as young adults [See Anderson et al., 2014].

MATERIALS AND METHODS

Participants were consecutive referrals of children under 37 months old with suspected ASD or a non-ASD developmental disorders to four North Carolina-based, state-funded autism centers (n = 113) and a specialty autism clinic at the University of Chicago (n = 79). Of the 213 original participants, three-quarters received ASD diagnoses at the initial age 2 visit [Anderson et al., 2007]. By the age 19 assessment, two thirds (n = 142) of the original sample and their families were still participating to some extent, with 120 participating in the age 19 in-person assessment. Attrition was not related to gender, diagnosis, or IQ at the initial assessment, but African American families with less education were lost to the study at a higher rate than Caucasian families and families with more education.

This study includes all 85 youths (92% male) who were diagnosed with ASD in early childhood and seen at age 19. The average ages at the first and last assessments were 2 years, 5 months (SD = 0.43) and 19 years, 1 month (SD = 1.08), respectively. Ethnic minorities, most of whom were African American, accounted for 24% of the sample, with a mix of children from rural and urban areas (North Carolina = 49%; Chicago = 51%). Participants with profound cognitive impairment (nonverbal IQ <25 at age 2) who received non-ASD diagnoses at age 2 but later received ASD diagnoses were excluded from the current analyses.

Procedures

Children and families completed a battery of face-to-face diagnostic and psychometric tests when the children were 2, 3, 5, 9, and 19 years, free of charge. Additional time points (e.g., ages 11 and 14) were available for some measures (VABS/Vineland II), and some children were seen at slightly different or additional time points due to follow up visits. At each face-to-face assessment, with the exception of age 3, an overall best estimate consensus diagnosis of autism spectrum disorder, other non-ASD diagnosis, or typical development/no diagnosis was based on all available information obtained during the assessment. At the age 19 assessment, a "typical" diagnosis required overall global functioning in the normal range of intelligence and in terms of a clinical judgment using social adjustment, restricted and repetitive behaviors, independence, and comorbid symptoms [see Anderson et al., 2014]. Details about the measures administered as part of the larger study, including all those considered in making best-estimate diagnoses, are described in other reports [e.g., Lord et al., 2006, Anderson et al., 2014]. Below we provide descriptions of the measures used in the current analyses.

Measures

The Autism Diagnostic Interview-Revised [ADI-R; Rutter et al., 2003] is a semi-structured standardized parent interview designed to differentiate children with ASD from those with non-ASD developmental disorders. The diagnostic algorithm uses scores based on historical information (i.e., "Most abnormal 4–5" scores or "Ever" scores), but because this study was focused on trajectories, we constructed scores for each time point based on current behaviors. As shown in Table I, a social-communication score for each time point was calculated by summing the "Current" item scores in the Nonverbal Communication and Reciprocal Social Interaction Domains of the ADI-R algorithm for all items that were included in interviews at each time of administration (ages 2, 3, 4, 5, 9, 19). For example, eye contact was not included because it is not coded for older children or adults as a "current behavior," and verbal items were not included because not all participants were verbal at all time points. Current social-communication deficit scores had a possible range of 0–30, where higher scores indicated greater abnormality. Two separate repetitive behavior scores were calculated for each time point, one representing repetitive sensory motor (RSM) behaviors, and one representing insistence on sameness (IS) behaviors [see Richler et al., 2010]. RSM and IS scores had possible score ranges from 0–10 and 0–8, respectively, where higher scores indicated greater abnormality.

Verbal IQ (VIQ) and nonverbal IQ (NVIQ) scores were derived from various psychometric tests following a pre-determined hierarchy of difficulty and appropriateness [see Anderson et al., 2014]. Social adaptation was assessed using the Vineland Adaptive Behavior Scales, known as Vineland II [Sparrow et al., 2005], a standardized, semi-structured, parent interview which yields domain scores in the areas of communication, daily living skills, and social skills, as well as an adaptive behavior composite. We used the standard score for social skills as a measure of social adaptation [Klin et al., 2007].

Analyses

Building on previous analyses [Anderson et al., 2014], the sample was divided into three groups based on IQ, diagnostic symptoms and independent functioning at age 19: ASD and VIQ < 70 (n = 53), ASD and VIQ ≥ 70 (n = 24), and Very Positive Outcome (VPO) (n = 8). Growth curve analyses with SAS MIXED procedure were used to examine changes in ADI-R social-

TABLE I. Current ADI-R Symptom Domains (Based on "Current" Item Scores; Scores of 3 Converted to 2)

Social communication (15 items; possible score range from 0–30)
Use of other's body 31
Pointing 42
Nodding 43
Head shaking 44
Gestures 45
Social smile 51
Showing 52
Offering to share 53
Sharing enjoyment 54
Offering comfort 55
Quality of social overtures 56
Range of facial expressions 57
Inappropriate facial expressions 58
Appropriateness of social response 59
Interest in children (for timepoints at which children were 9 years, 11 months or younger) OR friendships (for timepoints at which children were 10 years or older) 62, 65
Repetitive sensory motor (5 items; possible score range from 0–10)
Unusual preoccupations 67
Repetitive use of objects 69
Unusual sensory interests 71
Hand and finger mannerisms 77
Other complex mannerisms 78
Insistence on Sameness (4 items, possible score range from 0–8)
Compulsions and rituals 70
General sensitivity to noise 72
Abnormal idiosyncratic response to specific sensory stimuli 73
Difficulty with changes in routine 74

communication, ADI-R Repetitive Sensory Motor (RSM), ADI-R Insistence on Sameness, Verbal IQ, Nonverbal IQ, and Vineland Social Adaptation standard scores for the three groups. A separate intercept and slope was calculated for each child as a control for high correlations among repeated measures in the same individuals over time. The three groups were compared with respect to relative starting point at age 2 (intercept) and rate and pattern of change (linear and quadratic slopes). Age at testing was always covaried; VIQ was run as a covariate for all the non-IQ measures and was significant for each of them (Vineland Social Adaptation, ADI-R Social Communication Deficits, ADI-R RSM) except IS, but it did not change the slopes or any of the group differences and so is not represented further. Other measures that were included in previous analyses as covariates (gender, maternal education, ethnicity, occurrence of seizures, any psychopharmacological medication use) that had little effect (i.e., no evidence of main effects or interactions approaching significance; [Anderson et al., 2011]) were not included in order to maintain a reasonable number of parameters, given the sample sizes.

The estimates for both the covariance and β parameters were obtained by restricted maximum likelihood methods so that results would be less biased [Verbeke and Molenberghs, 2000]. To test for group differences in slopes and intercepts, we used *t* statistics for each parameter, calculated as the ratio of the parameter estimate divided by the standard error. To examine whether rate of change in each measure over time differed significantly from 0, we used *t*-tests for linear combinations of variables representing slopes.

Effect size for changes in mean scores over time was calculated using the standardized mean difference (SMD) method: SMD = (Time 1 behavior score—Time 2 behavior score)/pooled SD [Cohen, 1988]. We used the widely accepted guidelines of Cohen [1988] for interpreting the effect size, where 0.2 is small, 0.5 is medium, and 0.8 is large. Effect sizes in the present study were generally large, in part because variances were very low within each group. The fact that the variances were so low is also important in interpreting the validity of these three subgroups.

RESULTS

Previous Analyses

Previous analyses of the same sample included χ^2 to test for differences in means [Anderson et al., 2014]. The three groups did not differ in age at first or last testing, site, ethnicity, percent males, marital status of caregivers, maternal education, and diagnosis in preschool years (autism, PDD-NOS) or seizures (ever). Participants in the ASD IQ < 70 were more likely to have taken psychometric medications (68%) and to have received early intervention between ages 2 and 3 (93%) compared to the ASD IQ ≥ 70 (38% medication; 54% early intervention) versus the VPO group (none ever medicated; 100% early intervention). At age 2, there were differences between the ASD IQ < 70 group and the two other, higher IQ groups in verbal and nonverbal IQ, Vineland social adaptive scores, ADI-R social communication scores, RSM and IS, but these variables did not differ between the ASD ≥ 70 group and VPO. VPO was used as the reference group in the following analyses in order to compare the difference between a very positive outcome and continued functioning limited by ASD, with or without intellectual disability.

Trajectories in Intellectual Functioning

As shown in Table II and Figure 2, not surprisingly given the groupings we created, the two higher IQ groups (M VIQs of 53; M NVIQ's 81–83) differed from the less cognitively able group (ASD VIQ < 70) on VIQ (M VIQ = 29) and NVIQ (M NVIQ = 61) at age 2. Trajectories for VIQ for the two more able groups were quadratic, reflecting steady, quite remarkable improvements in verbal skills beginning at age 2 and continuing into the teen years followed by stable functioning at or above average levels. The difference in trajectory (age X group: $b = 0.11$, SE = 0.06) between the VPO group and the ASD VIQ ≥ 70 group approached significance, at $P < 0.10$, which, given the small sample sizes, is a call for consideration of a possible effect in further studies, but clearly needs to be replicated. Mean VIQ for the less cognitively able group (ASD VIQ < 70) did not change significantly from 2 to 19. It is important to note that the IQ differences at outcome are tautological because we defined the groups in part on the basis of verbal IQ. What is interesting here are the trajectories from early development, which were striking in their degree of improvement beginning from age 2 for about a third of our sample.

Trajectories for nonverbal IQ followed a linear pattern, significantly different from zero for all three groups. Slopes again differed for the two cognitively able groups and the less cognitively able participants, with steady increases over time for both the VPO and ASD VIQ ≥ 70 groups up to scores at average or above, and steady decreases for the ASD VIQ < 70 group, with nonverbal means moving from the mild range of intellectual disability to the moderate to severe range of intellectual disability.

Social Adaptation Scores (Vineland II VABS Social Domain)

Most interesting about the adaptive scores was that there was no group difference at intercept at age 2 at all, despite the relatively large differences on other measures and quadratic changes in all three groups. Not surprising, given our definition of outcome in terms of independence, are the relatively high (i.e., average range) adaptive scores of the VPO. However, what was unexpected is the slope of the increase to a mean of 97 by age 9 and 101 at 19 from a much lower mean score at age 2

TABLE II. Changes in Cognitive and Adaptive Abilities From Age 2 to 19 by Outcome Group

	Verbal IQ	Nonverbal IQ	Social adaptive skills
Predictors	Coefficient (S.E.)	Coefficient (S.E.)	Coefficient (S.E.)
Fixed Effects			
Intercept	53.65 (5.99)***	82.74 (5.26)***	65.39 (2.56)***
Age at Testing	0.87 (0.11)***	0.16 (0.04)***	0.31 (0.08)***
Group:			
Very Positive (VPO)[a]	—	—	—
VIQ > 70 ASD	0.30 (6.95)	1.37 (6.09)	4.39 (2.99)
VIQ < 70 ASD	−25.01 (6.44)***	−22.03 (5.64)***	−3.68 (2.75)
Linear Slopes:			
Age* VPO	—	—	—
Age* VIQ > 70 ASD	−0.26 (.13)*	−0.06 (.04)*	−0.32 (.09)***
Age* VIQ < 70 ASD	−0.82 (.12)***	−0.29 (.04)***	−0.58 (.08)***
Quadratic Slopes:[c]			
Age[b]	−0.29 (.05)***	—[b]	−0.01 (.03)
Age^{b,*} VPO	—	—	—
Age^{b,*} VIQ > 70 ASD	0.11 (0.06)$^{+}$	—	0.1 (0.04)$^{+}$
Age^{b,*} VIQ < 70 ASD	0.25 (0.05)***	—	0.2 (0.04)***
	Variance	Variance	Variance
Random Effects			
Intercept	192.03***	159.31***	9.04^{+}
Slope	0.001***	0.004**	0.003***

N = 85; $^{\star}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{+}P < 0.10$.

[a]Dashes indicate reference group.

[b]Dashes indicate parameter was omitted from final model.

[c]Quadratic slope parameters are multiplied by 100.

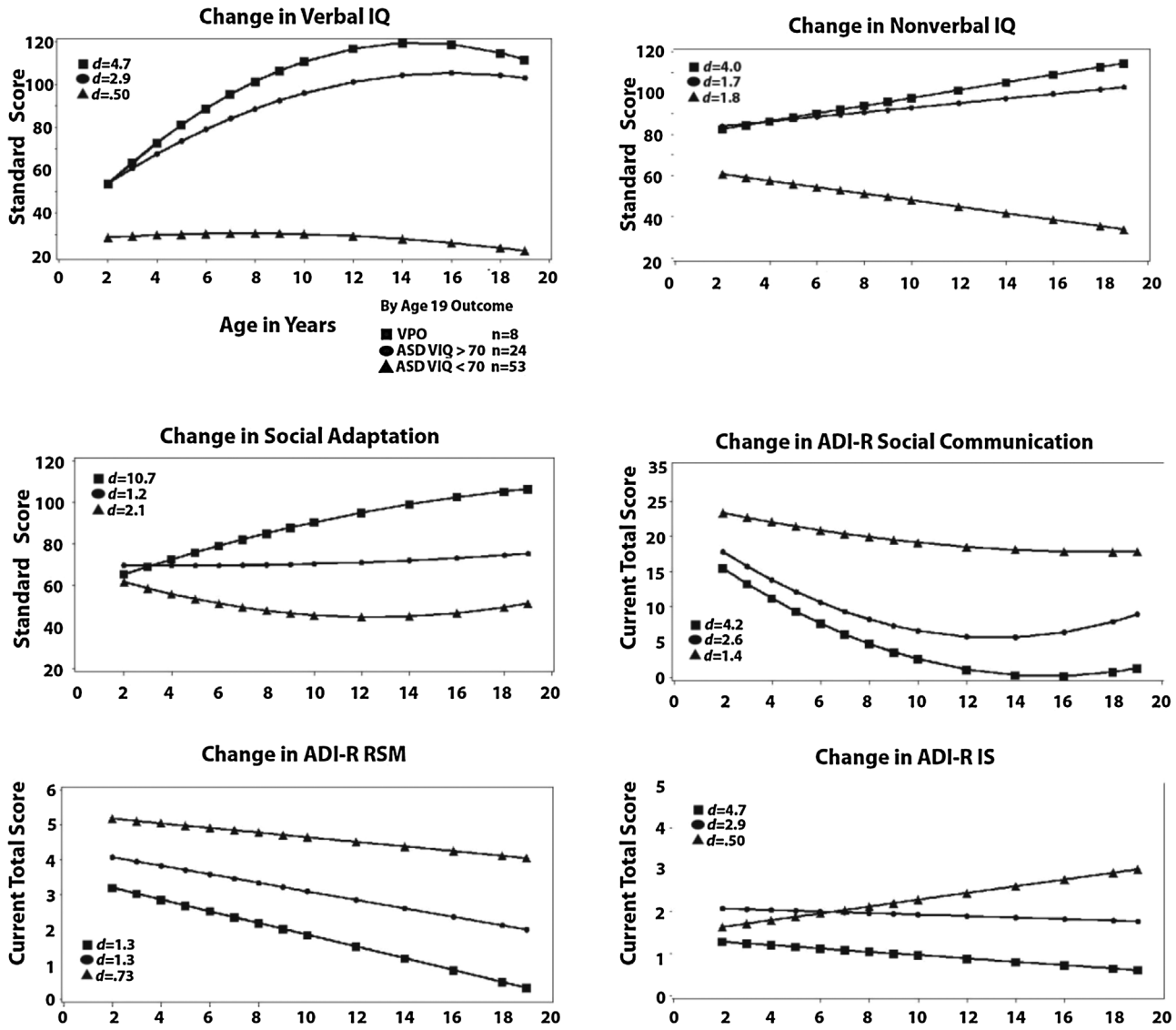

Figure 2. Developmental trajectories from 2 to 19 grouped by outcome.

(SS = 65). In contrast, the other more cognitively able group (ASD VIQ $\geq$ 70) made much slower progress from the same starting point to a mean of 80 at age 9 and 78 at 19 . The change for the ASD VIQ < 70 group was quite different, with steady decreases in standard scores into elementary school years and then a gradual increase into young adulthood. These patterns were all significantly different from each other and from no change. Although VIQ was a significant covariate, it did not change the slopes or the group differences.

Changes in ADI-R Social-Communication Scores

The ADI-R social-communication domain scores ranged from 0 to 30 possible, with higher scores indicating more abnormality (see Table I). As shown in Table III, intercepts for the less able group differed from VPO and the more cognitively able group, the latter of which did not differ from VPO scores at two; scores for the less cognitively able group decreased gradually following a quadratic pattern. Scores for the more cognitively able ASD group also decreased significantly but followed a linear pattern and were significantly different from the quadratic pattern of the VPO participants. It appears that the divergence of the VPO group and the more cognitively able group who continued to have ASD occurred later, after age 9, compared to the scores for Vineland Social Adaptation. Because we do not have ADI-R scores between 9 and 19, we cannot say when this change occurred for this measure.

TABLE III. Changes in ASD Core Features From Age 2 to 19 by Outcome Group

	ADI-R social communication	ADI-R RSM	ADI-R IS
Predictors	Coefficient (S.E.)	Coefficient (S.E.)	Coefficient (S.E.)
Fixed Effects			
Intercept	15.48 (1.86)***	3.20 (0.75)***	1.28 (0.51)*
Age at Testing	−0.19 (0.04)***	−0.01 (0.0)**	−0.00 (0.00)
Group:			
Very Positive (VPO)[a]	—	—	—
VIQ > 70 ASD	2.34 (2.17)	0.87 (0.87)	0.79 (0.59)
VIQ < 70 ASD	7.90 (2.00)***	1.99 (0.80)***	0.35 (0.55)
Linear Slopes:			
Age* VPO	—	—	—
Age* VIQ > 70 ASD	0.10 (0.05)*	0.00 (0.00)	0.00 (0.00)
Age* VIQ < 70 ASD	0.13 (0.05)**	0.01 (0.01)+	0.01 (0.003)**
Quadratic Slopes:[c]			
Age[b]	0.06 (0.01)**	—[b]	—[b]
Age[b,*] VPO	—	—	—
Age[b,*] VIQ > 70 ASD	0.01 (0.02)	—	—
Age[b,*] VIQ < 70 ASD	−0.04 (0.02)*	—	—
	Variance	Variance	Variance
Random Effects			
Intercept	13.04***	3.07***	1.31***
Slope	0.0002	0.0001*	0.0002***

N = 85; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; +$P < 0.10$.

[a]Dashes indicate reference group.

[b]Dashes indicate parameter was omitted from final model.

[c]Quadratic slope parameters are multiplied by 100.

Changes in ADI-R Repetitive Behaviors (RRBs)

Two subdomains of RRBs, Repetitive Sensory Motor behaviors (RSM) and Insistence on Sameness (IS) based on "current items" from each administration of the ADI-R were analyzed separately (see Table I). For RSM, there were significant differences at intercept between VPO who had the fewest RSM symptoms and ASD VIQ < 70 who had the most. As shown in Table III and Figure 2, linear slopes for all three groups indicated very gradual declines (i.e., improvements). Changes in these behaviors were much different than the quite marked changes in social communication deficits and social adaptation and for VIQ and NVIQ for both of the higher ability groups.

For Insistence on Sameness (IS), the only slope that changed significantly was for the participants with ASD VIQ < 70, where IS symptoms increased over time. There were no differences in intercept at two, but differences widened across the three groups as the children grew older. These groups were significantly different in previous analyses [Anderson et al., 2014] where we used normalized sets of all relevant items available at two and three for RRBs and then a different set at 19; rather than the single set of items available at all ages used here.

DISCUSSION

The present results offer geneticists and neuroscientists a number of behavioral phenotypes that provide a different approach to finding meaningful associations between behavior and outcomes in ASD than using static summary variables. We hope that this approach may also be useful to studies of the genetics of ASD and disorders that may overlap with ASD, such as syndromic or non-syndromic intellectual disability (ID) and Attention Deficit/Hyperactivity Disorder (ADHD), including individuals who do not necessarily meet standard ASD criteria.

One value of these trajectories is to help us determine which behaviors can be sensibly treated as categorical as opposed to those that are so continuous that division into subgroups is arbitrary and likely unreliable over time. Analyses of the distinctiveness of trajectories based on outcome provide us with evidence that, at least by school age, simple bimodal groupings of children with ASD can be created using verbal IQ, nonverbal IQ, social adaptive skills and parent-reported social-communication deficits. These groupings overlap but are not identical in membership. Moreover, the trajectories for more

cognitively able and less cognitively able children with ASD in these groups are clearly different from each other, though sometimes linear (NVIQ, ADI-R RSM and IS) and sometimes quadratic (VIQ, Vineland Social Adaptation, ADI-R Social Deficits). In addition, for Social Adaptation, Social-Communication Deficits and both forms of repetitive behavior, Repetitive Sensory Motor and Insistence on Sameness, trajectories sometimes starting in preschool, sometimes starting in early school age, and sometimes in later school age were different for the small group of participants with Very Positive Outcomes compared to young adults with ASD diagnoses at 19 who also had quite strong cognitive abilities. This offers the possibility of treating the VPO as a behavioral phenotype within ASD. Individuals with early diagnoses of ASD who have Very Positive Outcomes comprise a group that is not only defined by cognitive strengths, but also by early language [also see Tek et al., 2014] and repetitive sensory motor behaviors [Anderson et al., 2014] and later social behavior and insistence on sameness [also see Troyb et al., 2014] trajectories of improvement. How many other potential behavioral phenotypes, based on outcome, will eventually be defined for ASD, likely depends on the samples that we study and the variables we happen to measure. In any case, it seems clear that there is a statistically differentiable group of young adults with VPOs who have different trajectories on standardized measures, such as IQ and adaptive functioning, as well as social communication skills, than other individuals with ASD with higher IQs.

These and other analyses of both the same data and other samples indicate that significant changes in parent-reported and directly observed behavior occur in individuals with ASD into later adolescence and adulthood [Anderson et al., 2011; Smith et al., 2012; Howlin et al., 2013]. Therefore, attempts to link behavioral phenotypes with genetics may benefit from moving beyond cross-sectional data. In the present study, several of the trajectories were clearly differentiated by age 9, suggesting that for IQ, social adaptation, and RSM, longitudinal data from early into middle childhood may be sufficient to find effects. More detailed analysis of changes in receptive and expressive language in this sample [Pickles et al., 2014] found that, despite one small group of relatively "late bloomers" whose trajectories changed between 3 and 5, overall, there was little change in language trajectory after age 5 and most positive trajectories began to diverge from slower development from 2 to 3. This is not to say that there were not later changes in language development, but rather that there were no changes in relative level of delay after preschool years. On the other hand, the Fountain et al. [2012] study, with a much larger sample, but less detailed data, did find a significant number of what they called "bloomers" who had changes slightly later in preschool and early school age years. These differences call attention to limitations in the present study, which include a sample skewed to more frequently having intellectual disability compared to more recent U.S. samples [Center for Disease Control and Prevention, 2014], as well as our relatively small sample sizes here, particularly for the Very Positive Outcome group.

One explanation for why IQ often emerges as being associated with genetics findings may be its relative stability in school age populations compared to other behavioral constructs, though even IQ is not entirely stable [Bishop et al., 2014]. Other characteristics such as onset of seizures or attainment of early milestones are also appealing in this regard, though we have learned over the years that parent retrospective reports of these phenomena are subject to strong developmental factors and a potential source of misinterpretation [Hus et al., 2011; Jones et al., 2014]. From the data presented here, however, it is clear that attempts to draw links between genetics and behaviors described for children across a wide range of ages, without some attention to trajectory and level of development within individual children, may be misleading in a way that controlling for age effects at the time of data collection cannot address. This could also be why measures of whether RRBs ever occurred, as reported in the ADI-R, compared to current reports, have been more helpful than current scores in linkage studies. Nevertheless, because probands are still of different ages and at different points in trajectories at data collection in those studies, the difficulty is not completely avoided even when using a historical measure [Jones et al., 2014]. In a previous paper about a different sample [Bishop et al., 2006], we proposed that it was not necessarily as important to know IF a behavior was present (e.g., RSM or IS), but rather WHEN it was present, because of the clear interaction with NVIQ and age that exists for RRBs. For example, repetitive sensory motor behaviors in an intellectually able young child with autism may have a different meaning than repetitive sensory motor behavior that persists in an older child with or without significant delays.

Limited sample size and specific characteristics of this sample, in terms of including only children who were referred for possible autism at age 2, mean that it will be important to assess the degree to which similar trajectories are found in other samples of individuals with ASD, including more recently ascertained cohorts. Because we grouped the sample by outcome, the "end point" of the VPO group was predetermined, which also means that the slopes were different than previous analyses where we grouped children by how their trajectories clustered without specifying an endpoint [Richler et al., 2010; Gotham et al., 2012; Hus Bal et al., 2015]. Most important though is that these trajectories offer potential baselines to which individual slopes can be compared. It is possible that being "off" any of the usual trajectories for children with ASD, with or without intellectual disability, might be particularly useful as a unique phenotype for children with specific genetic or other etiologies. To this end, further refinement of the ASD diagnostic domains of social-communication or RRBs into more specific, reliably measured areas of

impairment will be useful for understanding where the trajectories of children with non-ASD diagnoses, such as language disorders, intellectual disability or ADHD, meaningfully diverge from children with ASD of the same age and level of cognitive ability.

A number of recent papers have described trajectories in early development of ASD [Yoder et al., 2009; Lord et al., 2012; Mayo et al., 2013; Chawarska et al., 2014; Luyster et al., 2014] and followed them into preschool years. They have shown that trajectories in very young children are even more variable within shorter periods of time than they are across much longer age spans, such as in the present paper. One difference is that most of these children did not have intellectual disabilities and many had milder symptoms than the two year-olds in our sample. A major conclusion is that the cases of "regressive autism" are not as well defined as previously expected, and that the course of early development in ASD is better characterized by variation in timing of the acquisition of prosocial behaviors, the emergence of "positive" autism symptoms, including repetitive sensory motor behaviors, plateaus in communicative development, and fairly frequent loss of social attention and engagement than a single across the board regressive shift [Ozonoff et al., 2010]. Nevertheless, other aspects of variation in these trajectories [Charwarska et al., 2014], particularly if they are shown to interact with responsiveness to treatment, are also of interest in terms of plasticity and neurobiological function.

In summary, our findings offer a number of potential behavior phenotypes for geneticists and other neurobiologists interested in autism and related disorders and developmental delays. Early trajectories in verbal IQ and nonverbal IQ are associated with what, by adulthood and even by middle childhood, will be very significant differences in functioning, but which do not account completely for differences in outcome in ASD. Linear trajectories in repetitive sensory motor behaviors beginning in early childhood also separate individuals with ASD with higher cognitive abilities by adulthood from those with lower IQs. Trajectories from preschool to school age years in social adaptation, and in social deficits and insistence on sameness into teen years and young adulthood, are related to significant differences in independent functioning and lack of comorbidity, at least with a sample of individuals with early diagnoses of ASD and no intellectual disability in adulthood. As we showed earlier in Figure 1, we are also hopeful, at least for restricted and repetitive behaviors, that measures that are less time consuming and difficult to administer than the ADI-R, such as the RBS-R [Bodfish et al., 1999], may present more scalable opportunities to measure such changes. The Vineland Adaptive Behavior Scales [Sparrow et al., 2005] also offers a parent reported measure of social adaptation that can provide critical information about social development. This information yielded is not the same as a detailed description of autism-specific social deficits, such as in the ADI-R, but is important in its own right. The Autism Diagnostic Observation Schedule [ADOS 2: Lord et al., 2000], because it is not dependent on parent report and is much less affected by level of verbal and cognitive function offers another alternative for documenting change in social deficits and skills over time [Gotham et al., 2012] but is limited, like the ADI-R, by the need for administration by a trained examiner. To date, other potentially useful instruments describing social and communicative functioning in ASD and related disorders, such as the Social Responsiveness Scale, 2nd Edition [SRS 2: Constantino and Gruber, 2012] and the Children's Communication Checklist-2 [Norbury et al., 2004], have not yet been shown to be sufficiently specific in describing social function to be valid measures of social skills or deficits [Charman et al., 2007; Aldridge et al., 2012; Hus et al., 2013]. It has been much more difficult to find instruments that provide empirically supported factors of social communication in ASD samples that are not highly correlated with other factors, such as repetitive behaviors, behavior problems, and IQ, than we would have expected [Berument et al., 1999; Frazier et al., 2014]. Thus, researchers cannot just choose items that sound like they correspond to domains such as social skills or RRBs from standardized measures and assume that they are independent of the other domain, age, IQ or behavior problems [Hus et al., 2013]. On the other hand, given the scalability of instruments such as the SRS and CCC-2, it is certainly worth testing if they have value in representing trajectories in more general behavior dysfunction and delay that, like Vineland II scores, in themselves may be quite important. Thus, there is still much to be learned about quantifying behavior, but we can also now see some clear directions for behavioral phenotypes based on trajectories of development.

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