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In Pursuit of Neurophenotypes: The Consequences of Having Autism and a Big Brain

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Abstract

A consensus has emerged that despite common core features, autism spectrum disorder (ASD) has multiple etiologies and various genetic and biological characteristics. The fact that there are likely to be subtypes of ASD has complicated attempts to develop effective therapies. The UC Davis MIND Institute Autism Phenome Project is a longitudinal, multidisciplinary analysis of children with autism and age-matched typically developing controls; nearly 400 families are participating in this study. The overarching goal is to gather sufficient biological, medical, and behavioral data to allow definition of clinically meaningful subtypes of ASD. One reasonable hypothesis is that different subtypes of autism will demonstrate different patterns of altered brain organization or development i.e., different neurophenotypes. In this Commentary, we discuss one neurophenotype that is defined by megalencephaly, or having brain size that is large and disproportionate to body size. We have found that 15% of the boys with autism demonstrate this neurophenotype, though it is far less common in girls. We review behavioral and medical characteristics of the large-brained group of boys with autism in comparison to those with typically sized brains. While brain size in typically developing individuals is positively correlated with cognitive function, the children with autism and larger brains have more severe disabilities and poorer prognosis. This research indicates that phenotyping in autism, like genotyping, requires a very substantial cohort of subjects. Moreover, since brain and behavior relationships may emerge at different times during development, this effort highlights the need for longitudinal analyses to carry out meaningful phenotyping.

Keywords

brain development; magnetic resonance imaging; megalencephaly; phenotype; subtypes

Introduction

Due to the rapid pace of research on autism spectrum disorder (ASD) over the last 20 years, much has been learned about its biological characteristics. Rather than the picture becoming clearer, however, a consensus has emerged that ASD is an incredibly complex and

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heterogeneous disorder [Constantino & Charman, 2016]. There would be little argument with the assertion that there are many causes and trajectories of ASD. One indication of the complexity comes from research on the genetic architecture of the disorder. Over the last 30 years or so, the field has moved from not knowing any genetic mutations that put an individual at risk for autism to the current state where at least 100 genes are known to confer risk of ASD [Geschwind & State, 2015; Sanders et al., 2015]. Current predictions are that ultimately more than 1,000 genes may be involved in the risk for ASD [De Rubeis et al., 2014]. It is also known that genetic risk may only account for half of the causes of ASD with environmental factors playing an equally prominent role [Hallmayer et al., 2011]. Similarly, there are a whole host of co-occurring conditions that differentiate subgroups of individuals with autism. On the order of 20–30% of individuals with autism will have co-occurring epilepsy [El Achkar & Spence, 2015; Spence & Schneider, 2009] during their lives and an equal number will have troubling gastrointestinal [Buie et al., 2010] or sleep [Accardo & Malow, 2015] issues. This enormous heterogeneity has seriously hampered efforts to develop effective pharmacological treatments for the core symptoms of ASD as attested to by failures of recent clinical trials [Erickson et al., 2014]. There is no doubt that identifying clinically meaningful subtypes of ASD would facilitate both the analysis of etiologies as well as more productive clinical trials of therapeutics [Beversdorf & Missouri Autism Summit, 2016].

The UC Davis MIND Institute launched the Autism Phenome Project (APP) in 2006. The overarching goal of this longitudinal project is to gather sufficient behavioral and biological information on a large enough population of children with autism starting in early childhood to identify clinically meaningful subtypes. The APP recruits children shortly after diagnosis —at 2–3 1/2 years of age—and involves a multidisciplinary and longitudinal analysis of affected children and their family members. There are very few exclusion factors for participation in the study, and both boys and girls are included. Children with a diagnosis of ASD are recruited, as well as a cohort of age-matched typically developing (TD) children. Thus far, approximately 400 families are participating in the study, which is ongoing. More information about the Autism Phenome Project can be found in previous publications [Libero et al., 2016; Nordahl et al., 2011, 2012; Ohta et al., 2016].

One of the unique features of this study is that all children undergo longitudinal magnetic resonance imaging studies of their brains. Many children are imaged at roughly 3, 4, and 5 years of age (Times 1, 2, and 3), and we are currently carrying out a fourth imaging session when children enter middle childhood (9–12 years old). While we are examining an abundance of data in a variety of ways, we have particularly focused on patterns of brain organization and development as one strategy of defining subtypes i.e., neurophenotyping. Given that ASD is a neurological disorder, one could reasonably hypothesize that alterations in brain organization or developmental trajectory might be strong biological indicators of autism subtypes that would be associated with different patterns of behavioral symptoms or co-occurring conditions. We have divided our cohort into a variety of subgroups based on differences in brain organization or development, but for the purpose of this article we will focus on one identifiable subtype—children with autism and very large brains.

Definition of Disproportionate Megalencephaly

It has been known for many years that children with ASD, as a group, have precocious growth of their heads and brains early in development [Courchesne et al., 2001; Lainhart et al., 1997; Piven et al., 1995; Redcay & Courchesne, 2005]. However, it was not known until recently what percentage of children with ASD demonstrate this neurophenotype. How one defines an enlarged brain is somewhat arbitrary, but we have elected to define this neurophenotype as disproportionate megalencephaly (ASD-DM) with inclusion based on having a total cerebral volume (defined as total brain minus the brainstem and cerebellum [Nordahl et al., 2011]) to height ratio that is 1.5 standard deviations above the mean of sex-matched typically developing controls in the Autism Phenome Project. We have used the ratio of brain size to height based on indications that body size is often larger in children with autism. When we formally evaluated this [Nordahl et al., 2011], we actually did not find that the children with enlarged brains were generally taller. Nonetheless, this is a more conservative way to define enlarged brain size. By this definition, at the first time point that MRIs are carried out, approximately 15% (N = 19 subjects to date[1]) of the boys with ASD in the APP have this neurophenotype [Nordahl et al., 2011]; the remainder of the boys with ASD have brains in the normal range[2] (ASD-N) or, for a small minority, had smaller than expected (microencephalic) brains (Fig. 1). Six percent of the typically developing boys had brain/height ratios in the megalencephalic range and we will return later to a discussion of the potential benefits of a large brain. Figure 1 presents these findings graphically and it is clear that while there is substantial overlap of the brain to height ratios for children with autism and typical development, the 15% of boys with disproportionate megalencephaly (red dots) form a substantial subgroup. Interestingly, far fewer girls with autism (4%) have ASD-DM. Because of this, and because we have data on a much larger cohort of males, the remainder of this paper will focus on the male subjects of the APP.

We have recently evaluated the distribution of brain volume to height in the population of boys in the Autism Phenome Project more comprehensively and over the first three imaging time points [Libero et al., 2016]. As indicated in Table 1, there are 19 boys in this category at Time 1, 15 at Time 2 and 11 at Time 3. At Time 1, the distribution of total cerebral volume to height is broader for the children with autism than for typically developing children (Fig. 2). It also appears that there is a shift in the distribution so that higher percentages of children with ASD have larger TCV/height ratios. We also asked the question of whether the enlarged brains in children with ASD-DM persisted across the 3–6 year age range. We found that the ASD-DM group had significantly greater TCV than the ASD-N and TD groups at Time 1 (F[2,171] = 39.25, P < .001), Time 2 (F[2,115] = 29.59, P < .001), and Time 3 (F[2,90] = 26.01, P < .001) (Table 1). In contrast, the ASD-N group did not significantly differ from the TD group for TCV at any of the three time points [Table 1; Libero et al., 2016]. Furthermore, the ASD-DM boys had a significantly greater rate of growth between Times 1 and 3 compared to the TD boys (t[205] = 2.52, P = .01) and a marginally increased rate of growth compared to the ASD-N boys (t[138] = 1.88, P = .062;

[1]Table 2 presents the numbers of subjects that make up the percentages reported here and throughout the article.
[2]While we used the abbreviation ASD-N for children with autism and normal-sized brains, we do not imply that there are no other alterations in the structure, connections, or functions of the brains in these children.

Fig. 3). Interestingly, the ASD-N boys did not significantly differ from TD controls in their overall rate of growth for TCV ($t[205] = 1.17$, $P = .24$) [Libero et al., 2016]. We have also evaluated whether the cerebral cortex is thicker and/or has more surface area in the ASD-DM group and our analyses clearly demonstrated that the cortex is not thicker but that there is more of it i.e., more surface area [Ohta et al., 2016]. This is not to say, of course, that the increased brain size is due entirely to cortical gray matter since previous studies had demonstrated enlargements of the white matter compartment as well [Nordahl et al., 2011].

We were also able to evaluate head circumference for the APP cohort based on data extracted from medical records as well as direct measurements during research visits (Fig. 4). We found that there were no group differences in the three groups (ASD-N, ASD-DM, and TD) in head circumference at birth. However, the ASD-DM group reached a significantly larger head size at three years of age compared to both the ASD-N ($t[126] = 4.40$, $P < .0001$) and TD ($t[65] = 6.01$, $P < .0001$) groups [Libero et al., 2016].

In the following sections, we will describe a variety of characteristics of all three groups of the boys when they received either their first MRI, at about 3 years of age (Time 1), or when they received their third MRI (Time 3) when they were around 5 years of age. Detailed descriptions of the characteristics of the Autism Phenome Project cohort as well as the methodology used to carry out magnetic resonance imaging have been presented in previous publications [Libero et al., 2016; Nordahl et al., 2011, 2012; Ohta et al., 2016]. It is important to point out that the three groups were very closely matched on age (Table 1; ASD-N: mean 37.6 months, SD 6.13; ASD-DM: mean 36.9, SD 5.44; TD: mean 36.0, SD 4.65).

Cognitive and Clinical Consequences of ASD-DM *IQ*

After identifying the megalencephalic subgroup, we attempted to determine whether the behavioral/developmental characteristics of the ASD-DM group differed from the other boys with ASD and from the TD boys. At Time 1, there was no significant difference in the mean developmental quotient (DQ) between the ASD-DM and ASD-N groups (Table 1; Tukey post hoc test $F[2,175] = 88.3$, $P = .28$) as measured by the Mullen Scales of Early Learning [Mullen, 1995]. Nor did we find that there was a difference in the percentage of boys with DQ less than 70 in the ASD-DM and ASD-N groups (Table 2; $P = .42$).

IQ was assessed again at Time 3, when the boys were approximately 5-years-old, using the Differential Ability Scales [Elliot, 2007]. By this time, the ASD-DM group had a significantly lower mean IQ compared to the ASD-N group (Table 1; $F[2,111] = 27.4$, $P = .03$). There was also a higher percentage of boys with IQ less than 70 in the ASD-DM group (63.6%), compared to the ASD-N group (31.3%) (Table 2; $P = .05$). Interestingly, this appeared to be due to the substantial improvement of over 20 points in IQ in the ASD-N group to a mean of 84. The ASD-DM group also had an improvement in IQ, although it was not as substantial (about 12 points), and the mean IQ at the later time point was 68—still in the range of intellectual disability (Table 1). Panels A and C of Figure 5 illustrates that the change over time in IQ scores was heterogeneous in both groups with some children showing substantial gains while others showed either no gain or losses between Time 1 and

Time 3. However, the mean IQ change in the ASD-N group was +14.7 whereas that for the ASD-DM group was only +1.53 (Fig. 5 panel A).

A longitudinal analysis of IQ using a linear mixed effects model showed that the ASD-N group had a significantly greater rate of change in IQ between Times 1 and 3 (Fig. 5D) compared to the ASD-DM group ($z = -2.36$, $P = .02$). The IQ in the ASD-N group increased significantly by 7.6% at a rate of 5.3 points in IQ per year whereas the IQ in the ASD-DM group increased minimally by 0.55% at a rate of 0.38 points in IQ per year. It appears that while the brain size of the ASD-DM group continues to increase at a significantly greater rate than the ASD-N group, this does not translate into an increase in IQ over time.

Substantial evidence demonstrates a positive correlation between brain size and IQ in unimpaired groups [Pietschnig, Penke, Wicherts, Zeiler, & Voracek, 2015]. We had previously found a similar correlation between hippocampal volume and IQ in a study of typical subjects that predated the APP [Schumann et al., 2007]. When we looked for this relationship between total cerebral volume and IQ in the typically developing boys within the APP, we find the expected positive correlation (Fig. 6; $r[47] = 0.35$, $P = .02$). However, for the ASD-DM group, there was no relationship between brain size and IQ ($r[17] = -0.18$, $P = .46$). In other words, the larger brain size in the ASD-DM group is not associated with enhanced intellectual functioning as it is in typically developing children. Even in the ASD-N group, there is no indication that a larger brain size correlates with higher IQ ($r[108] = 0.011$, $P = .91$). Since there are many biological processes that can lead to increased brain size, one could provisionally conclude from these findings that normal enlargement in typically developing children leads to enhanced cognitive processing whereas presumably pathological enlargement in the children with ASD confers no such advantage. This is obviously an oversimplification that needs refinement when more is known about the underlying neurobiology of brain overgrowth in ASD.

Autism Severity

We next evaluated whether the ASD-DM and ASD-N groups differed in autism severity. Utilizing the Autism Diagnostic Observation Schedule-Generic (ADOS-G) [DiLavore, Lord, & Rutter, 1995; Lord et al., 2000] we found no difference in ADOS severity scores at Time 1 or Time 3 (Table 1; Time 1: $P = .71$; Time 3: $P = .83$) between boys with ASD-DM and ASD-N. There was also no difference in mean change in ADOS severity scores over time (from Time 1 to Time 3). Moreover, within each ASD subgroup, there was no correlation between ADOS severity score and total cerebral volume to height ratio (Fig. 7; Time 1: ASD-DM $r[17] = -0.28$, $P = .24$, ASD-N $r[108] = -0.03$, $P = .72$; Time 3: ASD-DM $r[7] = -0.35$, $P = .36$, ASD-N $r[46] = 0.09$, $P = .56$).

Adaptive Functioning

The Vineland Adaptive Behavior Scales-II (VABS-II) was also collected at Time 1 and Time 3 (Table 3) [Sparrow & Cicchetti, 1985]. While a cross-sectional analysis of the Vineland Adaptive Behavior Composite scores at Time 1 and Time 3 did not demonstrate any significant mean differences between the ASD-DM and ASD-N groups, a linear mixed effects model showed that the ASD-DM group had a significantly different rate of change

between Times 1 and 3 compared to the ASD-N group ($z = -2.40$, $P = .016$). The Vineland Adaptive Behavior Composite score in the ASD-DM group decreased by 4.7% at a rate of 3.6 points per year whereas the ASD-N group remained unchanged over this time period (Fig. 8).

Language

One area that we did observe a significant difference in behavior was performance on language assessments. All of the APP subjects were grouped, based on the MSEL expressive language and receptive language raw scores, into children who had low language with few to no words (i.e., receptive language and expressive language scores of 16 or below), versus children who had some language. The latter ranged from having a stable lexicon of nouns to those who had a large vocabulary with receptive language or expressive language scores above 16. We found that there was a higher percentage of boys with ASD-DM who were in the low language group at Time 1 (Table 2; Fig. 9). Seventy-four percent of the boys in the ASD-DM group had low language versus 45% of the boys in the ASD-N group ($P = .03$).

Onset Status

A final behavioral observation was that a larger number of the ASD-DM group had a regressive component to the onset of their ASD (Fig. 10). We have used the term regression to mean a loss of previously acquired skills in language and/or social domains [Nordahl et al., 2011]. The occurrence of regression was based on parent reports via the Autism Diagnostic Interview-Revised [Lord, Rutter, & Le Couteur, 1994] and validated by the Early Development Questionnaire (see [Nordahl et al., 2011] for detailed description). While regression occurred in 52% of the boys with ASD-N, 84% of the ASD-DM boys had a regressive component to the onset of their autism ($P = .01$; Fig. 10).

Medical and Biological Characteristics of ASD-DM

Genetics

Analysis of potential genetic risk markers in the APP cohort is ongoing. In preliminary studies carried out in collaboration with the Eichler laboratory, one of the ASD-DM subjects was found to have a loss of function mutation of the CHD8 gene and was included in a recent paper highlighting the phenotypic characteristics of individuals with mutations of this gene [Bernier et al., 2014]. This rare mutation was not observed in any other of the ASD-DM cohort. While at least five other significant mutations were identified in the APP cohort, these were observed in the ASD-N rather than the ASD-DM group. Thus, while genetic studies are still at an early stage, we have not yet observed a consistent pattern of genetic alterations that differentiates the ASD-DM from the ASD-N groups.

Exposure to Maternal Antibodies

Because of our interest in the potential role of exposure to unique maternal antibodies as one etiology of ASD (Braunschweig & Van de Water, 2012; Fox, Amaral, & Van de Water, 2012; Martin et al., 2008), we investigated whether the proportion of boys whose mothers tested positively for the presence of antibodies associated with autism etiology [Braunschweig et al., 2008, 2013] was different in the ASD-DM and ASD-N groups. Van de

Water and colleagues have observed that as many as 23% of women that have had a child with autism demonstrate anti fetal brain autoantibodies [Braunschweig et al., 2013] and have proposed that these antibodies may produce a form of maternal antibody related autism. The majority of mothers involved in the APP have been evaluated for the presence of antibodies that identify proteins from the fetal brain at 37, 39, and 73 kDA. We have found that it was significantly more likely for the boys with ASD-DM to have been born to a mother that has these antibodies than the boys with ASD-N (Table 2 and Fig. 11). Nearly 18% of the ASD-DM boys had such an exposure whereas just 3% of the ASD-N boys did so ($P = .03$). This is consistent with an earlier study [Nordahl et al., 2013] that demonstrated that the brains of children exposed to autism-associated antibodies were larger than in children with autism that were not exposed. This is also of interest because a nonhuman primate model in which fetal monkeys were exposed to the same antibodies derived from women who have had children with autism also show enlarged brain size if male, but not if female [Bauman et al., 2013]. These results raise the possibility that exposure to these maternal antibodies may contribute to the enlarged size of the brains in some of the boys in the ASD-DM group, but also suggests that exposure to the antibodies does not necessarily lead to the megalencephalic neurophenotype.

Other Medical Issues

Because we have obtained comprehensive medical records for the children enrolled in the APP, we were able to investigate whether the ASD-DM group differed from the ASD-N group on a variety of medical factors. In sum, the ASD-DM group had neither more nor less occurrences of a large array of medical conditions ranging from febrile seizures, to eczema, to acid reflux. However, we did find a significant difference in the mean gestational age between the two groups (ASD-DM mean: 39.79 weeks, ASD-N mean: 38.73 weeks; $F[2,124] = 3.60$, $P = .03$). We also investigated gestational issues such as the presence of obesity and diabetes but these did not relate to group membership. In addition, maternal and paternal age were not significantly different for the ASD-DM and ASD-N groups (maternal age: $F[2,171] = 0.92$, $P = .40$; paternal age: $F[2,161] = 2.40$, $P = .09$).

Conclusions

Amassing sufficient behavioral and biological data to define clinically meaningful subtypes, or perhaps more conservatively variants, of autism spectrum disorder is a time and labor-intensive process. The goal of this endeavor is to employ biological stratification in order to facilitate subsequent analyses of etiology and to help to discover preventive measures and more targeted therapeutic interventions. It is not clear at this point what portion, or portions, of a multidimensional dataset will be most helpful in parsing autism spectrum disorder. In this Commentary, we have briefly summarized our efforts in the Autism Phenome Project to mine MRI data on the structure of the brain as one strategy for defining neurophenotypes. We have focused on the big brain form of autism in boys as an exemplar of this approach. But, we suspect that there may be several other differences in brain organization or development that will potentially define other neurophenotypes. For example, we have identified three patterns of growth of the amygdala in boys with autism [Nordahl et al., 2012] and suspect that they may be related to the emergence of symptoms of anxiety. We are

currently exploring this possibility as the APP children return to the MIND Institute for further testing in middle childhood. We are also currently recruiting additional females with autism into the APP in order to further explore whether girls exhibit a big brain phenotype and whether there are other sex-specific neurophenotypes.

In the effort to correlate patterns of brain organization with the symptoms of autism, a number of principles have emerged.

1. The correspondence between patterns of altered brain organization or growth and behavior is difficult to establish. Despite the substantial differences in total brain volumes of the brains in ASD-DM and ASD-N boys, we did not detect any differences in autism severity. But, we did find that the ASD-DM boys have slower gains in IQ and greater difficulties with expressive language.

2. The time course of altered brain development may not map onto the emergence of behavioral differences. A large majority of the ASD-DM boys had a regressive component to the onset of their autism. However, analysis of head circumference data indicates that their altered brain growth began at 4–6-months of life [Nordahl et al., 2011], long before their behavioral regression. Similarly, while disproportionate megalencephaly was already apparent at three years of age when the children were first assessed, there was no significant IQ difference in the ASD groups at this age. It was not until the children were 5-years-old that the IQ deficit in the ASD-DM boys became evident. This highlights the need for comprehensive longitudinal studies to carry out sensitive brain/behavior associations.

3. As the field has come to the realization that increasingly larger numbers of subjects must be recruited to understand the genetic architecture of ASD, it appears to be equally true for efforts at sensitive phenotyping. While nearly 200 boys with autism were involved in the MRI studies summarized in this Commentary, a substantial number by existing standards for ASD neuroimaging studies, this was barely sufficient to define the ASD-DM neurophenotype. The under-representation of girls with autism still leaves open the question as to whether this neurophenotype is really less common in girls with autism. There are also indications that the ASD-DM group is fractionating in terms of IQ over time; the majority are showing very little improvement whereas a minority have near normal IQ. This highlights the need for very large samples of children that are followed longitudinally to acquire the high quality MRI data that are essential to charting the altered brain growth associated with different forms of ASD.

4. Evaluation of the boys in the ASD-DM group are ongoing. But, the data acquired thus far suggest that the ASD-DM boys have more severe cognitive deficits and, therefore, a more guarded prognosis than the ASD-N boys. This is true even though our analyses indicate that they have received as much behavioral therapy as the ASD-N children. If this conclusion is validated in the continued follow-up studies of these children, it would suggest that they will need more intensive and perhaps different forms of intervention in order to maximize their potential and quality of life. We believe that this would be a clinically meaningful finding that

would go some way toward validating this approach and justifying the cost both to the funding agencies and to the families who have made the Autism Phenome Project a reality.

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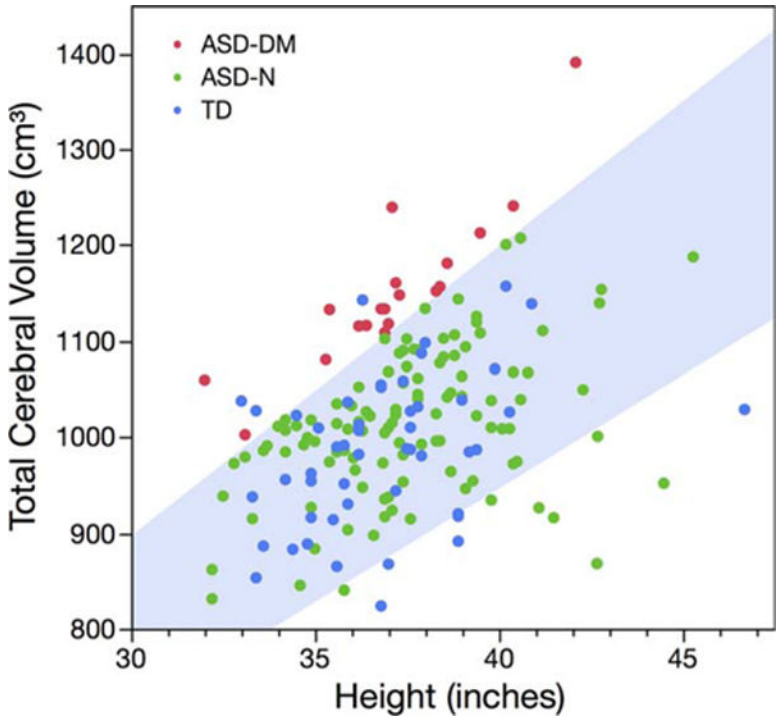

Figure 1.

Ratio of total cerebral volume to height for boys with ASD and disproportionate megalencephaly (ASD-DM, $N = 19$), boys with ASD and normal brain size (ASD-N, $N = 110$) and typically developing (TD, $N = 49$) boys at Time 1 in the Autism Phenome Project. The blue shaded region indicates values within 1.5 standard deviations of the typically developing mean. A TCV to height ratio above the upper boundary of this region is what we have defined as disproportionate megalencephaly.

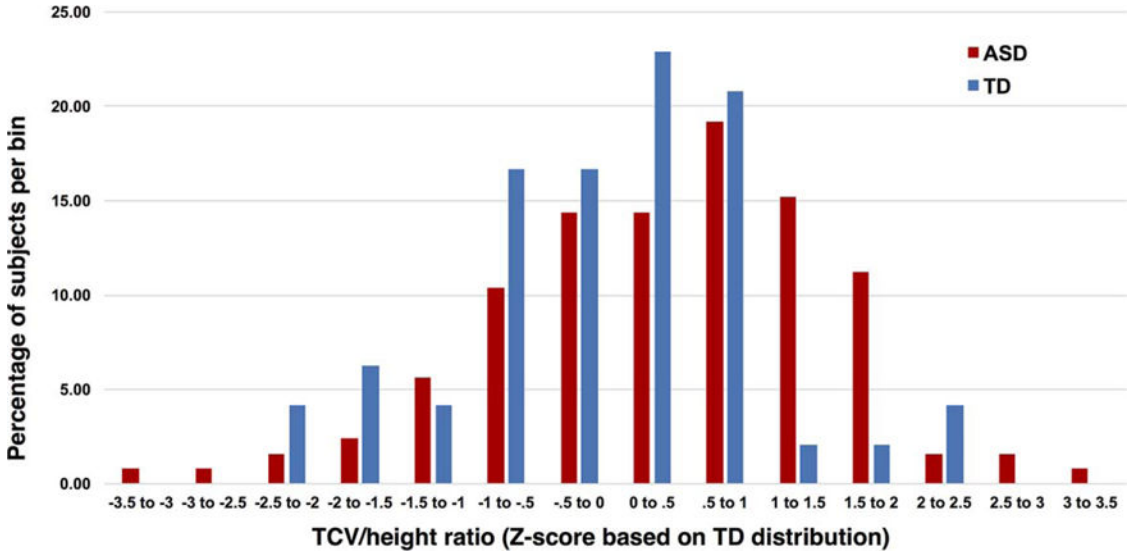

Figure 2.

Distribution of subject total brain volume (TCV) to height ratios and the percentage of APP boys (ASD and TD) in each bin. Reproduced from Libero et al. [2016].

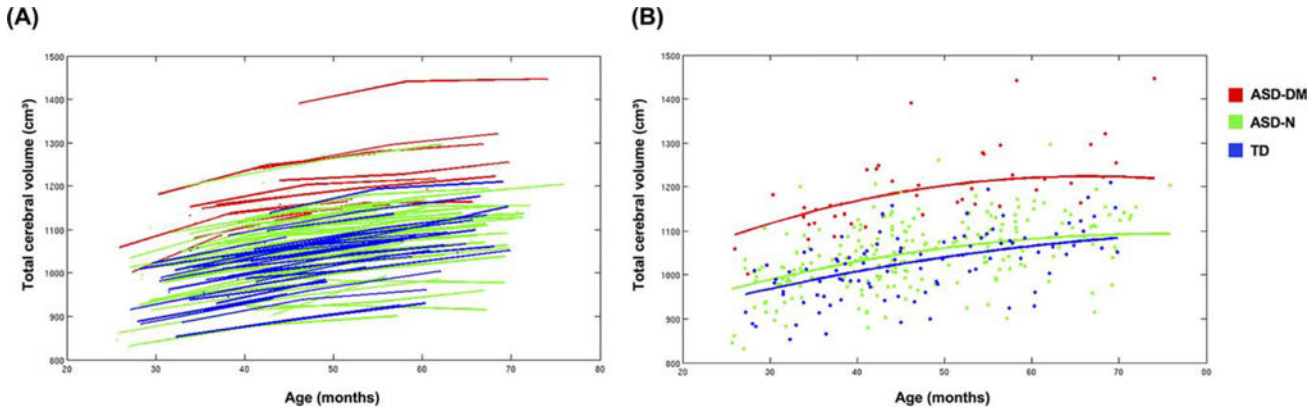

Figure 3.

Longitudinal analyses of total cerebral volume for subgroups. (**A**) Total cerebral volume data for the three subgroups: ASD-DM, ASD-N, and TD boys. Each line connects the data points from each individual subject. The ASD-DM group (red) falls in the upper end of the distribution, while there is almost complete overlap between the ASD-N (green) and TD (blue) participants. (**B**) Individual subject data are represented as individual data points. The lines represent the predicted growth trajectories of total cerebral volume for each of the three subgroups, ASD-DM, ASD-N, and TD. ASD-DM boys had significantly greater TCV at the earliest age and also a significantly greater rate of growth from ages 3 to 5 years, compared to ASD-N and TD boys. Reproduced from Libero et al. [2016].

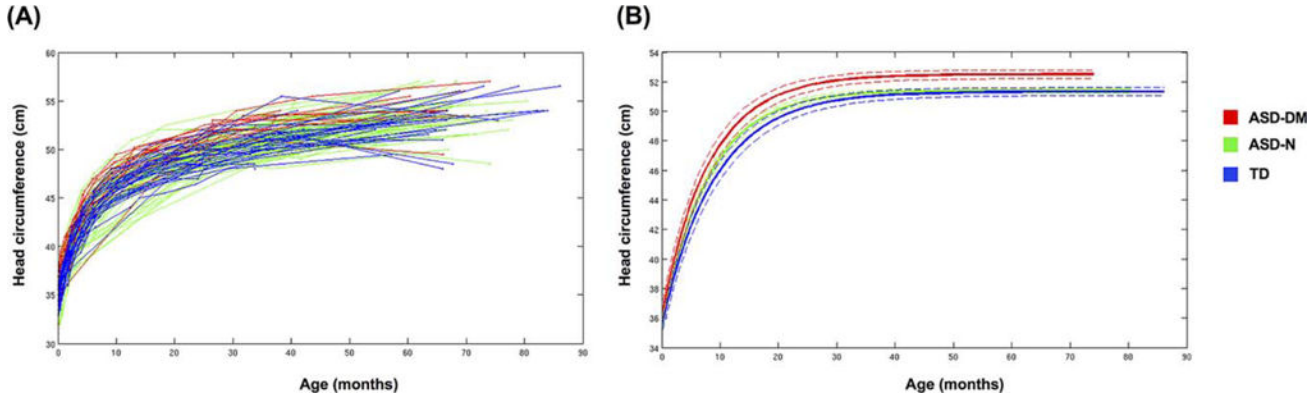

Figure 4.

Longitudinal analyses of head circumference for subgroups. (**A**) Data for head circumference for participants from each of the three subgroups: ASD-DM, ASD-N, and TD. Each line connects the data points from each individual subject. The ASD-DM group (red) fall in the upper end of the distribution, while there is a large amount of overlap between the ASD-N (green) and TD (blue) boys. (**B**) Predicted growth trajectories for head circumference for each of the three subgroups. Dashed lines indicate the lower and upper bounds of the 95% confidence intervals. The ASD-DM group did not differ in head circumference at birth, but reached a significantly larger head size compared to the ASD-N and TD boys. Reproduced from Libero et al. [2016] as in Figures 2 and 3.

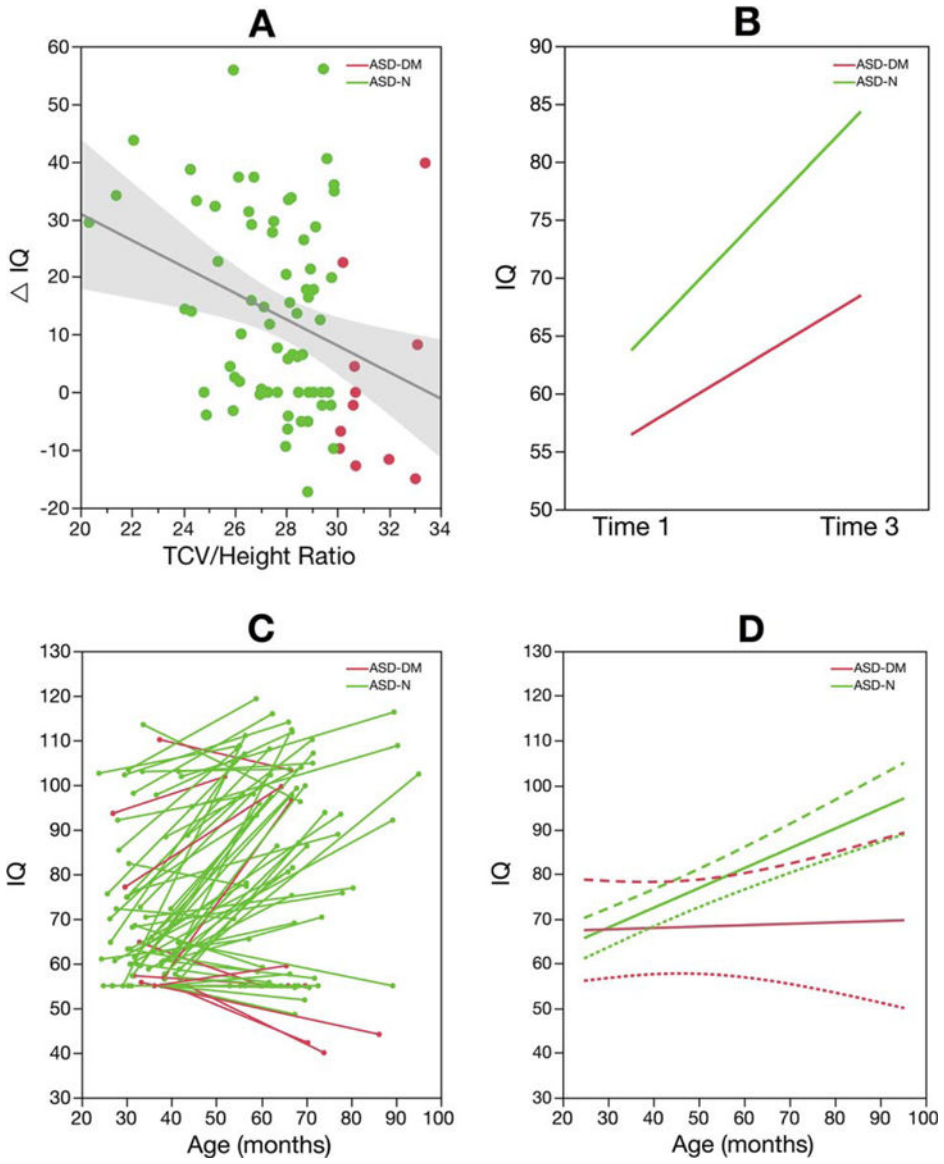

Figure 5.

IQ measurement was assessed using the Mullen Scales of Early (MSEL) Learning during the Time 1 evaluations and the Differential Ability Scales (DAS) during the Time 3 evaluations of the APP boys. (**A**) Change in IQ scores (utilizing standard scores from the MSEL and DAS) from Time 1 to Time 3 in the ASD-DM (red) and ASD-N (green) groups. Each dot represents an individual subject. The gray line illustrates the negative correlation ($t(76) = -0.315$, $P = .005$) between change in IQ scores and the TCV/height ratio among all ASD subjects. The gray region represents the 95% confidence interval of this relationship. (**B**) Mean increase in IQ from Time 1 to Time 3 in the ASD-DM and ASD-N groups. (**C**) Observed trajectories of IQ change for the ASD-DM and ASD-N groups. Each line connects the data points from each individual subject. (**D**) Predicted trajectories of IQ change for the ASD-DM and ASD-N groups (solid line). The dashed lines represent the upper limit of the 95% confidence interval for the two groups while the dotted lines represent the lower limit.

The ASD-N group had a significantly greater rate of IQ change compared to the ASD-DM group.

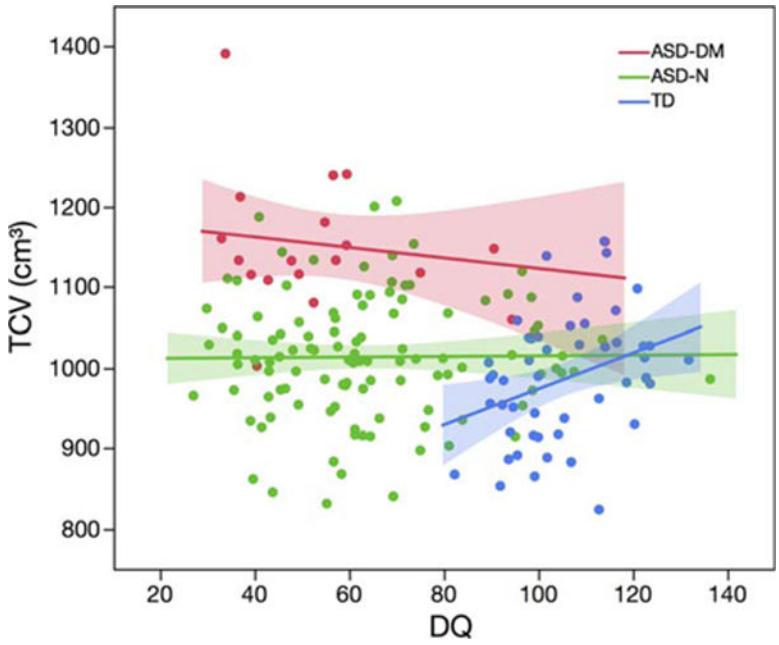

Figure 6.

Relationship between total cerebral volume (TCV) and developmental quotient (DQ) at Time 1. For the typically developing boys (in blue), there is a positive correlation ($r(47) = 0.35$, $P = .02$) between total cerebral volume and DQ. In contrast, for the ASD-DM group (in red) as well as the ASD-N group (in green) there is no significant association between brain size and DQ.

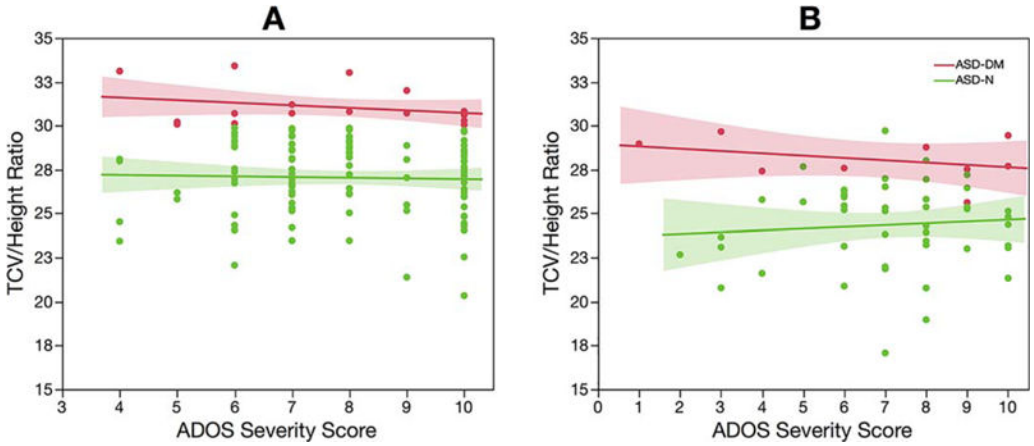

Figure 7.

Relationship between the TCV/height ratio and the ADOS severity score at Time 1 (**A**) and Time 3 (**B**) evaluations. No correlation was observed for either subgroup at either time point.

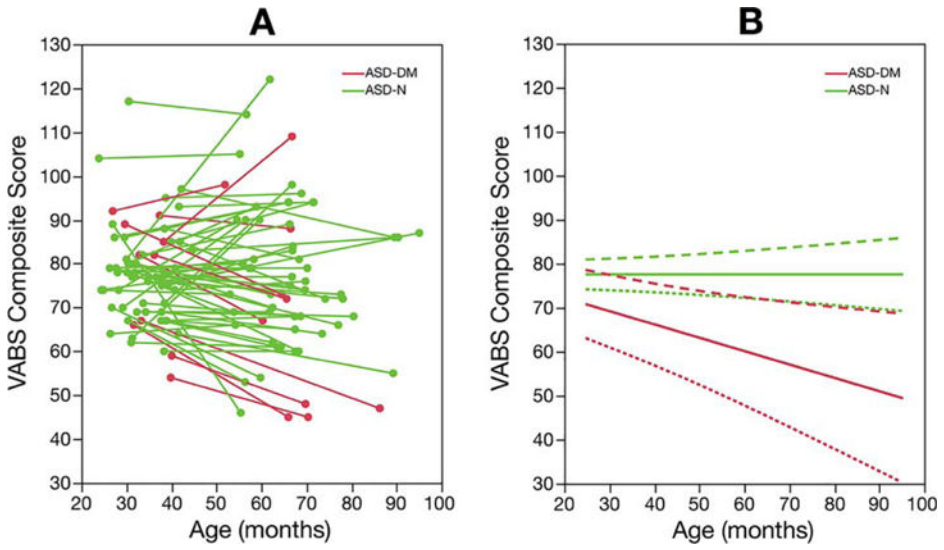

Figure 8.

(**A**) Observed trajectories of Vineland Adaptive Behavior Scales (VABS) Adaptive Behavior Composite scores between Time 1 and 3 for the ASD-DM and ASD-N groups. Each line represents an individual subject. (**B**) Predicted trajectories of VABS Adaptive Behavior Composite scores for the ASD-DM and ASD-N groups (solid line). The dashed lines represent the upper limit of the 95% confidence interval for the two groups while the dotted lines represent the lower limit. The ASD-DM group had a significant decrease in the rate of change compared to the ASD-N group.

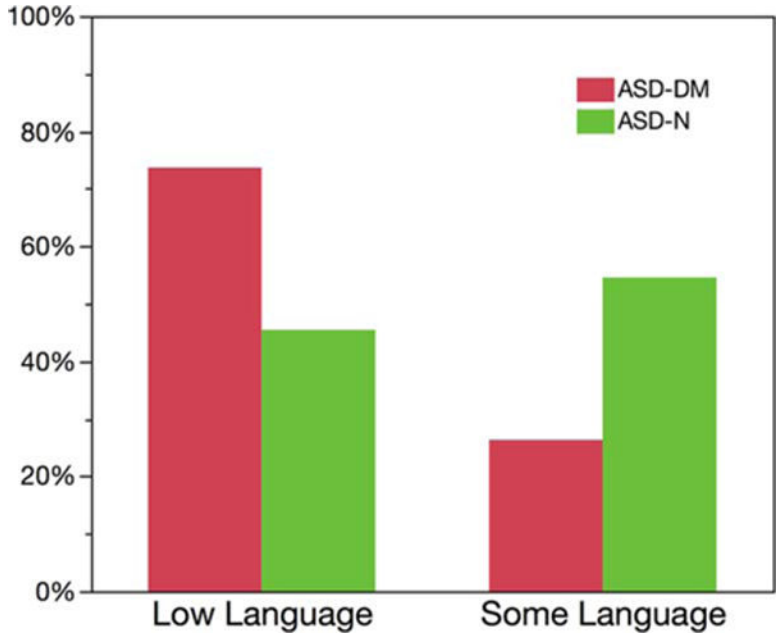

Figure 9.

The percentage of boys with ASD-DM (red) or with ASD-N (green) who were classified as having either low language or having some language ability.

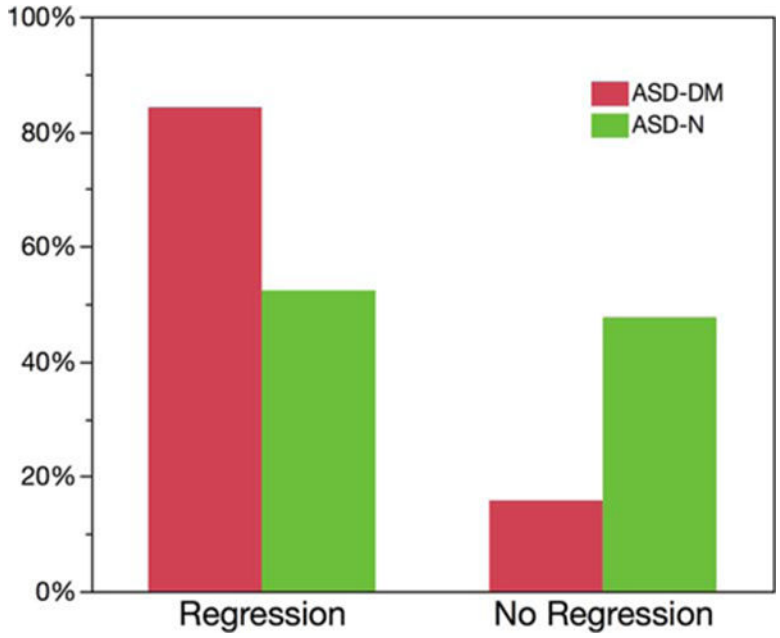

Figure 10.

The percentage of boys with ASD-DM (red) and ASD-N (green) who had a regressive versus no regressive component reported for the onset of their autism.

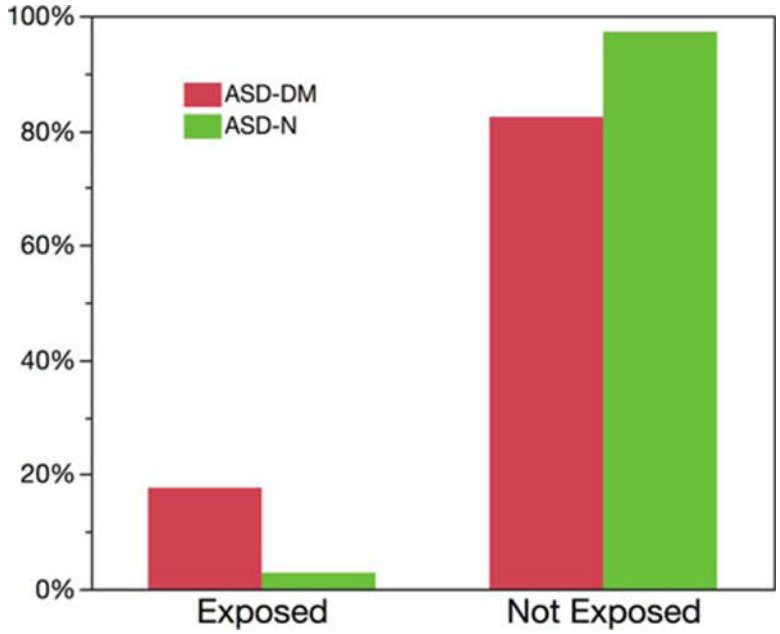

Figure 11.

The percentage of boys with ASD-DM (red) and ASD-N (green) who were, or were not, exposed to autism-related maternal antibodies.

Table 1

Participant Characteristics (Mean [Standard Deviation]) Collected for Each Group: ASD with Disproportionate Megalencephaly (ASD-DM), ASD with Normal-Sized Brains (ASD-N), and Typically Developing (TD)

	Time 1			Time 2			Time 3		
	ASD-DM	**ASD-N**	**TD**	**ASD-DM**	**ASD-N**	**TD**	**ASD-DM**	**ASD-N**	**TD**
N	19	110	49	15	69	39	11	67	36
Age, mo.	36.9 (5.44)	37.6 (6.13)	36.0 (4.65)	49.8 (6.79)	50.0 (5.54)	49.0 (4.62)	67.5 (8.48)	66.6 (9.54)	66.4 (10.3)
Total Cerebral Volume, cm^3	1151.68 (81.60) *†	1013.77 (75.65) *	986.27 (76.90) †	1208.02 (88.27) *†	1056.11 (76.68) *	1028.57 (80.88) †	1251.56 (82.06) *†	1086.63 (72.37) *	1065.04 (81.20) †
IQ	56.5 (22.42) †	63.8 (21.32) 0	105.6 (11.94) †0	–	–	–	68.4 (26.10) *†	84.2 (22.08) *0	108.8 (6.55) †0
Verbal IQ	48.5 (26.47) †	55.7 (26.31) 0	107.1 (12.52) †0	–	–	–	67.2 (27.08) *†	81.7 (22.91) *0	109.2 (8.85) †0
Nonverbal IQ	64.5 (20.52) †	71.9 (19.02) 0	104.1 (14.98) †0	–	–	–	69.6 (25.42) *†	86.8 (22.45) *0	108.3 (7.79) †0
ADOS-G Severity	7.8 (2.02)	8.0 (1.71)	–	–	–	–	7.0 (3.07)	7.2 (2.00)	–

IQ: Based on Mullen Scales of Early Learning at Time 1 and Differential Ability Scales at Time 3.

ADOS-G: Autism Diagnostic Observation Schedule-Generic.

Behavioral measures were not collected at Time 2.

Significant differences between: ASD-N and ASD-DM

*$P < .05$; ASD-DM and TD boys

†$P < .05$; ASD-N and TD boys

0$P < .05$.

Table 2

Comparison of Boys with ASD with Disproportionate Megalencephaly (ASD-DM) and Boys with ASD with Normal-Sized Brains (ASD-N)

	Time 1			Time 3		
	ASD-DM	**ASD-N**	***P*-value**	**ASD-DM**	**ASD-N**	***P*-value**
IQ			.42			<.05 *
<70	15 (79.0%)	74 (67.3%)		7 (63.6%)	21 (31.3%)	
≥70	4 (21.1%)	36 (32.7%)		4 (36.4%)	46 (68.7%)	
Verbal IQ			.28			.10
<70	16 (84.2%)	78 (70.9%)		7 (63.6%)	24 (35.8%)	
≥70	3 (15.8%)	32 (29.1%)		4 (36.4%)	43 (64.2%)	
Nonverbal IQ			.18			.09
<70	13 (68.4%)	57 (51.8%)		7 (63.6%)	22 (32.8%)	
≥70	6 (31.6%)	53 (48.2%)		4 (36.4%)	45 (67.2%)	
Language			.03 *			–
Little to no language	14 (73.7%)	50 (45.5%)		–	–	
Some or high language	5 (26.3%)	60 (54.6%)		–	–	
Regression			.01 *			–
Regression	16 (84.2%)	56 (52.3%)		–	–	
No regression	3 (15.8%)	51 (47.7%)		–	–	
Maternal Antibody			.03 *			–
Exposed	3 (17.7%)	3 (2.8%)		–	–	
Not exposed	14 (82.4%)	104 (97.2%)		–	–	

Significant difference between ASD-N and ASD-DM

* $P < .05$.

The groups have been divided based on IQ into those with Intellectual Disability (<70) and those without ID (≥70). Percentage of boys included in each of the groups identified in the left column is indicated in this table.

Behavioral measures were not collected at Time 2.

Table 3

Group Means (Standard Deviation) for the Vineland Adaptive Behavior Scales Collected for Each Group: ASD with Disproportionate Megalencephaly (ASD-DM), ASD with Normal-Sized Brains (ASD-N), and Typically Developing (TD)

	Time 1			Time 3		
	ASD-DM	**ASD-N**	**TD**	**ASD-DM**	**ASD-N**	**TD**
N	18	103	47	10	58	34
Adaptive Composite Score	73.3 (12.81)†	77.5 (11.29)^{0}	111.2 (13.26)$^{\dagger 0}$	69.2 (23.35)†	77.2 (15.78)^{0}	109.2 (10.36)$^{\dagger 0}$
Daily Living Skills	78.5 (14.58)†	80.8 (12.27)^{0}	107.8 (13.18)$^{\dagger 0}$	74.0 (26.28)†	80.4 (15.88)^{0}	108.4 (12.02)$^{\dagger 0}$
Communication	67.4 (16.55)†	74.9 (14.81)^{0}	111.2 (14.32)$^{\dagger 0}$	70.4 (28.51)†	81.5 (19.28)^{0}	110.3 (12.04)$^{\dagger 0}$
Socialization	74.9 (12.16)†	75.8 (11.23)^{0}	111.8 (13.41)$^{\dagger 0}$	71.1 (20.63)†	74.4 (19.09)^{0}	111.2 (11.44)$^{\dagger 0}$
Motor Skills	84.4 (12.59)†	89.8 (12.98)^{0}	107.5 (13.14)$^{\dagger 0}$	72.4 (20.54)†	82.8 (14.91)^{0}	102.1 (11.82)$^{\dagger 0}$

Behavioral measures were not collected at Time 2.

Significant differences between: ASD-N and ASD-DM

$^{*}P < .05$;

ASD-DM and TD boys

$^{\dagger}P < .05$;

ASD-N and TD boys

$^{0}P < .05$.