

Supplement 1

Description of Measures Included:

Cognitive Functioning:

Mullen Scales of Early Learning (Mullen, 1995; MSEL)

The MSEL was administered by a trained clinician at study entry to provide an estimate of verbal and nonverbal functioning. The MSEL is a standardized measure for young child 0-68 months of age. Four subscales were administered to provide measures of nonverbal (Visual Reception, Fine Motor) and verbal abilities (Expressive and Receptive Language). Ratio developmental quotients (average age equivalent/chronological age * 100) were calculated to provide nonverbal, verbal and combined IQ estimates.

Differential Abilities Scales-II (Elliot, 2007; DAS-II)

The DAS-II is a standardized measure of cognitive abilities children 2.5-17 years of age. At T3, most participants completed the core battery of the DAS-II Upper Early Years or School Age form. DAS-II verbal cluster, Special Nonverbal Composite and General Conceptual Ability scores provided estimates of verbal, nonverbal and combined IQ. Children who were unable to achieve basal scores on the DAS-II were administered the MSEL at Time 3, and ratio development quotients were calculated to provide nonverbal, verbal and combined IQ estimates.

Autism Diagnostic Observation Schedule (Lord et al., 2000; ADOS)

The Autism Diagnostic Observation Schedule is a semi-structured standardized observation administered by a trained clinician. Diagnostic classification is based upon exceeding a threshold in a combined Social Affect and Restricted and Restricted and Repetitive Behavior score, and severity scores provide estimates of social affect and restricted and repetitive behavior symptoms

that can be compared across ADOS modules (Gotham, Pickles, & Lord, 2009; Hus, Gotham, & Lord, 2014).

Autism Diagnostic Interview-Revised (Rutter, Le Couteur, & Lord, 2003; ADI-R)

The ADI-R is a clinician administered semi-structured caregiver interview, inquiring about past and current ASD symptoms. Diagnostic classification is based upon Language and Communication, Reciprocal Social Interaction and Restricted and Repetitive Patterns of Behavior algorithm total scores.

Vineland Adaptive Behavior Scales, Second Edition (Sparrow, Balla, & Cicchetti, 2005).

The Parent/Caregiver Rating Form of the Vineland Adaptive Behavior Scales (VABS-II) was completed by caregivers to assess adaptive behavior in Communication, Daily Living Skills and Socialization domains, yielding standard domain scores and a combined Adaptive Behavior Composite (ABC).

References for Measures

1. Elliott CD. Differential ability scales (2nd ed), Pearson, San Antonio, TX; 2007
2. Lord C, Risi S, Lambrecht L, Cook EH, Jr., Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*. 2000;30(3):205-223.
3. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. Autism diagnostic observation schedule: ADOS-2: Western Psychological Services; 2012.

4. Lord C, Rutter M, Le Couteur A. Autism diagnostic interview-revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders*. 1994;24(5):659-685.
5. Mullen EM. Mullen Scales of Early Learning. American Guidance Service, Inc; 1995.
6. Sparrow SS, Cicchetti DV, Balla DA. Vineland Adaptive Behavior Scales, Second Edition. San Antonio, TX: Pearsonl 2005.

Table S1. Parental Education, Family Income, Child Race and Ethnicity by Diagnosis and Sex

	TD-M	ASD-M	TD-F	ASD-F
	<i>n</i> = 60	<i>n</i> = 137	<i>n</i> = 50	<i>n</i> = 63
Maternal Education ^a (years), <i>M</i> (<i>SD</i>)	15.2 (2.0)	14.7 (1.9)	16.5 (2.2)	15.4 (2.4)
Paternal Education ^b (years), <i>M</i> (<i>SD</i>)	14.4 (2.1)	14.1 (2.2)	14.8 (2.4)	15.1 (2.8)
Family Income, <i>n</i> (%)				
\$49,000 or less	10 (16.7%)	23 (16.8%)	3 (6.0%)	13 (20.6%)
\$50,000-\$74,999	6 (10.0%)	15 (10.9%)	10 (20.0%)	10 (15.9%)
\$75,000-\$99,999	5 (8.3%)	11 (8.0%)	7 (14.0%)	7 (11.1%)
\$100,000-\$149,999	10 (16.7%)	5 (5.1%)	14 (28.0%)	6 (9.5%)
\$150,000 and above	8 (13.3%)	14 (10.2%)	5 (10.0%)	7 (11.1%)
Refused/Missing	21 (35.0%)	67 (48.9%)	11 (22.0%)	20 (31.7%)
Child Race, <i>n</i> (%)				
African American	1 (1.7%)	6 (4.4%)	1 (2.0%)	1 (1.6%)
Asian	3 (3.3%)	6 (4.4%)	2 (4.0%)	6 (9.5%)
White	43 (71.7%)	83 (60.6%)	33 (66.0%)	37 (58.7%)
Native American	–	1 (0.7%)	–	–
Multiracial	7 (11.7%)	18 (13.1%)	8 (16.0%)	7 (11.1%)
Not Provided/Missing	7 (11.7%)	23 (16.8%)	6 (12.0%)	12 (19.0%)
Child Ethnicity, <i>n</i> (%)				
Hispanic	10 (16.7%)	25 (18.2%)	12 (24.0%)	16 (25.4%)
Non-Hispanic	45 (75.0%)	89 (65.0%)	32 (64.0%)	36 (57.1%)
Not Provided/Missing	5 (8.3%)	23 (16.8%)	6 (12.0%)	11 (17.5%)

Abbreviations: TD: Typically Developing; TD-M: Typically Developing Male participants; ASD: Autism Spectrum Disorder; ASD-M: ASD Male participants; TD-F: Typically Developing Female participants; ASD-F: ASD Female participants.

^aMissing data: TD-M (*n* = 6), ASD-M (*n* = 22), TD-F (*n* = 4), and ASD-F (*n* = 11).

^bMissing data: TD-M (*n* = 11), ASD-M (*n* = 35), TD-F (*n* = 10), and ASD-F (*n* = 13).

Table S2. Summary of the Mixed Effects Model examining the Effect of Diagnostic Group and Sex on Trajectories of Left and Right Hippocampal Volumes (mm³), Unadjusted for Hemisphere Volume

Effect	Left			Right		
	Estimate	<i>SE</i>	<i>P</i> -value	Estimate	<i>SE</i>	<i>P</i> -value
Intercept	3407.60	34.59	<0.001	3604.14	36.26	<0.001
Age (months)	17.50	0.89	<0.001	14.96	1.03	<0.001
ASD Diagnosis	36.35	37.84	0.34	61.81	39.68	0.12
Female Sex	-226.03	37.69	<0.001	-228.91	39.54	<0.001
Age*ASD Diagnosis	-1.18	0.80	0.14	0.84	0.92	0.36
Age*Female Sex	-1.66	0.86	0.055	-1.92	0.99	0.053
Age*Age	-0.12	0.03	<0.001	-0.10	0.03	0.002

Note: Age was centered at 38 months. Thus, the intercept represents predicted hippocampal volume (in mm³) for 38 months old TD males.

Abbreviations: ASD: Autism Spectrum Disorder; SE: Standard Error; TD: Typically developing.

Table S3. Estimated Differences Between Children with ASD and Same Sex and Age Children with TD and 95% Confidence Intervals (CI) for Left and Right Hippocampus Volumes (mm³)

Age	Left			Right		
	Hemisphere Volume	Estimated Difference in Hippocampus Volume (95% CI)	<i>P</i> -value	Hemisphere Volume	Estimated Difference in Hippocampus Volume (95% CI)	<i>P</i> -value
29.3	460057	-41 (-110 to 28)	0.24	459811	-62 (-142 to 18)	0.13
29.3	491832	-4 (-67 to 60)	0.91	488411	-15 (-85 to 54)	0.66
29.3	508164	16 (-51 to 83)	0.64	522689	40 (-37 to 117)	0.31
34.5	441266	-81 (-166 to 4)	0.06	447367	-92 (-186 to 2)	0.055
34.5	476059	-40 (-105 to 26)	0.23	482670	-35 (-106 to 36)	0.33
34.5	537441	33 (-41 to 107)	0.38	540418	59 (-20 to 138)	0.14
37.9	461328	-68 (-144 to 7)	0.08	466421	-68 (-151 to 16)	0.11
37.9	485730	-39 (-103 to 24)	0.22	501367	-11 (-77 to 55)	0.74
37.9	533435	17 (-50 to 85)	0.62	539742	51 (-22 to 125)	0.17
41.1	465802	-74 (-151 to 3)	0.06	470669	-67 (-151 to 17)	0.12
41.1	518911	-11 (-71 to 50)	0.73	524289	20 (-45 to 85)	0.55
41.1	549531	26 (-47 to 98)	0.49	556396	72 (-7 to 151)	0.07
44.3	468571	-81 (-161 to -1)	0.047	466941	-79 (-171 to 13)	0.09
44.3	513244	-28 (-89 to 33)	0.36	511818	-6 (-72 to 59)	0.85
44.3	535316	-2 (-65 to 61)	0.96	534262	30 (-36 to 96)	0.37
47.4	482382	-75 (-151 to 0)	0.051	488476	-50 (-130 to 30)	0.22
47.4	510973	-41 (-104 to 21)	0.20	517198	-4 (-70 to 62)	0.91
47.4	542317	-4 (-68 to 60)	0.90	544136	40 (-27 to 107)	0.24
52.1	484186	-88 (-170 to -7)	0.03	479695	-73 (-168 to 21)	0.13
52.1	555198	-4 (-70 to 62)	0.90	554619	48 (-21 to 117)	0.17
52.1	576052	21 (-56 to 97)	0.59	587325	101 (14 to 188)	0.02
56.2	510114	-71 (-144 to 1)	0.055	512678	-28 (-106 to 50)	0.48
56.2	565563	-5 (-74 to 63)	0.88	569693	65 (-9 to 138)	0.08
56.2	581435	13 (-63 to 90)	0.73	595767	107 (18 to 196)	0.02
61.4	504845	-95 (-180 to -10)	0.03	510155	-42 (-131 to 47)	0.35
61.4	553334	-37 (-105 to 30)	0.28	551678	25 (-46 to 97)	0.49
61.4	581856	-3 (-77 to 70)	0.93	587780	84 (4 to 164)	0.04
68.3	516058	-105 (-195 to -14)	0.02	513304	-50 (-152 to 51)	0.33
68.3	552723	-61 (-135 to 13)	0.11	556743	20 (-58 to 99)	0.61
68.3	591029	-15 (-91 to 61)	0.69	600893	92 (6 to 177)	0.04

Note: Using the TD group, we divided the data in 10 age groups. For each of these age groups, we calculated 3 values of the hemisphere volume, using only TD children: lowest 20%, median, and highest 20%. We then estimated differences in hippocampus volume between children with ASD and same sex and age TD children at each age for each of the 3 values of hemisphere volume. For each age level, the first hemisphere value represents the lowest 20% value, the second the median, and the third the highest 20% hemisphere value. Results suggest that in

individuals with larger hemisphere volumes, ASD children had relatively larger hippocampi than same age and sex TD children of similar hemisphere volume, whereas in individuals with smaller hemisphere volumes ASD children had relatively smaller hippocampi than same age and sex TD children with similar hemisphere volume. Differences that reach significance are indicated in bold.

Table S4. Participant Behavioral Descriptive Information by Diagnosis/Neurophenotype Group

Time 1	TD-M (<i>n</i> = 60)			ASD-N (<i>n</i> = 120)			ASD-DM (<i>n</i> = 17)			TD-F (<i>n</i> = 50)			ASD-F (<i>n</i> = 63)		
	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>
VDQ	59	105.4	12.1	120	57.8	27.1	17	53.6	26.9	50	109.6	13.3	62	59.2	29.2
NVDQ	59	102.6	14.4	120	73.3	19.5	17	70.7	18.7	50	109.7	13.0	62	72.1	19.9
VABS-II ABC	54	110.4	13.3	111	77.4	11.2	15	77.1	11.4	43	111.3	12.2	57	70.8	10.2
ADOS CSS				119	7.5	1.6	16	7.3	2.1				63	7.5	1.8
ADI															
Communication Total				120	9.6	2.7	17	9.6	2.6				63	10.3	2.8
Social Total				120	16.7	4.0	16	17.3	4.8				63	18.1	4.0
Behavior Total				120	5.5	2.0	16	5.3	2.1				63	5.4	1.9
Time 3	TD-M (<i>n</i> = 29)			ASD-N (<i>n</i> = 42)			ASD-DM (<i>n</i> = 8)			TD-F (<i>n</i> = 20)			ASD-F (<i>n</i> = 13)		
	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>
VIQ	23	110.1	9.9	37	77.2	36.0	6	80.0	32.7	17	113.6	9.9	13	78.9	34.4
NVIQ	23	110.3	10.9	37	88.0	30.4	6	84.3	25.7	17	111.1	12.3	13	83.5	36.7
VABS-II ABC	23	111.6	10.8	33	76.7	17.2	6	84.5	16.7	16	115.7	12.4	12	70.3	10.8
ADOS CSS				38	7.2	2.1	6	5.8	3.3				13	6.7	2.2

Abbreviations: ASD: Autism Spectrum Disorder; ASD-F: ASD female participants; DM: Disproportionate Megalencephaly; ASD-DM: ASD male participants with DM; ASD-N: ASD male participants without DM; TD: Typically developing; TD-M: TD male participants TD-F: TD female participants; ADI: Autism Diagnostic Interview-Revised; ADOS CSS: Autism Diagnostic Observation Schedule, Calibrated Severity Score; VABS-II ABC: Vineland Adaptive Behavior Scales-II Adaptive Behavior Composite; NVDQ; Nonverbal developmental quotient; VDQ; Verbal developmental quotient; VIQ: Verbal IQ; NVIQ: Nonverbal IQ.

Table S5. Summary Statistics for Age, Hippocampal and Hemisphere Volumes (in mm³) at T1, T2, T3 by Diagnostic/Neurophenotype Group

	TD-M (<i>n</i> = 60)			ASD-N (<i>n</i> = 120)			ASD-DM (<i>n</i> = 17)			TD-F (<i>n</i> = 50)			ASD-F (<i>n</i> = 63)		
	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>
Time 1															
Age (months)	60	37.0	6.4	120	38.0	6.4	17	35.3	4.7	50	38.8	6.8	63	39.6	6.5
L HV	56	3359.6	289.9	119	3405.6	313.6	16	3657.3	365.7	47	3202.7	275.8	63	3221.8	351.2
R HV	56	3541.1	286.6	119	3617.3	344.3	16	3921.3	304.3	47	3390.3	288.5	63	3438.1	348.2
L Hemisphere	58	509233	42155	120	523361	38310	17	580709	33344	50	480206	34980	63	489477	46034
R Hemisphere	58	516059	42158	120	532127	38642	17	587308	30556	50	483933	35870	63	493670	45430
Time 2															
Age (months)	43	50.4	6.5	70	50.5	6.3	11	48.1	6.5	28	51.8	6.1	28	54.7	7.3
L HV	43	3584.8	298.6	70	3591.0	342.6	11	4008.0	310.6	28	3387.9	278.0	28	3466.8	329.4
R HV	43	3773.1	300.8	70	3810.1	351.7	11	4201.5	254.2	28	3576.9	268.0	28	3663.8	318.7
L Hemisphere	43	544535	45730	70	555526	39193	11	621058	44100	28	511083	40317	28	516098	42637
R Hemisphere	43	550377	45373	70	561891	40537	11	624296	39723	28	514474	41922	28	520072	40647
Time 3															
Age (months)	29	61.6	5.1	42	64.0	5.8	8	62.7	6.3	20	65.5	6.8	13	67.7	6.3
L HV	29	3786.0	272.8	42	3780.0	333.9	8	4213.1	288.9	20	3593.5	344.4	13	3605.4	397.5
R HV	29	3905.7	243.7	42	3985.8	408.3	8	4365.8	188.4	20	3725.9	303.8	13	3758.0	433.3
L Hemisphere	29	563830	40644	42	581973	34852	8	656505	30423	20	532488	35574	13	533040	45662
R Hemisphere	29	566987	40126	42	585954	35252	8	657652	28417	20	535660	36030	13	535763	44506

Abbreviations: ASD: Autism Spectrum Disorder; ASD-F: ASD female participants; DM: Disproportionate Megalencephaly; ASD-DM: ASD male participants with DM; ASD-N: ASD male participants without DM; L: Left; R: Right; HV: Hippocampal Volume; TD: Typically developing; TD-F: TD female participants; TD-M: TD male participants.

Table S6. Summary of the Mixed Effects Linear Models Examining the Effect of Disproportionate Megalencephaly (DM) Status, Diagnostic Group, Sex, and Hemisphere Volume on Left and Right Hippocampal Volumes (mm³)

Effect	Left			Right		
	Estimate	SE	P-value	Estimate	SE	P-value
Unadjusted Analyses						
Intercept	3396.64	33.73	<0.001	3591.42	35.19	<0.001
Female Sex	-202.07	37.13	<0.001	-200.81	38.77	<0.001
Age (months)	17.38	0.86	<0.001	14.93	1.03	<0.001
ASD Diagnosis	13.12	37.26	0.73	34.40	38.89	0.38
Age*Female Sex	-1.35	0.83	0.11	-1.84	1.00	0.07
Age*ASD Diagnosis	-1.58	0.78	.04	0.72	0.94	0.44
Age*Age	-0.12	0.03	<0.001	-0.10	0.03	0.002
DM Status	320.32	78.36	<0.001	368.2	81.63	<0.001
Age*DM Status	4.32	1.53	0.006	1.37	1.85	0.46
Adjusted Analyses						
Intercept	3336.54	28.36	<0.001	3511.26	31.00	<0.001
Female Sex	-68.21	32.57	0.04	-55.50	35.59	0.12
Age (months)	8.62	1.36	<0.001	7.45	1.51	<0.001
ASD Diagnosis	-24.69	31.10	0.43	-18.78	33.97	0.58
Hemisphere Volume (cm ³)	3.06	0.46	<0.001	2.73	0.51	<0.001
Age*Female Sex	-0.52	0.80	0.52	-1.17	0.97	0.23
Age*ASD Diagnosis	-3.32	1.36	0.02	-1.61	1.49	0.28
Age*Age	-0.04	0.03	0.16	-0.02	0.03	0.53
Hemisphere* ASD Diagnosis	1.03	0.56	0.07	1.45	0.62	0.02
DM Status	37.94	135.2	0.78	141.09	176.16	0.42
Age*DM Status	3.03	3.49	0.38	1.10	4.25	0.80
Hemisphere*DM Status	0.13	1.37	0.92	-0.45	1.73	0.80

Note: Age was centered at 38 months and hemisphere is centered at 498.9 cm³ (average hemisphere volume for 38 months old TD children). Thus, the intercepts represent predicted hippocampus volumes for 38 months old TD male children with hemisphere volume of 498.9 cm³. In these models the DM effect was entered as an interaction with diagnosis and sex. Thus, the effect for DM can be interpreted as the estimated difference in volume between ASD-DM boys and same-age ASD boys without DM. The interaction between DM and age is interpreted as the difference in linear growth rate between ASD-DM boys and same-age ASD boys.

Abbreviations: ASD: Autism Spectrum Disorder; DM: Disproportionate Megalencephaly; SE: Standard Error; TD: Typically developing.

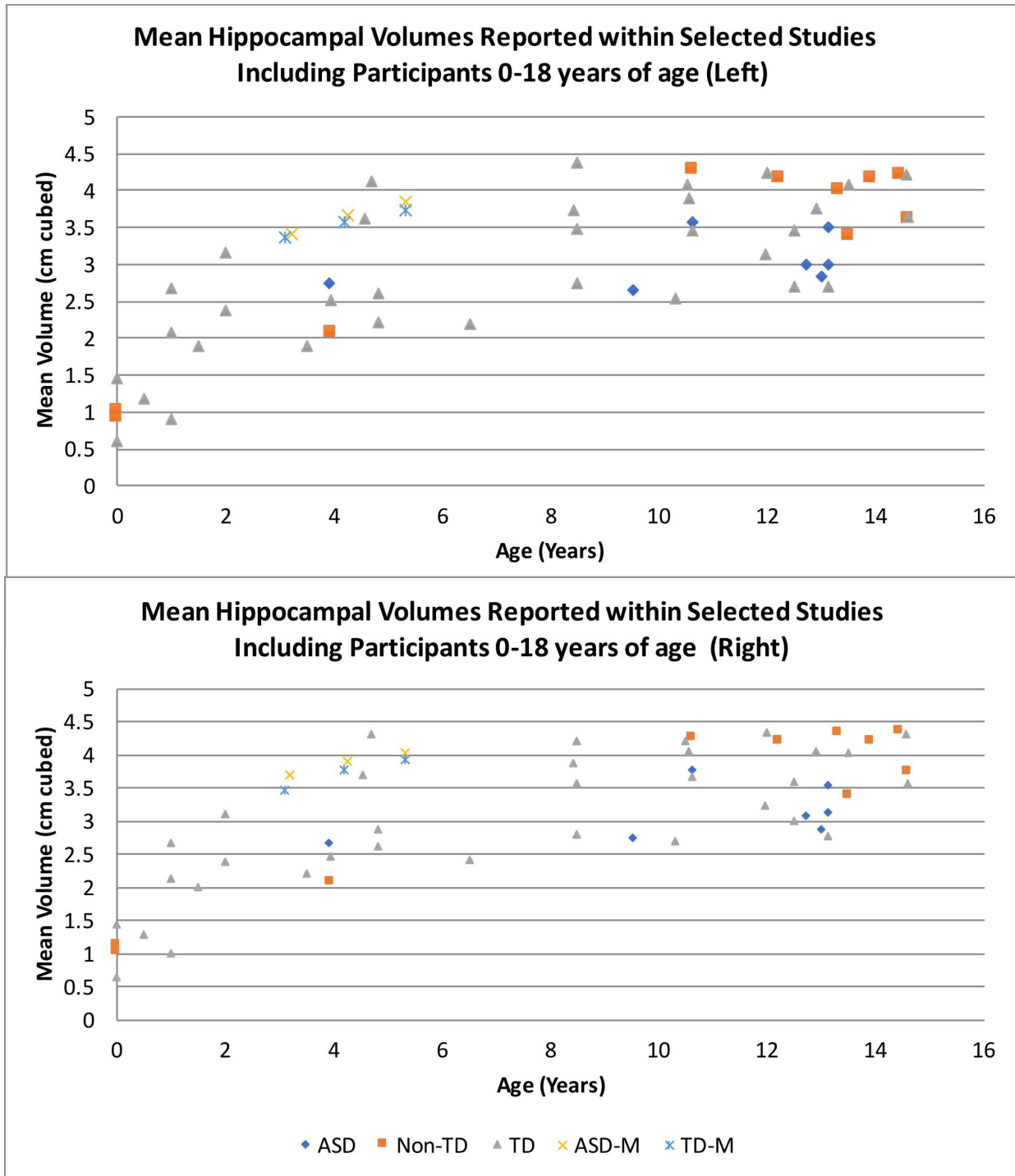
Table S7. Summary of the Mixed Effects Linear Models Examining Asymmetry

Effect	Estimate	SE	P-value
Intercept	3347.90	29.16	<0.001
Female Sex	-71.12	32.90	0.03
ASD Diagnosis	-29.34	31.96	0.36
Right Side	160.16	11.04	<0.001
Right Side*ASD Diagnosis	25.46	13.99	0.07
Hemisphere (cm ³)	2.64	0.45	<0.001
Hemisphere*ASD Diagnosis	1.49	0.53	0.005
Age (months)	8.70	1.35	<0.001
Age*ASD Diagnosis	-2.78	1.31	0.03
Age*Female Sex	-0.95	0.83	0.25
Age*Age	-0.03	0.03	0.25

Note: Age was centered at 38 months and hemisphere is centered at 498.9 cm³ (average hemisphere volume for 38 months old TD children). Thus, the intercept represents predicted left hippocampus volume for 38 months old TD males with hemisphere volume of 498.9 cm³.

Abbreviations: ASD: Autism Spectrum Disorder; SE: Standard Error; TD: Typically developing.

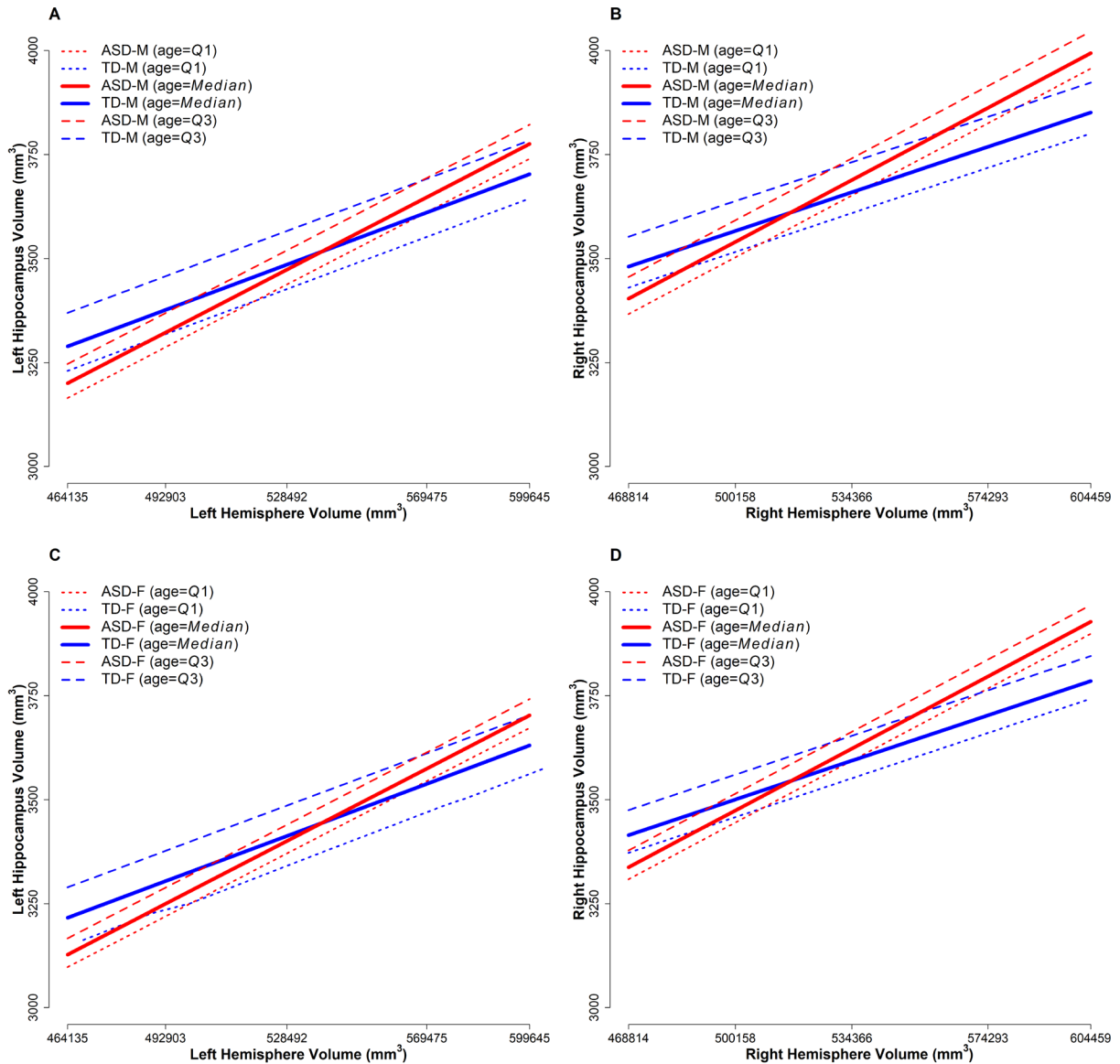
Figure S1. Other Studies of Hippocampal Volume in those with ASD and TD From Birth to Age 16: ASD-M and TD-M Represent the Current Study



Note: A list of studies reviewed for this Figure follows after Figure S3.

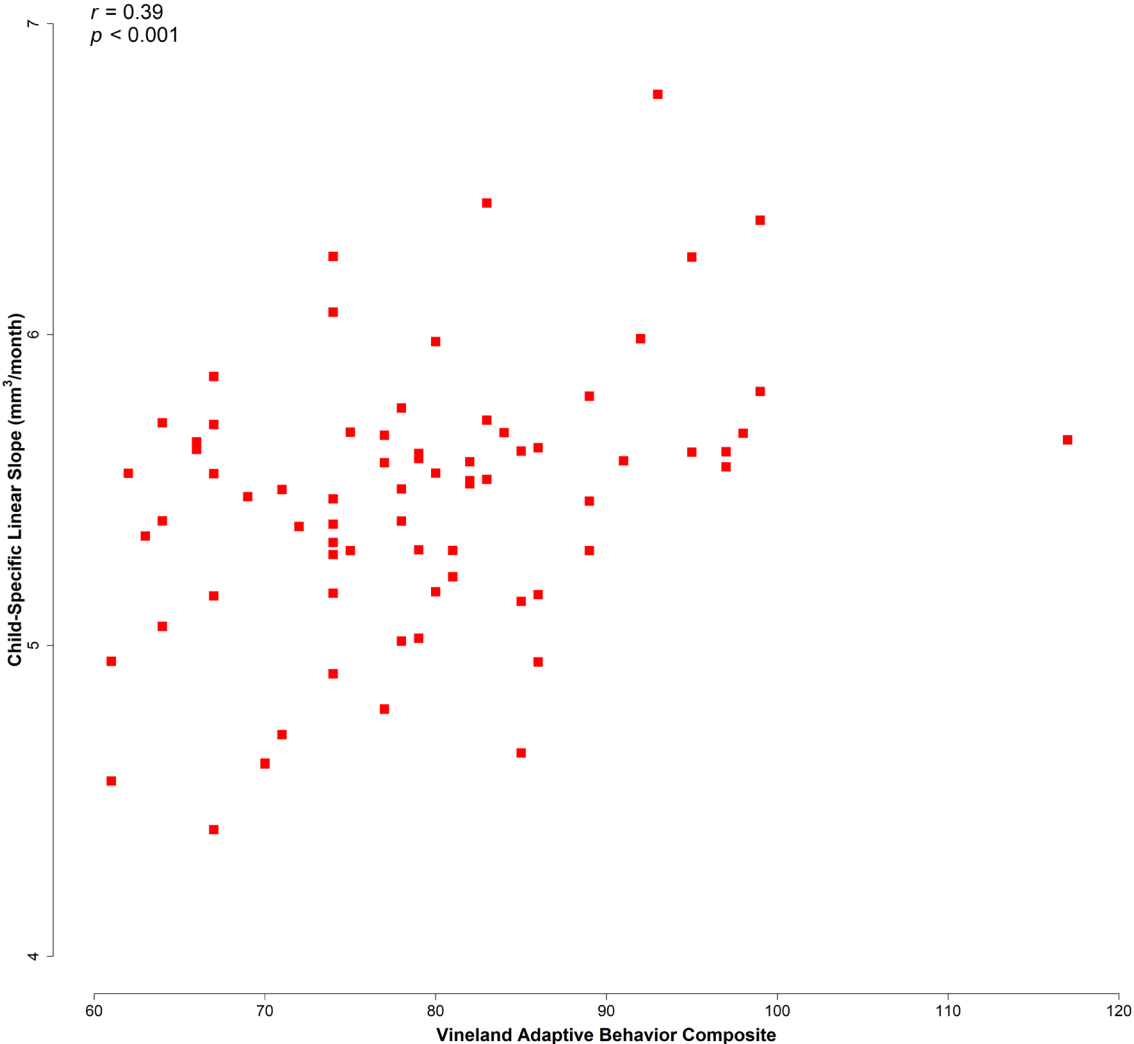
Abbreviations: ASD: Autism Spectrum Disorder; ASD-M: Male participants with ASD; SE: Standard Error; TD: Typically developing; TD-M: Male participants with TD.

Figure S2. Estimated Left and Right ASD and TD Hippocampus Volumes for Male and Female Children to Illustrate the Interaction Between Hemisphere Volume and Diagnosis.



Note: We calculated the first quartile ($Q1=38$ months), *Median* (45 months), and third quartile ($Q3=55$ months) for ages in our sample. At each of these three age levels, we used the adjusted models to calculate predicted hippocampal volumes at lowest 10%, first quartile, median, third quartile, and highest 10% values of hemisphere volume for male (panels A, B) and female (panels C, D) children with ASD and TD. Plots indicate that in individuals with larger hemisphere volumes, both male and female children with ASD had relatively larger hippocampi than same age and sex TD children of similar hemisphere volume, whereas in individuals with smaller hemisphere volumes both male and female ASD children had relatively smaller hippocampi than same age and sex TD children with similar hemisphere volume.

Figure S3. Scatterplot of Child-Specific Hippocampal Linear Slope Against T1 Vineland Adaptive Behavior Composite Score in Boys with ASD



Studies Reviewed for Figure S1

1. Albaugh MD, Orr C, Charani B, Althoff RR, Allgaier N, D'Alberto N, Hudson K, Mackey S, Spechler PA, Banaschewski T, Brühl R. Inattention and reaction time variability are linked to ventromedial prefrontal volume in adolescents. *Biological psychiatry*. 2017 Nov 1;82(9):660-8.
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5. Gilmore JH, Schmitt JE, Knickmeyer RC, Smith JK, Lin W, Styner M et al. Genetic and environmental contributions to neonatal brain structure: a twin study. *Hum Brain Mapp* 2010; 31: 1174–1182
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