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Original Article

Structural Magnetic Resonance Imaging-Based Brain Morphology Study in Infants and Toddlers With Down Syndrome: The Effect of Comorbidities

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ABSTRACT

Background: Down syndrome (DS) is the most prevalent chromosomal disorder characterized by intellectual disability, multiple organ anomalies, generalized muscular hypotonia, and characteristic physical features. The presence of DS-associated medical comorbidities has contributed to brain morphologic changes. The aim of this study was to evaluate brain morphologic characteristics during infant and toddler ages in patients with DS using structural brain magnetic resonance imaging.
Methods: Structural brain T1-weighted magnetic resonance images from participants with DS with complete chromosome 21 trisomy (n = 20; 1.6 ± 0.6 [mean ± standard deviation] years old) were analyzed using FreeSurfer. The measurements were compared with those of 60 gender- and age-matched neurotypical controls by Cohen's d statistic and unpaired *t* test with false discovery rate correction for multiple comparisons and analyzed using a univariate general linear model with the following DS-associated medical comorbidities: congenital cardiac disease, infantile spasms, and hypothyroidism.
Results: We identified 27 candidate measurements with large effect sizes (absolute d > 0.8) and statistically significant differences ($P < 6.9 \times 10^{-3}$). Among them were decreased volumes in bilateral cerebellar gray matter and right cerebellar white matter and brainstem and cortical abnormalities in the right superior temporal, right rostral anterior cingulate, and left rostral middle frontal gyrus, independent of comorbid effects. Only bilateral cerebellar gray matter volumes and brainstem volume showed differences between DS and healthy groups during infancy.
Conclusion: These results suggest that cerebellar gray matter and brainstem may represent the primary regions affected by the presence of an additional copy of chromosome 21.

Introduction

Down syndrome (DS, OMIM 190685), or trisomy 21, is the most prevalent chromosomal disorder with an incidence of over one per 700 live births.[1] DS is characterized by intellectual disability, multiple organ anomalies, generalized muscular hypotonia, and characteristic physical features such as a flat nasal bridge, upward-slanting palpable fissure, and single transverse palmar crease.[2] Cognitive impairment in DS is characterized by learning, memory, and speech or language problems.[1] Other major medical comorbidities include congenital cardiac disease (CCD) (40% to 50%), hearing loss (75%), eye disease (60%), thyroid disease (4% to 18%), and seizures (1% to 13%) such as infantile spasms (IS).[2] Prior neuropathological studies revealed several abnormalities in brain

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Conflict of interest: T.S., J.L., N.B., and E.T. declare no relevant conflicts of interest.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

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TABLE 1.
The Background of Down Syndrome and Neurotypical Control Participants

Category	Down Syndrome (N = 20)	Neurotypical Controls (N = 60)
No. of males (N [%])	13/20 [65%]	39/60 [65%]
Age in years (mean [S.D.])		
Of total participants	1.6 [0.6]	1.6 [0.5]
Of male participants	1.5 [0.5]	1.5 [0.5]
Of female participants	1.8 [0.8]	1.8 [0.6]
Congenital cardiac disease (N [%])	15/20 [75%]	0/60 [0%]
Infantile spasm (N [%])	3/20 [15%]	0/60 [0%]
Hypothyroidism (N [%])	6/20 [30%]	0/60 [0%]

development in DS such as neuronal migration, a hypocellular hippocampal dentate gyrus, and reduced cerebellar expansion during the late prenatal period.[3] In the cerebral cortex of fetal DS brains, delayed and disorganized cortical lamination[4] and low concentration of neurotransmitters[5] have been reported.

Neuroimaging has the potential to play an important role in further understanding neurological and neurodevelopmental impairments in DS. Although at least 14 structural brain magnetic resonance imaging (MRI) studies have been published so far, the majority of them focused on patients with DS older than five years.[1] The presence of CCD,[6] IS,[7] and congenital hypothyroidism,[8] which are often observed in patients with DS, have been associated with brain morphologic changes in prior structural brain MRI studies with non-DS patients. Infants with CCDs had smaller brain measures in the frontal lobe, parietal lobe, cerebellum, and brainstem.[6] According to a quantitative study, 71% of infants with IS had various developmental or acquired structural abnormalities.[7] Children with congenital hypothyroidism had regional thickening or thinning in cortical thicknesses.[8]

Moreover, patients with DS show accelerated aging both in clinical symptoms and structural brain MRI in adult ages.[9] Because IS occur in late infancy[2,7] and the risk of hypothyroidism increases with age,[2] brain morphology in DS is expected to be affected by comorbidities and aging. Therefore analysis of young children with DS that considers the effects of comorbidities may assist in accurately revealing abnormal brain morphology associated with DS. In this study, we explored brain morphology in patients with DS younger than three years, through a comparative analysis with neurotypical controls (NC) while considering the effects of comorbidities.

Methods

Participants

After approval by the Institutional Review Board at Boston Children's Hospital, we reviewed the electronic medical records from June 1, 2008, to February 24, 2016, to assemble a cohort of patients with DS. Gender- and age-matched NC were selected from our in-house database composed of electronic records of healthy participants without neurological disorders, neuropsychologic disorders, or epilepsy.[10] After excluding three individuals (see below), we used data from 20 patients with DS (13 males and seven females) and 60 NC participants (39 males and 21 females). The leading reasons for MRI examination in neurotypical controls were headaches (60%), to rule out intracranial pathologies (13%), vomiting (11%), and night awakenings (10%). The indications for MRI in the DS group were assessments of various diseases such as nystagmus, papilledema, spasmus nutans, and IS.

Structural MRI acquisition and processing

Both DS and NC participants ware imaged with the same model of clinical 3T MRI scanners (Skyra, Siemens Medical Systems, Erlangen, Germany) at the Boston Children's Hospital. Because of the clinical and retrospective nature of this study, there is variability in the pulse sequences employed to acquire T1-weighted volumetric examinations. Spatial resolution varied in the x and y directions from 0.219 to 1.354 mm (mean, 0.917 mm; S.D., 0.124 mm). Through-plane slice thickness varied from 0.500 to 2.000 mm (mean, 0.996 mm; S.D., 0.197 mm). After excluding low-quality images due to motion artifacts, DICOM files of T1-weighted volumetric examinations were accessed through the Children's Research and Integration System[11] and analyzed with the recon-all command on FreeSurfer version 5.3.[12] Through this process, 1573 regionally distributed measurements (463 for regional volume, 448 for surface area, and 662 for cortical thickness) were extracted from each imaging examination. The measurements were extracted using the brain atlases ("aseg.stats") for subcortical segmentation and ("aparc," "aparc.a2009s," and "aparc.DKT40") for automatic cortical parcellations.

Each FreeSurfer output from a T1 structural examination displayed with a labeled overlay map on FreeView (https://surfer.nmr.mgh.harvard.edu) was visually inspected for quality of regional segmentation results, and examinations were excluded from analysis despite manual corrections if FreeSurfer results were observed to substantially fail. Scans from three individuals with DS were excluded from this study because of failed FreeSurfer processing. After excluding these patients, there were 20 structural brain MRI examinations from 20 participants with DS. Age at MRI scans were not significantly different ($T(78) = -0.096$, $P = 0.92$) between DS and NC based on Student's t test (1.6 ± 0.6 and 1.6 ± 0.5 [mean $\pm$ S.D.] in both DS and NC, respectively).

Statistical analysis

The equality of means in each brain morphology measurement between DS and NC participants was evaluated with Cohen's

TABLE 2.
Global Brain Volumes in DS and NC Participants

Measurement	DS (N = 20) Mean [S.D.]	NC (N = 60) Mean [S.D.]	The Rate of DS/NC	t	Df	*P* value	Absolute Cohen's *d*
Category; aseg							
Estimated total intracranial volume (mm^2)	927,348 [171,768]	1,083,446 [195,133]	0.86	−3.2	78	0.0021	0.82
Total cortical GM volume (mm^2)	360,050 [104,933]	426,319 [118,838]	0.84	−2.2	78	0.029	0.57
Total cortical WM volume (mm^2)	219,022 [44,640]	247,444 [69,445]	0.89	−2.1	51.3	0.039	0.44
Total subcortical GM volume (mm^2)	37,461 [8274]	42,244 [9685]	0.89	−2.0	78	0.051	0.51

Abbreviations:
Df = Degree of freedom
DS = Down syndrome
GM = Gray matter
NC = Neurotypical controls
WM = White matter

TABLE 3.
Candidate Brain Morphologic Measurements in DS and NC Participants

Measurement	DS (N = 20) Mean [S.D.]	NC (N = 60) Mean [S.D.]	The Rate of DS/NC	t	Df	*P* value	Absolute Cohen's *d*
Annotation format; aseg							
Left cerebellar GM, volume (mm^3)	32,372 [5461]	47,260 [6653]	0.68	−9.0	78	9.3×10^{-14}	2.33
Right cerebellar GM, volume (mm^3)	32,007 [5489]	47,761 [6539]	0.67	−9.7	78	5.1×10^{-15}	2.50
Right cerebellar WM, volume (mm^3)	5924 [1036]	9149 [2288]	0.65	−8.6	70.8	1.4×10^{-12}	1.57
Brainstem, volume (mm^3)	9061 [1694]	12,399 [2069]	0.73	−6.5	78	6.5×10^{-9}	1.68
Annotation format; aparc							
Lh rostral middle frontal ThickStd (mm)	0.73 [0.08]	0.89 [0.17]	0.82	−5.8	70	2.1×10^{-7}	1.06
Rh rostral middle frontal ThickStd (mm)	0.74 [0.071]	0.89 [0.16]	0.83	−5.7	71.9	2.3×10^{-7}	1.04
Rh rostral anterior cingulate SurfArea (mm^2)	215.1 [73.3]	356.4 [146.0]	0.6	−5.6	65.7	4.5×10^{-7}	1.07
Rh rostral anterior cingulate GM, volume (mm^3)	865 [379]	1470 [639]	0.59	−5.1	56.4	4.6×10^{-6}	1.03
Rh superior temporal GM, volume (mm^3)	7000 [2152]	10,063 [3229]	0.70	−4.8	49.3	1.5×10^{-5}	1.02
Lh rostral anterior cingulate GM, volume (mm^3)	1080 [442]	1751 [848]	0.62	−4.4	60.1	4.0×10^{-5}	0.87
Rh supramarginal gyrus, SurfArea (mm^2)	2242 [465]	2883 [846]	0.78	−4.3	60.4	7.4×10^{-5}	0.83
Annotation format; aparc.a2009							
Lh inferior frontal sulcus, ThickStd (mm)	0.55 [0.11]	0.73 [0.22]	0.75	−4.9	64.2	7.9×10^{-6}	0.93
Lh anterior part of the cingulate gyrus and sulcus, SurfArea (mm^2)	772 [212]	1087 [392]	0.71	−4.5	61.2	2.8×10^{-5}	0.88
Rh supramarginal gyrus, SurfArea (mm^2)	1095 [178]	1492 [427]	0.73	−5.8	74.1	1.3×10^{-7}	1.04
Rh lateral aspect of the superior temporal gyrus, SurfArea (mm^2)	709 [146]	938 [254]	0.76	−4.9	57.7	7.2×10^{-6}	0.98
Rh inferior segment of the circular sulcus of the insula GM, volume (mm^3)	1223 [302]	1720 [416]	0.71	−4.9	78	4.8×10^{-6}	1.27
Rh inferior frontal sulcus, ThickStd (mm)	0.58 [0.10]	0.74 [0.21]	0.78	−4.6	66.2	2.3×10^{-5}	0.86
Rh transverse temporal sulcus, SurfArea (mm^2)	107 [36]	159 [65]	0.67	−4.4	56.5	4.3×10^{-5}	0.88
Lh frontal superior gyrus, SurfArea (mm^2)	2641 [505]	3399 [1056]	0.78	−4.3	68.1	6.0×10^{-5}	0.80
Annotation format; aparc.DKTatlas40							
Lh rostral middle frontal GM, ThickStd (mm)	0.74 [0.08]	0.89 [0.2]	0.82	−5.7	71.7	2.6×10^{-7}	1.03
Rh rostral middle frontal GM, ThickStd (mm)	0.73 [0.07]	0.88 [0.17]	0.83	−5.5	73.6	4.8×10^{-7}	0.99
Rh superior temporal GM, SurfArea (mm^2)	2745 [608]	3626 [967]	0.76	−4.8	52.5	1.5×10^{-5}	0.99
Rh rostral anterior cingulate GM, SurfArea (mm^2)	251 [77]	406 [149]	0.62	−6.0	64.3	1.2×10^{-7}	1.15
Rh rostral anterior cingulate GM, volume (mm^3)	1022 [406]	1695 [663]	0.60	−5.4	54.6	1.8×10^{-6}	1.10
Rh supramarginal GM, SurfArea (mm^2)	2140 [438]	2765 [790]	0.77	−4.4	59.9	4.3×10^{-5}	0.87
Lh caudal anterior cingulate GM, ThickStd (mm)	0.66 [0.18]	0.90 [0.28]	0.74	−4.3	51.7	8.3×10^{-5}	0.89
Lh rostral anterior cingulate GM, volume (mm^3)	1376 [643]	2217 [1066]	0.62	−4.2	55.2	9.6×10^{-5}	0.86

Abbreviations:
Df = Degree of freedom
DS = Down syndrome
GM = Gray matter
Lh = Left hemisphere
NC = Neurotypical controls
Rh = Right hemisphere
SurfArea = Surface area
ThickStd = Thickness standard deviation
WM = White matter

d statistic, Levene's test for equality of variances, and a two-tailed unpaired *t* test for two groups of samples with false discovery rate correction (q = 0.005) for multiple comparisons. We identified candidate measurements with large effect sizes (absolute d > 0.8) and statistically significant differences ($P < 6.9 \times 10^{-3}$). For each identified measurement, a univariate General Linear Model ($P < 0.05$) was constructed to evaluate the effects of binary or continuous covariates (age, gender, and presence of CCD, IS, and hypothyroidism). Critical values from the F-distribution calculation were determined to be F(0.05,6,73) = 2.22 and F(0.05,1,73) = 3.97 for the corrected model and each covariate, respectively. Statistical analysis was performed using IBM SPSS Statistics version 19 (IBM Corp. Armonk, NY, USA).

Results

Participants with DS in the current study showed complete 21 trisomy in all cases, CCD in 75% (atrioventricular canal defect in 35%, ventricular septal defect in 15%, and others in 25%), IS in 15%, and hypothyroidism in 30% (Table 1). The total volumes of the intracranial space, cortical gray matter (GM), cortical white matter (WM), and subcortical GM were not statistically significantly different in participants with DS and NC (Table 2).

Among measurements generated by the FreeSurfer recon-all pipeline, 19 brain morphologic measurements were identified for further analyses as the candidate measurements with large effect sizes and statistically significant *P* values (Table 3). The 19 candidate measurements included cerebellar volumes, brainstem volume, and some cortical measurements (surface areas, volumes, and S.D., of the thickness) (Table 3).

Univariate General Linear Model demonstrated that the presence of DS was an independent significant factor in the differences observed in bilateral cerebellar GM volumes, right cerebellar WM volume, brainstem volume, volume and surface area of the right rostral anterior cingulate cortex (ACC), volume of the right superior temporal cortex (STC), and S.D. of the cortical thickness of the left rostral middle frontal cortex (MFC). For these identified measurements, age was always a statistically significant covariate. The comparison between DS and NC was statistically significantly affected by gender, but not by the presence of any comorbidity (CCD, IS, and hypothyroidism) (Table 4). Bilateral cerebellar GM volumes and brainstem volumes have demonstrated marked disparity in the infantile period between participants with DS and NC (Fig A, B, and D). In the right cerebellar WM volume, right STC volume, and right rostral ACC volume, a gradually increasing difference between the two groups was observed as the age increased after about two years (Fig C, E, and F).

Discussion

We quantitatively evaluated brain morphology in infants and toddlers with DS using structural MRI. Our results showed

TABLE 4.
The Effects of Covariates on Candidate Brain Morphologic Measurements; Univariate General Linear Model

Measurement	Adjusted *R* Squire	Corrected Model	DS	Age	Gender	Congenital Cardiac Disease	Infantile Spasm	Hypothyroidism
Annotation format; aseg								
Left cerebellar GM, volume	.638	**F = 24.2**	**F = 10.8**	**F = 24.9**	F = 2.4	F = 2.2	F = 1.5	F = .31
		$\mathbf{P = 1.5 \times 10^{-15}}$	**P = .002**	$\mathbf{P = 4.0 \times 10^{-6}}$	P = .13	P = .14	P = .22	P = .58
Right cerebellar GM, volume	.682	**F = 29.2**	**F = 11.9**	**F = 32.1**	F = 3.6	F = 3.1	F = .38	F = 1.8
		$\mathbf{P = 1.5 \times 10^{-17}}$	**P = .001**	$\mathbf{P = 2.8 \times 10^{-7}}$	P = .06	P = .08	P = .54	P = .18
Right cerebellar WM, volume	.387	**F = 9.3**	**F = 9.0**	**F = 12.4 P = .001**	**F = 4.1**	F = .14	F = .13	F = 1.2
		$\mathbf{P = 1.4 \times 10^{-7}}$	**P = .004**		**P = .047**	P = .71	P = .72	P = .28
Brainstem, volume	.654	**F = 25.9**	**F = 9.6**	**F = 69.7**	F = 2.9	F = .68	F = .35	F = .68
		$\mathbf{P = 2.8 \times 10^{-16}}$	**P = .003**	$\mathbf{P = 3.1 \times 10^{-12}}$	P = .09	P = .41	P = .56	P = .41
Annotation format; aparc								
Lh rostral middle frontal ThickStd	.325	**F = 7.35**	**F = 5.6**	**F = 21.6**	F = .67	F = .16	F = .99	F = .26
		$\mathbf{P = 3.6 \times 10^{-6}}$	**P = .02**	$\mathbf{P = 1.5 \times 10^{-5}}$	P = .42	P = .70	P = .32	P = .61
Rh rostral middle frontal ThickStd	.226	**F = 4.6**	F = 3.4	**F = 9.9**	F = .26	F = .004	F = .17	F = .009
		$\mathbf{P = 3.2 \times 10^{-4}}$	P = .068	**P = .002**	P = .62	P = .95	P = .68	P = .92
Rh rostral anterior cingulate SurfArea	.517	**F = 14.8**	**F = 5.0**	**F = 55.2**	F = .76	F = .06	F = .04	F < .01
		$\mathbf{P = 7.3 \times 10^{-11}}$	**P = .03**	$\mathbf{P = 1.9 \times 10^{-10}}$	P = .39	P = .81	P = .84	P = .99
Rh rostral anterior cingulate GM, volume	.529	**F = 4.9**	F = 3.7	**F = 59.0**	F = .23	F = .27	F = .02	F = .008
		$\mathbf{P = 3.2 \times 10^{-4}}$	P = .057	$\mathbf{P = 6.6 \times 10^{-11}}$	P = .59	P = .61	P = .88	P = .93
Rh superior temporal GM, volume	.580	**F = 19.2**	**F = 5.4**	**F = 78.1**	F = .53	F = .03	F = 1.4	F = .19
		$\mathbf{P = 2.8 \times 10^{-13}}$	**P = .023**	$\mathbf{P = 3.7 \times 10^{-13}}$	P = .47	P = .86	P = .25	P = .67
Lh rostral anterior cingulate GM, volume	.471	**F = 12.7**	F = 3.3	**F = 57.1**	F = 0.1	F = .02	F = .02	F < .01
		$\mathbf{P = 8.7 \times 10^{-10}}$	P = .075	$\mathbf{P = 9.6 \times 10^{-11}}$	P = .82	P = .88	P = .90	P = .93
Rh supramarginal gyrus, SurfArea	.373	**F = 8.8**	F = .90	**F = 36.3**	**F = 4.2**	F = .23	F = .058	F = 1.9
		$\mathbf{P = 3.1 \times 10^{-7}}$	P = .35	$\mathbf{P = 6.3 \times 10^{-8}}$	**P = .044**	P = .63	P = .81	P = .17
Annotation format; aparc.a2009								
Lh inferior frontal sulcus, ThickStd	.176	**F = 3.8**	F = 2.1	**F = 7.8**	F = .20	F = .006	F = .73	F = .03
		P = .002	P = .16	**P = .007**	P = .66	P = .94	P = .40	P = .86
Lh anterior part of the cingulate gyrus and sulcus, SurfArea	.499	**F = 14.1**	F = 2.7	**F = 57.2**	F = .008	F = .40	F = 1.3	F = .06
		$\mathbf{P = 1.3 \times 10^{-10}}$	P = .10	$\mathbf{P = 9.3 \times 10^{-11}}$	P = .93	P = .53	P = .27	P = .82
Rh Supramarginal gyrus, SurfArea	.452	**F = 11.9**	F = 3.0	**F = 42.2**	**F = 10.6**	F = .02	F = .04	F = 2.3
		$\mathbf{P = 3.0 \times 10^{-9}}$	P = .09	$\mathbf{P = 8.7 \times 10^{-9}}$	**P = .002**	P = .89	P = .85	P = .14
Rh lateral aspect of the superior temporal gyrus, SurfArea	.404	**F = 9.9**	F = 1.8	**F = 36.1**	F = 1.2	F = .27	F = .42	F = .58
		$\mathbf{P = 5.6 \times 10^{-8}}$	P = .19	$\mathbf{P = 6.7 \times 10^{-8}}$	P = .28	P = .61	P = .52	P = .45
Rh inferior segment of the circular sulcus of the insula GM, volume	.331	**F = 7.5**	F = 3.8	**F = 15.8**	F = .08	F = .11	F = .08	F = .19
		$\mathbf{P = 2.7 \times 10^{-6}}$	P = .054	$\mathbf{P = 1.6 \times 10^{-4}}$	P = .78	P = .74	P = .79	P = .66
Rh frontal inferior sulcus, ThickStd	.162	**F = 3.5**	F = 2.1	**F = 8.4**	F < .01	F < .01	F = .73	F = .93
		$\mathbf{P = 3.9 \times 10^{-3}}$	P = .15	$\mathbf{P = 5.1 \times 10^{-3}}$	P = .95	P = .93	P = .40	P = .76
Rh transverse temporal sulcus, SurfArea	.340	**F = 7.8**	F = .33	**F = 28.5**	F = 1.7	F = .56	F = .36	F = 3.3
		$\mathbf{P = 1.7 \times 10^{-6}}$	P = .57	$\mathbf{P = 1.0 \times 10^{-6}}$	P = .20	P = .46	P = .55	P = .075
Lh frontal superior gyrus, SurfArea	.560	**F = 17.8**	F = 2.5	**F = 82.4**	F = .70	F = .74	F = .68	F = .91
		$\mathbf{P = 1.4 \times 10^{-12}}$	P = .12	$\mathbf{P = 1.3 \times 10^{-13}}$	P = .11	P = .68	P = .41	P = .34
Annotation format; aparc.DKTatlas40								
Lh rostral middle frontal GM, ThickStd	.293	**F = 6.4**	**F = 4.8**	**F = 18.7**	F = .99	F = .05	F = .32	F = .17
		$\mathbf{P = 1.7 \times 10^{-5}}$	**P = .03**	$\mathbf{P = 4.9 \times 10^{-5}}$	P = .32	P = .82	P = .57	P = .68
Rh rostral middle frontal GM, ThickStd	.173	**F = 3.8**	F = 3.1	**F = 6.4**	F = .08	F < .01	F = .31	F = .04
		P = .003	P = .08	**P = .013**	P = .77	P > .99	P = .58	P = .85
Rh superior temporal GM, SurfArea	.536	**F = 16.2**	F = 1.8	**F = 63.9**	F = .28	F = 1.0	F = .005	F = .36
		$\mathbf{P = 9.1 \times 10^{-12}}$	P = .18	$\mathbf{P = 1.5 \times 10^{-11}}$	P = .60	P = .32	P = .94	P = .55
Rh rostral anterior cingulate GM, SurfArea	.522	**F = 15.0**	**F = 6.3**	**F = 53.7**	F = .44	F = .02	F = .69	F = .046
		$\mathbf{P = 5.1 \times 10^{-11}}$	**P = .014**	$\mathbf{P = 3.0 \times 10^{-10}}$	P = .51	P = .89	P = .41	P = .83
Rh rostral anterior cingulate GM, volume	.554	**F = 16.9**	**F = 4.3**	**F = 63.2**	F = .13	F = .28	F = .05	F = .08
		$\mathbf{P = 4.9 \times 10^{-12}}$	**P = .042**	$\mathbf{P = 2.1 \times 10^{-11}}$	P = .72	P = .60	P = .83	P = .78
Rh supramarginal GM, SurfArea	.375	**F = 8.9**	F = .97	**F = 35.1**	**F = 5.1**	F = .30	F = .028	F = 1.8
		$\mathbf{P = 2.7 \times 10^{-7}}$	P = .33	$\mathbf{P = 9.4 \times 10^{-8}}$	**P = .027**	P = .58	P = .87	P = .18
Lh caudal anterior cingulate GM, ThickStd	.099	**F = 2.4**	F = 3.6	F = .25	F = 2.3	F = .011	F = .05	F = 1.2
		P = .033	P = .06	P = .62	P = .13	P = .92	P = .82	P = .27
Lh rostral anterior cingulate GM, volume	.501	**F = 14.2**	F = 3.2	**F = 62.1**	F < .01	F < .01	F = .012	F < .01
		$\mathbf{P = 1.2 \times 10^{-10}}$	P = .077	$\mathbf{P = 2.4 \times 10^{-11}}$	P = .95	P = .97	P = .91	P = .99

Abbreviations:
DS = Down syndrome
GM = Gray matter
NC = Neurotypical controls
Lh = Left hemisphere
Rh = Right hemisphere
SurfArea = Surface Area
ThickStd = Thickness standard deviation
WM = White matter
Bold numbers indicate values with a statistical significance. General Linear Model ($P < 0.05$) was constructed to evaluate the effects of binary or continuous covariates (age, gender, and presence of congenital cardiac disease, infantile spasms, and hypothyroidism). Statistically significant values from the F-distribution calculation were determined to be $F(0.05,6,73) = 2.22$ and $F(0.05,1,73) = 3.97$ for the corrected model and each covariate, respectively.

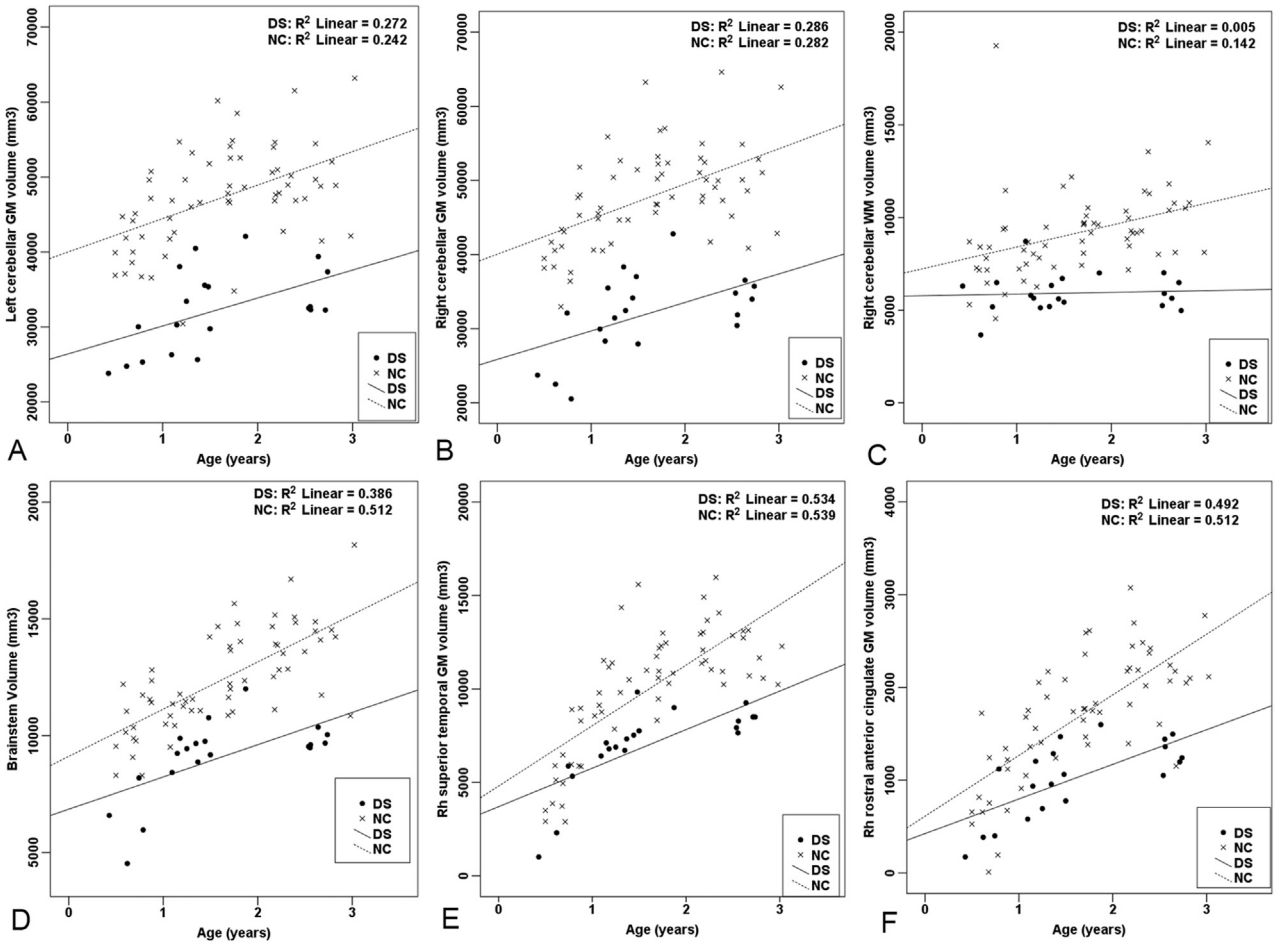

FIGURE. Scatter plots and regression lines (between age and volume) of left (A) and right cerebellar GM (B), right cerebellar WM (C), brainstem (D), right superior temporal GM (E), and right rostral anterior cingulate GM (F) in DS (closed circle and solid-line) and NC (x and dotted line) participants. DS, Down syndrome; NC, neurotypical controls; GM, gray matter; WM, white matter; Rh, right hemisphere.

decreased volumes in the bilateral cerebellar GM, right cerebellar WM, and brainstem, as well as cortical abnormalities in the right STC, right rostral ACC, and left rostral MFC in patients with DS. Although the timing of the comorbidities was different, such as CCD in early infancy, IS in late infancy, and hypothyroidism at any age, our analysis indicates that these differences in brain morphology develop over time. Only bilateral cerebellar GM volumes as well as the brainstem volume were observed as statistically significantly different between our two groups in infancy.

Prior brain morphologic studies in children with DS[13-20] demonstrated decreased volumes in the global cerebrum,[15,16,20] cerebellum,[13,18-20] and brainstem.[13,14,19] In contrast to findings of global brain volume loss, regionally preserved volumes in the temporal GM[19] and WM,[20] parietal GM[20] and WM,[19] and subcortical GM[20] were reported.

Only two studies have reported brain morphology of infants and toddlers with DS.[13,14] Gunbey et al. (2017) analyzed the structural brain MRI of 10 patients with DS with a mean age of 2.6 years and reported decreased volume in the brainstem, thalamus, basal ganglia, cerebellar cortex, right cerebellar WM, and corpus callosum.[13] An additional analysis assessed brainstem components in 32 patients with DS with a mean age of 3.7 years, and they noted a smaller pons in patients with DS compared with NC.[14] Similarly, pontine hypoplasia has been noted in adults with DS.[21] The findings of decreased volume in the cerebellar cortex and brainstem are consistent with our results. The "brainstem" in the FreeSurfer version 5.3 pipeline includes the medulla oblongata, the pons, the midbrain, and the superior cerebellar peduncle. However, the volumes of the substructures of "brainstem" are not generated by the recon-all command with FreeSurfer version 5.3. Therefore we could not reconfirm the pontine hypoplasia in DS. It is possible that the pontine hypoplasia often observed in DS is the leading abnormality of our findings.

The measurements in our study using FreeSurfer were extracted from multiple cortical automatic parcellations ("aparc," "aparc.a2009s," and "aparc.DKT40"). Thus brain measurements include some from overlapping regions in our datasets. These annotation formats were manually made with 34 cortical regions per hemisphere from 40 participants according to a sulcal approach, 74 cortical regions per hemisphere from 24 participants according to anatomical conventions, and 40 cortical regions per hemisphere from 101 participants according to a surface-based approach in the Desikan-Killiany Atlas ("aparc"), Destrieux Atlas ("aparc.a2009s"), and Desikan-Killiany-Tourville Atlas ("aparc.DKT40"), respectively. Because the pros and cons of these annotation atlases have not been established as a consensus, we analyzed all data from the three atlases.

Our results demonstrated DS-associated abnormal cortical development in the right rostral ACC, right STC, and left MFC. As we are reporting decreased cortical thickness variability in DS, we would like to assess whether or not this finding is associated with regional cortical dysfunction. Direct evidence that decreased

variation of regional cortical thickness is associated with regional cortical malfunction has not been reported. In our previous work using neurotypical controls, as the age increased from toddlers to adulthood, the S.D. of the left rostral MFC thickness decreased from 0.86 to 0.66 in males and from 0.85 to 0.68 in females.[10] Our results showed that patients with DS had decreased regional variation (S.D.) of cortical thicknesses when compared with NC. Although in typical development, decreased regional variation of cortical thickness with age may be related to normal brain maturation involving cortical folding, myelination, neural remodeling, and synaptic pruning,[22] it is unlikely that these maturation processes are accelerated in DS.[3] In our study, decreased S.D. of thicknesses of the right rostral ACC, right STC, and left MFC were observed in DS, which may be related to a decreased degree of neuronal migration and myelination in the cortex of those regions.[4] The ACC is a part of the limbic system. The dorsal part of the ACC is related to visual cognition and emotion such as anxiety.[23] The STC includes auditory association areas and plays a crucial role in the processing of auditory and visual speech information,[24] and internal timing when communicating with others.[25] The MFC plays a role in the downregulation of emotional responses,[26] reorienting of attention,[27] and handwriting symbolic codes such as letters and words.[28] The regional cortical changes observed in this study potentially contribute to intellectual dysfunction in DS found in the literature.

An additional perspective is that regional cortical changes might be secondary to cerebellar volume loss. The volumes of the bilateral cerebellar GM in DS were already abnormally decreased at birth (Fig) and continued to be significantly reduced compared with NC throughout the studied age period. The volumes of the right ACC and the right STC initially had similar values in DS and NC, with groupwise differences gradually increasing and demonstrating statistically significantly reduced volumes in DS in later developmental stages. Recent functional MRI studies revealed corticocerebellar functional networks of the ACC and STC with the cerebellum.[29-31] The hypoactivities in these corticocerebellar pathways due to the small cerebellum might contribute to decreased volume of the ACC and STC as secondary effects.

Limitations

In the current study, we did not include the assessment of neurocognitive functions in DS. Therefore, it is difficult to directly connect the findings in structural MRIs with cognitive dysfunction in DS. Some researchers have looked at potential pathways to connect structural differences and various neuroanatomic features with characteristic neurocognitive profiles in DS.[32-34] Although some patterns emerge as a population, prediction of individual neurocognitive outcomes based on neuroimaging is not currently possible. Therefore further research is needed in this area.

Furthermore, in this study, rates of comorbidities such as CCD and IS were higher than in prior prospective studies. The possible presence of selection bias (healthcare access bias) could not be excluded, because our study is retrospective and performed at a single medical facility. In addition, as a common issue among DS studies, our control population did not have comorbidities associated with DS. In the future, it would be important to use data from patients with such comorbidities without DS diagnoses (e.g., congenital cardiac disorders) to control for these comorbidities in DS.

An additional limitation of this study is that FreeSurfer[12] is not optimized for the youngest participants. As such, the rate at which FreeSurfer fails to extract measurements from clinical MRI examinations increases substantially for participants aged zero to eight months and the reliability of the results successfully produced by FreeSurfer on participants from this age range is not certain. FreeSurfer's reliability was assessed as reasonable for participants aged eight months or older,[10,35] at which point myelination contrast patterns have inverted so as to match the general pattern exhibited through the rest of life. Research aimed at overcoming the problem of FreeSurfer's applicability and reliability in very young populations is ongoing,[36] and developments in this venue will be incorporated into future work.

Conclusion

We analyzed structural MRI in infants and toddlers with DS, and found that cerebellar GM volumes and brainstem volume were reduced in infants with DS relative to NC, an effect that was independent of patient comorbidities. These results suggest that cerebellar GM and the brainstem might be the regions primarily affected by an extra copy of chromosome 21 during early brain development.

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