Altered Brain Structure in Infants with Turner Syndrome

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Abstract

Turner syndrome (TS) is a genetic disorder affecting approximately 1:2000 live-born females. It results from partial or complete X monosomy and is associated with a range of clinical issues including a unique cognitive profile and increased risk for certain behavioral problems. Structural neuroimaging studies in adolescents, adults, and older children with TS have revealed altered neuroanatomy but are unable to identify when in development differences arise. In addition, older children and adults have often been exposed to years of growth hormone and/or exogenous estrogen therapy with potential implications for neurodevelopment. The study presented here is the first to test whether brain structure is altered in infants with TS. Twenty-six infants with TS received high-resolution structural MRI scans of the brain at 1 year of age and were compared to 47 typically developing female and 39 typically developing male infants. Results indicate that the typical neuroanatomical profile seen in older individuals with TS, characterized by decreased gray matter volumes in premotor, somatosensory, and parietal-occipital cortex, is already present at 1 year of age, suggesting a stable phenotype with origins in the prenatal or early postnatal period.

Key words: infant, MRI, neuroimaging, Turner syndrome

Introduction

Females with Turner syndrome (TS), a well-defined genetic disorder resulting from the partial or complete loss of one of the sex chromosomes, represent a unique population for studying the effects of sex chromosomes and sex hormones on human brain development (Knickmeyer and Davenport 2011; Knickmeyer 2012). Affected individuals are haploinsufficient for genes that are normally expressed from both X chromosomes in females and also experience early loss of ovarian function, resulting in a postnatal developmental hormonal milieu that is estrogen- and androgen-deficient (Davenport et al. 2007). It is one of the most common human chromosomal abnormalities, occurring in approximately 1 in 2000 live female births (Jacobs et al. 1974; Nielsen and Wohlert 1991).

The most prominent physical features of the syndrome are short stature and pubertal delay, but individuals with TS also show a unique pattern of cognitive strengths and weaknesses. Girls and women with TS often exhibit specific deficits in visual-spatial functions (Murphy et al. 1994; Ross et al. 1996; Romans et al. 1998; Collaer et al. 2002; Rae et al. 2004; Hart et al. 2006), arithmetical abilities (Pennington et al. 1982; Rovet 1993; Temple and Carney 1996; Temple and Marriott 1998; Rae et al. 2004; Murphy et al. 2006), and executive functions (Murphy et al. 1994; Romans et al. 1998; Loesch et al. 2005; Green et al. 2015), with preserved or enhanced verbal ability (Temple and Carney 1996; Rae et al. 2004; Temple and Shephard 2012). Impairments in social skills and affective discrimination are common (Ross et al. 1997; Lawrence et al. 2003; Mazzola et al. 2006; Burnett et al. 2010; Hong et al. 2014; Lepage et al. 2014). Associated diagnoses may include attention deficit hyperactivity disorder (ADHD; present in approximately 25%; Russell et al. 2006; Green *et al.* 2015), specific learning disorders (dyscalculia present in approximately 75%; Mazzocco 2006), social communication and autism spectrum disorders (Hong et al. 2011), and developmental coordination disorder (Nijhuis-van der Sanden et al. 2003). As in other genetic syndromes, there is a high degree of individual variability in the somatic, cognitive, and psychosocial phenotypes that remains poorly understood.

Cognitive strengths and weaknesses presumably reflect changes in underlying neuroanatomy and function. Structural neuroimaging studies have consistently demonstrated a decrease in volume of parietal-occipital gray matter (GM; Brown et al. 2002, 2004; Cutter et al. 2006; Marzelli et al. 2011) and enlargement of the amygdala and orbitofrontal cortex (Good et al. 2003; Kesler et al. 2004; Cutter et al. 2006; Marzelli et al. 2011) in females with TS. Alterations in GM volume have also been reported in the insula, fusiform gyrus, posterior cingulate, inferior temporal gyrus, ventrolateral prefrontal cortex, anterior cingulate, hippocampus, basal ganglia, and around the superior temporal sulcus (Kesler et al. 2003; Cutter et al. 2006; Marzelli et al. 2011). These findings are of great interest, but previous studies have all been carried out in older children and adults; thus, they cannot address when in development these differences arise. Furthermore, older children and adults have often been exposed to many years of growth hormone or anabolic steroid therapy to enhance linear growth and exogenous estrogen therapy for feminization and health maintenance. Treatment may have produced some of the observed neural differences and it may have minimized or eliminated neural differences that were present before treatment began. For this reason, we have carried out the first structural neuroimaging study of infants with TS, prior to hormone therapy.

Materials and Methods

Subjects

A total of 26 females with TS (20 with complete X monosomy and 6 mosaic), 47 typically developing females, and 39 typically developing males are included in this study. All participants were approximately 1 year of age (see Table 1 for additional details). Participants with TS were recruited through the University of North Carolina (UNC) at Chapel Hill Pediatric Endocrinology and UNC Turner Syndrome Clinics, online advertisements with relevant support groups such as the Turner Syndrome Society, and mailings to health care providers both within and outside UNC, including genetic counselors, obstetricians, and pediatricians. Typically developing controls were drawn from an ongoing study of early brain development at UNC (Gilmore et al. 2007, 2012). Recruitment of controls occurred through community physicians, relevant clinics at UNC, including the general obstetrics clinics, and mass emails to the UNC community. Exclusion criteria for both groups included active substance or alcohol abuse or major medical illness in the mother during pregnancy (cancer and autoimmune disease); major psychiatric illness in the mother or the father (e.g., schizophrenia, schizoaffective disorder, bipolar disorder, and psychotic disorder not otherwise specified); extreme prematurity (birth prior to 32 weeks); and any major medical illness, learning delay, or congenital abnormality in the child not associated with a diagnosis of TS. Children were also excluded if health problems or metal implants precluded their participation in MRI. This study was approved by the Institutional Review Board of the UNC School of Medicine. Written informed consent was obtained from a parent or legal guardian prior to the study.

Image Acquisition

T1-weighted scans were acquired with either a Siemens Allegra head-only 3T scanner or a Siemens TIM Trio 3T scanner (Siemens Medical Supplies, Erlangen, Germany). Scan parameters for the Allegra were as follows: MP-RAGE time repetition (TR), 1900 ms; time echo (TE), 4.38 ms; flip angle, 7°; image resolution, $1 \times 1 \times 1$ mm. Scan parameters for the Trio were as follows: MP-RAGE TR, 1820–1900 ms; TE, 3.74–3.75 ms; flip angle, 7°; image resolution, $1 \times 1 \times 1$ mm.

Image Analysis

Brain tissue was classified as GM, white matter (WM), and cerebrospinal fluid (CSF) using an atlas-moderated iterative expectation maximization segmentation algorithm using the T_1 images as previously described (Knickmeyer et al. 2008), following standard reference space alignment and inhomogeneity correction. In addition, GM was parcellated into 90 regions by nonlinear warping of a neonatal adaptation of the Automated Anatomical Labeling (AAL) atlas template as previously described (Shi et al. 2011; Gilmore et al. 2012). With these methods, we obtained measures of intracranial volume (ICV), total GM, total WM, total CSF, and 90 regional GM volumes.

Developmental Assessment

All children completed the Mullen Scales of Early Learning (Mullen 1995) to assess general cognitive and motor development. The Mullen consists of five scales (gross motor, visual reception, fine motor, expressive language, and receptive

Table 1 Demographics, medical history, and cognitive performance of infants with TS and typically developing controls

Variable	TS		Female control		Male control		P
	N	Mean (SD) range	N	Mean (SD) range	N	Mean (SD) range	
Gestational age at birth (days)	26	268 (11) 246–286	47	277 (9) 259–295	39	274 (11) 241–289	0.0061
Birth weight (grams)	26	2804 (421) 2155–3925	47	3380 (433) 2340–4414	39	3386 (475) 2375–4562	<0.0001
Age at MRI (days)	26	389 (16) 359–419	47	383 (22) 339–439	39	382 (19) 343–422	0.2056
Maternal age (years)	25	30 (6) 22–43	47	31 (5) 21–41	39	31 (4) 21–42	0.5514
Paternal age (years)	24	33 (7) 24–51	47	33 (6) 22–51	37	33 (4) 23–40	0.8312
Maternal education (years)	24	15 (3) 6–20	47	16 (3) 9–23	39	16 (3) 10–22	0.2354
Paternal education (years)	23	14 (4) 3–23	46	16 (4) 8–22	39	16 (3) 9–22	0.0927
Total household income (dollars)	24	76 095 (69 420) 0–330 000	45	65 029 (41 482) 0–170 000	37	73 562 (47 868) 0–200 000	0.8229
Mullen early learning composite	24	100 (14) 72–120	47	120 (13) 84–144	39	122 (13) 78–150	<0.0001
Mullen gross motor*	19	42 (8) 31–58	47	58 (13) 37–80	39	57 (15) 20–80	<0.0001
Mullen fine motor*	24	54 (10) 22–66	47	64 (8) 44–77	39	66 (7) 49–80	<0.0001
Mullen visual reception*	24	52 (8) 38–66	47	64 (11) 37–80	39	65 (9) 41–80	<0.0001
Mullen expressive language*	24	48 (13) 20–71	47	61 (8) 42–78	39	60 (9) 33–80	<0.0001
Mullen receptive language*	24	45 (8) 31–60	47	52 (8) 31–69	39	54 (8) 31–77	<0.0001
	N	Percent	N	Percent	N	Percent	
Maternal ethnicity							0.0181
White	23	92	32	68	35	90	
Black	2	8	13	28	2	5	
Asian	0	0	2	4	2	5	
Paternal ethnicity							0.0567
White	23	92	32	68	32	86	
Black	2	8	13	28	3	8	
Asian	0	0	2	4	2	5	
Smoking							1.0000
Yes	1	4	3	6	2	5	
No	25	96	44	94	37	95	
Scanner							0.0160
Trio	13	50	11	23	7	18	
Allegra	13	50	36	77	32	82	

*T-scores; SD means standard deviation.

language) with their own age-group standardized normative T-scores and percentiles. The standardized T-scores of four scales (gross motor not included) are combined into an early learning composite similar to an IQ score. The Mullen has good standardization and reliability data, collected in two phases over an eight-year period in the 1980s, with median internal consistency scores ranging between 0.75 and 0.91, and test–retest correlations over 0.82 for 1–25 months.

Parent of Origin Analysis

To assign the parent of origin of the remaining X chromosome in individuals with nonmosaic TS, we extracted DNA from blood samples provided by the infants and their parents. Samples were available for 11 trios (infant, mother, and father) and 2 pairs (infant and mother). Samples were genotyped using the Illumina MEGA Array. We created two different algorithms for deriving the parent of origin in these families. The first required full trio data, and for each trio involved 1) identifying X chromosome variants with no missing genotypes and homozygous parental genotypes, one with allele A and one with allele B; 2) counting the total number of allele transmissions from mother and from father; 3) a two-sided binomial test of the proportion of mother/father transmissions under a null that 50% of transmissions will come from the mother. The second algorithm is useable on parent/child pairs. Here, for a particular parent/child pair we identified all variants where there is evidence of at least one nonreference allele in either the parent or child genotype. We then tested the null of a lack of nontransmission based on these genotypes, and tested this null using a one-sided binomial test versus an expected proportion of 0.01, which represents any nontransmissions in the data attributable to genotyping error. In cases where we rejected the null, there was considered to be sufficient evidence for the parent in the parent/child pair to not have contributed an X chromosome copy to the child. All classifications made with the parent/child algorithm were concordant with separate classifications made using the trio-based algorithm.

Statistical Analysis

Statistical analyses were performed using SAS statistical software, version 9.4. For demographic data, two-sided Fisher's exact tests were used to evaluate group differences in categorical variables. Two-sided nonparametric Kruskal–Wallis H tests were used for continuous variables. Subjects with missing data were excluded on a variable-by-variable basis. One-way analysis of covariance (ANCOVA) was used to test for differences in global

Table 2 Similar global brain volumes in infants with TS and typically developing controls

Brain volume	LSMean (SE) TS	LSMean (SE) control female	LSMean (SE) control male	P-value ANCOVA
ICV	892 235 (17 241)	926 152 (12 064)	940 315 (13 244)	0.1135
GM	611 636 (11 032)	636 502 (7719)	640 016 (8474.47)	0.1397
WM	213 653 (5977)	220 509 (4178)	228 199 (4587)	0.1500
CSF	66 946 (1956)	69 141 (1368)	72 101 (1502)	0.0980

SE means standard error.

and regional brain volumes between our three groups: females with TS (TS), typically developing females (control females), and typically developing males (control males). To achieve variance reduction, we included variables previously associated with imaging outcomes as covariates. For global brain volumes the following variables were included as covariates: birth weight, age at MRI, maternal education, paternal education, and scanner (Allegra or Trio). For regional GM volumes the following variables were included as covariates: ICV and scanner. For global brain volumes, the threshold for statistical significance was $\alpha = 0.05$ uncorrected. For regional GM volumes, the threshold for statistical significance was $\alpha = 0.05$ after performing an adjusted false discovery rate correction using the linear step-up method of Benjamini and Hochberg (1995) to control for multiple comparisons. If the overall test was significant post hoc tests (pairwise comparisons) were performed.

We also performed the following sensitivity analyses: 1) We repeated our primary analyses excluding individuals with non-monosomic TS ($N = 6$). 2) We repeated our primary analyses excluding individuals with evidence of a developmental delay (early learning composite score on the Mullen scales of learning less than 70 at any point from birth to 4 years of age; $N = 3$; all with TS). 3) We repeated our primary analysis excluding individuals born prematurely (gestational age at birth less than 37 weeks; $N = 9$; 4 with TS, 5 control males). 4) We repeated our primary analyses including demographic and medical history variables that differed between the three groups as covariates.

Finally, we performed the following exploratory analyses: 1) In order to assess whether variation in brain volumes contributed to variation in cognitive and motor skills, we calculated Pearson correlations between the Mullen early learning composite score (and T-scores on the Mullen subscales) and regional brain volumes that showed significant differences in our primary analysis. Correlations were run within each group and in the combined sample. We also used one-way ANCOVAs to test for differences in Mullen scores between our three groups, using each regional brain volume as a covariate; 2) a *t*-test for equal least squares (LS) means from linear models was used to determine whether regional brain volumes that showed significant differences in our primary analysis differed between TS infants who received their remaining X chromosome from their mother (maternal X; $n = 9$) or their father (paternal X; $n = 4$), adjusting for ICV and scanner.

Results

Demographic and Medical History Variables

There were significant differences between the three groups in gestational age at birth, birth weight, and maternal ethnicity. Infants with TS were born earlier and weighed less at birth than both male and female controls. Female controls were less likely to have a white mother than male controls and females with TS. In addition, a greater proportion of infants with TS were scanned on the Trio scanner (and less on the Allegra) as compared to both male and female controls (Table 1).

Global Brain Volumes

There were no significant differences between the three groups in ICV, total GM, total WM, or total CSF in our primary analysis or our sensitivity analyses (see Table 2 and Supplementary Tables S1–S4).

Regional Brain Volumes

The following regions showed significant differences between groups on the three-way ANCOVA after adjustment for multiple comparisons: right calcarine cortex, left calcarine cortex, left lingual cortex, right lingual cortex, right precentral gyrus, left precentral gyrus, left frontal inferior operon, left frontal inferior trigonal, right parahippocampal cortex, left superior temporal gyrus, right middle temporal gyrus, left Heschl's gyrus, and right supramarginal gyrus (see Table 3 and Fig. 1*a*). Post hoc *t*-tests indicated that TS females had smaller volumes than typical females in the right calcarine cortex, left calcarine cortex, left lingual cortex, right lingual cortex, right precentral gyrus, left precentral gyrus, left frontal inferior operon, left frontal inferior trigonal, and right middle temporal gyrus (see Table 3 and Fig. 1*b*). TS females had smaller volumes than typical males in right calcarine cortex, left calcarine cortex, left lingual cortex, right lingual cortex, and right precentral gyrus. TS females had larger volumes than typical males in right parahippocampal cortex, right superior temporal gyrus, and left Heschl's gyrus (see Table 3 and Fig. 1*c*). Typical females had larger volumes than typical males in right parahippocampal cortex, right superior temporal gyrus left Heschl's gyrus, left precentral gyrus, and the left frontal inferior trigonal (see Table 3 and Fig. 1*d*).

Results of our sensitivity analyses can be found in Supplementary Figs S1–S4. Reduced volumes of calcarine and lingual cortex in infants with TS, as compared to both typically developing females and typically developing males, were robust across all sensitivity analyses. A number of additional regions emerged as significant in sensitivity analysis 1 (excluding infants with nonmonosomic TS) including right anterior cingulate cortex (larger in infants with TS compared to typical females and males), left superior occipital gyrus and left postcentral gyrus (both smaller in infants with TS compared to typical females and males), left inferior temporal gyrus (smaller in infants with TS compared to typical females and smaller in typical males compared to typical females), right insular cortex, left posterior cingulate cortex, left hippocampus, left amygdala (all larger in infants with TS compared to typical males), and right Heschl's

Table 3 Significant differences in regional GM volumes between infants with TS and typically developing controls

Brain region	LS means (SE) TS	LS means (SE) control female	LS means (SE) control male	P-value ANCOVA (adjusted FDR)	Percent difference TS versus control female	Percent difference TS versus control male	Percent difference control male versus control female
Typical males = typical females > TS females							
Calcarine (right)	6675 (185)	7711 (144)	7483 (162)	0.0022	−13%****	−11%**	−3%
Calcarine (left)	7280 (206)	8188 (160)	7890 (180)	0.0363	−11%***	−8%*	−4%
Lingual (right)	8233 (170)	9193 (132)	8901 (148)	0.0022	−10%****	−8%**	−3%
Lingual (left)	8123 (153)	8987 (119)	8872 (134)	0.0022	−10%****	−8%***	−1%
Precentral (right)	8387 (184)	9115 (143)	8930 (160)	0.0390	−8%**	−6%*	−2%
Frontal inferior operon (left)	2741 (72)	3049 (56)	2927 (62)	0.0363	−10%***	−6%†	−4%
Supramarginal (right)	6013 (101)	6422 (79)	6246 (88)	0.0390	−6%**	−4%†	−3%
Typical females > typical males = TS females							
Precentral (left)	8895 (159)	9367 (124)	8842 (139)	0.0382	−5%*	+1%	−6%**
Frontal inferior trigonal (left)	5879 (123)	6347 (96)	6063 (108)	0.0390	−7%**	−3%	−5%*
Middle temporal (right)	15 130 (194)	15 629 (151)	14 944 (170)	0.0363	−3%*	+1%	−4%**
Typical females = TS females > typical males							
Parahippocampal (right)	4535 (90)	4367 (70)	4167 (79)	0.0390	+4%	+9%**	−5%*
Heschl (left)	1180 (37)	1131 (29)	1033 (33)	0.0390	+4%	+14%**	−9%*
Superior temporal (right)	10 345 (138)	10 420 (107)	9962 (120)	0.0390*	−1%	+4%*	−4%**

**** <0.0001, *** <0.001, ** <0.01, * <0.05, † <0.1 (uncorrected *P*-value post hoc *t*-test); FDR means false discovery rate.

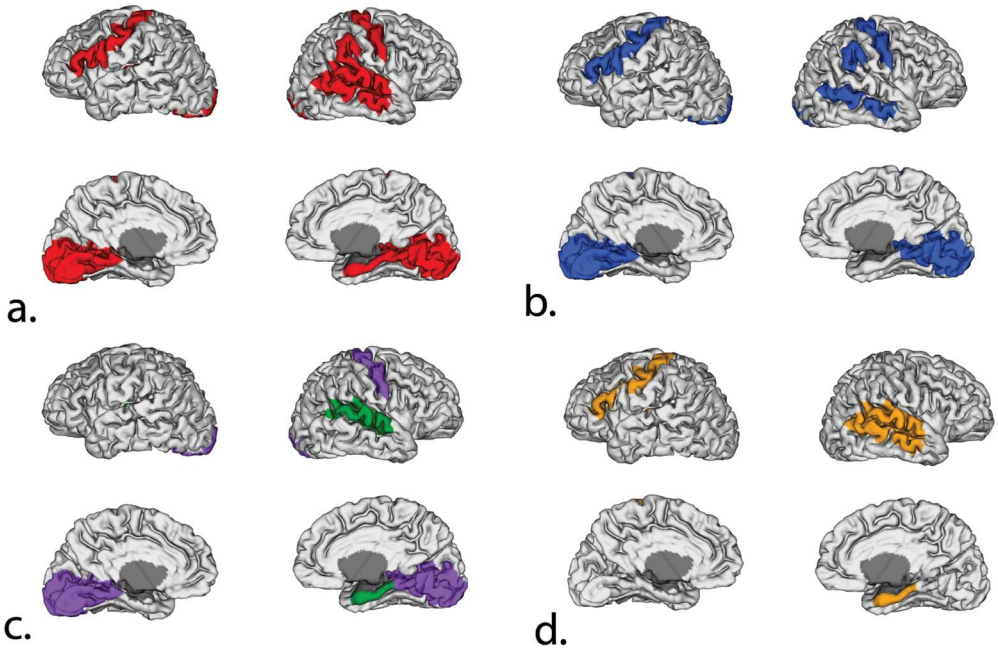

Figure 1. Significant differences in regional GM volumes between infants with TS and typically developing controls. (*a*) Results of three group ANCOVA on surface reconstruction; regions in red are significant after adjusted FDR correction. (*b*) Results of post hoc *t*-tests (TS females versus typical females); blue indicates TS females have significantly smaller volumes than typical females. (*c*) Results of post hoc *t*-tests (TS females versus typical males); purple indicates TS females have significantly smaller volumes than typical males; green indicates TS females have significantly larger volumes than typical males. (*d*) Results of post hoc *t*-tests (typical females versus typical males); orange indicates typical females have significantly larger volumes than typical males.

Table 4 Correlations between regional brain volumes identified in the primary analysis and scores on the Mullen Scales of Early Learning (combined sample and TS only)

Brain region	Mullen composite		Gross motor		Fine motor		Visual reception		Expressive language		Receptive language	
	Combined	TS	Combined	TS	Combined	TS	Combined	TS	Combined	TS	Combined	TS
Calcarine (right)	0.17†	0.11	0.19†	−0.10	0.16	−0.0004	0.13	−0.12	0.25**	0.45*	0.04	0.03
Calcarine (left)	0.11	−0.13	0.10	−0.10	0.10	−0.17	0.08	−0.12	0.18	0.18	−0.02	−0.40†
Lingual (right)	0.23***	0.46*	0.09	0.27	0.27**	0.38†	0.29**	0.18	0.28**	0.31	0.23*	0.62*
Lingual (left)	0.33***	0.15	0.24*	0.07	0.28**	0.17	0.31**	0.18	0.26**	−0.05	0.24*	0.31
Precentral (right)	0.19*	0.10	0.27**	0.05	0.14	0.11	0.24*	−0.29	0.12	0.32	0.14	0.17
Frontal inferior operon (left)	0.12	−0.06	0.15	0.09	0.18†	0.07	0.09	−0.0007	0.08	−0.13	0.07	0.06
Supramarginal (right)	−0.05	−0.35†	0.05	−0.25	−0.08	−0.24	0.02	−0.25	−0.05	−0.39†	−0.05	−0.003
Precentral (left)	0.07	0.08	0.13	0.24	0.10	0.12	0.06	0.03	0.08	0.10	0.01	0.14
Frontal inferior trigonal (left)	0.21*	0.30	0.27**	0.48*	0.15	0.34	0.20*	0.40†	0.19*	0.05	0.12	−0.004
Middle temporal (right)	−0.03	−0.35†	−0.003	−0.12	−0.02	−0.27	0.06	−0.20	−0.07	−0.30	−0.06	−0.17
Parahippocampal (right)	−0.07	−0.38†	−0.02	0.09	−0.04	−0.13	−0.03	−0.20	−0.10	−0.32	−0.04	−0.22
Heschl (left)	0.07	0.08	−0.09	−0.40†	0.05	−0.10	0.08	−0.04	0.03	0.18	−0.02	−0.24
Superior temporal (right)	0.03	−0.27	0.13	0.08	0.006	−0.17	0.03	−0.14	0.02	−0.22	0.04	−0.08

**** <0.0001, *** <0.001, ** <0.01, * <0.05, † <0.1 (uncorrected P-value); please note that no significant correlations were observed within typical males or typical females.

gyrus (larger in infants with TS compared to typical males and larger in typical females compared to typical males).

Exploratory Analyses

In the combined sample, scores on the Mullen Scales of Early Learning showed significant correlations with right calcarine, right and left lingual, right precentral, and left frontal inferior trigonal cortex (Table 4). However, no significant correlations are seen within typical males and females (data not shown). Several nominally significant correlations were identified in TS infants (Table 4). Specifically, right lingual cortex volumes were positively correlated with Mullen composite scores and T-scores for receptive language. Right calcarine volumes were positively correlated with expressive language T-scores, and volumes of the left frontal inferior trigonal were positively correlated with gross motor T-scores. None of these relationships would survive correction for multiple comparisons. Group differences in Mullen scores are still significant when including individual brain volumes as covariates (data not shown). We did not observe any significant differences between infants with TS and a maternal X chromosome and those with TS and a paternal X chromosome for brain regions that emerged as significant in our primary analysis (Table 5). In general, individuals with a maternal X chromosome had smaller regional volumes than those with a paternal X chromosome.

Discussion

We report results from the first quantitative neuroimaging study of infants with TS. Consistent with the literature in school age, adolescent, and adult individuals with TS (Brown et al. 2004; Kesler et al. 2004; Cutter et al. 2006; Marzelli et al. 2011; Green et al. 2014), we observed decreased GM volumes in premotor, somatosensory, and parietal-occipital cortex in comparison to typically developing females. When we restricted our analysis to individuals with monosomic TS, we also observed increased GM volumes in right insular cortex and left amygdala, similar to reports in older cohorts (Good et al. 2003; Kesler et al. 2004; Marzelli et al. 2011). Although post hoc comparisons for the latter two results were only significant when comparing TS females to typically developing males, examination of the least squares means (LSMean) suggests that TS females also have enlarged right insular cortex and left amygdala compared to typical females. Results suggest that many aspects of the neuroanatomical phenotype of TS are established in the prenatal and/or early postnatal period and remain relatively stable into adulthood. This is not entirely surprising given that the prenatal and early postnatal period is the foundational phase of human brain development, characterized by exuberant neurogenesis, neuronal migration, dendritic arborization, synaptogenesis, gyrification, myelinization, and waves of programmed cell death (Stiles and Jernigan 2010).

There were also some aspects of the neuroanatomical phenotype as described in older cohorts that were not present at this early age. In particular, we did not observe volume reductions in the cuneus or the superior parietal lobule, nor did we observe enlargement of the caudate or putamen. Our results suggest that volumetric reductions in primary visual cortex (centered around the calcarine sulcus) and secondary visual cortex (including the lingual gyrus) precede reductions in high-order visual-spatial processing areas. These findings may be relevant to the ongoing debate as to what foundational deficits might explain the broad

Table 5 Parent of origin of the remaining X chromosome exerts minimal/no effect on regional brain volumes

Brain region	LS means (SE) maternal X	LS means (SE) paternal X	LS mean difference (SE)	P-value test for equality of LS means	Percent difference maternal versus paternal X
Calcarine (right)	6266 (241)	6626 (361)	−360 (435)	0.4297	−6%
Calcarine (left)	6966 (248)	6584 (372)	383 (448)	0.4154	5%
Lingual (right)	7705 (312)	8204 (468)	−500 (565)	0.3994	−6%
Lingual (left)	7493 (313)	7810 (470)	−317 (567)	0.5894	−4%
Precentral (right)	7798 (195)	8112 (292)	−313 (352)	0.3969	−4%
Frontal inferior operon (left)	2465 (78)	2534 (117)	−68 (141)	0.6393	−2%
Supramarginal (right)	6008 (183)	5708 (274)	300 (331)	0.3875	4%
Precentral (left)	8315 (224)	8038 (336)	277 (405)	0.5106	3%
Frontal inferior trigonal (left)	5687 (162)	5796 (243)	−109 (293)	0.7184	−2%
Middle temporal (right)	14 440 (280)	14 703 (420)	−263 (506)	0.6166	−2%
Parahippocampal (right)	4201 (150)	4503 (225)	−302 (271)	0.2937	−7%
Heschl (left)	1071 (62)	1209 (93)	−137 (113)	0.2520	−12%
Superior temporal (right)	9838 (94)	10 013 (141)	−174 (170)	0.3310	−2%

range of visual-spatial and arithmetical difficulties exhibited by individuals with TS. Some researchers have hypothesized that the foundational deficit in girls with TS is altered development of spatiotemporal attention (Beaton et al. 2010). Others have suggested that impairments in executive function contribute to the emergence of cognitive difficulties in other domains (Lepage et al. 2011). The current study suggests that detailed assessments of the early stages of cortical visual processing in infants and toddlers with TS are warranted in order to understand how low-level computational processing or subtle differences in developmental timing might eventually produce the specific pattern of cognitive strengths and weaknesses observed in older individuals with TS.

We also note that while some researchers posit that the cognitive profile observed in individuals with TS is indicative of right hemisphere dysfunction (also referred to as nonverbal learning disorder or NVLD; Hepworth and Rovet 2000), the majority of neuroanatomical differences we observed were bilateral. Recent literature reviews suggest there is a TS-specific social and cognitive profile which may overlap with other constructs, such as NVLD, but which cannot be reduced to it (Hong et al. 2009; Knickmeyer and Davenport 2011; Gravholt et al. 2017). Our findings support the hypothesis that this social and cognitive profile reflects a relatively uniform dysfunction of the left and right hemispheres (Ganou and Grouios 2008) and is not exclusively tied to WM impairment.

In general, our results suggest that early interventions could be important for improving cognitive and psychosocial outcomes for individuals with TS. Parallels might be drawn to children with autism where behavioral interventions applied in preschool have clinically significant influences on symptoms (Dawson 2008; Odom et al. 2012; Kasari et al. 2014), and emerging evidence indicates earlier interventions have even greater impact (Harris and Handleman 2000; Rogers et al. 2014; Green et al. 2017). Similarly, early life interventions for disadvantaged children, such as high-quality preschool programs, show greater return on investment than later interventions (Masten and Cicchetti 2010). Of course, this assumes that the neuroanatomical phenotypes we observed are linked in a meaningful way to behavioral function. In order to begin exploring this question, we tested whether scores on the Mullen Scales of Early Learning correlated with the 13 regional brain volumes showing significant group differences in our primary analysis. In evaluating these results, it is important to keep in mind that the Mullen scales assess general cognitive and motor ability; the procedure is not designed to target the specific constructs that are disrupted in older individuals with TS. In addition, although we observed significant differences between infants with TS and typical males and females, this did not necessarily reflect poor performance in the TS group. Instead, it appears to reflect exceptionally high performance in our control sample. For a more thorough analysis of early cognitive development in TS, readers are referred to another manuscript from our group (Pretzel et al., unpublished data).

Several significant correlations between key brain volumes and outcomes on the Mullen were observed in the combined sample, but this is likely a consequence of strong group differences between infants with TS and typical infants in both brain volume and Mullen scores. No significant correlations are seen within typical males and females. There are a few nominally significant correlations within infants with TS, although these would not survive correction for multiple comparisons. Larger studies are needed to confirm whether variation in regional brain volumes in infants with TS contributes to individual differences in cognitive, language, and motor development, and whether such findings vary with brain development over time. The current results suggest that variation in lingual and calcarine cortex, as well as the frontal inferior trigonal, may be relevant. Group differences in Mullen scores remain significant when including these individual brain volumes as covariates, which suggests that these particular brain volumes do not mediate the influence of TS diagnosis on Mullen scores.

One of the unique features of the current study is the inclusion of both a female and a male control group. Neuroanatomical differences in girls with TS may arise through a variety of mechanisms including haploinsufficiency of genes on the X chromosome, failure to express parentally imprinted genes, the uncovering of X chromosome mutations, gonadal steroid deficiency (Knickmeyer 2012), and changes in methylation of autosomal genes (Sharma et al. 2015). Comparing girls with TS to both male and female controls provides suggestive evidence regarding potential underlying mechanisms. A pattern of results where typical males = typical females ≠ TS females suggests the action of genes expressed from the inactive X chromosome in typical females that have homologues on

the Y chromosome. These are primarily located in an area called the pseudoautosomal region (PAR; Carrel and Willard 2005). In the current study, we observed this pattern of results for calcarine and lingual cortex, the right precentral and supramarginal gyri, and the left frontal inferior operon. A pattern where typical males = TS females ≠ typical females suggests the effect of genes that escape X-inactivation but have no functionally equivalent Y homologues (most likely these would be outside the PAR). However, this result would also be compatible with an estrogen mediated effect or an imprinted X-linked gene. In the current study, we observed this pattern of results for the left precentral gyrus, left frontal inferior trigonal, and the right middle temporal gyrus. Analyses comparing infants with TS and a maternal X chromosome to infants with TS and a paternal X chromosome did not provide strong evidence for an imprinted X-linked gene, but these results must be considered with caution given the small sample size for this exploratory analysis. Finally, a pattern where typical females = TS females ≠ typical males suggests a testosterone-mediated effect but would also be compatible with social/experiential factors associated with sex. We observed this pattern for right parahippocampal cortex, right superior temporal gyrus, and left Heschl's gyrus.

In conclusion, the current study represents an essential first step in constructing a developmental model of TS. Strengths of the study include the comprehensive analysis of brain structure, the inclusion of both male and female controls, and the careful consideration of mosaicism, developmental delay, and prematurity in our sensitivity analyses. Limitations include the moderate sample size. Although comparable to many studies carried out in older individuals with TS, our analyses may be underpowered to detect subtle differences in brain volume. Correlations between brain volumes and Mullen scores and results on parent of origin effects in particular must be considered as exploratory. Future studies ought to take a longitudinal approach, beginning prenatally or in early infancy, and incorporate neuroimaging, behavioral assessment, and evaluation of relevant clinical parameters such as endogenous hormone levels, growth failure and its treatment, cardiovascular malformations (and related surgeries), thyroid autoimmunity, and hearing issues. Ultimately, a better understanding of early development in girls with TS could lead to new interventions aimed at normalizing adverse ontogenetic pathways. It will also facilitate comparisons to other developmental conditions that may overlap with TS, including ADHD, dyscalculia, and autism, informing the question of whether interventions developed for non-TS populations will have similar, positive effects for girls with TS. Finally, a better understanding of the pathways leading to sexually dimorphic brain development will allow us to clarify how and why the sexes show differential vulnerability to certain psychiatric disorders and open up new possibilities for sex-tailored interventions and therapeutics.

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