Psychiatry Research: Neuroimaging 313 (2021) 111299

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

Cortical gray matter structure in boys with Klinefelter syndrome

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ARTICLE INFO

Keywords:
Brain structure
Klinefelter syndrome
XXY
Testosterone
MRI
Genetics

ABSTRACT

Klinefelter syndrome (KS, 47,XXY) is a common sex chromosome aneuploidy in males that is associated with a wide range of cognitive, social and emotional characteristics. The neural bases of these symptoms, however, are unclear. Brain structure in 19 pre- or early-pubertal boys with KS (11.5 ± 1.8 years) and 22 typically developing (control) boys (8.1 ± 2.3 years) was examined using surface-based analyses of cortical gray matter volume, thickness and surface area. Boys in the KS group were treatment-naïve with respect to testosterone replacement therapy. Reduced volume in the insula and dorsomedial prefrontal cortex was observed in the KS relative to the TD group, as well as increased volume in the parietal, occipital and motor regions. Further inspection of surface-based metrics indicated that whereas KS-associated increases in volume were driven by differences in thickness, KS-associated reductions in volume were associated with decreases in surface area. Exploratory analyses additionally indicated several correlations between brain structure and behavior, providing initial support for a neural basis of cognitive and emotional symptoms of this condition. Taken together, these data add support for a neuroanatomical phenotype of KS and extend previous studies through clarifying the precise neuroanatomical structural characteristics of that give rise to volumetric alterations.

1. Introduction

Klinefelter syndrome (KS) is the most common sex chromosome aneuploidy (47,XXY), affecting as many as 1 in 500 males (Smyth et al. 1998). Typical physical characteristics of the condition include androgen insufficiency, tall stature, gynecomastia and impaired spermatogenesis. A variety of cognitive, social and emotional features also accompany KS, including language and learning problems, executive dysfunction, poor concentration, low self-esteem, increased shyness, worry and depressed mood (Herlihy et al., 2011). Such features often go underappreciated, yet are frequently cited as being the greatest clinical concern by parents (Bourke et al., 2014).

To better understand the nature of KS-associated alterations in behavior, investigators have turned to the use of neuroimaging to examine brain structural patterns in this population. Findings from our group (Bryant et al., 2011; Hong et al., 2014; A. J. Patwardhan et al., 2002) and others has documented a variety of KS-related reductions in gray matter, including in the temporal lobe (Bryant et al., 2011; DeLisi et al., 2005; Giedd et al., 2007; Goddard et al., 2016; Itti et al., 2006; Patwardhan et al., 2000; Skakkebæk et al., 2014), insula (Bryant et al., 2011; Goddard et al., 2016; Hong et al., 2014; Shen et al., 2004; Skakkebæk et al., 2014), and inferior frontal cortex (Bryant et al. 2012; DeLisi et al., 2005; Goddard et al., 2016; Skakkebæk et al., 2014). Anomalous increases in cortical gray matter have also been noted in the sensorimotor (Bryant et al., 2011; Hong et al., 2014; Savic et al. 2014), cuneus/precuneus (Hong et al., 2014; Skakkebæk et al., 2014) and parietal-occipital cortices (Bryant et al. 2012; Hong et al., 2014; Skakkebæk et al., 2014). Importantly however, there are inconsistencies in findings, with many studies reporting no alterations in these regions (DeLisi et al., 2005; Giedd et al., 2007; Goddard et al., 2016; Itti et al., 2006; Shen et al., 2004).

Investigations of brain-behavior correlations have similarly mixed findings. Reduced left temporal lobe volume in KS was correlated in one report with poorer performance on language-related tasks (Itti et al., 2006). Others however have reported no observable correlations between brain and behavioral measures (Bryant et al., 2011; Skakkebæk

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https://doi.org/10.1016/j.pscychresns.2021.111299
Received 1 February 2021; Received in revised form 27 April 2021; Accepted 29 April 2021
Available online 4 May 2021

et al., 2014) or did not include an examination of these associations (DeLisi et al., 2005; Giedd et al., 2007; Goddard et al., 2016; Hong et al., 2014). Clarification of the relation between structural alterations in the brain and cognitive and psychosocial difficulties nevertheless represent a critical research goal, since the severity of behavioral difficulties can vary considerably between affected individuals, and can negatively influence long-term academic, adaptive (Boada et al., 2009) and psychological functioning (Bruining et al., 2009). Indeed, early identification and intervention for cognitive, social and emotional difficulties represents a pressing research priority for individuals with KS and for treating healthcare providers.

Factors contributing to inconsistencies in findings include age, androgen treatment status and neuroimaging analysis methods. Notably, most studies have included participant samples that have varied with respect to pubertal status and to history of testosterone supplementation, both of which have a significant effect on brain structure (Blakemore et al., 2010; Foland-Ross et al., 2019; Giedd et al., 2006; Patwardhan et al., 2000; Samango-Sprouse et al. 2013, but see Skakkebæk et al., 2014b). Moreover, existing work has largely relied on traditional voxel based morphometry (VBM) and manual region-of-interest (ROI) based methods for the quantification of cortical gray matter volume. In contrast to these approaches, newer surface-based modeling packages can measure the components that make up gray matter volume: cortical thickness and surface area. These two individual metrics follow different neurodevelopmental trajectories (Fjell et al., 2015; Hogstrom et al., 2013), have distinct genetic influences (Panizzon et al., 2009; Winkler et al., 2010) and exhibit unique associations with cognitive functions and psychiatric conditions (Noble et al., 2015; Schnack et al., 2015; Vuoksimaa et al., 2016). Moreover, the biological processes that drive surface area are separate from those that drive thickness (Geschwind et al. 2013; Rakic 1988). Thus, studies that incorporate measures of cortical thickness and surface area in addition to volume are likely to yield additional clarity into the underlying neural characteristics of different clinical conditions and their associated behaviors.

Given these issues, we conducted a rigorous analysis of KS-associated alterations in cortical gray matter structure using a surface-based analysis of cortical volume, thickness and surface area. To control for the effects of puberty and testosterone replacement therapy, we focused on pre- or early-pubertal males who had not yet initiated hormone supplementation. We hypothesized that males with KS would exhibit reduced cortical gray matter in the insula, temporal and frontal cortices relative to TD males, as well as increased cortical gray matter in the parietal and sensorimotor regions. Additionally, we explored whether KS-associated alterations in cortical gray matter were correlated with differences in cognitive and behavioral symptoms associated with this condition.

2. Methods

2.1. Participants

The study was approved by the Stanford University's institutional review board. Boys provided written assent, and a parent provided written informed consent. Tanner staging was performed by a trained physician. A total of 19 boys with KS (11.5 ± 1.8 years) and 22 TD boys (8.1 ± 2.3 years) were included in the study. Details regarding recruitment and eligibility criteria can be found in the Supplement.

2.2. Cognitive and behavioral testing

Parents completed the Behavioral Assessment System for Children, Second Edition (BASC-II; Reynolds 2004) to provide information on their child's emotions and behavior, and the Behavior Rating Inventory of Executive Function (BRIEF; Gioia et al., 2000), to provide information relating to their child's executive functioning. Children were administered the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler 2003), as well as the Wide Range Assessment of Visual Motor Abilities to index visual-motor skills (WRAVMA; Adams et al. 1995), the Wide Range Achievement Test, Fourth Edition, to index academic skills (WRAT; Wilkinson et al. 2006), and the Developmental NEuroPSYchological Assessment (NEPSY, Second Edition), to index a variety of cognitive skills including executive functioning/attention, language, memory/learning, sensorimotor functioning, visuospatial processing and social perception (Korkman et al., 2007). Age-normed composite *T* scores were compared between KS and TD groups using independent samples *t* tests. Scores exhibiting a significant difference between groups were fed into secondary correlation analyses to test for brain-behavior associations.

2.3. MRI acquisition and analysis

Imaging data were acquired using a Signa 3.0 T whole-body MR system (GE Medical Systems, Milwaukee, WI) and processed using the FreeSurfer software package (version 5.3, http://surfer.nmr.mgh.harvard.edu). Details on MRI data acquisition can be found in the Supplement.

Statistical analyses were conducted to assess group differences in cortical gray matter volume, thickness and surface area. For each of these analyses, a general linear model (GLM) was fit at each vertex with the structural measure as the dependent variable and diagnostic group (KS, TD) as the independent variable. Age and total cortical tissue volume were included as covariates, centered to the sample mean. Correction for multiple comparisons was conducted using Monte-Carlo simulation.

2.4. Associations between cortical gray matter structure and cognition

Associations between brain and behavior were examined separately within the KS and TD groups using bivariate correlation analyses. After checking distributions with the Shapiro-Wilks test, associations between normally distributed variables were assessed by Pearson correlation. Non-normally distributed data was analyzed using Spearman correlation. Structural metrics were adjusted for age and total cortical tissue volume. To reduce the number of comparisons and because volume represents the direct product of thickness and surface area (Fischl et al. 2000), we constrained these exploratory analyses to volume measurements. Bonferonni correction for multiple comparisons was conducted across the number of brain regions tested. Due to the exploratory nature of these analyses however, we did not correct for the number of behavioral tests or sample groups.

3. Results

3.1. Participants

Participants in the KS and TD groups did not differ with respect to age, $t(39) = -0.607$, $p = 0.547$, total cortical tissue volume, $t(39) = 1.686$, $p = 0.100$, or Tanner stage, $t(39) = 0.440$, $p = 0.663$ (**Table 1**).

Comparisons of standardized scores from behavioral assessments indicated significant differences between the two groups (**Table 2**), including measures of intelligence (Full Scale IQ, Perceptual Reasoning

Table 1
Participant characteristics.

	KS	TD	*p* value
N	19	22	–
Age (years)	8.5 ± 2.1	8.1 ± 2.3	0.547
Tanner stage	1.1 ± 0.3	1.2 ± 0.4	0.663
Total cortical tissue volume (cm^3)	992 ± 79	1041 ± 103	0.100

KS, Klinefelter syndrome. TD, typically developing. Values for the KS and TD groups are means ± standard deviation.

Table 2
Behavioral and cognitive functioning differences between groups.

Assessment	KS	TD	*p* value
WISC-IV			
Full scale Intelligence Quotient (FSIQ)[a]	94.7 ± 14.2	111.5 ± 8.5	**< 0.001**
Perceptual Reasoning Index (PRI)[a]	102.3 ± 13.4	113.2 ± 11.5	**0.008**
Verbal Comprehension Index (VCI)[a]	95.3 ± 14.6	116.5 ± 14.5	**< 0.001**
Processing Speed Index (PSI)[a]	92.4 ± 12.5	96.3 ± 12.1	0.321
Working Memory Index (WMI)[a]	88.0 ± 15.9	102.3 ± 9.3	**0.003**
BASC-2			
Behavioral Symptoms Index[b]	55.6 ± 12.5	47.1 ± 5.7	**0.012**
Adaptive Skills[a]	43.7 ± 10.6	50.3 ± 7.9	**0.030**
Externalizing Problems[b]	54.1 ± 11.8	49.3 ± 8.3	0.145
Internalizing Problems[b]	54.2 ± 15.8	45.9 ± 9.9	**0.050**
BRIEF			
Behavioral Regulation Index[b]	55.4 ± 13.4	45.7 ± 7.5	**0.009**
Metacognition Index[b]	59.6 ± 14.5	49.9 ± 11.0	**0.021**
Global Executive Composite[b]	58.5 ± 14.3	48.0 ± 9.5	**0.011**
NEPSY-II*			
Comprehension of Instructions[a]	10.1 ± 2.5	12.0 ± 2.0	**0.020**
Response set[a]	8.4 ± 3.6	11.3 ± 2.6	**0.028**
Memory for faces (delayed)[a]	10.1 ± 2.9	11.8 ± 2.0	**0.048**
Narrative memory (free and cued recall)[a]	8.3 ± 3.0	10.7 ± 3.4	**0.035**
WRAVMA			
Visual-Motor Integration Composite[a]	94.6 ± 13.3	104.0 ± 11.4	**0.027**
WRAT			
Word reading[a]	101.5 ± 11.1	111.5 ± 12.9	**0.016**
Spelling[a]	101.5 ± 16.4	111.8 ± 12.2	**0.048**
Math[a]	102.6 ± 13.2	107.9 ± 10.3	0.188
Sentence comprehension[a]	90.3 ± 30.5	113.4 ± 35.4	0.061
Reading composite[a]	98.1 ± 13.7	117.8 ± 13.6	**0.001**

KS, Klinefelter syndrome. TD, typically developing. WISC-IV, Wechsler Intelligence Scale for Children, 4th Edition. BASC-2, Behavior Assessment System for Children 2nd Edition. BRIEF, Behavior Rating Inventory of Executive Functioning. NEPSY, A Developmental NEuroPSYchological Assessment. WRAVMA, Wide Range Assessment of Visual Motor Abilities. WRAT, Wide Range Achievement Test.

* See Supplement for more information. A, higher scores indicate better performance. B, higher scores indicate worse performance.

Index, Working Memory Index on the WISC-IV; *ps* < 0.01), visuospatial capabilities (Visual-Motor Integration Composite of the WRAVMA; $p = 0.027$), emotion and behavior (Behavioral Symptoms Index, Internalizing Problems composite and Adaptive Skills composite of the BASC-2; *ps* < 0.031), executive functioning (Behavioral Regulation Index, Metacognition Index and Global Executive Composite on the BRIEF, Response Set subtest on the NEPSY; *ps* < 0.0289), memory (Memory for Faces and Narrative Memory subtests on the NEPSY; *ps* < 0.049) and verbal skills (Verbal Comprehension Index on the WISC-IV, Word Reading and Spelling subtests of the WRAT; Comprehension of Instructions on the NEPSY; *ps* < 0.049).

3.2. Statistical analysis of a main effect of group on cortical gray matter structure

Group differences in cortical structure are presented in Table 3 and Fig. 1. Vertex-based analyses of cortical gray matter volume indicated decreased volume in the KS relative to the TD group in the left and right insula and left dorsomedial prefrontal cortex. Increased volume in the KS group was observed in a cluster in the left occipital cortex that included the precuneus, cuneus, pericalcarine cortex and lingual gyrus, a cluster in the right parietal cortex that included the paracentral cortex and precuneus, and a cluster in the right precentral gyrus.

Vertex-based analyses of cortical gray matter thickness indicated increased thickness in the KS relative to the TD group in 3 regions: a cluster that encompassed the left parahippocampal gyrus, a cluster that included the left occipital and parietal cortex and a third cluster that included the right precentral and parietal cortex. No areas of decreased thickness were observed in the KS relative to the TD group.

Finally, vertex-based analyses of pial surface area indicated decreased surface area in the KS relative to the TD group in the left and right insula, the left dorsomedial prefrontal cortex and the left fusiform gyrus. Increased surface area was observed in the KS relative to the TD group in the left occipital cortex. This cluster included the pericalcalarine cortex and lingual gyrus.

Posthoc analyses of regional measures of volume, thickness and surface area using a multivariate GLM indicated that the main effect of group across all regions remained significant when controlling for FSIQ in addition to age and total cortical tissue volume.

3.3. Secondary analyses of an association between structure and behavior

Exploratory correlations between assessment and brain measures were performed separately within the KS and TD groups. Within the KS group, increased volume in the left dorsomedial prefrontal cortex was

Table 3
Regional differences in cortical gray matter structure between groups.

Region	KS	TD	x/y/z			Cluster Size (mm^2)	*p* value
Volume (mm^3)							
Left insula	5667 ± 319	6366 ± 393	−26	24	−6	2233	0.017
Right insula	4694 ± 435	5534 ± 493	33	4	7	1879	0.043
Left dorsomedial prefrontal cortex	4354 ± 589	5176 ± 491	−7	34	50	1893	0.028
Left occipital cortex	10,872 ± 1515	9541 ± 1387	−12	−67	35	4735	<0.001
Right precentral gyrus	4533 ± 636	4081 ± 557	40	−11	43	1854	0.039
Right parietal/occipital cortex	12,723 ± 1303	10,646 ± 1326	3	−33	65	4656	<0.001
Thickness (mm)							
Left parahippocampal gyrus	2.819 ± 0.212	2.596 ± 0.169	−35	−23	−23	2260	0.018
Left parietal/occipital cortex	2.248 ± 0.114	2.062 ± 0.123	−47	−28	53	4632	<0.001
Right parietal/occipital cortex	2.319 ± 0.130	2.128 ± 0.148	62	−10	29	11,363	<0.001
Surface Area (mm^2)							
Left insula	2297 ± 207	2567 ± 260	−26	24	−6	2917	0.002
Right insula	1468 ± 114	1710 ± 181	37	−13	2	2140	0.015
Left dorsomedial prefrontal cortex	1674 ± 140	1870 ± 181	−7	34	50	2312	0.011
Left fusiform	2060 ± 242	2500 ± 417	−43	−65	−19	2809	0.002
Left occipital cortex	1770 ± 264	1635 ± 253	−13	−91	4	1932	0.017

KS, Klinefelter syndrome. TD, typically developing. Values for the KS and TD groups are means ± standard deviation. X/Y/Z, MNI coordinates of the peak significance within the cluster. Significance values indicate the cluster wise *p* value, corrected for multiple comparisons.

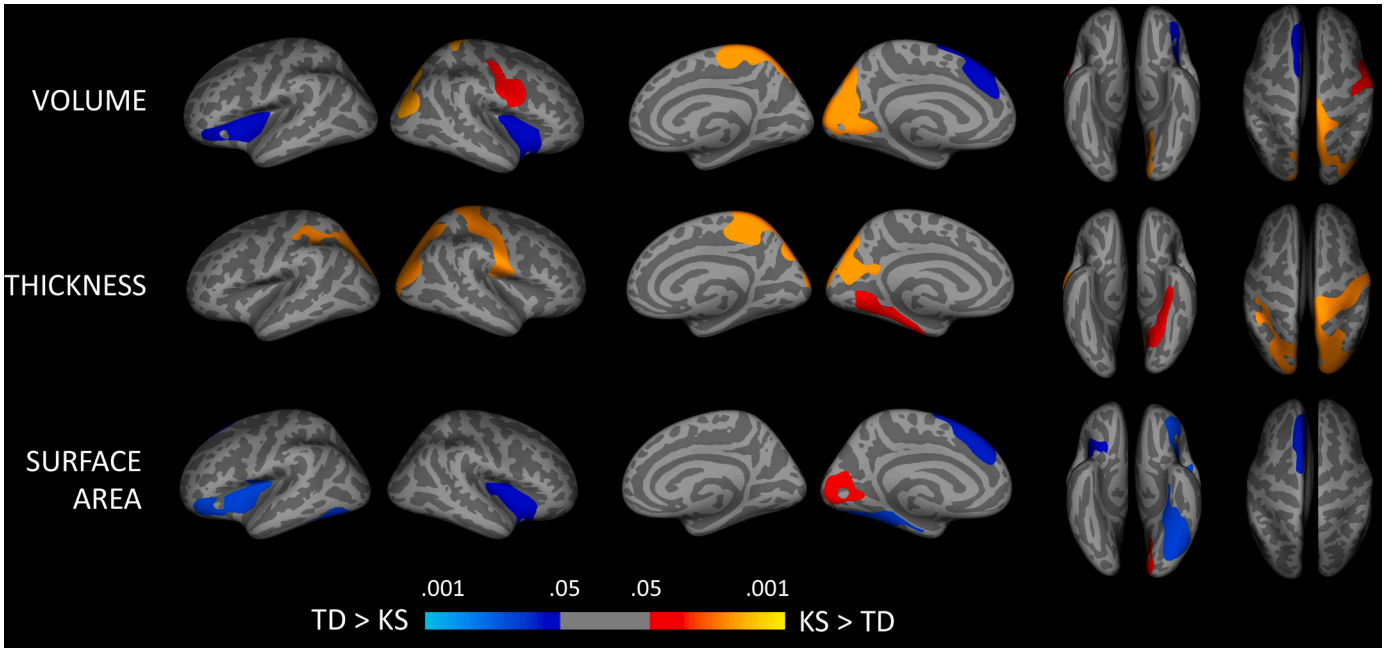

Fig. 1. Corrected statistical significance maps showing areas of reduced (blue) and increased (orange/red) cortical gray matter metrics in boys with KS relative to typically developing (TD) boys. Colors are corrected significance (p) values, shown on the inflated surface of the average template.

correlated with higher FSIQ (WISC, $r = 0.679$, corrected $p = 0.006$), better adaptive skills (BASC-2 Adaptive Skills Composite, $r = 0.586$, corrected $p = 0.048$), fewer behavioral regulation problems (Behavioral Regulation Index of the BRIEF, $r = -0.619$, corrected $p = 0.030$), and improved visuomotor abilities (WRAVMA Visual-Motor Integration Composite, $r = 0.634$, corrected $p = 0.048$). Increased parietal volume in the right hemisphere was also correlated in the KS group with fewer behavioral problems (Behavioral Symptoms Index of the BASC-2, $r = -0.600$, corrected $p = 0.043$). No associations between assessment scores and brain measures were observed within the TD group. Fisher's r-to-z transformation indicated that correlation differences between groups were not significantly different, $ps > 0.05$.

4. Discussion

This study was conducted to examine KS-associated alterations in cortical gray matter volume, thickness and surface area in a sample that was carefully recruited to avoid the confounds of puberty and testosterone supplementation. Using advanced surface-based procedures, we observed reduced volume in the insula and dorsomedial prefrontal cortex in the KS relative to the TD group, as well as increased volume in the parietal-occipital and sensorimotor regions. Further inspection of these differences indicated that whereas reductions in volume were associated with decreases in surface area, increases in volume were associated with greater regional cortical thickness. Finally, exploratory analyses indicated correlations between structure and behavior, suggesting a neural basis for KS-associated alterations in emotional and cognitive function (Temple et al. 2003; van Rijn et al. 2018). Taken together, these data add support for a neuroanatomical phenotype of KS and extend previous studies through clarifying the specific aspects of cortical morphometry that may underlie volume alterations in this genetic condition.

A unique strength of the present study is the careful selection of the study sample. The influence of pubertal fluctuations testosterone and other sex hormones on brain structures been well documented in typical development (Bramen et al., 2011). Restricting our examination of brain structure to pre- or early-pubertal boys that have not yet begun treatment with testosterone replacement therapy therefore controls for the confounding influence of this hormone on gray matter structure. Indeed, because testosterone deficiency in KS typically begins at or after the onset of puberty (Salbenblatt et al., 1985, but see Gravholt et al., 2018), alterations in cortical gray matter observed here may likely be due to the genetic components of this condition. Support for this interpretation comes from Savic and Arver (2014), who found that sensorimotor cortical thickness was reduced in XY adult males compared with both XX adult females and XXY adult males, indicating an X-chromosome gene-dosage effect. Future studies that track whether testosterone supplementation in adolescents influences KS-associated differences in cortical gray matter structure is not yet known and is the focus of ongoing studies by our group.

Our finding of reduced insula volume in boys with KS are strikingly consistent with the small extant literature examining structural alterations in males with this condition (Bryant et al., 2011; Hong et al., 2014; Shen et al., 2004; Skakkebæk et al., 2014). This area of the brain is well recognized for its role in the identification, experience and regulation of emotions, as well as social functioning and empathy (Namkung et al., 2017; Singer 2006) – behaviors that are notably affected in KS (Boone et al., 2001; Ratcliffe 1999; van Rijn et al. 2006, 2008, 2014a, 2014b). Unlike previous studies however, the current investigation found that volume reductions in this region of XXY males were driven by alterations in surface area.

Indeed, use of surface-based analyses in the current study serves as a unique contribution to the literature. This method can identify spatially overlapping patterns of alterations in thickness and surface area – metrics that comprise cortical gray matter volume and that are genetically and phenotypically independent from one another (Panizzon et al., 2009; Winkler et al., 2010). While the precise neurobiological factors that drive each metric have yet to be understood, available evidence indicates that one determinant of surface area is the number of cortical columns (Rakic 1988). This number does not change following birth, despite significant increases in surface area seen in childhood (Hill et al., 2010). However, surface area is also influenced by the spacing between columns, and inter-columnar neuropil (Buxhoeveden et al., 2001). Additional research that tests whether these or other factors directly underlie the reductions in cortical gray matter volume and surface area observed here are needed.

Our observations of increased volume of the sensorimotor, cuneus and parietal-occipital areas of boys with KS are also remarkably

consistent with those of previous studies (Bryant et al., 2011, 2012; Hong et al., 2014; Savic et al. 2014; Skakkebæk et al., 2014). In contrast to the insula, however, increased volume in these regions appear driven by differences in cortical thickness. Available research finds that across widespread areas of the cortex, there is an inverse relation between thickness and neuronal density (Cahalane et al., 2012; la Fougère et al. 2011). Thickness increases in the sensorimotor, cuneus and parietal-occipital cortices of boys with KS therefore may be driven by a reduction in neuron number. Alternatively, increased thickness in these regions may occur in the absence of an increase in number of neurons, leading to a decrease in density. It is also possible, however, that increased thickness in these regions may be the result of reduced myelination of cortical axons. Neurodevelopmental studies of typically developing youth, for example, find that regional cortical thinning during adolescence is tightly coupled with the expansion of white matter and an increased organization of cortical axons (Alemán-Gómez et al., 2013; Vandekar et al., 2015). Thus, cortical thinning at puberty is not entirely the result of reductions in the size or number of neuron cell bodies or their synaptic processes, but rather by an increase in the myelin coating of fibers in lower cortical layers (Sowell et al., 2004; Toga et al., 2006). Increased proliferation of myelin into the inner periphery of the cortical neuropil, in turn, leads to a change in the MR signal value from gray matter in young children to white matter in adolescents and young adults. Whether increased thickness in the sensorimotor, cuneus and parietal-occipital cortices of boys with KS is the result of reduced neuron number, decreased myelination of cortical axons or another neurobiological process remains to be clarified. The functional significance of these alterations also remains to be understood. Increased gray matter in this region, for example, may reflect a relative sparing of these regions and their associated functions. In line with this formulation, visuospatial abilities are relatively unaffected in KS (Gravholt et al., 2018). Inconsistent with this interpretation, however, are observations of reduced sensorimotor function in KS (Verri et al., 2010).

The current study is the first, to our knowledge, to observe reductions in left dorsomedial prefrontal and fusiform gray matter in boys with KS. The latter area represents a key neural structure subserving the perception of social and emotional signals as well as structural features from human faces (Schultz et al., 2003). Thus, volumetric and surface area reductions in fusiform gray matter may underlie a reduced capacity of males with KS to recognize faces or identify emotional facial expressions (van Rijn et al. 2018). The dorsomedial prefrontal cortex, in turn, subserves a wider range of higher-order functions, including decision-making (Venkatraman et al. 2012), social processing (Lieberman et al., 2019) and emotion regulation (Downar et al. 2013). Volume reductions in this area may therefore contribute to KS-associated alterations in socio-emotional and executive functioning (Temple et al. 2003; van Rijn et al. 2018). In line with this possibility, exploratory correlations indicated increased volume of this region was associated with better adaptive skills (e.g., social skills, leadership skills, study skills, functional communication skills), improved behavioral regulation (e.g., ability to shift cognitive set and modulate emotions and behavior via appropriate inhibitory control), higher IQ, and increased visuomotor capabilities in XXY males.

A unique strength of the present study is the careful selection of the study sample. Limiting our sample to pre- and early-pubertal males that have not been administered testosterone replacement therapy avoids potential confounds relating to androgen effects on the brain (Foland-Ross et al. 2019; Nguyen et al., 2013; Patwardhan et al., 2000). A primary limitation, however, regards sample size. The small number of participants in our study may have limited our power to detect group differences in brain structure and/or correlations with behavior. Second, while we speculate that regional alterations in thickness and surface area drove local volume differences between the two groups, additional testing is needed to confirm this conclusion. Third, the two groups differed with respect to IQ. Although our findings remained significant when IQ was added to our statistical model, additional research is needed to tease apart the influence of IQ from KS on cortical structure. Finally, although we limited our exploratory correlation analyses to specific domains affected in KS and corrected for multiple comparisons, we cannot exclude the possibility of Type II error.

In summary, in a carefully selected sample of boys designed to control for the potentially confounding effects of testosterone, we observed widespread alterations in cortical gray matter volume in pre- and early-pubertal boys with KS. Further inspection of surface-based metrics indicated that KS-associated increases in cortical gray matter volume were associated with increases in cortical thickness, and further, that reductions in volume were coupled with decreases in surface area. Exploratory analyses additionally indicated correlations between brain structure and behavior, providing initial support for a neural basis of cognitive and emotional symptoms of this condition. Taken together, these data add support for a neuroanatomical phenotype of KS and extend previous studies through clarifying the precise structural characteristics of cortical morphometry that may give rise to alterations in gray matter volume. Future studies that replicate and build upon these findings in larger samples are warranted, as are investigations that tease apart the influence of X-chromosome dosage and testosterone replacement therapy on the brain in boys with this genetic condition.

Declaration of Competing Interest

The authors report no conflicts of interest.

Acknowledgements

The authors thank the participants and their families. This work is supported by funding from the National Institute of Mental Health (NIMH; MH099630) and the National Institute of Child Health and Human Development (HD092847, HD049653). We also gratefully acknowledge AXYS for their assistance and support with subject recruitment, and Alexandra Ishak for help with data collection and management.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2021.111299.

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