Hemispheric Module-Specific Influence of the X Chromosome on White Matter Connectivity: Evidence from Girls with Turner Syndrome

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Abstract

Turner syndrome (TS) is caused by the congenital absence of all or part of one of the X chromosomes in females, offering a valuable human "knockout model" to study the functioning patterns of the X chromosome in the human brain. Little is known about whether and how the loss of the X chromosome influences the brain structural wiring patterns in human. We acquired a multimodal MRI dataset and cognitive assessments from 22 girls with TS and 21 age-matched control girls to address these questions. Hemispheric white matter (WM) networks and modules were derived using refined diffusion MRI tractography. Statistical comparisons revealed a reduced topological efficiency of both hemispheric networks and bilateral parietal modules in TS girls. Specifically, the efficiency of right parietal module significantly mediated the effect of the X chromosome on working memory performance, indicating that X chromosome loss impairs working memory performance by disrupting this module. Additionally, TS girls showed structural and functional connectivity decoupling across specific within- and between-modular connections, predominantly in the right hemisphere. These findings provide novel insights into the functional pathways in the brain that are regulated by the X chromosome and highlight a module-specific genetic contribution to WM connectivity in the human brain.

Key words: functional connectivity, module, structural connectivity, the X chromosome, Turner syndrome

Introduction

Accumulating evidence has revealed a strong linkage between the X chromosome and the development of the human brain and cognition (Turner 1996; Zhao and Gong 2017). However, researchers have not clearly determined how the X chromosome influences specific brain structures and functions, particularly in humans, which is of particular importance for elucidating sex-specific differences in the human brain and cognition.

Turner syndrome (TS) is caused by the congenital absence of all or part of one of the X chromosomes in females (Sybert and McCauley 2004). This condition is therefore a naturally occurring human “knockout model” of the X chromosome, offering a valuable opportunity to investigate the functioning
patterns of the X chromosome in the human brain. By applying multimodal MRI techniques to individuals with TS, previous studies have reported a number of TS-related abnormalities in various brain phenotypes (see review: Zhao and Gong 2017). For instance, a decrease in the gray matter (GM) volume around the parietal and occipital lobes and increased cortical thickness around the temporal lobe have been repeatedly observed in patients with TS (Murphy et al. 1993; Kesler et al. 2003; Xie et al. 2015). During cognitive tasks (e.g., visuospatial processing and working memory tasks), patients with TS exhibit abnormal profiles of functional activity or connectivity, mainly around the frontal and parietal lobes (Habericht et al. 2001; Hart et al. 2006; Bray et al. 2011). In addition, several studies revealed abnormalities in the white matter (WM), mainly around the parieto-occipital, frontoparietal, and sensorimotor pathways, indicating a disruption of structural/anatomical connectivity in patients with TS (Holzapfel et al. 2006; Yamagata et al. 2012; Xie et al. 2015). These WM-related findings, however, were uniformly obtained by voxel-based or regional analyses of MRI metrics of volume or microstructural properties, and researchers have not determined whether the organizational patterns of WM connections across brain regions are disrupted in patients with TS.

Revealing the organizational patterns of WM connections (also referred to as brain network analysis) provides important insight into the structure and function of the human brain (Sporns 2013). Typically, all regional WM connections are first estimated using MRI tractography, resulting in a network model for each human brain. Network analysis approaches are then applied to quantify the organizational principles of all these WM connections. An important observed attribute of WM connection patterns is their modular organization (Hagmann et al. 2008; Bassett et al. 2010, 2011); WM connections are organized into a set of modules/subnetworks within the human brain. The modular structure is evident at birth and becomes refined during adolescence and adulthood (Huang et al. 2015; Lim et al. 2015; Baum et al. 2017). In addition, the modularity of the WM network and its modular structure are disrupted in the brains of patients with various diseases, such as Alzheimer’s disease, schizophrenia, and autism (Alexander-Bloch et al. 2010; de Haan et al. 2012; Shi et al. 2013), indicating a fundamental role of WM modules in the organization of the human brain. Moreover, the modular organization of WM connections is likely controlled by genetics and underlies cognitive functions (Stam and van Straaten 2012). Intriguingly, the X chromosome is associated with the structure or specific properties of modules/subnetworks within human brains. Some recent resting-state fMRI studies showed module/subnetwork-specific disruption of intrinsic functional connectivity (FC) in patients with TS (Green et al. 2018; Xie et al. 2017). These FC-based findings imply that the disruption of WM connectivity in patients with TS occurs in a module-specific manner, a hypothesis that has not yet been tested.

The present study aims to empirically investigate whether and how the WM modules are disrupted in patients with TS. Notably, a few theories have suggested a differential impact of the X chromosome on the two hemispheres (Geschwind and Galaburda 1985; Crow 2002). Consistent with these findings, a number of whole-brain analyses have revealed either left- or right-lateralized brain abnormalities (Holzapfel et al. 2006; Yamagata et al. 2012; Xie et al. 2015), as well as changes in brain asymmetry in individuals with TS (Rezaie et al. 2009; Leroy et al. 2015). Based on these results, we hypothesized that X chromosome loss results in a selective disruption of hemisphere WM modules, possibly accounting for specific cognitive deficits in patients with TS. In addition, altered hemispheric asymmetries of specific WM modules are expected in patients with TS. We acquired a multimodal MRI dataset and performed intelligence quotient (IQ) assessments in two groups, patients with TS and female controls, to test these hypotheses. The WM connectivity and its coupling with FC were comprehensively analysed at the hemispheric modular level.

Materials and Methods

Participants

Twenty-two girls with TS presenting a non-mosaic 45XO karyotype (age range: 10.2–17.6 years) were recruited from the China-Japan Friendship Hospital and Peking Union Medical College Hospital. Age-matched healthy controls (HCs) (21 girls; age range: 9.94–17.5 years) were recruited from the local community and parent networks. Each patient’s karyotype was confirmed by a standard cytogenetic assessment of peripheral blood. All girls with TS included in the current study have “classical” TS, as the entire second X chromosome is absent in all cells; this karyotype is referred to as non-mosaic 45XO karyotype TS, accounting for ~50% of TS cases in females (Zhao and Gong 2017). Patients with TS typically exhibit severe physical manifestations, including a short stature, gonadal dysgenesis, and infertility (Ranke and Saenger 2001; Hong and Reiss 2014), as well as cognitive deficits in multiple cognitive domains, such as visual-spatial ability, mathematical processing, social cognition (Hong et al. 2009). Among the various TS karyotypes, the 45XO karyotype usually presents the most typical TS phenotypes.

Twenty of the patients with TS had received growth hormone (GH) treatments, and only two were on estrogen replacement (ER) therapy. All participants were right-handed and had no history of neurological or psychiatric disorders. Additionally, no visible abnormalities (e.g., WM hyperintensity) were observed on the MRI scans, which were examined by an experienced radiologist. For each participant, informed written consent was obtained from her legal guardian, and the travel and accommodation expenses for participating in this study were reimbursed. The research protocol was approved by the Research Ethics Committee of Beijing Normal University.

Cognitive Assessment

The IQ assessment was performed within 2 days before or after the MRI scan. The Chinese version of the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV), was applied to participants aged 9–17 years, and five primary index scores were generated for each participant: the full-scale intelligence quotient (FSIQ), verbal comprehension index (VCI), perceptual reasoning index (PRI), processing speed index (PSI), and working memory index (WMI).

MRI Acquisition

All MRI scans were performed using the same 3 T Siemens Tim Trio MRI scanner in the Imaging Center for Brain Research, Beijing Normal University. Straps and foam pads were used to fix the head of each subject to minimize head movement.

Diffusion-Weighted Images

Diffusion-weighted images (DWIs) were axially acquired using a single-shot echo planar imaging-based sequence: coverage of the whole-brain; 62 axial slices; repetition time (TR), 8000 ms; echo time (TE), 89 ms; 30 optimal non-linear diffusion-weighted
directions with \( b = 1000 \text{s/mm}^2 \) and one additional image without diffusion weighting (i.e., \( b = 0 \text{s/mm}^2 \)); average, 2.2-mm slice thickness; acquisition matrix, 128 \( \times \) 128; and 2.2 \( \times \) 2.2-mm in-plane resolution.

Resting-State fMRI

During resting-state fMRI (rs-fMRI) scanning, all participants were instructed to relax with their eyes closed, while remaining awake and not thinking systematically. Thirty-three axial slices covering the whole-brain were acquired using the following echo planar imaging sequence: TR, 2000 ms; TE, 30 ms; flip angle, 90°; slice thickness/gap, 3.5/0.7 mm; acquisition matrix, 64 \( \times \) 64; 3.1 \( \times \) 3.1-mm in-plane resolution; and 200 volumes.

High-Resolution T1-Weighted Images

High-resolution 3D T1-weighted images were sagittally acquired using a magnetization prepared rapid gradient echo (MPRAGE) sequence: 144 sagittal slices; TE, 3.39 ms; TR, 2530 ms; inversion time (TI), 1100 ms; 1.33-mm slice thickness with no gap; acquisition matrix, 256 \( \times \) 256; and 1 \( \times \) 1-mm in-plane resolution. These images were used for better alignment and anatomically-constrained tractography methods.

Hemispheric WM Connections and Modules

MRI Preprocessing and Tractography

DWIs were preprocessed to correct for eddy current-induced distortions and simple head motions using the PANDA pipeline toolbox (Cui et al. 2013) by calling the modules of the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/). Within each voxel, the diffusion tensor was first fitted, and the fractional anisotropy (FA) was calculated. Furthermore, fiber orientation distributions (FODs) were estimated using constrained spherical deconvolution (CSD) with a harmonic order of 6 and default parameters (Tournier et al. 2007). Whole-brain fiber tracking was then implemented using the second-order integration over FODs (iFOD2) algorithm (Tournier et al. 2012) in MRtrix3 (http://www.mrtrix.org/), with the application of the anatomically-constrained tractography (ACT) framework (Smith et al. 2012). The ACT incorporates prior anatomical segmentation information into the tractography procedure, substantially improving the biological accuracy of fiber reconstruction (Smith et al. 2012). Regarding the ACT-related tissue segmentation, each subject’s T1 image was first transformed into the native diffusion MRI space by linearly aligning it to the native FA image; next, it was segmented into tissue partial volume maps (PVMs) of brain WM, GM (cortical and subcortical GM), and cerebrospinal fluid (CSF) using FSL tools (Smith et al. 2004). The fiber-tracking parameters incorporated into ACT were: step size = 1.1 mm, maximum curvature = 45°/step, length = 2.2–250 mm, and FOD threshold = 0.1. For each subject, 100 million streamlines (i.e., tractograms) were initially generated through seeding from the ACT-generated GM-WM interface (GMWMI) and then filtered to 10 million streamlines through the spherical deconvolution informed filtering of tractograms (SIFT) method, which substantially improves the quantitative nature of all reconstructed streamlines (Smith et al. 2013).

Regional WM Connections

The Brainnetome Atlas (http://atlas.brainnetome.org) was used to parcellate all GM into a set of GM regions (Fan et al. 2016). This fine-grained, cross-validated atlas is based on connectivity and is therefore favored for regional connectivity and brain network analyses (Dresler et al. 2017; Cui and Gong 2018). This atlas contains 246 GM regions, including 210 cortical and 36 subcortical regions (123 in each hemisphere). The original Brainnetome Atlas in the Montreal Neurological Institute (MNI) space was transformed into the native diffusion space, as previously described (Gong et al. 2009). Briefly, T1 images were first non-linearly normalized to the MNI space using FSL tools. Then, the inverted non-linear transformations along with the previously estimated linear transformations obtained from the ACT process were sequentially applied to the atlas in the MNI space, resulting in a whole-brain GM parcellation with 246 regions for each subject in the native diffusion MRI space.

For each subject, WM connections between each pair of Brainnetome regions were determined using the SIFT-processed tractograms (Figure 1A). First, each streamline was assigned to the closest region within a sphere of a 2-mm radius centered at its endpoint. Then, the set of streamlines linking each region pair was derived from the tractogram. Given these large-scale regions, a plausible assumption is a consistent yes-no pattern for the existence of fibers between each pair of regions across all subjects. Any detected streamlines linking a particular pair of regions only in a portion of subjects are likely to be spurious (e.g., due to noisy data and algorithm errors) and therefore were discarded in those subjects. This process led to a binarized population-level network skeleton that was applied to all subjects (8575 WM connections in total). The strength/weight of each WM connection was defined using the fraction of streamlines (FSe), which is analogous to the connection strength in retrograde-tracer-based studies (Donahue et al. 2016). The strong correlation between this MRI-derived measure and traditional tracer-based connection strength has been verified using monkey brains (Donahue et al. 2016). Specifically, the FSe is calculated as the fraction of streamlines linking two regions relative to the number of streamlines extrinsic to those regions. The following equation was used:

\[
\text{FSe}(i, j) = \frac{\sum_{x=1}^{N} \text{NOS}(i, x)}{\sum_{x=1}^{N} \text{NOS}(i, x) + \sum_{y=1}^{N} \text{NOS}(y, j)} - \text{NOS}(i, j)
\]

where \( x \neq i \) and \( y \neq j \); \( N \) is the total number of regions (i.e., \( N = 246 \)); and \( \text{NOS}(i, j) \) is the number of streamlines connecting region \( i \) and region \( j \).

Hemispheric Module Identification

According to the criterion described above, two 123 \( \times \) 123 FSe-weighted networks/matrices were generated for each subject, each representing a hemispheric WM network of the human brain. We applied a k-means clustering method to each population-averaged hemispheric matrix/network to identify the WM modules within each hemisphere (Sporns and Betzel 2016), resulting in K modules (clusters). The optimized clustering number (K) was determined by calculating the Calinski-Harabasz index (Calinski and Harabasz 1974).

Experimental Design and Statistical Analysis

Topological Efficiency of Hemispheric Networks and Modules

Topological efficiency measures have been widely used to represent the capacity for parallel information processing within a network or module (Latora and Marchiori 2001, 2003). Importantly, these parameters are conceptually preferred to assess the topological architecture of brain networks/modules (Achard and Bullmore 2007). In the current study, two commonly used...
topological efficiency measures were calculated using the Guetna package (Wang, Wang et al. 2015): global efficiency (gE) and local efficiency (locE). Specifically, gE is a global measure of the information-transferring ability of the entire network/subnetwork, which is defined as follows (Latora and Marchiori 2001):

$$gE = \frac{1}{N(N-1)} \sum_{i \in G} \sum_{j \in G} \frac{1}{L_{ij}}$$

where $L_{ij}$ is the shortest path length between nodes i and j, and N is the number of nodes in graph G. In contrast, locE quantifies the efficiency of information flow within the local environment and reflects the average ability of a network/subnetwork to tolerate faults (Latora and Marchiori 2001); this measure is defined as follows:

$$locE = \frac{1}{N} \sum_{i \in G} gE(G_i)$$

where $G_i$ is the subgraph composed of the nearest neighbors of node i and the connections among them. The nearest neighbors in $G_i$ are defined only in the context of graphs/networks, without any spatial constraints in the brain.

For the hemispheric networks and within-hemisphere modules, we calculated both gE/locE and the mean connectivity strength (mSC, representing the network/modular wiring cost). We statistically compared the gE, locE, and mSC values between the TS and HC groups using two-sample t-tests to evaluate whether and how X chromosome loss influences hemispheric WM networks of within-hemisphere modules. Notably, network efficiency parameters were significantly correlated with brain size (Yan et al. 2011). Both age and hemispheric intracranial volume (ICV) were therefore included as covariates in the statistical models. During the comparison of modules, the Bonferroni method was used to correct for multiple comparisons at the $P < 0.05$ significance level.

Asymmetries Between the Two Hemispheres

For the TS and HC groups, we first tested the within-group hemispheric asymmetries in gE, locE, and mSC using paired t-tests, where age and hemispheric ICV were included as covariates (Zhong et al. 2017; Wei et al. 2018). The asymmetry index (AI) value, which is defined as $AI = (R - L)/(R + L)$, and the absolute AI value, which represents only the degree of asymmetry regardless of the direction of asymmetry, were further compared between the two groups using two-sample t-tests, with age, whole-brain ICV and the difference in hemispheric ICV serving as covariates. Again, the Bonferroni correction was applied ($P < 0.05$).

Mediation Analysis from X Chromosome Loss to Modular Deficits to Cognitive Impairment

We employed mediation analyses to determine whether the observed deficits in hemispheric networks or modules plays a mediating role in the effects of X chromosome loss on cognitive impairment. In the mediating models, the group factor (HC/TS),...
The influence of X chromosome loss on coupling between structural and functional connectivity

Functional image preprocessing was performed using the DPABI toolbox (Yan et al. 2016). Briefly, the first 10 volumes were discarded to allow the magnetization to approach dynamic equilibrium and the participants to adapt to the scanner. The remaining volumes were then corrected for time offsets between slices due to interleaved acquisition and then realigned to the first volume to correct for interscan head motion. Individual anatomical T1 images were coregistered to the corresponding functional images and segmented into GM, WM and CSF using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Subsequently, the normalized information derived from the T1 segmentation procedure was employed here to normalize the resulting rs-fMRI scans to the standard MNI space, and then the images were resampled to a 3-mm isotropic resolution. Next, the normalized rs-fMRI images were linearly detrended and temporally bandpass filtered (0.01–0.1 Hz) to minimize the effects of low-frequency drift and high-frequency physiological noise. Several nuisance covariates were regressed out from the time course of each voxel, including head motion profiles (Friston 24-parameter model) and the global mean signal, WM signal, and CSF signal (Fox et al. 2005). Notably, three HC were excluded from this analysis because their head motion exceeded 2 mm or 2° in a specific direction. Finally, the rs-fMRI data from 22 patients with TS (mean age: 13.8 years; SD: 2.4 years) and 18 HCs (mean age: 14.2 years; SD: 2.3 years) were entered into our structural connectivity (SC)–FC coupling analysis.

For each Brainnetome region, a mean blood oxygen level-dependent (BOLD) time series was extracted. As usual, the strength of FC was quantified by the Pearson correlation of the BOLD time series between each pair of regions. Due to our interest in SC-FC coupling, the FC between any two regions lacking a WM connection was excluded from our analysis. We applied Fisher’s r-to-z transformation to the FC r values to improve the normality.

We estimated the correlation between the SC (i.e., WM connectivity) and FC strength/weight across subjects to evaluate the SC-FC coupling of each connection. For either the HC or TS group, a Pearson correlation r value was computed for each connection and then transformed to Fisher’s z value. Notably, for either the HC or TS group, both positive and negative z values were observed (i.e., anticorrelations between SC and FC). The amount (HC: 52.3% of connections, TS: 49.7% of connections) and amplitude of these negative z values are comparable to the positive z values, and therefore, they are unlikely to be merely noise. The interpretation for the biological meaning of these observed SC-FC anticorrelations however is difficult. Nevertheless, the simple consideration of a negative SC-FC correlation as a lower degree of coupling than a positive SC-FC correlation is obviously problematic. We used the absolute z values in the subsequent statistical comparisons to avoid a direct comparison between the positive and negative SC-FC coupling values. Here, a larger absolute value indicates a tighter coupling between SC and FC, regardless of the coupling direction.

We applied paired t-tests to compare the absolute z values across all hemispheric connections between the HC and TS groups and to determine whether the X chromosome influences SC-FC coupling within a hemisphere. Furthermore, similar tests were performed to test group differences in SC-FC coupling across within- or between-module connections. Given the analysis of multiple modules, the Bonferroni method was also used to correct for multiple comparisons.

Results

Demographics and Cognitive Assessment

As summarized in Table 1, no significant group differences in age or ICVs were observed. Patients with TS consistently exhibited significantly lower values for the five IQ scores than HCs.

Hemispheric WM Modules

In each hemisphere, the k-means clustering algorithm was applied with multiple predefined K values (2 to 8). According to the Calinski–Harabasz index, the optimized number of clusters (K) was 4 for the left hemisphere and 2 for the right hemisphere (see Supplementary Figure S1). Nevertheless, a K value of four clusters is actually another optimized choice for the right hemisphere, and the corresponding modular composition matched well with the left hemisphere; only three pairs of homotopic regions were assigned into different modules in the two hemispheres (Table 2). We therefore chose four clusters for each hemisphere to ensure the comparability of modules between the two hemispheres. Under this choice, the following within-hemisphere modules were identified: (1) a left/right frontal-insular-subcortical nuclei (frontal-INS-SN) module, mainly consisting of regions within the frontal and insular lobes, as well as regions of the basal ganglia and thalamus; (2) a left/right temporal-subcortical nuclei (temporal-SN) module, mainly consisting of regions within the temporal lobe, as well as regions of the amygdala and hippocampus; (3) a left/right parietal module, mainly consisting of regions within the parietal lobe; and (4) a left/right occipital module, mainly consisting of regions within the occipital lobe.
Table 1 Demographics and cognitive assessment

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Table 2 Composition of the four identified modules for each hemisphere

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Group Differences in Hemispheric Networks/Modules

We first evaluated the group differences in the developmental trajectory by testing an “age × group” interaction effect, but significant results were not obtained for any measures. Next, according to the between-group comparison, girls with TS exhibited significantly lower values for hemispheric gE (left hemisphere: t(39) = 3.88, P = 0.0004; right hemisphere: t(39) = 2.99, P = 0.005, two-sample t-tests) and locE (left hemisphere: t(39) = 2.21, P = 0.033; right hemisphere: t(39) = 2.17, P = 0.036; two-sample t-tests), but not mCS (left hemisphere: t(39) = 0.87, P = 0.392; right hemisphere: t(39) = 1.3, P = 0.202; two-sample t-tests), than HCs.

Regarding the hemispheric modules, mCS did not differ in any modules. Both the left and right parietal modules exhibited significantly reduced gE (left parietal module: t(39) = 3.02, P = 0.004; right parietal module: t(39) = 3.61, P = 0.0009; two-sample t-tests) and locE (left parietal module: t(39) = 2.77, P = 0.008; right parietal module: t(39) = 3.88, P = 0.0004; two-sample t-tests) in the TS group, but significant group differences in either gE or locE for any other modules were not observed (Figure 2). We repeated the statistical comparisons of gE and locE after controlling for mCS to assess the potential confounding effect of mCS on these efficiency findings, and the results essentially remained unchanged.
The Mediation of Hemispheric Modular Deficits in the Effect of X Chromosome Loss on Cognitive Impairments in Girls with TS

We next conducted a mediation analysis in which the group factor, hemispheric/modular efficiencies, and IQ scores were taken as the predictor, mediator, and outcome, respectively, to determine whether the aforementioned topological deficits in hemispheres or bilateral parietal modules may mediate the effect of X chromosome loss on specific cognitive impairments in patients with TS. As specified above, significant group differences (i.e., significant path c, total effects) in all IQ scores (Table 1) and bilateral hemispheric and parietal modular efficiency (i.e., significant path a) were observed. Among all the possible paths b (two hemispheres × five IQ scores and two modules × five IQ scores), only the efficiency of the right parietal module showed a significant correlation (corrected P < 0.01) with the WMI score (locE: \( \beta = 5994.1, P = 0.0001, 99\% CI = [2310.28, 9677.92] \); gE: \( \beta = 5928.2, P = 0.0001, 99\% CI = [2274.18, 9582.12] \)). Consequently, only two candidate mediating pathways were identified: from X chromosome loss to gE or locE deficits of the right parietal module and then to WMI impairment. In both models, the direct effect (i.e., path c') became non-significant after controlling for the mediators (locE: \( \beta = 7.78, P = 0.087, 99\% CI = [-4.25, 19.76] \); gE: \( \beta = 8.28, P = 0.067, 99\% CI = [-3.63, 20.19] \)). Finally, the bootstrap simulation (n = 5000) confirmed the significant indirect effect \( a \times b \) for both topological efficiencies (locE: 99\% CI = [1.15, 21.12], \( P < 0.01 \); gE: 99\% CI = [0.94, 21.22], \( P < 0.01 \)). Therefore, both local and global efficiency in the right parietal module were considered to significantly mediate the effect of the X chromosome on working memory performance, as illustrated in Figure 3.

Asymmetries Between the Two Hemispheres

As shown in Table 3, significant within-group asymmetries were observed between the two hemispheres in both gE/locE and mCS. For all measures of hemispheric networks and modules, the TS group exhibited the same directions of asymmetry (leftward or rightward) as the HC group. However, neither the AI nor the absolute AI of any hemispheric network and modular measures showed any significant group differences, indicating the robustness of these asymmetric patterns between patients with TS and HCs.

Between-Group Differences in SC-FC Coupling

As illustrated in Figure 4A, a Pearson correlation coefficient was computed for each WM connection, representing the degree of SC-FC coupling in either the HC or the TS group. The statistical results for group comparisons of the SC-FC coupling are summarized in Table 4 and Figure 4B. For both the left and right hemispheres, the TS group showed significantly decreased SC-FC coupling across the entire hemisphere compared with the HC group (left hemisphere: \( t(3546) = 5.09, P = 4 \times 10^{-7} \); right hemisphere: \( t(3588) = 9.38, P = 10^{-20} \); paired t-tests). We compared within- and between-module connections to further localize this decrease in SC-FC coupling in patients with TS. In
the left hemisphere, only the set of connections within the frontal-INS-SN module showed decreased SC-FC coupling in the TS group ($t(956) = 4.23, P = 0.00002$, paired $t$-test). In the right hemisphere, significant decreases in SC-FC coupling were observed across connections within the frontal-INS-SN module ($t(996) = 5.33, P = 10^{-7}$, paired $t$-test). In addition, the right parietal module exhibited a marginally significant decrease ($t(301) = 2.78, P = 0.006$, paired $t$-test, but the result did not survive the...
Bonferroni correction). The SC–FC coupling within the right hemisphere also showed significant decreases across connections between the frontal-INS-SN and the other three modules (temporal-SN module: \( t(426) = 3.48, P = 0.0005 \); parietal module: \( t(758) = 3.97, P = 0.00008 \); occipital module: \( t(98) = 3.64, P = 0.0004 \); paired t-tests), as well as between the temporal-SN and occipital modules (\( t(243) = 4.37, P = 0.00002 \), paired t-test).

Because three pairs of homotopic regions belonged to different modules in the two hemispheres, we repeated the analyses described above by applying the modular composition from either the left or the right hemisphere to the two hemispheres. The statistical results remained unchanged (Supplementary Tables S1–S3). In addition, we repeated the aforementioned SC–FC coupling analyses on the FC measures without applying the global signal regression (GSR) to assess the impact of GSR during fMRI preprocessing on our SC–FC findings. The results of the statistical analyses were largely consistent with our main findings, showing significantly decreased SC–FC coupling in patients with TS, e.g., across connections within the entire left or right hemisphere, the left or right frontal-INS-SN module, and the right parietal module.
Topological Disruption of Selective Hemispheric Modules in Girls with TS

Using voxel-based, tract-based, or region-based analyses, several MRI studies reported widespread anomalies in WM morphological or microstructural parameters in patients with TS (Holzapfel et al. 2006; Yamagata et al. 2012; Xie et al. 2015). Complementary to these focal methods, assessments of organizational patterns of WM connections across the brain (i.e., brain network topology) provide another avenue to understand brain structure and function from a systematic network perspective (Hagmann et al. 2008; Bullmore and Sporns 2009; Gong et al. 2009). To the best of our knowledge, the current study represents the first investigation of the topological pattern of WM connections in patients with TS. By focusing on WM connectivity patterns in hemispheric modules, the present study provides more spatially specific information than whole-brain network analyses. The modular architecture is an important organizational principle of the human brain, putatively facilitating locally segregated processing of specialized functions while conserving wiring length for rapid information integration among different modules (Meunier et al. 2010).

Specifically, four modules were identified in each hemisphere, and WM connections within a module were much stronger and denser than connections between modules. These identified modules correspond well to the four main lobes, suggesting the biological plausibility of module identification. Importantly, only the bilateral parietal modules showed a disrupted topological efficiency, i.e., aberrant information segregation and integration within these modules, in patients with TS. This module-selective disruption indicated spatial specificity in the effect of the X chromosome on the organizational patterns of WM connections. The topological abnormalities of the bilateral parietal modules may relate to the decrease in parietal GM volume in patients with TS, the most consistent finding in the TS literature (Zhao and Gong 2017). Although the causality between GM atrophy and the observed disorganization of WM connections within the parietal lobe is difficult to disentangle,
both findings strongly indicate a central role for the parietal lobe in the functional pathways controlled by the X chromosome in the nervous system.

Regarding the observed module-selective topological disruptions in patients with TS, we have not yet clearly determined whether they are caused by a direct genetic or indirect hormonal effect of the X chromosome. Nevertheless, some previously reported findings offer important clues related to this issue. Some evidence supports a direct genetic effect on the parietal abnormalities in patients with TS. For instance, Hong and colleagues simultaneously investigated both patients with TS (45X0) and Klinefelter’s syndrome (47XXXY) and found that the increase in the X chromosome copy number was associated with a relative decrease in parietal GM volume (Hong et al., 2014). In addition, Lepage and colleagues studied two TS groups stratified by estrogen treatment, estrogen-naive and estrogen-treated groups, and found that the reductions in parietal GM/WM and surface area persisted in young to adolescent girls even after the estrogen treatment (Lepage et al., 2013). On the other hand, a few neuroimaging studies also revealed a substantial role for gonadal hormones in the maturation of the parietal region by showing decreased GM volumes during puberty (Giedd et al., 1999; Neufang et al., 2009; Peper et al., 2009), in favor of the possibility of a hormonal effect on the parietal deficits in patients with TS. Taken together, our observed disruption of the right parietal modules is likely associated with a combination of both direct genetic and indirect hormonal effects.

While we were unable to determine the exact X-linked genes underlying the disruption of parietal modules in patients with TS in the present study, there are a few possible candidates whose linkage with the parietal lobe have been reported. One candidate X-linked gene is the MECP2 gene that regulates the cortical surface area of multiple parietal regions, such as supramarginal, precuneus, paracentral, and inferior parietal cortices, in humans (Joyner et al., 2009). In addition, decreased GM volumes in dorsal parietal regions were observed in girls with Rett syndrome who carried mutations in the MECP2 gene (Carter et al., 2008). Another candidate gene accounting for the parietal module anomalies is the FMR1 gene that encodes the fragile X mental retardation protein (FMRP) (Crawford et al., 2001). Studies of females with fragile X syndrome observed lower FA in WM tracts linked to parietal sensory-motor areas and a larger parietal GM volume than HCs (Barnea-Goraly et al., 2003; Gothelf et al., 2008), supporting a role for the FMR1 gene in the parietal lobe. Notably, these genes are essentially speculative candidates, and specific experimental assessments are required in the future to determine whether our observed disruption of the right parietal modules can be attributed to abnormalities in these genes in patients with TS.

Notably, we did not observe significant TS-related changes in the topological asymmetry of any of the four modules, which is unexpected to some degree. Based on the influential Geschwind–Galaburda hypothesis and other genetic theories (Geschwind and Galaburda 1985; Crow 2002; Corbally 2009), a close link between the X chromosome and human brain asymmetry/lateralization should exist. Consistent with these hypotheses, altered volumetric asymmetry was reported in patients with TS (Rezaie et al., 2009). Contradictory results also exist; e.g., robust human brain asymmetries in cortical thickness were observed among patients with X chromosome aneuploidies (Lin et al., 2015). This result is consistent with our negative results for asymmetries. Given these mixed findings, more investigations of brain asymmetries in patients with TS are needed.

**Topological Disruption of WM Mediates the Effect of X Chromosome Loss on Cognitive Impairment in Girls with TS**

Regarding the observed disruption of bilateral parietal modules in patients with TS, it is important to determine its cognitive/behavioral consequences. Parietal regions have been strongly associated with working memory abilities (Jonides et al., 1998; Berryhill and Olson 2008; Jones and Berryhill 2012). Intense functional activation around parietal regions has been reliably observed during various working memory tasks (Wager and Smith 2003). In particular, patients with damage in the right parietal lobe exhibited severe impairments in both visual and verbal working memory compared with uninjured controls (Ravizza et al. 2005; Berryhill and Olson 2008; Berryhill et al. 2010; Jones and Berryhill 2012). Accordingly, multiple studies using transcranial direct current stimulation (tDCS) reported alterations in working memory performance after right parietal tDCS (Berryhill et al. 2010; Jones and Berryhill 2012). These results consistently indicate an important mnemonic role of the right parietal lobe. Consistent with these findings, the present study revealed a significant correlation between the topological efficiency of the right parietal module and WMI scores in both TS and HC groups.

As described in other studies (Hong and Reiss 2014; Xie et al., 2017), the WMI scores of the patients with TS were significantly decreased in the present study. During working memory tasks, TS-related abnormalities in functional activation/connectivity mainly occurred around the parietal and frontal regions (Haberecht et al. 2001; Hart et al. 2006; Bray et al., 2011), implying that parietal deficits contribute to the TS-related working memory impairment. Using mediation analyses, we confirmed the following functional pathway: X chromosome loss leads to deficits in the right parietal module and subsequent working memory impairment in patients with TS.

A mediating pathway is very useful for developing directional/causal hypotheses (Preacher et al. 2007). To date, pairwise associations among the X chromosome, brain, and cognition (i.e., X chromosome-cognition, X chromosome-brain, and brain-cognition relationships) have been reported in patients with TS, but an entire X chromosome-to-brain-to-cognition pathway remains unreported. Here, we revealed the first TS-based neural mediating pathway linking the X chromosome to cognitive performance: X chromosome loss in patients with TS impairs working memory performance by damaging WM connectivity patterns of the right parietal module. However, causation from this observed mediating pathway should be cautiously inferred, given the nature of our cross-sectional comparison in the present study. A convincing causal inference from mediation pathways requires temporal precedence, but in our case, it is impossible to conduct a longitudinal study before and after knocking-out the X chromosome in humans. Nevertheless, this pathway is of great value by providing clues to answer two important questions: (1) What neural circuits are regulated by the X chromosome to modulate working memory performance, and where do these functions occur? (2) What is the behavioral consequence of the X-linked effects on the parietal lobes in humans? Finally, this observed pathway is unlikely to be the only pathway underlying the X chromosome-working memory relationships; namely, other mediating pathways from TS to...
working memory deficits exist, in which structural or functional brain measures that were not examined in the current study may serve as mediators.

**Effects of the X Chromosome on SC–FC Coupling**

Very recently, a few studies reported a disruption in resting-state FC in patients with TS (Green et al. 2018; Xie et al. 2017), complementary to the well-observed structural connectivity abnormalities in this population. Notably, tight coupling between SC and FC have been reported at multiple scales (Wang, Dai et al. 2015). For example, interhemispheric FC is almost completely eliminated after removing the interhemispheric WM tract, i.e., callosotomy (Johnston et al. 2008). Importantly, this SC–FC coupling is dynamic during normal development (Magnann et al. 2010) and disrupted in patients with various neurological diseases (Skudlarski et al. 2010; Sun et al. 2017). Compared with those brain diseases, TS is also associated with decreased SC–FC coupling, mainly involving the frontal-INS-SN module and showing lateralization towards the right hemisphere.

It is unclear why this SC–FC decoupling was largely associated with the right frontal-INS-SN module. One possible explanation might relate to the relatively prolonged developmental period of the frontal lobe, which increases its vulnerability to hormonal influences, experience, and the environmental milieu during the protracted period of development (Royall et al. 2002). In healthy children, developmental changes in SC–FC coupling have been observed for intrahemispheric frontal tracts, which are weak or nonexistent in children but become obvious in adults (Supakar et al. 2010; Uddin et al. 2011). Our observed SC–FC decoupling around the frontal lobe in patients with TS may be attributed to the impaired developmental trajectories related to the abnormal adolescent hormone levels in these regions due to X chromosome loss. Regardless of the underlying mechanism, these results suggest a genetic or hormonal basis for the observed SC–FC coupling, particularly around the frontal lobe, which may be useful for interpreting the changes in SC–FC coupling under various conditions.

Notably, although the direct statistical analysis of the AI or absolute AI values did not show any significant difference between the TS and HC groups, the observed mediating pathway and SC–FC decoupling both showed a strongly rightward lateralization: the mediation pathway was detected only in the right hemisphere. Based on these findings, the X chromosome may contribute to human brain structure–function pathways/couplings in a hemisphere-specific way rather than by directly acting on the asymmetry pattern of brain structure or function per se. Future studies are warranted to evaluate this hypothesis.

Finally, a few limitations should be addressed. First, due to the difficulties we faced in recruiting girls with TS, the sample size of the present study is small, although the actual number is comparable to or even slightly greater than that in other TS studies. The limited statistical power might account for some negative results, e.g., no asymmetry changes in patients with TS. In addition, the SC–FC coupling was estimated using cross-subject correlations within each group, and the small sample size may bias the correlations. Next, given the limited scanning time, the diffusion MRI in the present study adopted a single shell protocol (b-value = 1000 s/m²) with 30 encoding directions. While this diffusion MRI acquisition was proven to be suitable for the CSD method (Tournier et al. 2007), multishell diffusion MRI data with more diffusion encoding directions improve the CSD fitting and subsequent tractography (Jeurissen et al. 2014), and therefore is becoming more frequently applied. Finally, some confounding factors, including GH use, ER treatment, family environment, and X-linked imprinting, were not controlled or assessed because of the limited sample size or the lack of related information. Future studies with larger sample sizes and advanced diffusion MRI protocols are warranted to verify our results and evaluate the potential effects of these confounding factors.

**Supplementary Material**

Supplementary material is available at Cerebral Cortex online.

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