Resting-state functional connectivity in drug-naive pediatric patients with Tourette syndrome and obsessive-compulsive disorder

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A B S T R A C T

Previous studies in cohorts of Tourette syndrome (TS) or obsessive-compulsive disorder (OCD) patients have not clarified whether these two disorders represent two clinical conditions or they are distinct clinical phenotypes of a common disease spectrum. The study aimed to compare functional connectivity (FC) patterns in a pediatric drug-naive cohort of 16 TS patients without any comorbidity (TS), 14 TS patients with OCD (TS + OCD), and 10 pure OCD patients as well as 11 matched controls that underwent resting state fMRI. Via independent component analysis, we examined FC in the basal ganglia (BGN), sensorimotor (SMN), cerebellum (CBN), frontoparietal (FPN), default-mode (DMN), orbitofrontal (OBFN), and salience (SAN) networks among the above cohorts and their association with clinical measures. Compared to controls, TS and TS + OCD patients showed higher FC in the BGN, SMN, CBN and DMN and lower FC in the FPN and SAN. The TS and TS + OCD groups showed increased FC in all networks. In contrast to controls, OCD patients exhibited increased FC in the BGN, SMN, CBN, DMN, FPN, and SAN. OCD patients also showed higher FC in CBN and FPN when compared with TS and TS + OCD patients both separately and as one group. Tic severity negatively correlated with FC in CBN and FPN in the TS group, while the compulsiveness scores positively correlated with the same two networks in OCD patients. Our findings suggest common FC changes in TS and TS + OCD patients. In contrast, OCD is characterized by a distinctive pattern of FC changes prominently involving the CBN and FPN.

1. Introduction

Tourette syndrome (TS) is a neurodevelopmental disorder defined by the occurrence of multiple motor and phonic tics (American Psychiatric Association, 2013). Tics affect about 0.5–0.8% of children and have a typical onset at 4–6 years (Robertson et al., 2017; Scharf et al., 2015). Although the current diagnostic definition for TS does not include obsessive-compulsive disorder (OCD), TS and OCD tend to display a phenomenological overlap. First, TS is frequently associated with obsessive-compulsive symptoms (Robertson et al., 2017). Second, TS and OCD patients are known to share a common genetic susceptibility (Mathews and Grados, 2011). Third, both TS and OCD patients exhibit repetitive behaviors, whose labelling into complex tics or compulsions may be clinically challenging at times (Mansueto and Keuler, 2005). Accordingly, TS and OCD may share similar pathophysiological features, including common patterns of brain activity responsible for repetitive behaviors. In keeping with this hypothesis, patients manifesting TS with OCD may reflect an intermediate clinical phenotype characterized by overlapping pathophysiological features. Alternatively, TS and OCD may reflect independent disorders arising from different pathophysiological mechanisms (Mancini et al., 2018; Miguel et al., 1995; Mirabella et al., 2020). As a result, the frequently observed obsessive-compulsive symptoms in TS patients may reflect pathophysiological mechanisms that differ from those underlying pure OCD. To address this issue, an appropriate methodological approach would be to compare possible brain functional changes in patients with TS without comorbidity, TS with OCD symptoms (TS + OCD), and pure OCD.

Brain functional changes may be investigated by means of resting-state functional magnetic resonance imaging (rs-fMRI), which, by monitoring oscillations in the blood oxygen level dependent (BOLD) signal across time, allows the identification of specific brain resting-state networks (RSNs) which are visualized as spatial maps representing the brain’s resting-state functional connectivity (FC) (Fox and Raichle, 2007).

Previous rs-fMRI studies in TS have focused on adult cohorts with or without different types of psychiatric comorbidities and with variable levels of tic severity. In TS without comorbidity, resting-state FC abnormalities have been reported in the cerebellum (CBN), the orbitofrontal (OBFN), and the salience (SAN) networks (Yue et al., 2012). In an OCD sample, Mesulam and colleagues (2000) showed reduced FC in the default-mode (DMN) and increased FC in the salience (SAN) networks. In addition, FC changes have been previously reported in the orbitofrontal (OBFN) and the salience (SAN) networks (Herscovitch et al., 1999; Mathews and Grados, 2011). In an OCD sample with a comorbid tic disorder,increased FC in the salience (SAN) network and decreased FC in the default-mode (DMN) network was found (Herscovitch et al., 1999).

In TS, increased FC in the DMN and decreased FC in the salience (SAN) and frontoparietal networks (FPN) were reported (Herscovitch et al., 1999). In a study focusing on pediatric TS patients, increased FC in the DMN and decreased FC in the salience (SAN) and frontoparietal networks (FPN) were reported (Herscovitch et al., 1999). However, in a recent study, increased FC in the CBN, DMN, FPN, and SAN was reported (Mansueto et al., 2015). Additionally, an increased FC in the FPN was found to be associated with tic severity (Mansueto et al., 2015). Taken together, these findings suggest that pediatric TS patients may exhibit increased FC in the CBN, DMN, FPN, and SAN, which may be associated with tic severity.

In contrast, OCD is characterized by a distinctive pattern of FC changes prominently involving the CBN and FPN. OCD patients showed increased FC in the CBN and FPN compared to controls (Herscovitch et al., 1999). In an OCD sample with a comorbid tic disorder, increased FC in the CBN and FPN was reported (Herscovitch et al., 1999). In a recent study, increased FC in the CBN and FPN was found to be associated with tic severity (Mansueto et al., 2015). Additionally, an increased FC in the FPN was found to be associated with tic severity (Mansueto et al., 2015).

In this study, we aimed to compare functional connectivity (FC) patterns in a pediatric drug-naive cohort of 16 TS patients without any comorbidity (TS), 14 TS patients with OCD (TS + OCD), and 10 pure OCD patients as well as 11 matched controls that underwent resting state fMRI. Via independent component analysis, we examined FC in the basal ganglia (BGN), sensorimotor (SMN), cerebellum (CBN), frontoparietal (FPN), default-mode (DMN), orbitofrontal (OBFN), and salience (SAN) networks among the above cohorts and their association with clinical measures.
exposure to chronic pharmacological treatments. Additional confounders (e.g. differences in sample size or the presence of comorbidities) have further contributed to the heterogeneous findings insofar reported. Therefore, it is still unclear whether FC abnormalities previously demonstrated in adult TS patients reflect neural adaptation processes or instead depend on primary pathophysiological abnormalities (Bohlhalter, 2006; Neuner et al., 2014; Peterson et al., 1998). Given the neurodevelopmental nature of TS and early-onset OCD, studies on pediatric patients could be particularly valuable in elucidating the primary FC disruptions in both disorders (Huyser et al., 2009).

A handful of rs-fMRI studies examining FC in pediatric drug-naive patients with TS without comorbidities have shown increased brain activity within the basal ganglia network (BGN, Cui et al., 2014) and in vision-related areas (Liu et al., 2017a) as compared to healthy controls. In contrast, decreased FC has been found in the frontal, parietal and cingulate cortices, as well as in the insular cortex (Cui et al., 2014a; Liu et al., 2017a; Wen et al., 2016). Other studies on pediatric TS patients exploring functional network topology have reported widespread abnormalities within the thalamo-cortico-striato-frontal networks (Church et al., 2009; Openneer et al., 2020). However, those studies are hindered by the inclusion of both medicated/unmedicated patients with varying profiles of comorbidities and wide age ranges. Furthermore, none of the previous studies have ever looked at differences between pediatric drug-naive TS patients with and without OCD, making it impossible to infer which abnormalities relate to TS, OCD, or both. Another brain region has been recently gained attention in TS, i.e., the cerebellum. More recently, several studies have hinted about the salient role of the cerebellum in the tic disorder (Caligiore et al., 2017; McCarroll et al., 2013; Neuner et al., 2014). Previous functional studies in drug-naive pediatric and adult TS cohorts have demonstrated decreased cerebellar activity and reduced cortico-cerebellar connectivity in TS patients with respect to controls (Liu et al., 2017a; Ramkiran et al., 2019). Additionally, a recent seed-based rs-fMRI study using the caudate nucleus as the region of interest has shown that adult TS patients exhibit weaker connectivity between caudate and cerebellum compared to healthy persons (Bhiyram et al., 2020). All in all, these findings highlight the involvement of highly complex networks in tic pathophysiology as opposed to models of single-network dysfunction and advocate the need to further inspect the involvement of the cerebellum in TS.

Regarding OCD, previous rs-fMRI studies have mainly examined adult patients and have reported FC changes in the orbitofronto-striato-thalamic circuitry, cingulate cortex (Anticevic et al., 2014a; Calzà et al., 2019; Fan et al., 2017; Peng et al., 2013; Yang et al., 2016; Zhu et al., 2016), and cerebellum (Anticevic et al., 2014a; Peng et al., 2013; Zhang et al., 2019). However, only a few studies have focused on pediatric patients with OCD, and these studies have been hindered by the relatively small sample sizes and the variable medication status (Bernstein et al., 2016; Weber et al., 2014). A rs-fMRI study using region-of-interest analysis approach (via seeds placed in the thalamus and striatum) in an OCD cohort of both children and adults, demonstrated that fronto-striato-thalamic loops showed decreased FC at an early disease stage. At the same time both children and adults increased FC between the dorsal striatum and medial frontal cortex. However, the cohort investigated was rather heterogeneous in that it included both medicated and unmedicated patients (Fitzgerald et al., 2011). In addition, two resting-state FC studies in pediatric drug-naive OCD patients have also highlighted the presence of cingulate cortex FC alterations in the disorder (Gruner et al., 2014; Weber et al., 2014).

To date, no previous rs-fMRI study has directly compared FC changes in drug-naive pediatric patients with TS, TS + OCD, and OCD. The current study therefore aimed to investigate FC patterns in the above patient cohorts along with age-matched controls using an automated hypothesis-free approach, i.e. independent component analysis (ICA). This approach has the potential to add new relevant insights into the pathophysiology of TS and OCD by clarifying: i) whether drug-naive pediatric patients with TS, TS + OCD, and OCD differ in terms of FC in specific RSNs; ii) whether TS + OCD patients are characterized by overlapping or independent FC changes when compared to TS or OCD patients; and iii) whether FC changes correlate with clinical measures in each patient cohort. The approach of our study focused on pediatric patients might provide clinicians and researchers with relevant information. First, it may help to differentiate neural correlates of different symptom dimensions, thereby detailing the pathophysiological relationship between two highly comorbid disorders. Second, it may pave the way for longitudinal studies to further analyze FC changes over time, to evaluate the modulations related to the disease course severity and treatment.

2. Methods

2.1. Participants

From a consecutive series of 70 children, a group of 51 subjects were included in this study: 16 patients with TS (15 males, mean age: 9.7 ± 2.1), 14 with TS + OCD (10 males, mean age: 10.2 ± 2.1), 10 with OCD (7 males, mean age: 10.9 ± 2.5), and 11 children with episodic tension headache who were headache-free during the MRI scan (controls) (2 males, mean age: 9.9 ± 1.3 years). A total of 19 children were excluded due to head movement during the scan (n = 10) or inability to complete the MRI exam (n = 9). Of note, the total YGTSS scores of the excluded participants did not significantly differ from that of the included participants. All subjects were recruited from the child and adolescent neuropsychiatry outpatient clinic at the Department of Human Neurosciences, Sapienza University of Rome, Italy.

Inclusion criteria were as follows: a) drug-naivety; b) not having received any behavioral treatment; c) right-handed (as assessed by the Edinburgh Handedness Inventory, Bryden, 1977); and d) having a normal cognitive profile (IQ ≥ 70). Exclusion criteria were as follows: a) comorbidity with attention deficit hyperactivity disorder (ADHD), autism spectrum disorder, schizophrenia, or developmental disabilities; or b) contraindications to MRI.

All subjects underwent a cognitive evaluation by means of the Wechsler Intelligence Scale for Children III (WISC-III) full scale. TS and OCD diagnoses were made according to DSM-5 criteria by a neuropsychiatrist experienced in TS, OCD, and related comorbidities. The severity of tics and OCD symptoms was assessed using the Yale Global Tic Severity Scale (YGTSS) (symptom severity scale: max. 50, without impairment score) (Leckman et al., 1989) and Children’s Yale-Brown Obsessive Compulsive Scale (CYBOCS) (Goodman et al., 1989), respectively. Importantly, all TS and TS + OCD patients had a YGTSS scores above 14, thus showing a moderate to severe symptomatology. Typically, patients with YGTSS above 14 seek medical treatment and this represents a common inclusion criterion for clinical studies including, e.g., the ongoing clinical trial assessing the efficacy and of online delivered behavioral treatment for children and adolescents with tic disorders (ORBIT trial) (Hall et al., 2019).

The presence of other developmental disorders including ADHD or psychiatric disorders other than OCD was ruled out by means of the Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime version (K-SADS-PL) parental interview administered to both parents. The anamnestic interview of all participants included items on family history. The parents or guardians of participants provided written informed consent. The study was approved by the institutional review board and conformed to the Declaration of Helsinki.

2.2. MRI acquisition

All subjects underwent a 3T MRI scan at Sapienza University of Rome, Italy. MRI was performed with the 3.0T MR scanner (Verio, Siemens AG, Erlangen, Germany) with a 12-channel head coil designed for parallel imaging (GRAPPA). A multplanar TI-weighted localizer
image with section orientation parallel to the subcallosal line was acquired at the start of each MRI examination. Noise reduction head-phones were used for attenuation of scanner noise. MRI protocol included the following sequences: a) high-resolution 3D, T1-weighted (3DT1) MPRAGE: TR = 1900 ms; TE = 2.93 ms; flip angle = 9°; field of view [FOV] = 260 mm²; matrix = 256 x 256; 176 sagittal slices 1 mm thick; no gap; b) dual-turbo spin-echo, proton density (PD) and T2-weighted images: TR = 3320 ms; TE = 10/103 ms; FOV = 220 mm; matrix = 384 x 384; 25 axial slices 4 mm thick; 30% gap; c) rs-fMRI: repetition time [TR] = 3000 ms; echo time [TE] = 30 ms; flip angle = 89°, 64 x 64 matrix; 50 contiguous axial slices 3 mm thick; 140 vol; acquisition time = 7 min. Before being positioned in the scanner, participants were instructed to lie down relaxed, awake, and with the eyes closed.

2.3. MRI data analysis

Images were analyzed using FMRIB Software Library (FSL) tools (https://www.fmrib.ox.ac.uk/fsl/fslwiki/FSL). Brain Extraction Toolbox (BET) FSL was used to skull strip three-dimensional T1-weighted structural images. Cortico-spinal fluid (CSF), grey matter (GM), and white matter (WM) segmentation masks were created via FAST (FMRIB’s Automated Segmentation Tool). In order to minimize potential confounders introduced by the differences in cortical thickness, surface area, and folding between children and adult brains, an age-specific template was generated via the CerebroMatic toolbox (Wilke et al., 2017), including age and gender as nuisance covariates.

The following pre-processing steps were administered via FSL FEAT (FMRIB-Expert Analysis Tool) after discarding the first three volumes to maintain a steady-state rs-fMRI signal: a) head motion correction using MCFLIRT; b) slice time correction; c) spatial smoothing using a Gaussian kernel of 8 mm full width at half maximum (FWHM); e) high-pass filter with a threshold of 100s. Furthermore, movements and artefacts were removed using ICA-AROMA (Automatic Removal of Motion Artefacts), which recognizes and removes motion-related independent components from rs-fMRI data (Pruim et al., 2015), out-regressing WM and CSF time series, and filtering the resultant functional images, band-pass at [0.01–0.09] Hz. Individual denised functional images were spatially registered with their respective 3D T1 images and then finally normalized into the customized T1 template space using FSL: FLIRT (FMRIB’s Linear Image Registration Tool,FLIRT) and FNIRT (FMRIB’s Non-Linear Image Registration Tool), respectively. A multisession temporal concatenation approach in FSL-MELODIC (Beckmann et al., 2005) was implemented, which decomposed the data into 30 independent components.

2.4. Statistical analysis

Kruskal-Wallis test and post-hoc Mann Whitney U test were performed to assess between-group differences with respect to age. Chi-square test was also used to check for gender distribution between groups. Differences between TS, TS + OCD, and OCD with respect to clinical scores were analyzed with Mann-Whitney U test. Analyses were performed with SPSS (Statistical Package for the Social Sciences).

Using FSL Randomise (5000 permutations), non-parametric statistics were performed to investigate FC differences between pairs of groups (unpaired two-sample t-test two-tailed, alpha = 0.05) and to compute correlations between FC and clinical scores in patients. Randomise is a non-parametric permutation algorithm which enables modelling and inference using a standard GLM design setup (Winkler et al., 2014). Age and gender were included as nuisance variables. In each RSN, correlation analysis was performed between FC and clinical scores via a general linear model compensating for age and gender. Statistical significance was set at p < 0.05, FDR corrected for multiple comparisons. Demographic and clinical data of participants are summarized in Table 1.

3. Results

Kruskal-Wallis test revealed that, TS, TS + OCD, OCD, and controls did not statistically differ in terms of age (H (3) = 2.575, p = 0.46). Chi-square test revealed uneven gender distribution between TS patients and controls (χ2 [1, N = 27] = 15.9, p < 0.001) and between OCD patients and controls (χ2 [1, N = 21] = 48.9, p < 0.001). There were significant differences in clinical measures between the TS, TS + OCD, and OCD groups. Post-hoc Mann-Whitney U test revealed that YGTSS scores were higher in the TS (U = 1.0, p < 0.001) and TS + OCD (U = 1.0, p < 0.001) than the OCD group. In addition, as expected, OCD patients had higher CYBOCS scores than TS patients (U = 0, p < 0.001). Moreover, we found significant differences in the CYBOCS scores of TS and TS + OCD patients (U = 0, p < 0.001, see Table 1).

3.1. Functional connectivity changes

ICA decomposed the data into 30 spatial components. The neuroanatomically-relevant RSNs (i.e. representing brain activity after the removal of physiological and non-physiological noise, e.g. respiratory, vascular, and motion artefacts) were identified by visually matching them against a previously published dataset of 20 canonical RSNs (Smith et al., 2009). Seven RSNs that were found to be impaired either in TS or in OCD in previous studies were selected for further analysis (Cui et al., 2014b; Gruner et al., 2014; Liu et al., 2017b; Weber et al., 2014). These RSNs included the basal ganglia (BGN), cerebellum (CBL), frontoparietal (FPN), default-mode (DMN), orbitofrontal (OBF), salience (SAN), and sensorimotor (SMN) networks. Dual regression was applied to regress the group ICA maps to subject-specific spatial maps.

TS patients exhibited higher FC than controls in four RSNs: the BGN (right thalamus and left putamen), SMN (bilateral precentral gyrus), CBL (right Crus I), and DMN (right precuneus and left frontal medial cortex). They also showed lower FC than controls in two RSNs: the FPN (right middle frontal and superior parietal gyrus) and SAN (right insula, right superior temporal gyrus) (Fig. 1). TS + OCD patients exhibited a FC pattern very similar to TS patients without comorbidity (Fig. 2). Therefore, we merged the two subgroups of TS patients into a single group (TS/TS + OCD) for further analyses (Fig. 3). When considering TS and TS + OCD as one group (TS/TS + OCD), FC was still higher in the aforementioned four RSNs and lower in the remaining two RSNs than controls.

OCD patients showed functional abnormalities in the same six RSNs, but in contrast to both TS and TS + OCD patients, all RSNs showed higher FC than controls. Higher FC was observed in the BGN (bilateral thalamus and left pallidum), SMN (right SMA and left precentral gyrus), CBL (bilateral Crus I and left lobule VI), DMN (right precuneus and left frontal medial cortex), FPN (right middle frontal gyrus, bilateral superior parietal lobule), and SAN (anterior cingulate gyrus and left insula-planum polare) (Fig. 4). One of the seven RSNs considered, the OFN, showed no FC differences between groups. Brain regions exhibiting altered FC between TS, TS + OCD, TS/TS + OCD, and OCD with respect to controls are depicted in Table 2.

The direct comparison between OCD and TS subgroups showed significant and consistent differences in two RSNs, the CBN and FPN. OCD patients exhibited higher FC in the CBN (right Crus I, left Crus II, and left lobule VI) and FPN (right middle frontal gyrus and bilateral superior parietal lobule) than TS patients (Fig. 5a). Similarly, OCD patients exhibited higher FC in the CBN and FPN than TS + OCD patients (Fig. 5b). Lastly, when directly comparing OCD patients with the TS/TS + OCD group, FC was higher in those RSNs (Fig. 5c). Brain regions exhibiting altered FC between patient groups i.e. TS, TS + OCD, TS/TS + OCD with respect to OCD patients are reported in Table 2.

3.2. Correlations between FC and clinical scores

FC within two RSNs, the CBN and FPN, negatively correlated with...
Table 1
Demographic variables and clinical characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>TS (n = 16)</th>
<th>TS + OCD (n = 14)</th>
<th>OCD (n = 10)</th>
<th>Ctrl (n = 11)</th>
<th>TS vs Ctrl</th>
<th>TS vs OCD</th>
<th>TS + OCD vs Ctrl</th>
<th>TS + OCD vs OCD</th>
<th>TS vs TS + OCD</th>
<th>OCD vs Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.7 ± 2.1</td>
<td>10.2 ± 2.1</td>
<td>10.9 ± 2.5</td>
<td>9.9 ± 1.3</td>
<td>p &lt; 0.001</td>
<td>p = 0.18</td>
<td>p = 0.43</td>
<td>p = 0.46</td>
<td>p = 0.44</td>
<td>p = 0.31</td>
</tr>
<tr>
<td>Male/Female</td>
<td>15/1</td>
<td>10/4</td>
<td>7/3</td>
<td>2/9</td>
<td>p &lt; 0.001</td>
<td>p = 0.10</td>
<td>p = 0.08</td>
<td>p = 0.94</td>
<td>p = 0.10</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>YGTSS score</td>
<td>17.5 ± (0-50)</td>
<td>18.1 ± 10.8</td>
<td>0.8 ± 1.7</td>
<td>-</td>
<td>p &lt; 0.001</td>
<td>-</td>
<td>p &lt; 0.001</td>
<td>p = 0.72</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CYBOCS score</td>
<td>0.25 ± (0-40)</td>
<td>16.4 ± 6.1</td>
<td>18.6 ± 7.5</td>
<td>-</td>
<td>p &lt; 0.001</td>
<td>-</td>
<td>p = 0.62</td>
<td>p &lt; 0.001</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean (M) ± standard deviation (SD).

YGTSS: Yale Global Tic Severity Scale.
CYBOCS: Children’s Yale-Brown Obsessive-Compulsive Scale.
OCD: Obsessive Compulsive Disorder.
TS: Tourette syndrome without Obsessive Compulsive Disorder symptoms.
TS + OCD: Tourette syndrome with Obsessive Compulsive Disorder symptoms.
Ctrl: controls.

Tic severity was evaluated by summing the motor and phonetic tics (without impairment scores) as per the YGTSS guidelines. Significant p values are highlighted in bold font (p < 0.05).

* Age difference were assessed via Mann Whitney (U) test.
* Gender differences were assessed via chi square (χ²) test.
* Mann Whitney (U) test was used to evaluate differences in patient cohort with respect to clinical scores.

YGTSS scores in the TS/TS + OCD group. By contrast, FC positively correlated with CYBOCS in OCD patients (Fig. 6). In addition, the TS/TS + OCD group showed a positive correlation of FC within the BGN and DMN with YGTSS (Supplementary material). When TS and TS + OCD patients were analyzed separately, the positive correlation between BGN FC and YGTSS was still significant in both groups. Differently, the negative correlation between CBN FC and YGTSS was significant only in TS patients, whereas TS + OCD patients showed a negative correlation between FPN FC and YGTSS. Finally, no significant correlation was observed between FC within any RSN and CYBOCS scores in TS + OCD patients. FC correlations with TS, TS + OCD, and OCD patient’s clinical scores are reported in Table 3.

4. Discussion

To the best of our knowledge, this is the first study comparing FC alterations between drug-naïve children with TS, TS + OCD, OCD, and age-matched controls. To overcome possible confounding, we carefully selected a cohort of children without any comorbid disorders known to be associated with these conditions, e.g. ADHD. Moreover, the pediatric and drug-naïve nature of our cohort allowed us to minimize confounding factors such as age, disease duration, and chronic pharmacological treatment.

In the present study, children with TS and TS + OCD exhibited the same pattern of increased FC in four RSNs (the BGN, SMN, CBN, and DMN) and decreased FC in two RSNs (the FPN and SAN), when compared to controls. With respect to controls, OCD patients showed FC alterations in the same six RSNs as TS and TS + OCD patients, though they had higher FC in all six RSNs. OCD patients had higher FC in two RSNs, CBN and FPN, as compared to TS and TS + OCD patients both separately and as one TS/TS + OCD group. When exploring the relationship between FC of CBN and FPN and clinical scores, we found that FC in both the CBN and FPN negatively correlated with YGTSS scores in TS and positively correlated with CYBOCS scores in OCD.

4.1. FC changes in TS, TS + OCD, and OCD

BGN and SMN. FC within the BGN and SMN increased in the TS, TS + OCD, and OCD groups with respect to age-matched controls. Increased FC in the thalamus, lentiform nucleus, SMA, and primary motor cortex...
supports the traditional model of cortico-striato-thalamo-cortical (CSTC) motor loop disruption in TS, which is considered a hallmark of TS pathophysiology (Albin and Mink, 2006; Leckman, 2002; Suppa et al., 2014, 2011). These findings are consistent with previous rs-fMRI studies in TS that have reported a central involvement of the BGN in tic pathophysiology, both in children (Cui et al., 2014b) and adults (Ramkiran et al., 2019; Wang et al., 2011). In keeping with this evidence, the positive correlation of the FC of the right thalamus with YGTSS scores indicates that higher BGN connectivity is associated with greater tic severity. Besides, our findings strengthen the role of basal ganglia and sensorimotor cortical dysfunction in OCD, as FC in the BGN and SMN increased in this cohort. This evidence is consistent with that of previous studies (Armstrong et al., 2016; Calzà et al., 2019; Fitzgerald et al., 2011; Sakai et al., 2011) and has been linked to the altered processing of action monitoring and error detection in OCD (Bonini et al., 2014; Malhy et al., 2005; Stern et al., 2011a).

CBN. We found increased FC in the right Crus I in TS. Cerebellar involvement in TS has been reported in clinical, neurophysiological, and neuroimaging studies (Bohlhalter, 2006; Ramkiran et al., 2019; Sigurdsson et al., 2020; Tobe et al., 2010). One such rs-fMRI study in children with TS reported decreased regional homogeneity within the right cerebellum (Liu et al., 2017a) while another study investigating whole brain functional network topology in TS reported reduced cerebellar-cortical connectivity (Ramkiran et al., 2019). Differences in patient clinical features or in the methodological approach used (ICA vs. a regional homogeneity method or graph theory analysis) may account for inconsistencies between studies. Moreover, while we did not find generalized cerebellar functional abnormality, we found a specific...
Cerebellar involvement, however, differs between the two disorders in OCD patients, consistent with previous rs-fMRI studies (Anticevic et al., 2012; Voogd, 2014). Overall, the present study provides evidence of the cerebellar’s role in the pathophysiology of both TS and OCD. Cerebellar involvement, however, differs between the two disorders in terms of both functional connectivity and its association with clinical severity, as discussed below.

**DMN.** Within the DMN, we found increased FC in the precuneus and left medial prefrontal cortex (mPFC) both in TS and OCD. The DMN has been found to be disrupted in a wide range of neuropsychiatric disorders and has been implicated in processes related to mind-wandering or task-independent thoughts (Mason et al., 2007). Within this network, rs-fMRI abnormalities have been reported in both TS (Fan et al., 2018) and OCD patients (Fan et al., 2017; Jang et al., 2010) with unclear results. Of note, former studies in TS and OCD have focused on between-network connectivity and pointed out an increased connectivity between the DMN and other networks, particularly the FPN (Fan et al., 2017, 2018; Stern et al., 2012). The altered coupling between DMN and FPN has been variably interpreted either as a sign of the inability in OCD to disengage from internal thoughts when performing everyday tasks requiring attention to the outer environment (Stern et al., 2012), or as the attempt in TS to enhance monitoring of the outer world due to long-term struggling and coping with inappropriate acts (Fan et al., 2018).

**FPN.** In the FPN, TS and OCD patients exhibited FC changes in opposite directions with decreased FC in TS and increased in OCD within the middle frontal gyrus and superior parietal cortex. In TS patients, decreased FC in the frontal and parietal cortices has already been reported in pediatric drug-naïve patients (Buse et al., 2016; Church et al., 2009; Cui et al., 2014b). The FPN is considered a core system underlining attention control (Ptak, 2012; Scolari et al., 2015) and serving attentional gating, shifting and information retaining to perform rapid adaptive control tasks (Oosenbach et al., 2008). Given the involvement of such structures in adaptive action control (Zanto and Gazzaley, 2013), this finding may explain defective control over volitional actions observed in TS (Cavanna and Nani, 2013) whereas in OCD it may reflect an overactive cognitive control over behavior, as occurs in obsession-compulsion pairing (Viard et al., 2005; Vries et al., 2019).

**SAN.** Similar to the FPN, brain areas in the SAN also showed opposite FC changes in TS and OCD, with decreased insular FC in TS and increased insular FC in OCD. The insula has been associated with social cognitive fitness, and decreased FC in TS, consistent with previous studies (Liu et al., 2017b) may be in tune with evidence of an impairment in social cognition in these patients (Vicario and Martino, 2018). Moreover, the pathological role of the insula is supported by previous MRI studies showing that structural and functional abnormalities in the insula are related to premonitory urges (Jackson et al., 2020; Tinaz et al., 2015). Conversely, in OCD some areas of the SAN, namely the anterior cingulate gyrus and left insula, displayed increased FC. This is consistent with previous reports (Hou et al., 2012; Ping et al., 2013; Yang et al., 2010; Zhang et al., 2011). The functional involvement of the limbic system in OCD may underpin the heightened emotional processing linked to arousal and negative emotional states experienced by these patients (Stern et al., 2011b).

**4.2. Resting-state fMRI differences between TS and OCD**

Comparison between the three groups of patients showed that: i) the FC of children with TS and TS + OCD did not show any significant difference; ii) the FC of children with TS and TS + OCD significantly differed from those with OCD in two RSNs, the CBN and FPN.

Both TS and OCD patients showed increased FC in the CBN, though FC was significantly higher in OCD than in TS patients. Crucially, CBN FC changes were anti-correlated with YGSS scores and positively correlated with CY-BOCS scores. These correlations suggest a different functional role of FC changes in the two disorders. Since higher FC in the CBN was associated with lower tic severity in TS, CBN FC may reflect neuroplastic mechanisms likely involved in the modulation of tic expression. Conversely, the direct correlation between FC and OCD clinical severity suggests that increased cerebellar FC could represent an abnormal mechanism of neuropsychiatric affecting symptoms’ severity. Thus, our evidence suggests the CBN is a relevant component of the network underpinning the pathophysiology of TS and OCD. Further research is needed to elucidate its precise interactions at a multi-network level and explaining its role in the modulation of symptoms.

In addition, FPN FC showed opposite changes in that it decreased in TS patients and increased in OCD patients. In TS, decreased FPN connectivity may result in impaired cognitive control over volitional actions, provoking tics and tic-like compulsions. Conversely, in OCD increased FPN connectivity may reflect an overactive cognitive control over behavior, typically occurring in the obsession-compulsion pairing.

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**Fig. 4.** Significant differences in functional connectivity (FC) in six resting state networks (RSNs) between Obsessive compulsive disorder patients (OCD) and controls: Within each RSN, areas of increased FC are shown in red, areas of decreased FC in blue. Red and blue bars represent t values. The binarized masks in yellow represent the six RSNs. Compared with controls, OCD patients showed increased functional connectivity in five RSNs: a) BGN (Basal ganglia network): bilateral thalamus, left putamen. b) SMN (Sensorimotor network): right supplementary motor area and left precentral gyrus. c) CBN (Cerebellar network): bilateral Crus I and left lobule VI. d) DMN (Default mode network): right precuneus and left medial frontal cortex. e) FPN (Frontoparietal network): right middle frontal gyrus, bilateral superior parietal cortex. f) SAN (Salience network): Anterior cingulate gyrus and left insula (Planum polare). Results were FDR corrected (p < 0.05) for multiple comparisons.
Table 2
Altered resting state functional connectivity (rs-FC) between all groups.

<table>
<thead>
<tr>
<th>Networks</th>
<th>F/(p-value)</th>
<th>TS vs Ctrl</th>
<th>TS + OCD vs Ctrl</th>
<th>TS/TS + OCD vs Ctrl</th>
<th>Two sample t-test</th>
</tr>
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<tr>
<td></td>
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<td>Cluster size</td>
<td>MNI coord</td>
<td>T/p-value</td>
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<td>2.11/0.002</td>
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</table>

(continued on next page)
Thus, the changes in FPN FC may be exploited as a neural marker to distinguish TS from OCD.

Although speculative at this stage, the different involvement of the FPN, thus of brain areas related to action monitoring and control, might underpin some phenomenological differences of the two disorders. Obsessive-compulsive symptoms in TS patients, as opposed to OCD patients, are mostly represented by non-cognitive repetitive phenomena, i.e. tic-like compulsions and other movement-related sensory events “just right” and “just so” requirements. Conversely, in OCD...
compulsions are generally associated with underlying cognitive processes (i.e. an obsession, (Miguel et al., 2000, 1995). Moreover, some types of OC symptoms are more prevalent in patients with TS compared to the broader OC phenomenology observed in OCD (Cath et al., 2001). It is possible to speculate that the decreased FPN connectivity observed in TS may result in impaired cognitive control over volitional actions, in that it is disrupted by tics and tic-like compulsions. Conversely, in OCD increased FPN connectivity may reflect an overactive cognitive control over behavior, typically occurring in the obsession-compulsion pairing.

4.3. Limitations

This study has few limitations that deserve emphasis. First, no online tic measurement was collected during rs-fMRI acquisition, thus possibly masking the exact neural underpinnings of TS. Second, gender distribution differences between patients and controls potentially may have influenced the results. However, as gender was included as a nuisance covariate in FC analysis, we believe that gender distribution is unlikely to influence our results. Third, our study cohort consisted of only 51 patients. Such a small sample size may make the study prone to Type II errors. Moreover, previous pediatric studies with small sample size were able to detect only major effects and the studies might have succumbed to type-II errors. Hence the comprehensive interpretation of the results was affected (Cui et al., 2014b; Liu et al., 2017b; Mirabella et al., 2020;...
Declaration of competing interest


Cavanaugh, A.E., Nani, W., 2013. Tourette syndrome and consciousness of action. Tremor Hyperkinetic Mov. 3.


