GILLES DE LA TOURETTE SYNDROME: CROSS-CULTURAL PERSPECTIVES WITH A FOCUS ON THE ASIA-PACIFIC REGION

EDITED BY: Valsamma Eapen and Yukiko Kano
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GILLES DE LA TOURETTE SYNDROME: CROSS-CULTURAL PERSPECTIVES WITH A FOCUS ON THE ASIA-PACIFIC REGION

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Editorial: Gilles de la Tourette Syndrome: Cross-Cultural Perspectives With a Focus on the Asia-Pacific Region

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Keywords: Gilles de la Tourette syndrome, OCD, neurodevelomental disorders, tic discorder, Asia - Pacific

Editorial on the Research Topic

Gilles de la Tourette Syndrome: Cross-Cultural Perspectives With a Focus on the Asia-Pacific Region

Gilles de la Tourette Syndrome (GTS) is a neurodevelopmental disorder characterized by the presence of multiple motor and one or more vocal tics of more than one-year duration (1). Most people with GTS also experience associated psychiatric co-morbidities and other challenges, leading to significant social impact and poor quality of life (2). Once thought to be rare, tic disorders are now recognized to be relatively common, with an estimated prevalence of 1% in children and adolescents in the majority of cultures of the world (1). However, GTS is often under-recognized, and a significant cause of hidden disability. Epidemiological, phenomenological, and genetic studies have demonstrated broad overlap between GTS and commonly occurring co-morbidities such as Obsessive-Compulsive Disorder (OCD) and Attention Deficit Hyperactivity Disorder (ADHD) and less commonly Autism Spectrum Disorder (3–5). Further, it has been proposed that OCD may represent an alternative phenotypic expression of the putative GTS genes with a gender-dependent difference in expression in that male family members typically present with tics while female members present more often with OCD (6). Recent research has also shown that only around one in ten patients with GTS have "pure" GTS with only motor and vocal tics, while the rest have a number of co-morbidities (7). The precise mechanisms underlying GTS are yet to be revealed, however, research suggest the involvement of a number of neurodevelopmental genes and the neurexin trans-synaptic connexus (NTSC) (8).

While GTS presents in all ethnic and cultural groups worldwide, there has been an overrepresentation of GTS literature from European and North American perspectives. However, given the heterogenous nature of the disorder, the influence of race, culture and environmental factors on symptom expression merits further exploration; the focus of this special issue. In this regard, it has been shown that, while GTS is seen less frequently in some cultures, in all cultures where it has been reported, the phenomenology is similar, highlighting the biological underpinnings of the disorder while there is also some evidence to suggest there may be some variations in the occurrence of associated behaviors and co-morbidities (9). A study of clinical patients from the UK and the United Arab Emirates for example found the rates of occurrence of OCD and ADHD to be similar in the two cohorts thereby emphasizing the biological and genetic link with these conditions but a much higher prevalence of Oppositional Defiant Disorder and Conduct Disorder in the UK sample suggesting the role of environmental and cultural differences in the occurrence of such co-morbidities (10). Further, cultural differences have been observed in the level of distress and dysfunction caused by GTS with Caucasian patients exhibiting less

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distress and impairment than in Latinos (11). It is therefore important to elucidate the differences in clinical presentation, impact of symptoms and the range of treatments that may be effective—some of which are more or less acceptable in different settings. Thus, cross-cultural perspectives and research have an important place in both helping clinicians work with people with GTS in specific cultural settings, but also in deepening our understanding of the disorder by observing it within different contexts as illustrated in this special issue.

Regarding phenomenology, Matsuda et al. in their study have examined whether awareness of premonitory sensation could assist in tic suppression and explored the relationship between tic-associated sensation and the potential subtypes or variants of such sensation. Further, they developed the "Rumination and Awareness Scale for tic-associated sensations" (RASTS), a new questionnaire to assess the intensity of the somatosensory hyperawareness and the patient's ability to identify signals of emerging tics. The findings indicate that aversive tic-associated somatosensory experiences do not facilitate tic suppression but the awareness of when the tic will occur prior to them experiencing the tic was correlated with the ability to suppress tics. Age-wise analysis found that the correlation between the premonitory awareness and tic suppression was significant only in children with GTS. The authors propose the possibility that premonitory awareness may gain aversive valence only in adulthood, for those where tic symptoms persist. This may have clinical implications in the behavioral management of tics.

In a 4 year follow up study of GTS, Kano et al. found that tics and associated sensory phenomena as well as global functioning had not changed much in this period but they observed significant improvement in obsessive compulsive symptoms (OCS). They also reported significant correlation between sensory phenomena and past symptoms of tics and OCS. The sample size being small, the results need replication.

Liu et al. has reported on the Expert Consensus on Diagnosis and Treatment of Tic Disorders in China, developed by the Chinese Child Neurology Society (CCNS). This provides a comprehensive account on the approach to clinical diagnosis of Tic Disorders along with therapeutic options such as educational, psychological, and pharmacological interventions, including traditional Chinese medicine and acupuncture.

Most genome-wide association studies (GWAS) have been carried out in populations of European descent leading to a disparity in our understanding of the genetics of complex traits between populations. For many conditions with complex genetic traits, gene regulation is extremely critical. While it is well-known the consistent enrichment of regulatory variants among trait-associated variants, the exact effects of these key variants across populations is unclear. In this regard, Yuan et al. has described higher variant allele frequency of CLCN2 G161S genotype in patients with tics and GTS compared to control population in Chinese Han population. However, this variant has not been reported in the currently available public databases, suggesting this may be a population specific finding.

Lou et al. functional alterations between GTS and healthy children in Chinese population using frequency-specific regional homogeneity (ReHo) and found that GTS patients showed decreased ReHo in the right operculum, increased ReHo in the left precentral gyrus and increased connectivity of the right superior frontal gyrus within the left executive control network. In addition, a significantly negative correlation was found between Yale Global Tic Severity Scale (YGTSS) vocal score and ReHo values of the right operculum in the highest frequency bands, while a significant positive correlation was found between YGTSS motor score and altered connectivity of the right superior frontal gyrus. The study also observed altered connectivity within the executive control network of GTS children alongside frequency-specific abnormal alterations of ReHo in the whole brain. Further research is indicated to examine the neural importance and clinical applications of these findings.

Zhao et al. have reported on an exploratory pilot study of Fecal microbiota transplantation and suggested that this helped shift the composition of the gut microbiota with restoration of B.coprocola which in turn correlated with tic symptom improvement. However, given the small sample size of this study, further research in this area is indicated before any conclusions can be made.

Clarke et al. in their paper using pathway analysis has implicated mitochondrial dynamics, structure and function (MDSF) in GTS which in turn has a role in neuronal circuitry development, synaptic connectivity, and neurotransmission. Given the sensitivity and responsiveness of mitochondria to environmental cues and their intimate role in neuronal development and function, they may be instrumental in mediating the nature of phenotypic presentation and the degree of phenotypic penetrance.

Thus, while genetic and biological factors are critical in conferring the risk to the development of GTS, environmental, and cultural factors may impact on how the tics and associated behaviors present, and are perceived, the rate of occurrence of associated co-morbidities, as well as the help seeking and referral options.

AUTHOR CONTRIBUTIONS

VE has conceptualized and wrote the manuscript.

REFERENCES

1. Robertson MM, Eapen V, Singer HS, Martino D, Scharf JM, Paschou P, et al. Gilles de la Tourette syndrome. *Nat Rev Dis Primers*. (2017) 3:16097. doi: 10.1038/nrdp.2016.98
2. Eapen V, Cavanna AE, Robertson MM. Comorbidities, social impact and quality of life in Tourette syndrome. *Front Psychiatr*. (2016) 7:97. doi: 10.3389/fpsyt.2016.00097
3. State MW. The genetics of child psychiatric disorders: focus on autism and Tourette syndrome. *Neuron*. (2010) 68:254–69. doi: 10.1016/j.neuron.2010.10.004

4. O'Rourke JA, Scharf JM, Yu D, Pauls DL. The genetics of Tourette syndrome: a review. *J Psychosom Res*. (2009) 67:533–45. doi: 10.1016/j.jpsychores.2009.06.006
5. Kadesjo B, Gillberg C. Tourette's disorder: epidemiology and comorbidity in primary school children. *J Am Acad Child Adolesc Psychiatry*. (2000) 39:548–55. doi: 10.1097/00004583-200005000-00007
6. Eapen V, Pauls DL, Robertson MM. Evidence for Autosomal dominant gene transmission in Tourette syndrome. *Br J Psychiatry*. (1993) 162:593–6. doi: 10.1192/bjp.162.5.593
7. Eapen V, Robertson MM. Are there distinct subtypes in Tourette Syndrome (TS)? Pure-Tourette syndrome versus Tourette syndrome- Plus and simple versus complex tics. *Neuropsychiatric Dis Treat*. (2015) 11:1431–6. doi: 10.2147/NDT.S72284
8. Clarke RA, Lee S, Eapen V. Pathogenetic model for Tourette syndrome delineates overlap with related neurodevelopmental disorders including Autism. *Transl Psychiatry*. (2012) 2:e158. doi: 10.1038/tp.2012.75
9. Robertson M, Eapen V, Cavanna AE. The International prevalence, epidemiology and clinical phenomenology of Tourette syndrome; a cross cultural perspective. *J Psychosomatic Res*. (2009) 67:475–83. doi: 10.1016/j.jpsychores.2009.07.010
10. Eapen V, Robertson MM. Clinical correlates of Tourette's disorder across cultures: a comparative study between the United Arab Emirates UK. *Prim Care Companion J Clin Psychiatry*. (2008) 10:103–7. doi: 10.4088/PCC.v10n0203
11. Mathews CA, Herrera Amighetti LD, Lowe TL, van de Wetering BJ, Freimer NB, Reus VI, et al. Cultural influences on diagnosis and perception of Tourette syndrome in Costa Rica. *J Am Acad Child Adolesc Psychiatry*. (2001) 40:456–63. doi: 10.1097/00004583-200104000-00015

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ORIGINAL RESEARCH
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Changes in Sensory Phenomena, Tics, Obsessive–Compulsive Symptoms, and Global Functioning of Tourette Syndrome: A Follow-Up After Four Years

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Sensory phenomena and related features of Tourette syndrome are related to poorer quality of life. Therefore, sensory phenomena might also have a negative impact on global functioning. However, the influence of sensory phenomena, tics, and obsessive–compulsive symptoms (OCS) on global functioning after several years of usual treatment has not been investigated. Twenty out of 45 Japanese patients with Tourette syndrome who had previously undergone an evaluation of these clinical features were assessed again after an average of four years. We conducted a panel of assessments for premonitory urges, broader sensory phenomena, tic severity, OCS, and global functioning. Based on Pearson's correlation coefficient, current global functioning was significantly negatively correlated with previous tics and marginally negatively correlated with previous broader sensory phenomena. Current global functioning was marginally correlated with change in tics. Change in global functioning was significantly correlated with change in OCS and marginally correlated with change in premonitory urges. Due to the small sample size, it was not possible to use a multiple regression analysis to conclude that sensory phenomena, tics, and OCS predict global functioning in adolescents and adults with TS. However, it was suggested that further investigation of this relationship would be meaningful.

Keywords: sensory phenomena, tics, Tourette syndrome, obsessive–compulsive symptoms, global functioning

INTRODUCTION

Tourette syndrome (TS) is a childhood-onset neurodevelopmental disorder characterized by multiple motor and vocal tics (1). Aside from tics, the crucial symptoms of TS are "sensory phenomena," which include urges to move that often precede tics (i.e., premonitory urges) and a strong desire to have things be "just right" (2).

Sensory phenomena in TS have often been investigated in relation to frequent comorbid symptoms such as obsessive–compulsive symptoms (OCS) and global functioning. For instance, one study indicated that sensory phenomena, especially premonitory urges, were significantly correlated with vocal tics and OCS in adults with TS (3). Another study showed that sensory phenomena were significantly correlated with clinical symptoms, particularly OCS and anxiety (4). Among sensory phenomena, "just right" perception is an important feature of tic-related OCD. Our previous studies showed that premonitory urges and broader sensory phenomena including "just right" perception are different in terms of their relationships with OCS dimensions (5) and clinical course after Deep Brain Stimulation (6).

Sensory phenomena, especially premonitory urges were significantly and negatively correlated with quality of life (QOL) in previous research (3, 4). QOL is evaluated as one's perception of broader health, whereas global functioning represents psychological, social, and occupational functions assessed by behaviors. Despite this difference between QOL and global functioning, the relationship between sensory phenomena and global functioning seems to be similar to that between sensory phenomena and QOL. In a previous study, we found that both premonitory urges and broader sensory phenomena were significantly positively correlated with total tics, vocal tics, and OCS, while premonitory urges were significantly and negatively correlated with global functioning (5).

One study showed that premonitory urges, tic severity, and family history of TS in childhood were predictors of poorer QOL in adults with TS (7). However, there have been no investigations of the influence of whole sensory phenomena and their related features (e.g., tics and OCS) on global functioning during the clinical course of TS. We believe that such an investigation would provide a deeper understanding of TS as well as better treatment and support. Thus, in this study, we described changes in sensory phenomena as well as tics, OCS, and global functioning after several years of usual treatment for TS. Based on these data, we examined whether previous sensory phenomena, tics, and OCS would predict current global functioning. We also examined whether changes in sensory phenomena, tics, and OCS over the course of treatment would predict current global functioning as well as change in global functioning. Based on previous findings, we hypothesized that more severe previous sensory phenomena, tics, and OCS would linearly predict poorer current global functioning.

METHODS

Participants

Participants were recruited from the 45 patients with TS who had participated in our previous study (5). Out of the 45 patients, we asked 22 to participate in the current study, but two patients did not accept our request. Of the remaining 23 patients, we were unable to ask 15 to participate because they had changed hospitals or had completed or dropped out of treatment. An additional eight patients were not asked to participate because they visited the hospital rarely or irregularly (n = 5), had undergone deep brain stimulation (n = 2), or were in an unstable condition (n = 1). Therefore, the participants of the study consisted of 20 patients with TS (16 men and 4 women; age range, 17–53 years; mean = 30.2; SD = 11.2) who had fully completed all study instruments. All participants had been diagnosed with Tourette's disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (8). Participants also met the DSM-5 (fifth edition) criteria for Tourette's disorder (1). The participants were recruited between January 2014 and May 2015, and the mean time since previous study participation was 4.0 years (SD = 0.61, range: 3–5 years). Data obtained from these participants in the previous study were also used in analyses.

The Institutional Ethical Committee of the University of Tokyo Hospital approved this study. Written informed consent was obtained from all adult participants and the parents of participants aged 19 years old or younger. Psychologists with clinical experience of TS and sufficient ability to perform an assessment following instrument administration training conducted all the interviews.

Instruments

To facilitate comparison between the studies, the same instruments used in our previous study to assess sensory phenomena, tics, and global functioning (5) were utilized in the current study. Assessment of sensory phenomena included administration of the University of São Paulo Sensory Phenomena Scale (USP-SPS) (5, 9) and the Premonitory Urge for Tics Scale (PUTS) (10). The USP-SPS is a clinician-rated scale that assesses presence or absence of sensory phenomena in five types, including "just right" perception, and measures severity of broader sensory phenomena on three ordinal scales focusing on frequency, distress, and interference, with six anchor points. The USP-SPS total score (0–15) is obtained by combining these scores. The PUTS is a 9-item self-report scale that measures severity of premonitory urges for tics. The PUTS total score (9–36) is obtained by summing the scores for all items.

Tics were evaluated using the Yale Global Tic Severity Scale (YGTSS) (11, 12), which is a clinician-rated scale that measures severity of motor and vocal tics as well as impairment due to tics. The YGTSS total tics score (0–50) is obtained by summing the scores for motor and vocal tics on five ordinal scales focusing on

number, frequency, intensity, complexity, and interference, with six anchor points. The YGTSS global severity score (0–100) is obtained by summing the total tics score (0–50) and the impairment score (0–50). Global functioning was evaluated using the Global Assessment of Functioning (GAF) scale (8).

Comorbid obsessive–compulsive disorder (OCD) and attention-deficit/hyperactivity disorder (ADHD) were diagnosed according to the DSM-IV-TR criteria, in addition to TS. Information about medication use was obtained from patients' psychiatrists and medical records. In the current study, OCS were evaluated using the Yale-Brown Obsessive–Compulsive Scale (Y-BOCS) (13, 14), which is a clinician-rated scale that measures severity of obsessions and compulsions on five ordinal scales with five anchor points. The Y-BOCS total score (0–40) was obtained by summing the scores for the obsessions (0–20) and compulsions (0–20) scales.

Statistical Analyses

All statistical analyses were performed using PASW Statistics 18.0 (i.e., SPSS). The Shapiro-Wilk test showed that the assessment scores in this study were normally distributed, except for the current YGTSS impairment score. A paired t-test was used to compare the scores on all instruments between the previous and current studies. In addition, the Wilcoxon rank sum test was used for comparison of YGTSS impairment score between the two studies. After examining the associations between previous and current sensory phenomena, tics, and OCS, the correlations of current global functioning with previous sensory phenomena, tics, and OCS were examined. In all the association analyses, total score was used for USP-SPS, global severity score for YGTSS, and total score for Y-BOCS. The score changes in sensory phenomena, tics, OCS, and global functioning were defined as the differences between current and previous scores. The interrelationships between the score changes in clinical characteristics and current global functioning, as well as the score change in global functioning, were also examined. For these examinations, Pearson's correlation coefficients were calculated. The standard $p<0.05$ level of significance was used. When we examined correlations between previous and current USP-SPS, PUTS, YGTSS, and Y-BOCS in *Correlations Between Previous and Current Sensory Phenomena, Tics, and OCS*; correlations between previous USP-SPS, PUTS, YGTSS, and Y-BOCS, and current GAF in *Correlations Between Previous Sensory Phenomena, Tics, and OCS, and Current Global Functioning*; correlations between change in USP-SPS, PUTS, YGTSS, and Y-BOCS, and current GAF in *Correlations Between Changes in Sensory Phenomena, Tics, and OCS, and Current Global Functioning*; and correlations between changes in USP-SPS, PUTS, YGTSS, and Y-BOCS, and change in GAF in *Correlations Between Changes in Sensory Phenomena, Tics, and OCS, and Change in Global Functioning*; the Bonferroni correction was applied and the level of significance changed to $p<0.0125$.

RESULTS

Before analyzing data of the current study, we compared age, sensory phenomena, tics, OCS, and global functioning in the previous study between the 20 current study participants and the 25 non-participants by an independent sample t-test. Fisher's exact test was used to compare gender differences between the two groups. Although we found that Y-BOCS total scores were significantly higher in the latter group ($p = 0.02$), no other significant differences were found ($p = 0.12$–0.93).

Description of Sensory Phenomena, Tics, OCS, Global Functioning, Comorbidity, and Medication in the Current Study

In the current study, the mean total scores for the USP-SPS and PUTS were 5.0 and 10.2, respectively (**Table 1**). Out of five types of sensory phenomena, rate of Energy build-up decreased the most between two studies (**Table 2**). Counting change in each person, the proportion without change was highest at 95.2% for

TABLE 1 | Participants' demographic and clinical characteristics.

		Previous	Current	*t*-value	*p*-value
Age (years)		26.1 (11.2; 13–49)	30.2 (11.2; 17–53)	21.5	0.00
USP-SPS	Total	6.2 (3.1; 0–12)	5.0 (3.2; 0–12)	–1.7	0.11
	Frequency	3.0 (1.6; 0–5)	2.3 (1.5; 0–5)	–1.4	0.18
	Distress	1.9 (1.0; 0–4)	1.7 (1.1; 0–4)	–1.2	0.23
	Interference	1.3 (1.3; 0–4)	1.0 (1.2; 0–4)	–1.2	0.23
PUTS		10.6 (6.6; 1–25)	10.2 (6.1; 0–26)	–0.3	0.74
YGTSS	Total tics	21.3 (8.1; 7–37)	20.9 (9.0; 8–37)	–0.4	0.76
	Motor tics	10.1 (6.2; 0–22)	9.9 (7.4; 0–22)	–0.3	0.75
	Vocal tics	11.2 (4.2; 4–19)	11.1 (4.6; 0–17)	–0.1	0.89
	Impairment	22.0 (11.1; 0–40)	21.0 (13.3; 0–40)	–0.4	0.67 [a]
	Global severity	43.3 (17.9; 7–77)	41.9 (21.1; 8–77)	–0.4	0.66
Y-BOCS	Total	11.7 (8.9; 0–29)	7.8 (7.3; 0–25)	–2.8	0.01
	Obsessions	5.7 (4.4; 0–14)	4.2 (4.0; 0–14)	–1.4	0.17
	Compulsions	6.1 (5.4; 0–15)	3.6 (3.4; 0–11)	–3.0	0.008
GAF		64.7 (11.1; 45–86)	62.4 (10.5; 43–83)	–1.1	0.28

Data reported as: Mean (SD; range).

USP-SPS, University of São Paulo Sensory Phenomena Scale; PUTS, Premonitory Urge for Tics Scale; YGTSS, Yale Global Tic Severity Scale; Y-BOCS, Yale-Brown Obsessive–Compulsive Scale; GAF, Global Assessment of Functioning.

[a]*When the Wilcoxon rank sum test was used for comparison between the two studies, the p value became 0.642.*

TABLE 2 | Participants' number with each type of sensory phenomena.

	Previous	Current
A. Physical sensation	14 (70%)	12 (60%)
A1. Tactile	10 (50%)	8 (40%)
A2 Muscle-joint	8 (40%)	9 (45%)
B. "Just-right" perception	11 (55%)	10 (50%)
B1. Visual	5 (25%)	5 (25%)
B2. Auditory	5 (25%)	4 (20%)
B3. Tactile	7 (35%)	7 (35%)
C. Feeling of incompleteness	8 (40%)	6 (30%)
D. Energy build-up	9 (45%)	4 (20%)
E. Urge only	11 (55%)	9 (45%)

Auditory "just-right" perception, and lowest at 52.4% for Tactile sensation.

The mean scores on the YGTSS were 20.9 for total tics, 21.0 for impairment due to tics, and 41.9 for global severity. The mean GAF score was 62.4. None of these scores differed significantly between the current and previous studies. The mean total score of the Y-BOCS was 7.8 in the current study, which was significantly lower than that in the previous study ($p = 0.01$).

In the current study, six (30%) participants had comorbid OCD and four (20%) had comorbid ADHD. All participants were taking some form of psychotropic drug. Specifically, all participants were on antipsychotics, including aripiprazole ($n = 12$), risperidone ($n = 4$), and haloperidol (n = 4). Nineteen were on other drugs, including antidepressants ($n = 13$, of which 11 were on selective serotonin reuptake inhibitors), clonazepam ($n = 6$), anxiolytics ($n = 2$), and clonidine ($n = 1$). There were only slight differences in medication between the current and previous studies, although the number of participants on antidepressants increased from 9 to 13.

Correlations Between Previous and Current Sensory Phenomena, Tics, and OCS

Previous USP-SPS total scores and PUTS total scores were significantly correlated with previous YGTSS global severity scores and Y-BOCS total scores ($p<0.001$, $p = 0.026$, respectively, for USP-SPS; $p<0.001$, $p = 0.002$, respectively, for PUTS). Current USP-SPS total scores were significantly correlated with current YGTSS global severity scores ($p = 0.009$), while current PUTS total scores were marginally correlated with current YGTSS global severity scores ($p = 0.024$). Both current USP-SPS total scores and PUTS total scores were significantly correlated with current Y-BOCS total scores ($p = 0.002$, $p = 0.002$, respectively). Previous USP-SPS total scores were significantly correlated with current Y-BOCS total scores ($p = 0.006$) and marginally correlated with current YGTSS global severity scores ($p = 0.048$). Previous PUTS total scores were marginally correlated with current Y-BOCS total scores ($p = 0.019$).

Correlations Between Previous Sensory Phenomena, Tics, and OCS, and Current Global Functioning

Current GAF scores were significantly negatively correlated with previous YGTSS global severity scores ($p = 0.005$) and marginally negatively correlated with USP-SPS total scores ($p = 0.023$) (**Table 3**).

Correlations Between Changes in Sensory Phenomena, Tics, and OCS, and Current Global Functioning

Current GAF scores were marginally correlated with the change in YGTSS global severity scores ($p = 0.042$; **Table 4**).

Correlations Between Changes in Sensory Phenomena, Tics, and OCS, and Change in Global Functioning

The change in GAF scores was significantly correlated with the changes in Y-BOCS total scores ($p = 0.009$) and marginally correlated with the change in PUTS total scores ($p = 0.023$) (**Table 5**).

DISCUSSION

The adolescents and adults with TS in this study demonstrated significant improvement in OCS after an average of four years of usual treatment, although their sensory phenomena, tics, and global functioning showed little change. In these participants, sensory phenomena were significantly correlated with tics and OCS in the past. Broader sensory phenomena were significantly correlated with tics and OCS, and premonitory urges were

TABLE 3 | Correlations between previous sensory phenomena, tics, and OCS, and current global functioning.

	Current GAF	
	r	*p*
Previous USP-SPS	–0.505	0.023
Previous PUTS	–0.327	0.186
Previous YGTSS	–0.602	0.005
Previous Y-BOCS	–0.373	0.127

USP-SPS, University of São Paulo Sensory Phenomena Scale; PUTS, Premonitory Urge for Tics Scale; YGTSS, Yale Global Tic Severity Scale; Y-BOCS, Yale-Brown Obsessive–Compulsive Scale; GAF, Global Assessment of Functioning; OCS, Obsessive–Compulsive symptoms.

TABLE 4 | Correlations between changes in sensory phenomena, tics, and OCS, and current global functioning.

	Current GAF	
	r	*p*
Change in USP-SPS	0.073	0.759
Change in PUTS	0.289	0.229
Change in YGTSS	0.458	0.042
Change in Y-BOCS	0.192	0.459

USP-SPS, University of São Paulo Sensory Phenomena Scale; PUTS, Premonitory Urge for Tics Scale; YGTSS, Yale Global Tic Severity Scale; Y-BOCS, Yale-Brown Obsessive–Compulsive Scale; GAF, Global Assessment of Functioning; OCS, Obsessive–Compulsive symptoms.

TABLE 5 | Correlations between changes in sensory phenomena, tics, and OCS, and change in global functioning.

	Change in GAF	
	r	*p*
Change in USP-SPS	–0.264	0.275
Change in PUTS	0.518	0.023
Change in YGTSS	0.383	0.105
Change in Y-BOCS	0.630	0.009

USP-SPS, University of São Paulo Sensory Phenomena Scale; PUTS, Premonitory Urge for Tics Scale; YGTSS, Yale Global Tic Severity Scale; Y-BOCS, Yale-Brown Obsessive–Compulsive Scale; GAF, Global Assessment of Functioning; OCS, Obsessive–Compulsive symptoms.

significantly correlated with OCS in the present. These findings were similar to our previous study (5). Broader sensory phenomena in the past were significantly correlated with OCS in the present. The influence of previous sensory phenomena on current OCS seems to be slightly stronger than that on current tics.

We found that previous tics were significantly negatively correlated with current global functioning and previous broader sensory phenomena were marginally correlated with current global functioning, based on Pearson's correlation coefficient. These results were mostly consistent with our expectations, particularly the relationship between previous tics and current global functioning. On the other hand, previous premonitory urges were not correlated with current global functioning. This seems to be different from a previous study that found that premonitory urges predicted QOL (7), although a similar relationship between premonitory urges and global functioning was expected. A possible reason for this difference is related to study participants. Specifically, in Cavanna et al.'s study, the participants were children or adolescents at the first assessment, and they had poor or insufficient recognition of premonitory urges, whereas all the participants in our study had obtained sufficient recognition of their premonitory urges.

We did find that change in tics was marginally related to current global functioning. Improvement of tics would alleviate tic-related impairment of life, and then improve global functioning. In a previous study, subjective satisfaction with tic control was positively correlated with life satisfaction and QOL (15). If tic control results in improvement of tics, increased life satisfaction might have a positive impact on global functioning. We found that change in premonitory urges was marginally related to change in global functioning, although the relationship between previous premonitory urges and current global functioning was not significant. The difference between the two findings suggests that when change in premonitory urges is large, it may influence global functioning. In addition, significant improvement of OCS over the clinical course might affect the relationship between changes in OCS and global functioning.

The current study has several limitations. First, all the participants had regularly visited a single specialty clinic for TS and related disorders for several years, which led to a small, potentially biased sample. Moreover, due to the small sample size, sufficient statistical power was not achieved in certain instances. In addition, we applied Bonferroni correction only for association analyses. Second, we could not examine influence by comorbid ADHD, depression, anxiety, and other symptoms, although previous studies have suggested it (16) and recommended comprehensive assessment (17). Third, the possible influence of change in medication during the clinical course on clinical characteristics and global functioning was not examined. Finally, the small sample size did not allow use of a multiple regression analysis, which would be necessary to draw firm conclusions.

Due to these limitations, it was not possible to conclude that sensory phenomena, tics, and OCS predict global functioning in adolescents and adults with TS; however, the findings suggested that further investigation of this relationship would be meaningful. During the clinical course, premonitory urges and broader sensory phenomena might have slightly different effect on global functioning. In the future, researchers might recruit a larger number of patients to further clarify these findings.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The Institutional Ethical Committee of the University of Tokyo Hospital approved this study. Written informed consent was obtained from all adult participants and the parents of participants aged 19 years old or younger.

AUTHOR CONTRIBUTIONS

YK was in charge of research design and writing the manuscript. MF was in charge of data collection and analysis. NK was in charge of data collection. NM was in charge of data analysis and reviewing the manuscript. MN and TK were involved in discussion about the research design and reviewed the manuscript.

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REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders. 4th Edn.* Washington DC: Author (2000). text revision.
2. Houghton DC, Capriotti MR, Conelea CA, Woods DW. Sensory phenomena in Tourette syndrome: Their role in symptom formation and treatment. *Curr Dev Disord Rep* (2014) 1:245–51. doi: 10.1007/s40474-014-0026-2
3. Crossley E, Cavanna AE. Sensory phenomena: Clinical correlates and impact on quality of life in adult patients with Tourette syndrome. *Psychiatry Res* (2013) 209:705–10. doi: 10.1016/j.psychres.2013.04.019
4. Eddy CM, Cavanna AE. Premonitory urges in adults with complicated and uncomplicated Tourette syndrome. *Behav Modif* (2014) 38:264–75. doi: 10.1177/0145445513504432
5. Kano Y, Matsuda N, Nonaka M, Fujio M, Kuwabara H, Kono T. Sensory phenomena related to tics, obsessive-compulsive symptoms, and global functioning in Tourette syndrome. *Compr Psychiatry* (2015) 62:141–6. doi: 10.1016/j.comppsych.2015.07.006
6. Kano Y, Matsuda N, Nonaka M, Fujio M, Kono T, Kaido T. Sensory phenomena and obsessive-compulsive symptoms in Tourette syndrome following deep brain stimulation: Two case reports. *J Clin Neurosci* (2018) 56:199–201. doi: 10.1016/j.jocn.2018.06.046
7. Cavanna AE, David K, Orth M, Robertson MM. Predictors during childhood of future health-related quality of life in adults with Gilles de la Tourette syndrome. *Eur J Paediatr Neurol* (2012) 16:605–12. doi: 10.1016/j.ejpn.2012.02.004
8. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders. 5th Edn.* Arlington, VA: American Psychiatric Association (2013).
9. Rosario MC, Prado HS, Borcato S, Diniz JB, Shavitt RG, Hounie AG, et al. Validation of the University of São Paulo Sensory Phenomena Scale: Initial psychometric properties. *CNS Spectr* (2009) 14:315–23. doi: 10.1017/S1092852900020319
10. Woods DW, Piacentini J, Himle MB, Chang S. Premonitory Urge for Tics Scale (PUTS): Initial psychometric results and examination of the premonitory urge phenomenon in youths with tic disorders. *J Dev Behav Pediatr* (2005) 26:397–403. doi: 10.1097/00004703-200512000-00001
11. Inoko K, Nishizono-Maher A, Tani S, Kano Y, Kishimoto J, Hayakawa N, et al. Reliability and validity of a Japanese version of the Yale Global Tic Severity Scale: A preliminary study. *Jpn J Child Adol Psychiatry* (2006) 47:38–48.
12. Leckman JF, Riddle MA, Hardin MT, Ort SII, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: Initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry* (1989) 28:566–73. doi: 10.1097/00004583-198907000-00015
13. Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, et al. The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. *Arch Gen Psychiatry* (1989) 46:1006–11. doi: 10.1001/archpsyc.1989.01810110048007
14. Nakajima T, Nakamura M, Taga C, Yamagami S, Kiriike N, Nagata T, et al. Reliability and validity of the Japanese version of the Yale-Brown Obsessive-Compulsive Scale. *Psychiatry Clin Neurosci* (1995) 49:121–6. doi: 10.1111/j.1440-1819.1995.tb01875.x
15. Matsuda N, Kono T, Nonaka M, Fujio M, Kano Y. Self-initiated coping with Tourette's syndrome: Effect of tic suppression on QOL. *Brain Dev* (2016) 38:233–41. doi: 10.1016/j.braindev.2015.08.006
16. Evans J, Seri S, Cavanna AE. The effects of Gilles de la Tourette syndrome and other chronic tic disorders on quality of life across the lifespan: A systematic review. *Eur Child Adolesc Psychiatry* (2016) 25:939–48. doi: 10.1007/s00787-016-0823-8
17. O'Hare D, Helmes E, Reece J, Eapen V, McBain K. The differential impact of Tourette's syndrome and comorbid diagnosis on the quality of life and functioning of diagnosed children and adolescents. *J Child Adolesc Psychiatr Nurs* (2016) 29:30–6. doi: 10.1111/jcap.12132

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Premonitory Awareness Facilitates Tic Suppression: Subscales of the Premonitory Urge for Tics Scale and a New Self-Report Questionnaire for Tic-Associated Sensations

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Awareness of premonitory urge in Tourette syndrome (TS) may facilitate tic suppression; however, previous studies have not supported this observation. We aimed to clarify the relationship between tic-associated sensation and tic suppression by identifying the subtypes of tic-associated sensations, including the Premonitory Urge for Tics Scale (PUTS). We developed a new questionnaire called "Rumination and Awareness Scale for tic-associated sensations" (RASTS) to assess the two additional aspects of tic-associated sensations: the intensity of somatosensory hyperawareness and the ability to identify signals of emerging tics. Sixty-two individuals with TS participated in the study (mean age = 19.2 ± 10.3 years). All participants completed the RASTS, PUTS, and Tic Suppression Scale. Of all participants, 41 were evaluated by the Yale Global Tic Severity Scale (YGTSS), while another group of 41 completed both the Leyton Obsessional Inventory-Child Version (LOI-CV) and the Tics Symptom Self-Report (TSSR). Factor analyses including nine items of the PUTS and the RASTS were conducted, and their relationships with patients' tic suppression ability were examined. The results support using RASTS for the two supposed dimensions (rumination about sensation and premonitory awareness) for assessing the two different tic-associated sensations, and PUTS for three dimensions for assessing the two types of quality of premonitory urges and intensity of premonitory urges. Premonitory awareness correlated with tic suppression ability. Conversely, rumination about sensation, PUTS total score, and the three subscales of PUTS correlated with obsessive-compulsive symptoms. In summary, being aware of signals for emerging tics facilitated self-initiated tic suppression,

while ruminative tic-associated sensations did not. This study provides new insights into behavioral therapy for tics by identifying two distinct aspects of tic-associated sensations that include premonitory urges.

Keywords: Tourette syndrome, tics, suppression, premonitory urge, behavioral therapy

INTRODUCTION

Following Bliss, a physician with Tourette syndrome (TS), describing his own experience of sensory phenomena as "unfulfilled sensations that precede, accompany, and follow tics" in 1992 (1), many studies have revealed that premonitory urges are common phenomena in individuals with TS (1–5). Recently, premonitory urges have been examined in relation to tic suppressibility. Premonitory urges are often assessed by using the Premonitory Urge for Tics Scale (PUTS) (3). Using PUTS, three studies showed that premonitory urges did not correlate with tic suppressibility. Ganos et al. did not find a correlation between tic suppression ability and PUTS in 15 adults with TS (6), neither did Müller-Vahl et al., in 22 adults with TS (7). Conelea et al. examined predictors of tic suppressibility in 99 youth with tic disorders and did not find a significant correlation between tic suppressibility and PUTS (8). These three studies assessed tic suppressibility by comparing the tic frequency in "free tic" (no suppression) *vs*. "tic suppression" (instructed to maximally suppress their tics) conditions. In addition, Banaschewski and colleagues assessed the tic suppression ability of 254 children and adolescents with TS, and asked whether they felt a pre-sensation immediately before tics. Only 37% of patients with TS reported premonitory urges; however, 64% reported that they could suppress tics (9). Moreover, Woods et al. found no positive correlation between the nine-item PUTS total score and the self-reported tic suppressibility (PUTS item 10) in youth with TS, when they developed the PUTS scale (3), while dropping item 10 to calculate the total PUTS score. These studies indicate that premonitory urges are not necessary prerequisites for tic suppression.

However, awareness of premonitory urges seems to facilitate tic control in behavioral therapy. Awareness training for the premonitory urge is an essential technique in Habit Reversal Training (HRT). HRT is a core component of the Comprehensive Behavioral Intervention for Tics (CBIT), and CBIT has been proved effective for reducing tic symptoms in a large randomized control study in children and adolescents (10), and adults (11). Indeed, 18% of the 132 participants with TS reported that awareness of the premonitory urge helped them suppress their tics (2). Himle et al. evaluated the real-time subjective premonitory urge strength using the "urge thermometer" and investigated its relationship with tic suppression in five children and adolescents with TS (12). One out of five participants could not suppress her tic symptoms in the reinforced tic suppression conditions compared to the baseline conditions, while a relatively stable and low premonitory urge was observed during both base-line and tic suppression conditions. The other four participants suppressed their tics in the tic suppression conditions and reported a higher premonitory urge compared to the female participant during both conditions. In addition, Raines et al. found modest positive correlations with the nine-item PUTS total scores and self-reported tic suppressibility (PUTS item 10) in youth with TS (13). These studies imply that awareness of premonitory urge may facilitate tic suppression.

Inconsistent results may rise from a large variety of tic-associated sensations. As noted above, many studies evaluated premonitory urges by using the PUTS (3, 6–8, 13). The PUTS is a brief self-reported scale with good internal consistency, temporal stability, and concurrent validity in children and adolescents (3, 13), and adults (14), and with a good convergent validity in adults (15). Prior studies conducted a factor analysis of PUTS and indicated a unidimensional structure (3, 14), two structures (13), or three structures (15). Brandt et al. conducted a factor analysis, which included the 10 PUTS scores and an average real-time subjective premonitory urge intensity score during 5-min experiments (15), and showed three factors: one appearing to measure the urge intensity; one sensory quality of urges; and one subjective control. This suggests that PUTS might assess more than one dimension and it may be worthwhile exploring its different subscales. Raines found similar structures for PUTS and suggested that one factor represents the specific urge qualities while another represents the general urge experiences (13). Brandt et al. emphasized the need to investigate the underlying dimensions of premonitory urges in future studies (15).

Therefore, we aimed to explore the dimensions of tic-associated sensations and examine their relationship with tic suppression. First, we conducted a factor analysis of PUTS and explored the dimensions of the premonitory urge. Inconsistent findings of dimensions of premonitory urge have made it difficult to comprehend the underlying dimensions of tic-associated sensations. Second, we developed a new questionnaire to assess the other two aspects of tic-associated sensations, which PUTS does not assess, and examined the relationship with the tic suppression ability. PUTS of nine items includes six items for describing the different sensory qualities of urge, such as itchiness and tense of energy (item 1–6), and two items for describing how frequent the urges come with their tics (item 7 and 8), as well as the patients' relief experienced following an executed tic (item 9) (3). Although PUTS is a commonly used scale for the tic-associated detail quality of sensory phenomena description, we also propose two additional important aspects of tic-associated sensations, including the somatosensory hyperawareness severity (rumination about sensation) and the ability to identify signals of emerging tics (premonitory awareness).

We focused not only on the pre-tic sensations but also on the ruminative tic-associated sensations, which may last following

the tics. Kane, a graduate student with TS, noted that the term, "pre-tic" sensation is a misnomer as the TS feeling is acute and omnipresent (16). Kane also described the tic-associated sensations as "enduring somatosensory bombardment" and "somatosensory hyper attention." As noted above, Bliss described his sensory phenomena as "unfulfilled sensations that precede, accompany, and follow tics" and did not focus only on the pre-tic sensation (1). However, following Leckman et al., using the term "premonitory urge" (2), many researchers have focused only on the pre-tic sensations (3–9) while underestimating the ruminative tic-associated sensations that may last even after the tics. The University of São Paulo Sensory Phenomena Scale (USP-SPS) evaluates sensory phenomena occurring before or during the performance of repetitive behaviors (17). Although USP-SPS includes broader sensory phenomena including the premonitory urge, the scale was first developed to assess the sensory phenomena in OCD and includes the sensory phenomena before compulsions (17). Therefore, we created a new scale to assess the somatosensory hyperawareness severity associated with tic symptoms, evaluated by how individuals with TS ruminate the tic-associated sensations (rumination about sensation). This ruminative sensation may be affected by obsessive-compulsive symptoms, as previous studies reported that obsessive-compulsive symptoms correlate with premonitory urges (3, 5, 18).

The second additional aspect of tic-associated sensation is the ability of signal identification for the emerging tics (premonitory awareness). Some individuals with TS have noted that they can tell when a tic will occur and suppress it before it emerges; however, they do not feel any specific sensation. Indeed, Banaschewske et al. showed that several participants could suppress tics without reporting the premonitory urge (9). During HRT, practitioners encouraged patients to identify signals for the emerging tics to initiate a competing response before the tic's emergence (11). We assumed this signal of emerging tics to be related to the tic suppression ability.

Therefore, we created a new scale to assess the two aspects of tic-associated sensation (premonitory awareness and rumination about sensation). We also examined each trait concerning the tic suppression ability. We hypothesized that premonitory awareness correlates significantly with the tic suppression. In contrast, we hypothesized that PUTS is not related to the tic suppression ability, as previously reported (6–8), but correlates with the obsessive-compulsive symptoms, consistent with other reports (3, 5, 18). We hypothesize that rumination about sensation correlates with PUTS and obsessive-compulsive symptoms, and does not correlate with the tic suppression ability.

METHODS

Participants

Participants were recruited from 2013 to 2016 in three steps (**Figure 1**). First, 59 patients with TS aged between 9 and 30 and their parents were recruited from the University of Tokyo Hospital from 2013 to 2014. Out of these, 26 patients with TS (44%) and the child participants' parents participated following a full explanation of the study. Second, under the approval of the Tourette syndrome Association of Japan (TSAJ), 154 questionnaires were mailed to TSAJ members; consequently, 32 questionnaires (21%) returned from individuals with tic disorders and their parents. Third, 45 participants with TS aged between 13 and 53 were recruited from the University of Tokyo Hospital, from 2015 to 2016. Of these participants, 22 patients with TS (49%) participated following a full explanation

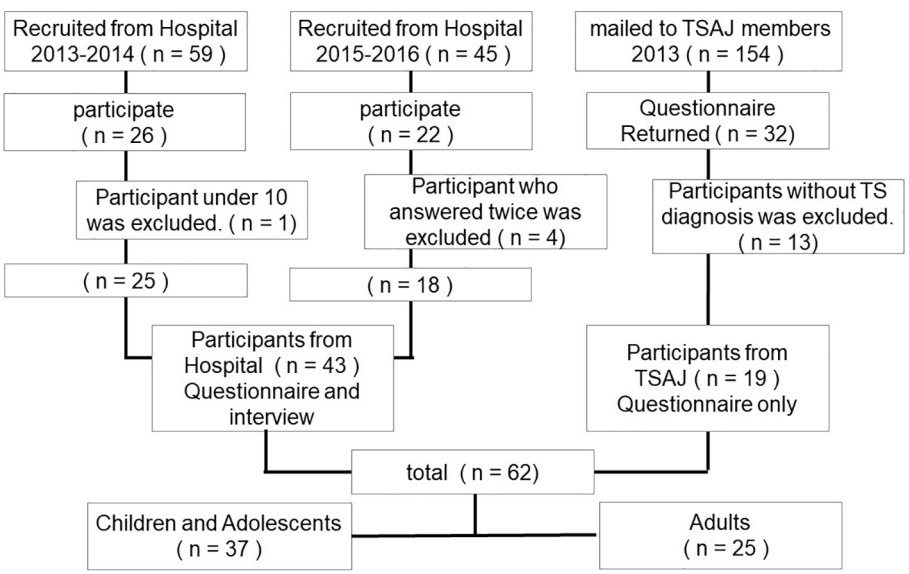

FIGURE 1 | Participants flow-chart.

of the study. Patients from the University of Tokyo Hospital had been diagnosed with TS according to the Diagnostic and Statistical Manual, Fourth Edition, Text Revision (DSM-IV-TR) criteria by a child psychiatrist (19). We excluded participants under the age of 10 years. For participants from TSAJ members, we excluded questionnaires that did not report a TS diagnosis. In addition, four clinic participants answered the questionnaire twice; consequently, we excluded the 2nd questionnaire. Taken together, a total of 62 questionnaires were analyzed (43 clinic participants and 19 non-clinic participants; 50 men, 12 women, mean age = 19.2 years, *SD* = 10.3 years, range = 10–53 years). For the TSAJ participants and 22 of the clinic participants, their parents also answered a questionnaire, including the TSSR and diagnostic information (n = 41). We also evaluated YGTSS (20) for 41 participants recruited from the hospital. Regarding the clinic participants, written informed consent was obtained from the adults and parents of participating children, and assent was obtained from the children. For non-clinic participants, a response to the questionnaire that included a full explanation of the study was recognized as informed consent. The institutional ethical committee of the University of Tokyo Hospital approved the study protocol.

Measures

The questionnaire consisted of RASTS, the Tic Suppression Scale, PUTS, and LOI-CV for participants with TS and TSSR, and diagnostic information for the parents of the participants with TS. YGTSS was evaluated for the participants from the hospital.

Rumination and Awareness Scale for Tic-Associated Sensations

This scale was designed to assess the two distinct aspects of the tic-associated sensation. We developed RASTS as follows: a set of questions were developed based on the clinical interviews with patients, consultation with experts, and a review of the relevant literature regarding the premonitory awareness and rumination about sensation. We referred to a negative rumination scale (21) in order to develop items evaluating somatosensory hyper-awareness severity. Participants answered 14 items (7 items for each; see **Supplementary Material**), and each item was scored from 0 to 5. To facilitate the participants in recalling their tic-associated sensations, they first completed the PUTS, in which detailed examples of description of tic-associated sensations were described.

The Tic Suppression Scale

We used a list of questions that addressed the three aspects of the tic suppression (see **Supplementary Material**) as follows: frequency (three items; Cronbach's $\alpha = 0.87$), suppression ability (six items; Cronbach's $\alpha = 0.92$), and subjective discomfort (three items; Cronbach's $\alpha = 0.87$). Participants responded using a 4-point Likert-type scale ranging from 0 to 3. These items were also used in our previous study (22), except for the tic suppression ability which was improved by adding four extra items that evaluated the distinct tic suppression ability aspects in this study. The validity and usefulness of the Japanese version of the Tic Suppression Scale was tested by demonstrating a negative correlation with a motor tic interference scale of the YGTSS and a positive correlation with overall satisfaction with tic control (23).

Premonitory Urge for Tics Scale

The PUTS is a nine-item self-reported scale that measures the sensations before the emergence of tics, and each item is scored from 1 to 4 (3). The total score (range: 9–36) is obtained by summing all of the nine items. The Japanese version of the scale was designed using rigorous methods, including translation and back translation, and with sufficient internal and concurrent validity (18).

The Yale Global Tic Symptoms Scale

The presence and severity of tics were evaluated in the recruited clinic participants using the Japanese version of the Yale Global Tic Symptoms Scale (YGTSS) (20). This version has previously been proven valid and reliable (24). On this scale, the motor and vocal tics were evaluated separately (0–25) on 5 ordinal scales, and a total tic symptom score was obtained by summing the individual scores (0–50). The current impairment due to tics was additionally assessed (0–50). The global severity score was determined (0–100) as the sum of the total tics score and the impairment score.

The Tic Symptom Self Report

The Tic Symptom Self Report (TSSR) is a 40-item self- or parent-rated scale measuring the tic severity, with items scored from 0 to 3. We adopted the parent-rated version (25). Participants rated the frequency and intensity of the list of symptoms (20 for motor tics and 20 for vocal tics); then, a summed tic severity score was calculated (0 to 120). The validity and usefulness of the Japanese version of the TSSR was tested by demonstrating a high correlation with the YGTSS (26). We used the TSSR to only compare the tic symptoms between the clinic patients and the TSAJ members.

The Leyton Obsessional Inventory Child Version

The Leyton Obsessional Inventory Child Version (LOI-CV) is a self-reported 20-item scale measuring the severity of obsessive-compulsive symptoms (27). In this inventory, participants report whether they experience any of the 20 symptoms that have been previously reported, as well as how these symptoms interfere with their daily activities. Both the validity and reliability of the Japanese version of the LOI-CV have been demonstrated (28).

Other characteristics, including the participants' age, sex, diagnoses of TS, attention deficit hyperactive disorder (ADHD), and obsessive-compulsive disorder (OCD) was obtained from the parents' reported questionnaire for the participants from TSAJ. For the participants recruited in the hospital, comorbid OCD and ADHD were diagnosed based on the DSM-IV-TR criteria, as in the case of TS (19).

Statistical Analysis

Statistical analyses were performed using the SPSS software version 25.0. Differences were considered statistically significant if $p < 0.05$. Exploratory factor analysis for the RASTS was conducted. In addition, we conducted an exploratory factor analysis to explore the PUTS dimensions. Spearman's rank correlation coefficients were calculated to examine the relationships between each clinical characteristic, as some scales were not normally distributed, including the premonitory awareness, rumination about sensation, tic suppression frequency, and subjective discomfort according to the Shapiro-Wilk's normality test (29). We applied Bonferroni corrections for multiple comparisons. To examine the relationship between the three scales of tic-associated sensation (PUTS, premonitory awareness, and rumination about sensation) and tic suppression ability, the significance level was divided by three ($p < 0.017$). To examine the relationship between the three tic-associated sensation scales and the obsessive-compulsive symptoms, the significance level was further divided by three ($p < 0.017$). Missing values were replaced by estimated values calculated by regression analysis using other items on the scale. We conducted a factor analysis of PUTS and Spearman's rank correlation by the age group (children and adolescents for age ≤17 and adult for age ≥18) to find the possible effect of age. To explore the effect of comorbid disorders, we conducted a correlation analysis of tic suppression and tic-associated sensations by the two groups (participants without comorbid disorders or participants with ADHD or OCD).

RESULTS

There was no significant difference in the tic severity (evaluated by the TSSR) between the clinical participants (mean = 13.4, *SD* = 9.3) and TSAJ members (mean = 18.8, *SD* = 12.1, t (39) = –1.64, p = 0.11). Therefore, we did not separate the clinical participants and TSAJ members in the following analysis. **Table 1** describes the participants' demographic and clinical data for the total group, the children and adolescent group, and the adult group. There were no significant differences in the PUTS, two RASTS scales, LOI-CV, total tic symptom score, and the global severity score of YGTSS between the children and adolescent participants and the adult participants, when using the t-test. Impairment scores of YGTSS and the ability to suppress tics were higher in the adult group compared to the children and adolescent group (t (39) = –2.4, p = 0.02, t (60) = –2.8, p = 0.007, respectively). Gender differences and comorbid disorders (ADHD and OCD) were compared between the two groups using the chi-square test, which indicated no significant differences (p = 0.24–0.51).

Factor Analysis for Rumination and Awareness Scale for Tic-Associated Sensations

An exploratory factor analysis (maximum likelihood method: MLM) with an oblique rotation method was conducted to determine the factor structure of the 14 items of RASTS. Consideration of the criteria of the absolute value of the eigenvalues, visual screening test, and theoretical interpretability of the factors suggested a two-factor solution. One primary factor accounted for 34.4% of the variance in the data and another factor accounted for an additional 22.3% of the variance. Therefore, we conducted an MLM factor analysis in which we specified a two-factor solution. Items were removed according to the following criteria: a) items should exceed 0.4 on the corresponding factor and b) items should not exceed 0.25 on another factor. Consequently, three items were removed from the RASTS as they did not meet these criteria (items 8, 9, and 11). Another

TABLE 1 | Demographic and clinical data for the participants.

	Total			Children and adolescents			Adults		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Age	19.2	(10.3)	10–53	12.9	(2.4)	10–17	28.5	(10.5)	18–53
PUTS	21.9	(6.9)	9–35	21.2	(6.9)	9–34	22.9	(6.9)	10–35
Rumination and Awareness Scale for tic-related sensation									
Rumination about sensation	2.3	(1.6)	0–5	2.3	(1.6)	0–5	2.2	(1.6)	0–5
Premonitory awareness	3.6	(1.3)	0.6–5	3.4	(1.4)	0.6–5	3.8	(1.3)	1–5
Tic Suppression Scale									
Frequency to suppress	1.6	(0.9)	0–3	1.4	(0.9)	0–3	1.9	(0.8)	0.3–3
Ability to suppress	1.5	(0.8)	0–3	1.3	(0.8)	0–2.8	1.8	(0.7)	0.4–3
Discomfort from suppression	1.9	(0.9)	0–3	1.8	(0.9)	0–3	1.9	(0.9)	0–3
YGTSS									
Tic symptoms	21.1	(7.7)	5–37	19.4	(7.2)	5–30	23.4	(7.8)	11–37
Impairments	19.0	(10.7)	0–40	15.7	(9.0)	0–30	23.3	(11.4)	0–40
LOI-CV	22.7	(13.5)	2–53	22.2	(13.8)	4–53	24.1	(13.1)	2–43
TSSR	15.9	(10.9)	0–40	16.2	(11.0)	0–40	14.9	(11.2)	2–30
Gender distribution (male/female)	50/12			31/6			19/6		
Comorbidity									
OCD	15	24%		7	19%		8	32%	
ADHD	17	27%		9	24%		8	32%	

n = 62 for total (n = 37 for the children and adolescents, n = 25 for adults), n = 41 for YGTSS, LOI-CV, TSSR.

MLM exploratory factor analysis with oblique rotation was conducted with the remaining 11 items, where 2 factors accounted for 39.2 and 25.6% of the variance, respectively, and 64.8% of the variance cumulatively. The first factor with six items was labeled "rumination about sensation" and the second factor with five items was labeled "premonitory awareness" (**Table 2**). Both measures indicated adequate internal consistency, with Cronbach's α = 0.91 for the rumination about sensation (α = 0.91 both in the young and adult sample) and Cronbach's α = 0.79 for premonitory awareness (α = 0.80 in the young sample and α = 0.79 in the adult sample). The correlation between the two factors was measured at −0.03 (p = 0.83).

Factor Analysis for Premonitory Urge for Tics Scale

Cronbach's α across nine items of the PUTS were acceptable in the total sample (α = 0.85), young sample (α = 0.85), and adult sample (α = 0.87). An exploratory factor analysis (maximum likelihood method) with an oblique rotation method was conducted to determine the factor structure of the nine items of PUTS, indicating three factors. One factor accounted for 36.4% of the variance, two factors accounted for 56.4% of the variance, and three factors accounted for 65.2% of the variance. For total participants, items 1, 2, and 3 loaded on the first factor, items 1, 7, and 8 loaded on the second factor, and items 4, 5, and 9 loaded on the third factor. The same exploratory factor analysis was conducted for the young participant group and the adult participant group, resulting in a similar result (**Table 3**). For the young sample, items 1, 2, and 3 loaded on the first factor, items 1, 7, and 8 loaded on the second factor, and items 4, 5, and 9 loaded on the third factor. For the adult group, items 1, 2, and 3 loaded on the first factor, items 7 and 8 loaded on the second factor, and items 1, 4, 5, and 9 loaded on the third factor.

Correlation Between Premonitory Urge and Rumination and Awareness Scale for Tic-Associated Sensations

The rumination about sensation and the three subscales of the PUTS score and total PUTS score significantly correlated with each other, while there were no significant correlations between

TABLE 2 | Factor analysis of Rumination and Awareness Scale for tic-associated sensations (RASTS).

Item		Mean (SD)		Factor 1	Factor 2
Rumination about sensation					
12. Sometimes, I just keep feeling bothered by the sensation for over 30 min without a break.		1.69	(1.98)	0.88	−0.08
14. Often, I just can't stop focusing on a sensation.		2.06	(2.00)	0.87	0.05
10. Sometimes, I feel bothered by a sensation all day long.		1.50	(1.85)	0.82	−0.06
4. Once the sensation has emerged, I will just keep being bothered by it.		2.71	(1.95)	0.75	−0.01
6. Sometimes, I focus on the sensation for dozens of minutes.		2.40	(1.91)	0.75	−0.02
1. After the sensation emerges, I'm likely to be bothered by it repeatedly, again and again.		3.18	(1.60)	0.67	0.10
	Mean	2.26	(1.57)	Range: 0–5	
Premonitory awareness					
13. I notice tics before they appear, tics do not happen automatically.		3.52	(1.85)	0.08	0.94
5. Before I experience a tic, I know what kind of tic it will be.		3.74	(1.74)	0.11	0.67
2. When the tic is about to appear, I can often notice it.		4.03	(1.41)	0.14	0.64
7*. I often experience a tic without noticing it.		3.39	(1.99)	−0.21	0.61
3*. In some cases, I don't notice that the tic has appeared.		3.27	(1.92)	−0.21	0.49
	Mean	3.59	(1.33)	Range: 0.6–5	

The refined 11-item Rumination and Awareness Scale for tic-associated sensations (RASTS) is presented here, with three items were removed; n = 48; factors were extracted using maximum likelihood method (MLM) analysis with Oblique rotation. Items are presented with their loadings onto their assigned factor.
**Items for reverse scoring.*

TABLE 3 | Factor analysis of Premonitory Urge for Tics Scale (PUTS).

	Total			Children and adolescent group			Adult group		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
Item 1 Right before I do a tic, I feel like my insides are itchy.	**0.48**	**0.40**	−0.03	**0.44**	**0.54**	−0.12	**0.43**	−0.02	**0.40**
Item 2 Right before I do a tic, I feel pressure inside my brain or body.	**0.79**	0.08	−0.11	**0.74**	0.18	−0.07	**0.79**	−0.09	−0.05
Item 3 Right before I do a tic, I feel "wound up" or tense inside.	**1.03**	−0.18	0.03	**1.07**	−0.20	0.07	**1.08**	0.06	−0.22
Item4 Right before I do a tic, I feel like something is not "just right."	−0.02	0.05	**0.82**	−0.07	0.11	**0.77**	0.09	−0.05	**0.89**
Item 5 Right before I do a tic, I feel like something isn't complete.	0.03	0.09	**0.92**	0.06	0.09	**0.93**	0.08	0.19	**0.78**
Item 6 Right before I do a tic, I feel like there is energy in my body that needs to get out.	0.16	0.29	0.15	0.18	0.30	0.16	0.07	0.23	0.30
Item 7 I have these feelings almost all the time before I do a tic.	0.03	**0.86**	0.06	−0.02	**1.00**	0.02	0.01	**1.04**	−0.07
Item 8 These feelings happen for every tic I have.	−0.10	**0.96**	−0.10	−0.08	**0.76**	0.04	−0.08	**0.76**	0.12
Item 9 After I do the tic, the itchiness, energy, pressure, tense feelings, or feelings that something isn't "just right" or complete go away, at least for a little while.	−0.08	−0.11	**0.55**	−0.01	−0.10	**0.61**	−0.27	−0.01	**0.52**

Total, n = 62; for children and adolescents, n = 37; for adult, n = 25. The largest loading factors are written in bold font.

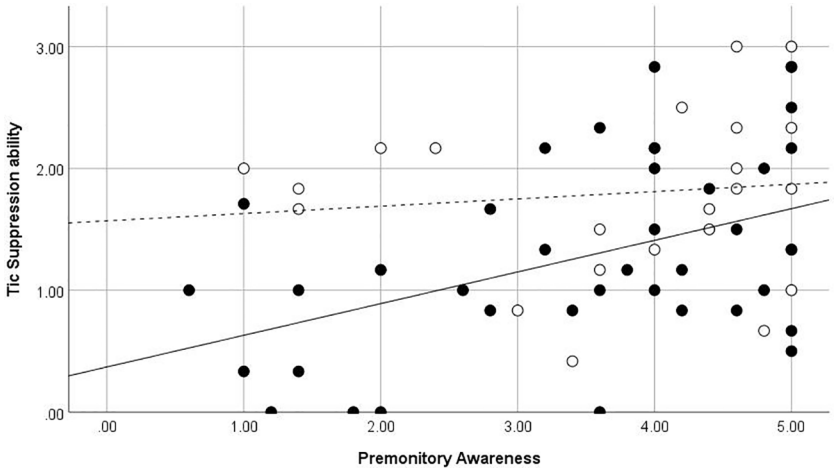

FIGURE 2 | Premonitory Awareness and Tic Suppression ability. ●, Children and adolescents, straight line (—) is for regression line in children and adolescent, dot line (- - -) is for regression line in adult participants ○, Adults. Regression line in adult participants.

the premonitory awareness and other tic-associated sensation scales (rumination about sensation, three subscales of PUTS score, or PUTS total scores) (**Table 4**). The rumination about sensation and PUTS scores were significantly correlated with the LOI-CV score (ρ = .50, p <.001; ρ = .52, p <.001, respectively), while the premonitory awareness score did not correlate with the LOI-CV score (ρ = .11, p = .50). In addition, the rumination about sensation and PUTS scores were significantly correlated with YGTSS total tic symptom score (ρ = .35, p = .02; ρ = .32, p = .04, respectively), while the premonitory awareness score was not correlated with the YGTSS total tic symptom score (ρ = −.21, p = .18). These correlation analyses were conducted in the adult and the young group separately, and they exhibited similar correlation patterns.

Correlation Between Tic Suppression and Premonitory Urge and Rumination and Awareness Scale for Tic-Associated Sensations

The premonitory awareness score was significantly correlated with the tic suppression ability (ρ = .34, p = 0.008). The rumination about sensation and PUTS scores did not correlate with the tic suppression ability (ρ = −.25, p = .052; ρ = −.22, p = .09, respectively), consistent with our hypothesis (**Table 5**). In the young group, the premonitory awareness score was significantly correlated with the tic suppression ability (ρ = .39, p = .019). However, the premonitory awareness score was not significantly correlated with the tic suppression ability in the adult group (ρ = .19, p = .38). **Figure 2** shows the scatter plot of the relationship between the premonitory awareness and rumination about sensation and the tic suppression ability for the adult and young groups. In the groups without OCD or ADHD (n = 37), rumination about sensation and PUTS scores significantly correlated negatively with the tic suppression ability (ρ = −.41, p = .012; ρ = −.40, p = .015, respectively), and the premonitory awareness correlated with tic suppression ability (ρ = .36, p = .028) although p score was slightly higher than 0.017 (modified significant level). In the group with ADHD or OCD diagnosis (n = 25), the PUTS, premonitory awareness, and rumination about sensation did not correlate with the tic suppression ability (p = .17~.99).

TABLE 4 | Spearman's Correlation between Premonitory Urge for Tics Scale (PUTS) and Rumination and Awareness Scale for tic-associated sensations (RASTS).

	Rumination about sensation	PUTS Total	PUTS Physical	PUTS Intensity	PUTS Just right	LOI-CV	Age	YGTSS Symptoms	YGTSS Total severity
Premonitory awareness	−.03	.00	−.09	.02	.10	.11	.28*	−.21	−.19
Rumination about sensation		.72***	.49***	.64***	.59***	.50***	−.11	.35*	.29
PUTS total			.73***	.84***	.82***	.52***	.08	.32*	.38*
PUTS physical				.48***	.33**	.48**	.07	.17	.25
PUTS intensity					.62***	.37*	.03	.25	.34*
PUTS just right						.41**	.14	.45**	.41**
LOI-CV							.17	.28	.18
Age								.10	.21

For LOI-CV and YGTSS, n = 41; for items expect for LOI-CV and YGTSS, n = 62.
PUTS, Premonitory Urge for Tics Scale; LOI-CV, Leyton Obsessional Inventory Child Version; RASTS, Rumination and Awareness Scale for tic-associated sensations.
**p < 0.05, **p < 0.01, ***p < 0.001.*

TABLE 5 | Spearman's correlation between tic-associated sensations and tic suppressibility.

		Tic suppression scale			
		Frequency	**Suppression ability**	**Subjective discomfort**	**Satisfaction with tic control**
Total	Premonitory awareness	.12	.34**	−.21	.04
	Rumination about sensation	.33**	−.25	.56***	−.38**
	PUTS total	.30*	−.22	.61***	−.36**
Children and adolescents	Premonitory awareness	.06	.39*	−.25	.09
	Rumination about sensation	.25	−.31	.54***	−.53***
	PUTS total	.28	−.30	.55***	−.45**
Adult	Premonitory awareness	.17	.19	−.23	.00
	Rumination about sensation	.40	−.09	.63***	−.14
	PUTS total	.27	−.12	.66***	−.23

Total, n = 62; for children and adolescent group, n = 37; for adult group, n = 25.
*PUTS, Premonitory Urge for Tics Scale, * p < 0.05, **p < 0.01, ***p < 0.001.*

DISCUSSION

Premonitory Urge in Japanese Participants

Premonitory urge in Tourette syndrome has not been fully investigated in Asia. The PUTS score of our result was 21.9 out of 36 (21.2 for children and adolescents and 22.9 for adults). This score is similar to the initial report (18.5 for children and adolescents) (3) and the following reports [19.6 for children and adolescents, (13), 21.5 for adults, (14)], which are reported in the USA. Moreover, this score is similar to a previous Japanese study (18.6 for children and adolescents and adults) (18). Consistent with previous reports (3, 14, 18), our study showed a moderately positive correlation between the PUTS scores and the YGTSS total tic symptom score and obsessive-compulsive symptoms. These results indicate that Japanese individuals with TS feel similar premonitory urges compared to individuals with TS in Western cultures.

Dimensions of Tic-Associated Sensations

Factor analysis of the 9 item PUTS revealed three factors, which is consistent with a previous study that conducted a factor analysis of the 10 item PUTS. Items loading on the first factor (items 1, 2, and 3) assess whether participants with TS feel "itchy," "pressure," or "tense" before a tic, potentially reflecting the physical quality of the premonitory sensations. Items loading on the second factor (items 7 and 8) assess the extent to which the participants had these "feelings almost all the time" before tics and "for every tic," which are related to the overall intensity of the premonitory urges. Item 1 also loaded on the first factor in the total group and the young group. Items loading on the third factor (items 4, 5, and 9) assess whether participants feel "not just right" or "something isn't complete" before a tic, or how much relief of sensation comes after the tic occurs. Our results showed that the first and second factors reflect the quality of the premonitory urge and the intensity of premonitory urges, respectively, similar to the previous factor analysis of PUTS (13, 15). In contrast to a previous study (15), our third factor also includes the just right sensation (items 4 and 5) in addition to the relief from the premonitory urge following the tic (item 9). This result may partly come from a different number of items that were used for the factor analysis. We used nine items of PUTS, consistent with the original version. Brandt et al. used all 10 items of PUTS and their original real-time urge intensity score (15) whereas Raines et al. used 8 items of PUTS while excluding item 9 (13).

Correlation analysis between PUTS and our newly created tic-associated sensations scale suggested that the three PUTS subscales and ruminative tendency toward tic-associated sensations correlated with each other; however, pre-tic awareness was not related to any other tic-associated sensations. Upon conducting an exploratory analysis, two items including the "sensation", were excluded from the premonitory awareness factor (item 9, "I experience more tics with a prior sensation than I do tics without warning," and item 11, "Often, I feel some kind of sensation before the tic appears."), and items not including the sensations but referring their awareness before the tics were retained. It indicates that individuals with TS do not have to feel any concrete sensations before tics to tell when their tic will occur.

Relation Between Tic-Associated Sensations and Tic Suppression Ability

Consistent with previous studies (6–8), PUTS scores were not correlated with tic suppression ability. PUTS scores were significantly correlated with obsessive-compulsive symptoms, consistent with previous reports (3, 5, 18). Rumination about tic-associated sensation was not correlated with the tic suppression ability but was significantly correlated with obsessive-compulsive symptoms. In the group of participants without ADHD nor OCD, PUTS and rumination about sensation was negatively correlated with the tic suppression ability. Therefore, we concluded that aversive tic-associated somatosensory experiences do not facilitate tic suppression or even make the tic suppression more difficult. In contrast, the ability to tell when tic will occur before participants experience tics was correlated positively with the tic suppression ability, and this awareness may help tic suppression.

When the analysis was conducted by the age group, the correlation between the premonitory awareness and tic suppression was significant only in the group with the TS children. An additional different relationship between the urges and tic suppression was observed in the different habituation of the premonitory urge in CBIT practice between the children and adults with TS (30). Houghton et al. examined the habituation of premonitory urge for the CBIT responders and the CBIT non-responders with chronic tic disorders and revealed that adult CBIT

responders exhibited some degree of premonitory urge severity reductions; however, child CBIT responders failed to show any significant reduction in the premonitory urges (30). The authors partially explain these differences by hypothesizing on the development and maintenance of the premonitory urges. First, children with tics may fail to recognize the urges or experience them as non-aversive; however, as tics continue to occur and increase in severity, they result in aversive consequences i.e., their urges being associated with tics acquires aversive valence (3, 30, 31). We created a premonitory awareness scale to evaluate the more neutral aspects of tic-associated sensation. Indeed, premonitory awareness did not correlate with the tic severity and obsessive-compulsive symptoms, and the subjective discomfort from tic suppression. However, it may be possible that premonitory awareness gains aversive valence as participants get older, affecting the tic-suppression ability in older participants.

Limitation and Future Directions

Some limitations should be noted. First, the participants were recruited through only one specialty clinic for TS and related disorders or members of TSAJ, which may have led to biased and small sample size. Further, we included both pediatric and adult patients from the clinic. Although we divided all participants by age group and conducted the analyses by age group, the number of participants in each group was small. As Banaschewski et al. demonstrated, awareness of premonitory sensory phenomena develops with advancing age (9). Further studies with an increased number of participants for both children and adolescents are required to clarify the effect of developmental steps on the relationship between the tic-associated sensations and tic suppression. Second, we evaluated the tic suppression ability and premonitory awareness by using self-reported measures. We asked the participants to report RASTS and fill in the tic suppression questionnaire only once. Further studies are required to ensure test-retest reliability and concurrent validity to use the experimental evaluation of both the tic suppression ability and the ability to realize signals of emerging tics. Third, we recruited adult participants with TS mainly from the hospital, and this may lead to a possible confounding factor of the effect of age on the relationship between the tic-associated sensation and tic suppression. A higher percentage of ADHD in adults compared to children and adolescents may be caused due to this recruitment method. Fourth, we used the LOI-CV to evaluate the obsessive-compulsive symptoms. Although the relationship between the obsessive-compulsive symptoms and PUTS scores were consistent with those reported in previous studies, the results concerning obsessive-compulsive symptoms should be interpreted with caution. Further studies using Y-BOCS (32) to evaluate obsessive-compulsive symptoms are required.

In conclusion, to the best of our knowledge, this is the first study to examine the relationship between the tic suppression ability and the premonitory awareness and rumination about sensation. Premonitory awareness was not correlated with any other tic-associated sensations; conversely, PUTS and rumination about sensation correlated with each other. Our results indicate that the severity and frequency of tic-associated sensations (evaluated by PUTS and rumination about sensation) do not facilitate the tic suppression. In contrast, the awareness of signals for emerging tics facilitates the self-initiated tic suppression. This study provides additional insights into the mechanisms underlying the effectiveness of behavior therapy by clarifying two distinct aspects of tic-related somatosensory sensation that involve premonitory urges; repeated somatosensory phenomenon that is bothersome to individuals with TS and signals for emerging tics that may facilitate tic suppression in HRT. Future studies are required to examine these two distinct aspects of tic-associated sensations in the context of behavioral therapy.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The institutional ethical committee of the University of Tokyo Hospital approved the study protocol. Written informed consent was obtained from the adults and parents of participating children, and assent was obtained from the children for clinic participants. For non-clinic participants, a response to the questionnaire that included a full explanation of the study was recognized as informed consent.

AUTHOR CONTRIBUTIONS

NM: conceptualization/design, data acquisition, analysis, data interpretation, draft the manuscript, MaiN, MF, and YK: data acquisition, critical revision of the manuscript, TK and MarN: critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00592/full#supplementary-material

REFERENCES

1. Bliss J, Cohen DJ, Freedman DX. Sensory experiences of Gilles-de-la-Tourette Syndrome. *Arch Gen Psychiatry* (1993) 37:1343–7. doi: 10.1001/archpsyc.1980.01780250029002
2. Leckman JF, Walker DE, Cohen DJ. Premonitory urges in Tourettes Syndrome. *Am J Psychiatry* (1993) 150:98–102. doi: 10.1176/ajp.150.1.98
3. Woods DW, Piacentini J, Himle MB, Chang S. Premonitory urge for tics scale (PUTS): Initial psychometric results and examination of the premonitory urge phenomenon in youths with tic disorders. *J Dev Behav Pediatr* (2005) 26:397–403. doi: 10.1097/00004703-200512000-00001
4. Kwak C, Dat Vuong K, Jankovic J. Premonitory sensory phenomenon in Tourette's syndrome. *Mov Disord* (2003) 18:1530–3. doi: 10.1002/mds.10618.
5. Crossley E, Cavanna AE. Sensory phenomena: clinical correlates and impact on quality of life in adult patients with Tourette syndrome. *Psychiatry Res* (2013) 209:705–10. doi: 10.1016/j.psychres.2013.04.019.
6. Ganos C, Kahl U, Schunke O, Kuhn S, Haggard P, Gerloff C, et al. Are premonitory urges a prerequisite of tic inhibition in Gilles de la Tourette syndrome? *J Neurol Neurosurg Psychiatry* (2012) 83:975–8. doi: 10.1136/jnnp-2012-303033.
7. Muller-Vahl KR, Riemann L, Bokemeyer S. Tourette patients' misbelief of a tic rebound is due to overall difficulties in reliable tic rating. *J Psychosom. Res* (2014) 76:472–6. doi: 10.1016/j.jpsychores.2014.03.003
8. Conelea CA, Wellen B, Woods DW, Greene DJ, Black KJ, Specht M, et al. Patterns and predictors of tic suppressibility in youth with tic disorders. *Front Psychiatry* (2018) 9:188. doi: 10.3389/fpsyt.2018.00188.
9. Banaschewski T, Woerner W, Rothenberger A. Premonitory sensory phenomena and suppressibility of tics in Tourette syndrome: developmental aspects in children and adolescents. *Dev Med Child Neurol* (2003) 45:700–3. doi: 10.1017/s0012162203001294.
10. Piacentini J, Woods DW, Scahill L, Wilhelm S, Peterson AL, Chang S, et al. Behavior therapy for children with tourette disorder a randomized controlled trial. *JAMA* (2010) 303:1929–37. doi: 10.1001/jama.2010.607
11. Wilhelm S, Peterson AL, Piacentini J, Woods DW, Deckersbach T, Sukhodolsky DG, et al. Randomized trial of behavior therapy for adults with tourette syndrome. *Arch Gen Psychiatry* (2012) 69:795–803. doi: 10.1001/archgenpsychiatry.2011.1528.
12. Himle MB, Woods DW, Conelea CA, Bauer CC, Rice KA. Investigating the effects of tic suppression on premonitory urge ratings in children and adolescents with Tourette's syndrome. *Behav Res Ther* (2007) 45:2964–76. doi: 10.1016/j.brat.2007.08.007
13. Raines JM, Edwards KR, Sherman MF, Higginson CI, Winnick JB, Navin K, et al. Premonitory Urge for Tics Scale (PUTS): replication and extension of psychometric properties in youth with chronic tic disorders (CTDs). *J Neural Transm* (2018) 125:727–34. doi: 10.1007/s00702-017-1818-4.
14. Reese HE, Scahill L, Peterson AL, Crowe K, Woods DW, Piacentini J, et al. The premonitory urge to tic: measurement, characteristics, and correlates in older adolescents and adults. *Behav Ther* (2014) 45:177–86. doi: 10.1016/j.beth.2013.09.002.
15. Brandt VC, Beck C, Sajin V, Anders S, Munchau A. Convergent Validity of the PUTS. *Front Psychiatry* (2016) 7:51. doi: 10.3389/fpsyt.2016.00051.
16. Kane MJ. Premonitory urges as attentional tics in Tourettes-Syndrome. *J Am Acad Child Psy* (1994) 33:805–8. doi: 10.1097/00004583-199407000-00005.
17. Rosario MC, Prado HS, Borcato S, Diniz JB, Shavitt RG, Hounie AG, et al. Validation of the University of São Paulo Sensory Phenomena Scale: initial psychometric properties. *CNS Spectr* (2009) 14:315–23. doi: 10.1017/s1092852900020319.
18. Kano Y, Matsuda N, Nonaka M, Fujio M, Kuwabara H, Kono T. Sensory phenomena related to tics, obsessive-compulsive symptoms, and global functioning in Tourette syndrome. *Compr Psychiatry* (2015) 62:141–6. doi: 10.1016/j.comppsych.2015.07.006.
19. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders. 4th ed*. Washington, DC: American Psychiatric Publishing (2000).
20. Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Psy* (1989) 28:566–73. doi: 10.1097/00004583-198907000-00015.
21. Ito T, Agari I. Development of the negative rumination scale and its relationship with depression. *Japanese J Couns Sci* (2001) 34:31–42. (in Japanese).
22. Matsuda N, Kono T, Nonaka M, Fujio M, Kano Y. Self-initiated coping with Tourette's syndrome: Effect of tic suppression on QOL. *Brain Dev* (2016) 38:233–41. doi: 10.1016/j.braindev.2015.08.006
23. Matsuda N, Nonaka M, Kono T, Fujio M, Kano Y. Tic symptoms and tic suppressiblity in tourette's syndrome. *58th Japanese Soc Child Adolesc Psychiatry* (2017) 262. (in Japanese).
24. Inoko K, Nishizono-Maher A, Tani S, Kano Y, Kishimoto J, Hayakawa N, et al. Reliability and validity of a Japanese version of the Yale Global Tic Severity Scale: A preliminary study. *Jpn J Child Adolesc Psychiatr* (2006) 47 (Supplement):38–48.
25. Scahill L, Leckman JF, Schultz RT, Katsovich L, Peterson BS. A placebo-controlled trial of risperidone in Tourette syndrome. *Neurology* (2003) 60:1130–5. doi: 10.1212/01.wnl.0000055434.39968.67.
26. Nonaka M, Matsuda N, Kono T, Fujio M, Kano Y. Utilization of Tic Symptom Self Report; Testing validity and utilization for cognitive behavior therapy. *54th Japanese Soc Child Adolesc Psychiatry* (2013) 428. (in Japanese).
27. Berg CZ, Whitaker A, Davies M, Flament MF, Rapoport JL. The survey form of the Leyton Obsessional Inventory-Child Version: norms from an epidemiological study. *J Am Acad Child Psy* (1988) 27:759–63. doi: 10.1097/00004583-198811000-00017
28. Hayashi Y, Yoshihashi Y, Okada R, Tani I, Onishi M, Nakajima S, et al. Investigation on obsessive-compulsive tendencies of japanese children and adolescents: Using a Japanese version of the Leyton Obsessional Inventory-Child Version (LOI-CV-J). *Japanese J Child Adolesc Psychiatry* (2012) 53(1):1–10.
29. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* (1965) 52:591–611. doi: 10.2307/2333709
30. Houghton DC, Capriotti MR, Scahill LD, Wilhelm S, Peterson AL, Walkup JT, et al. Investigating habituation to premonitory urges in behavior therapy for tic disorders. *Behav Ther* (2017) 48:834–46. doi: 10.1016/j.beth.2017.08.004
31. Capriotti MR, Espil FM, Conelea CA, Woods DW. Environmental factors as potential determinants of premonitory urge severity in youth with Tourette syndrome. *J Obsess-Compuls Rel* (2013) 2:37–42. doi: 10.1016/j.jocrd.2012.10.004
32. Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, et al. The Yale-Brown Obsessive Compulsive Scale. 1. Development, Use, and realiability. *Arch Gen Psychiatry* (1989) 46:1006–11. doi: 10.1001/archpsyc.1989.01810110048007

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Current Status, Diagnosis, and Treatment Recommendation for Tic Disorders in China

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Tic disorders (TD) are a group neuropsychiatric disorders with childhood onset characterized by tics, i.e. repetitive, sudden, and involuntary movements or vocalizations; and Tourette syndrome (TS) is the most severe form of TD. Their clinical manifestations are diverse; and are often associated with various psychopathological and/or behavioral comorbidities, including attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), anxiety, depression, and sleep disorders. Individual severity and response to treatment are highly variable, and there are some refractory cases, which are less responsive to conventional TD treatment. TD/TS are also common in the Chinese pediatric population. To help improve the understanding of TD for pediatricians and other health professionals, and to improve its diagnosis and treatment in China, the Chinese Child Neurology Society (CCNS) has developed an *Expert Consensus on Diagnosis and Treatment of TD in China*, which is based on our clinical experience and the availability therapeutic avenues. It is focused on clinical diagnosis and evaluation of TD and its comorbidities, psychological and educational intervention, nonpharmacological therapy, pharmacological treatment, including traditional Chinese medicine and acupuncture, as well as prognosis in children with TD in China. A summary of the current status of TD and up-to-date diagnosis and treatment recommendations for TD in China is presented here.

Keywords: tic disorders, Tourette syndrome, diagnosis, pharmacological treatment, behavioral therapy, comorbidity, expert consensus, Chinese population

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INTRODUCTION

Tic disorders (TD) are a group of common neuropsychiatric disorders with onset in childhood and adolescence, characterized by sudden, rapid, recurrent, nonrhythmic movements or vocalizations, including some simple forms, such as eye blinking, facial grimacing, and throat clearing; and some complex forms, such as body twisting, coprolalia (uttering socially inappropriate words, such as swearing) or echolalia (repeating the words or phrases of others) (1–3). TD is often associated with various psychopathological and/or behavioral comorbidities, including attention deficit hyperactivity disorder (ADHD), obsessive-compulsive behavior (OCB) or disorder (OCD), anxiety, depression, and problems with impulse control and sleep (1, 3–8).

TD was first described by a French physician Dr. Jean-Marc Gaspard Itard in 1825 (9) to report patients with involuntary motor tics with echolalia and coprolalia, and then named as Gilles de la Tourette syndrome (often shortened as Tourette syndrome, TS) by Dr. Jean-Martin Charcot in 1885 after his resident Dr. Georges Gilles de la Tourette (10). However, subsequent clinical experience has found that there are more patients with involuntary tics without echolalia and coprolalia, thus only TD. Notably, TS only represents the most severe form of TD in the current DSM-5 diagnostic criteria (2); but it is still often used to describe TD in the Western clinical practice and literature (3). Interestingly, some experts in the field recently suggested that a unified diagnosis of tic spectrum disorder (TSD), similar to autism spectrum disorder (ASD) in DSM-5, would be more appropriate and practical to diagnose individuals with a primary TD; while also reducing stigmatization by discarding the 'Tourette Syndrome' title" (11).

TS diagnosis was introduced to China in the early 1980s after several Chinese pediatric neurologists and psychiatrists first went abroad as visiting scholars and learned about the Western diagnosis and treatment of TD (12). In the Chinese literature and clinical practice, "tic-coprolalia syndrome" was initially used as an equivalent for TS, but "tic disorder" has been gradually used to replace "tic-coprolalia syndrome", to include the majority of tics without coprolalia, as well as avoiding stigmatization of children suffering from "tic-coprolalia syndrome". In Chinese culture, the manifestation of tics is considered as embarrassing, and coprolalia is socially unacceptable. However, this situation has ameliorated in recent years through the campaign of public education, such as public educational programs and social media spread of medical knowledge and scientific facts about TD (12). For example, August 3 has been set as the TD Day in China since 2012 to raise the public awareness of TD.

Epidemiological studies have shown that TD is equally common across all races, ethnicities, and populations, with transient and mild tics affecting as many as 20% of school-age children (13, 14); while chronic TD and TS affect 0.3–5.0% and 0.3–1.0% of school-age children, respectively in various Western populations (3, 6, 8, 13–16). TD is likewise a quite common neuropsychiatric disorder in the Chinese pediatric population as well (12). A meta-analysis of 13 epidemiological studies between 1992–2010 in China showed that the prevalence of combined TD, provisional TD, chronic TD, and TS was 6.1% (95% CI: 0.036–0.100), 1.7% (95% CI: 0.009–0.031), 1.2% (95% CI:0.007–0.022), and 0.3% (95% CI: 0.001–0.008), respectively (17), which are comparable with the prevalence reported in other world populations. Currently, more than 20% of the 1.4 billion people living in China are in the 0–18 age bracket (https://www.unfpa.org/data/world-population-dashboard). Therefore, it is estimated that more than 10 millions of children and adolescents in China suffer from some mild form of TD, and up to 1 million with TS (12, 17).

In China, children with TD are often mainly under the care of general pediatricians and pediatric neurologists. Pediatric psychiatrists are usually consulted for or in charge of more severe cases with mental comorbidities (12). However, presently child psychiatry is still a discipline in its nascent stage in China with less than 500 available qualified pediatric psychiatrists, and most of them practice in big cities, such as Beijing, Shanghai, Guangzhou, and Wuhan (12). Nevertheless, there is no strict tiered referral healthcare system in China and the patients with TD could seek medical service anywhere in China.

As one of the main bodies of physicians treating children with TD in China, the Chinese Child Neurology Society (CCNS), with ~2,300 members from 31 provinces and regions (18), formed a consortium working on TD and reached an agreement in 2013 to jointly improve the diagnosis and management of TD/TS nationwide; and subsequently developed a Chinese version of expert consensus on diagnosis and treatment of TD in children (19), and further revised it in 2017 (20). Recently, the Chinese Child Neurology Society Tic Disorders Consortium have collectively reviewed the current updates in the field and revised the latest Chinese version of TD diagnosis and treatment, based on the most recent recommendations from the American Academy of Neurology for treatment of TD/TS, and feedback from colleagues in China. Here we present an updated English version of this Chinese experts' opinions on current status of TD, and its diagnosis and treatment in China, which are mainly established on the commonly adopted Chinese national (19–22) and international practices and standards (15, 16, 23–28).

PATHOPHYSIOLOGY OF TD

TD used to be considered as a mysterious illness, and the cause was mostly unknown (9, 10). However, decades of clinical observation and basic research have suggested that TD is a spectrum of neurodevelopmental disorders. The pathogenesis of TD could be due to a combination of genetic, immunological, psychological, and environmental factors. The links between the pathophysiology and clinical symptoms probably lie in the disinhibition of the cortical-striatum-thalamus-cortical circuits (4, 29–31). An imbalance of inhibitory–excitatory signals in these circuits is considered as the molecular mechanism to produce the tics and related symptoms. For example, overactivity of striatal dopamine or postsynaptic dopamine receptor hypersensitivity could produce tic symptoms (8, 29, 31, 32). Multiple neurochemical and neurotransmitter abnormalities have been implicated in TD/TS, most notably dopaminergic, adrenergic,

GABAergic, and glutamatergic pathways (3, 4, 31). More recently, histaminergic (33, 34) and endogenous cannabinoid pathways have been associated with TD/TS through genetic, pharmacological, and brain imaging studies as well (35, 36).

Furthermore, recent extensive genetics, neuroimaging, and neurophysiology research have demonstrated that TD/TS with or without comorbidities of ADHD and or OCD are not distinct disorders but instead arise from common neurodevelopmental abnormalities of parallel cortical-striatal-thalamo-cortical circuits, which regulate initiation, selection, execution, learning, and reinforcement of intended movements, thoughts, behaviors, and moods (30, 31, 37). While tics could arise from dysregulation of the sensorimotor and oculomotor loops, OCB/OCD symptoms may stem from dysregulation of the anterior cingulate and lateral orbitofrontal loop, and ADHD symptoms could be due to dysregulation of the dorsolateral prefrontal loop (30, 31, 37).

Previous studies have suggested that TD/TS is highly inheritable and the heritability is up to 0.77, but no definitive TS causal gene has been identified (38, 39). Recently, a largest-ever genome-wide association and family co-segregation studies with 4,819 TS case subjects and 9,488 control subjects have found only one significant locus (***FLT3*** on chromosome 13, rs2504235, with odds ratio = 1.16), and TS polygenic risk scores could significantly predict both TS and tic spectrum disorders status in the population-based sample (40). Moreover, a meta-analysis of eight psychiatric disorders in 232,964 cases and 494,162 controls, including independent ADHD, OCD, and TS samples, detected 109 loci associated with at least two psychiatric disorders, and 23 loci with pleiotropic effects on four or more disorders. These loci are enriched among genes highly expressed in the brain and play prominent roles in neurodevelopmental processes (41). These findings indicate that TD/TS may be highly polygenic in nature, and TD/TS and its comorbidities may share some overlapped genetic origins, pathogenic pathways, and underlying neural circuits.

CLINICAL CHARACTERISTICS OF TD

Age of Onset and Sex Differences

Tics mostly begin before 18 years of age, typically between 4–8 years old, and the mean age at onset is around 6 years old (42). The tics increase in severity to a peak around 10–12 years old, and then gradually decrease and some remit in late adolescence and young adulthood (4, 6–8, 15, 16, 43).There are rare cases of adulthood onset TD, which is not within the scope of this proposal.

TD and its various subtypes are more common in boys than girls, and the ratio of male to female is estimated to be 3–4:1 (4, 6–8, 14, 42). In a meta-analysis of TD in China, the boy to girl ratio ranged from 2.22 to 3.68 for transient TD, 1.57–2.79 for chronic TD, and 2.17–10.6 for TS, which are in line with the global reports of sex difference in TD/TS (17).

Clinical Manifestation of Tics

The word "tic" is evolved from the French word "tique", meaning a sudden, aimless, fast, and rigid muscle contraction (9, 10). Tics are divided into motor tics and vocal tics. Motor tics are rapid contraction of the fingers, face, neck, shoulders, trunk, and limbs. Vocal tics are the contraction of the oropharynx, throat, and respiratory muscles, and the sound is produced through the airflow in the nose, mouth and throat.

Motor tics or vocal tics can be further divided into two categories: simple and complex, depending on the duration of tics and part(s) of body and group(s) of muscles involved. Simple tics involve brief activation of single muscles or a localized muscle group and manifesting as simple movement or sound; while complex tics activating more muscle groups and manifesting as a goal-directed or purposeful-like movements, or sounds of word or phrase.

Practically any body-muscle may be involved in tics, and there is wide variability in tic phenomenology. Nevertheless, certain tics occur much more frequently than others, and the most common tic is eye blinking. Some descriptive examples of tics are given in **Table 1**, and some video demonstrative examples could also be found in the supplementary online files from the reference (31). Tic symptoms usually start from the face, gradually spread to the muscles of the head, neck, and shoulder, and then to the trunk and upper and lower extremities.

Tics are mostly involuntary but could also be voluntarily held temporarily, particularly in older children. However, voluntary tic suppression could result in a tic "buildup", followed by a sense of relief when the tics are finally carried out (44). Sometimes in older children with longer disease course, after motor tic or vocalization, another action could quickly occur in an attempt to hide or disguise the tics, making the clinical manifestations more complex and challenging to recognize (20).

Forty to fifty-five percent of children report a premonitory urge before motor tic or vocalization, which is an urge-for-action

TABLE 1 | Manifestation and classification of tics.

Tic Type	Simple Tic	Complex Tic
Motor Tic	Blink of eye/oblique eye, frown, eyebrows, open mouth, loll tongue, tapir mouth/crooked mouth, lick lips, crumpled nose, nod/raise/shake/swivel head, torticollis, shrug shoulders, move fingers/toes, rub hands, clench fist, move wrists, lift/stretch/internal rotate arms, stretch/shake legs, step/pedal foot, extend/bend knees, extend/bend coxa, lift chest, hold abdomen, twist waists, and so on.	Lift eyebrows and wink, make faces, eyeball rotate, knob fingers, swing/clap hands, wave arms, stab action, flick limbs, hit chest with fists, bend waist, mandible touch the knee, twist trunk, move up and down, squat, kneel posture, kick legs, knee joint, stamp foot, jump, hop, throw, beat, touch, sniff, touch the hair, walk in circles, walk backwards, and so on.
Vocal Tic	Single-tone, sniffing, clearing throat, roaring, humming, coughing, squeaky sound, screaming, shouting, grunting, spitting, whistling, sucking, barking, tweeting, and so on.	Single word/phrase/clause/sentence, repeat single word or phrase, repeat sentence, imitate speech, obscene language, and so on.

to a perceived local sensory stimulus or sensation or discomfort. Such a premonitory urge could manifest as local pressure, itching, pain, hot, or cold sensation, or other strange feelings, which are also called sensory tics (32, 45, 46). Sensory tic is considered as a premonitory symptom that will disappear after the tics, especially in older children (4, 32, 46). Motor tic or vocal tic could be related to relieving such premonitory urge or local discomfort. Premonitory urge is a characteristic feature of TD, and its awareness and control increase with age (47).

Echopraxia (involuntary repetition or imitation of another person's actions), echolalia (repetition of other people's vocalizations), and palilalia (repetition of the last word or phrase said by the patient) are present in some patients with TD. However, it is noteworthy that echophenomena (echopraxia and echolalia) are essential developmental elements in social learning up to the age of 28–36 months. So only when their persistence beyond this developmental age should prompt diagnostic consideration for a neuropsychiatric disorder, including TD (48). Copropraxia, the involuntary making of obscene gestures, and coprolalia, inappropriate, and out-of-context swearing, are complex forms of motor and vocal tics, respectively. They have been strongly associated with TD/TS, but relatively uncommon in clinical practice, affecting less than 30% of TD/TS patients (47, 49).

Clinical Course of TD

The tics in one individual can change from one form to another, and new forms of tics could emerge during the disease course, but usually manifest as some specific stereotype during a particular time period (50). The frequency and intensity of tics could also fluctuate significantly during the disease course, and new tic symptoms can replace old tic symptoms or superimpose on old tic symptoms.

Tics usually occur in bouts, and tic symptoms often wax and wane during the disease course (4, 43), and they can also be aggravated or mitigated by some stimuli. For example, common factors exacerbating tics include stress, anxiety, anger, shock, excitement, fatigue, infection, and being reminded, while common factors reducing tics include attention concentration, relaxation, emotional stability, and sleep (43, 45). Exercises, especially those involving fine motor movements, such as dancing or sports activities, are often associated with tic attenuation as well (43, 45).

Comorbidity of TD

About half of the children with TD and more than 80% of patients with TS suffer from at least one comorbid psychopathological or behavioral disorder(s) and about 60% TS patients suffer from two or more, which are known as comorbidities (4, 42, 43, 51), e.g., ADHD, OCB or OCD, learning difficulties, anxiety, depression, sleep disorders (52, 53), self-harm behavior or self-injurious behavior (SHB or SIB) (54), conduct disorder (55), rage attacks, or explosive outbursts (32, 56–58). Among them, ADHD is the most common comorbidity, followed by OCD, affecting approximately 50–60% and 36–50% of the patients with TD/TS, respectively (4, 43). There is also a sex difference in the incidence of TD/TS comorbidity. Usually, ADHD, learning difficulties, conduct disorder, and rage attacks are more common in boys, while OCD and SHB/SIB happen more often in girls (4, 43, 47, 51). Comorbidity increases the complexity and severity of TD (4, 43, 47), affects the healthy development of children's learning, social adaptation, personality, and psychological quality, and adds much more difficulties and challenges to the diagnosis, treatment, and prognosis of the illness (59).

DIAGNOSIS OF TD AND CLINICAL ASSESSMENTS

Extensive clinical observation and research have shown highly variable clinical manifestations, severity, and comorbidities in patients with TD. Such high clinical variability has created significant challenges in clinical diagnosis and management, as well as for clinical research. In the last two decades, better understanding of this group of disorders and collaborative efforts have facilitated the development of standardized diagnostic procedures and criteria for tics and related disorders. For example, the DSM-IV-TR in 2000 (60), the Chinese Classification of Mental Disorders 3rd Edition (CCMD-3) in 2001 (61), the DSM-5 in 2013 (2), and the ICD-11 in 2018 (62) all have specific criteria for the diagnosis of TD and related medical conditions. The CCMD-3, DSM-5, and ICD-11 diagnostic criteria for tics are almost the same. Currently, the DSM-5 is mostly used in clinical practice around the world, including China. Notably, the older versions of diagnostic criteria had a discrepancy in terms of tic-free period regarding the 1-year duration for diagnosis of chronic TD and TS, i.e., CCMD diagnostic criteria specified no remission period of >2 months in 1-year period for chronic TD and TS. In comparison, the DSM-IV-TR diagnostic criteria specified no remission period of > 3 months, which could be a contributing factor for variation of prevalence in the previous reports. Nevertheless, the current commonly used DSM-5 criteria have no limitation on remission or symptom-free periods.

At present, descriptive clinical diagnostic methods are mainly used to identify children with tic symptoms and associated mental and behavioral manifestations. Therefore, a detailed inquiry of the medical history and careful observation of the tic manifestation and its associated abnormal cognition and behaviors are the prerequisite for a correct diagnosis. A thorough medical history should include mother's medication during pregnancy, birth history, early development, and past medication use by the patient, etc. plus a complete psychosocial and family history to detect psychiatric and or neurological conditions in relatives. A thorough physical, neurological, and psychiatric examination are critical to identifying any potential causal factor, and symptoms and signs of accompanying medical conditions. Please refer to **Figure 1** for essential diagnostic steps.

International Diagnostic Criteria

According to the clinical characteristics and course of the illness, TD can be divided into three types according to DSM-5, i.e., Tourette syndrome (TS), chronic TD, and provisional TD.

TS: (1) both multiple motor and one or more vocal tics, but the motor and vocal tics do not necessarily appear at the same time. (2) onset before age 18. (3) after the first onset of tics, the frequency of tic can increase or decrease, while the duration of the tic symptoms is more than one year. (4) tic symptoms are not caused by certain drugs or substances or other medical conditions.

Chronic TD, previously known as persistent TD: (1) single or multiple motor or vocal tics, but not both motor and vocal tics at the same time. (2) onset before age 18. (3) since the first onset of tics, the frequency of tics can increase or decrease, while the duration of the disease is more than one year. (4) tic symptoms are not caused by any medication or substance or any other medical conditions. (5) fail to meet the diagnostic criteria for TS.

Provisional TD, previously known as transient TD: (1) single or multiple motor and/or vocal tics but not both motor and vocal tics at the same time. (2) onset before age 18. (3) the duration of tics is less than one year. (4) tic symptoms are not caused by any medication or substance or any other medical conditions. (5) fail to meet the diagnostic criteria for chronic TD or TS.

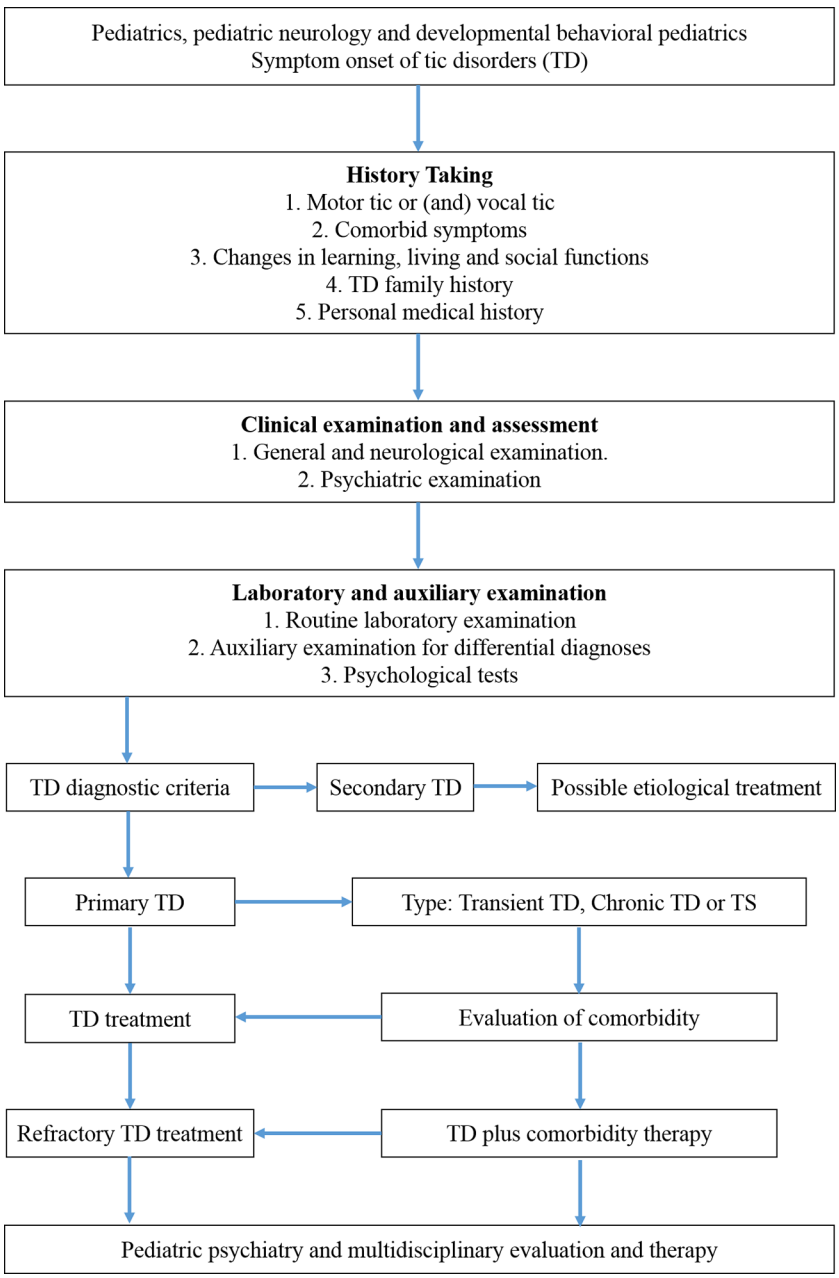

FIGURE 1 | Diagnostic and Treatment Roadmap of TD.

There is a certain continuity between the three types, with transient TD can develop into chronic TD, while chronic TD can also transit into TS. Some patients do not fall into any of the above categories; they belong to other TD, such as adult-onset TD or late-onset TD, and any other unspecified TD.

Refractory TD is a new concept gradually formed in pediatric neurology/psychiatry in recent years, and there is no clear definition yet. It is generally accepted to consider as refractory TD when a severe case of TS has been treated with classical anti-TD medications, such as tiapride and haloperidol or aripiprazole, for more than 1 year without satisfactory results (63).

Differential Diagnoses and Auxiliary Exams

First of all, tic symptoms should be differentiated from epileptic seizures, substance or medication-induced dyskinesia, chorea, dystonia, to name a few (32, 64). The presence of a premonitory urge with relief following the tic movement, the ability to suppress the tic movement, as well as the waxing and waning pattern support the diagnosis of TD. Furthermore, tics occur when the motor function of the involved muscle is normal; and tics are sudden, brief, and repetitive, and happen temporarily and episodically (32, 64).

Secondly, the majority of TD cases are primary TD or idiopathic, in which tics are the main clinical manifestation and no direct cause could be identified. Hence there are no specific biomarkers nor diagnostic tests for primary TD. Neurologic examination is usually normal for patients with primary TD. However, "soft" neurologic signs may present, including impaired fine movement coordination and motor restlessness, especially in children with ADHD (31).

In general, electroencephalogram (EEG), neuroimaging, psychological test and laboratory examination are not required to support the diagnosis for primary TD. The results of such an examination could show nonspecific abnormalities; and they are mainly used to assist the diagnosis of comorbidity or exclude the possibility of other diseases. In a small number of children with tic-like symptoms, EEG could show background slow waves or asymmetry, or paroxysmal epileptiform discharges, which is helpful to identify any active brain pathology or concomitant or mistaken seizure disorders. Video-EEG is commonly available in most major cities of China with affordable cost therefore it could be considered a routine test to exclude epileptic seizures and other neurological conditions (20).

Skull CT or MRI examination in some patients with TD could show smaller caudate nucleus, slightly thinner frontal and occipital cortex, mild ventricular enlargement, or deeper lateral fissure, and other nonspecific structural changes (65). Therefore, brain imaging examination could mainly be used to exclude any structural lesion of the basal ganglia and other relevant brain regions if suspected (65).

However, it is important to keep in mind that a variety of medical conditions and acquired factors could also cause tics or tic-like symptoms (8, 29, 46, 64). According to the previous studies, the following medical conditions and disorders could present tics or tic-like movements as the main or part of the clinical manifestations (8, 29, 46, 64): (1) genetic syndromes, in which tics or tic-like symptoms are only part of, but not the primary clinical manifestation, such as Down's syndrome, Fragile X syndrome, tuberous sclerosis complex, and neuroacanthocytosis; (2) infectious diseases, such as streptococcal infection, encephalitis, neurosyphilis, Creutzfeldt-Jakob disease; (3) intoxicating factors, such as carbon monoxide, mercury, or bee poisoning; (4) medication side effects, such as methylphenidate, pemoline, amphetamine, cocaine, carbamazepine, phenobarbital, phenytoin, and lamotrigine; (5) other factors, such as stroke, and head trauma.

In such above-mentioned situations, the patients will present repetitive, patterned, but aimless and inattentive tics or tic-like movements, or seemingly stereotypy movements. Careful observation, medical history and physical examination could differentiate them from primary TD/TS (32, 66). A secondary TD should be suspected when tics present in much older children, start abruptly, or rapidly worsen over days to weeks, or occur in patients with other neurologic signs or symptoms. Systematic screening and specific auxiliary tests should be ordered to exclude the above-mentioned causes if suspected. The laboratory blood tests of antistreptolysin "O" (ASO), erythrocyte sedimentation rate, rheumatoid factor, virus antibody, trace elements, and ceruloplasmin are helpful to identify some common causative factors or for differential diagnoses (46, 67). Consultation or referral to specialised health professionals could help solving some complicated cases of secondary TD with some rare primary disease conditions.

Tics Severity Assessment

The severity of tics and its associated comorbidities and functional impairment are also highly variable. Tics could be simply divided into mild, moderate, and severe cases based on simple clinical observation. Mild cases refer to light tic symptoms, which do not affect children's normal lives, learning, or social activities. Moderate cases refer to frequent tic symptoms, which somehow interfere with children's normal functions and social activities. Severe cases refer to very frequent tic symptoms, which significantly impair children's lives, education, and social activities.

Nevertheless, it is highly recommended to use a standardized instrument to objectively, quantitatively, and systematically evaluate the severity in order to monitor the disease course and treatment effect. There are several different tools that have been developed to measure the severity of TD and its associated psycho-social-behavioral comorbidities and impairments (32), such as the Gilles de la Tourette Syndrome Health-Related Quality of Life Scale (68) and the Premonitory Urge for Tics Scale (69). One of the most commonly used tic severity measurements is the Yale Global Tic Severity Scale (YGTSS) (70).

YGTSS is based on a semi-structured clinical interview and consists of three parts. The first part consists of checking items of motor/vocal tic symptoms. The second part is a score-system to assess the severity of motor and vocal tics separately in five dimensions, including tic numbers, frequency, intensity, complexity, and interference. The third part is the scale of functional impairments in self-esteem, social interaction, study, or work of children with TD. The total score of YGTSS (maximum rating 100) is obtained by summing up the scores

of motor and vocal tics and functional impairment. TD cases with less than 25 of YGTSS total scores are considered as mild, 25–50 scores as moderate, and more than 50 scores as severe.

Besides, the number of comorbidities is also highly associated with the overall severity of TD, with more comorbidities, more severe the cases are (46). Repeated measures of the YGTSS can help systematically monitoring the disease course and evaluating response to treatment. A recent study has further proved its utility in assessing tic severity in children and adults with some minor revisions, i.e., YGTSS-Revised (links.lww.com/WNL/A423) (71).

Diagnosis of Comorbidity

In any case, a careful evaluation of the common comorbidities is an essential component of the TD assessment. MINI Kid 5.0 (Mini-International Neuropsychiatric Interview for Children and Adolescents) is a short, structured diagnostic interview for DSM-IV and ICD-10 psychiatric disorders in children and adolescents (72). It has been translated and validated in Chinese language and is highly recommended by the Chinese pediatric psychiatric experts for diagnoses of psychiatric comorbidities of TD (73).

TD/TS With ADHD

ADHD is characterized by an enduring pattern of developmentally inappropriate inattention or hyperactivity and impulsive behavior. ADHD is reported to affect about 50% (range of 21%–90%) of referred patients with TD/TS, compared to 2%–12% in the general pediatric population (5, 74). ADHD symptoms (inattentiveness, hyperactivity-impulsivity, or both) usually precede the onset of tics by 2–3 years (5, 74). Comorbid ADHD may contribute to behavioral disturbances, such as aggressiveness, disruptive behaviors, poor school performance and social adaptation, and problems with executive function, as well as increased emotional problems and functional impairments (5, 74). Therefore, a coexisting ADHD and its health burden should be screened for every patient with TD (32) through MINI Kid 5.0 (72, 73). Thorough evaluation could be performed by using pediatric ADHD rating scales within the Child Behavior Checklist (CBCL), and the Children's version of the Connors ADHD Rating Scale (CAARS) if needed (5, 32, 45).

TD/TS With OCD

OCD is characterized by the occurrence of obsessions, which manifest as recurrent and intrusive thoughts, ideas, images, or impulses; and the occurrence of compulsions, which are repetitive behaviours or mental acts sought to prevent or reduce anxiety or distress. The DSM-5 criteria for OCD require that obsessions, compulsions, or both occupy at least 1 h per day or cause significant clinical distress or functional impairment.

A lifetime comorbid diagnosis of OCD is present in about 50% of patients with TS (5). To a lesser degree, OCB usually presents as a need for order or routine, and a requirement for things to be symmetric or in specific order or pattern, e.g., repeated checking or reordering or counting, rituals, and forced touching (5). OCB occurs in 20%–60% of patients with TS, compared to 0.5%–3.6% of healthy children and adolescents. OCB/OCD often emerges during early adolescence, several years after the onset of tics, frequently accompanied by a higher frequency of aggressive behaviors (5). The most recommended instrument to capture the full range of obsessive and compulsive symptoms and assess its severity in children is the Children's Yale-Brown Obsessive–Compulsive Scale (CY-BOCS in children), entailing 58–80 items on obsessive and compulsive symptoms and 10 items on obsessive and compulsive severity (32).

TD/TS With Anxiety and Depression

The presence of generalized anxiety disorder in subjects with TD/TS has been reported in the range from 19%–80%, with a high-risk period for anxiety issues starts at age 4, and a high-risk period for mood disorders begins at age 7 (47, 75). The presence of depression in patients with TS has been positively correlated with an earlier onset, greater severity, and a longer duration of tics (31, 45, 64, 75). Anxiety and depression should be routinely screened in children and youth with TD/TS by MINI Kid 5.0 (72, 73), and properly assessed using the Multidimensional Anxiety Scale for Children (MASC) and the Children's Depression Inventory (CDI) when the symptoms are prominent and intervention is needed (75).

TD/TS With Disruptive Behaviors and Potentially Life-Threatening Symptoms

Disruptive behaviors, including episodic outbursts, rage, aggression, and impulse control problem, are common in patients with TD/TS, e.g., episodic behavioral outbursts and anger control problems have been reported in 25%–70% of TS populations (31, 45), which should be recognized during history taking and considered when planning for intervention and therapy.

The risk of premature death was reported to be higher among individuals with TD (mortality rate ratio, 2.02; 95% CI, 1.49–2.66) and with TS (mortality rate ratio, 1.63; 95% CI, 1.11–2.28), compared with controls in a large population-based prospective cohort study in Denmark (76). When having complex and greater severity of motor symptoms, plus the presence of two or more behavioral comorbidities, particularly OCB/OCD, the patients could result in tic-related injuries, self-injurious behaviors (SIB), uncontrollable violence and temper, and suicidal ideation/attempts (31). Notably, copropraxia and coprolalia have been reported highly associated with SIB in patients with TD/TS (47). Such high-risk symptoms and behaviors need to be recognized during psychiatric assessment, and these patients need immediate medical attention and intervention to prevent severe consequences (54, 77).

THERAPEUTIC APPROACHES

There have been some consensus TD/TS treatment recommendations in the past decade in the Western countries, based on some experts experience and the clinical evidence, such as the European Clinical Guidelines for Tourette syndrome and other TD (15, 23, 24, 32, 78), the Canadian guidelines for the evidence-based treatment of TD (16, 25, 26), and the most recent American Academy of

Neurology Practice Guideline Recommendations for Treatment of Tourette syndrome and Chronic TD (28).

The establishment of an effective therapeutic plan requires careful initial assessment of tics, determining the presence of co-occurring psycho-social-behavioral issues, and clarifying the resulting impairment of each issue. Many children and adolescents with TD do not require intervention or treatment for their tics if their tics do not interfere with daily life or school activities. In China, there are also variations in terms of availability of cognitive-behavioral therapy (CBT) and pharmacological treatments, as well as clinicians' experiences to justify the clinical guidelines in different places and conditions. Therefore, treatment decisions should be based on an individual patient's needs, available resources, experience of the treating clinician, guided by the recommendations from the experts and professional organizations in the fields.

Target symptoms should be identified before treatment starts, i.e., the most influential symptoms on patients' daily life, study, or social activities. Tics are usually the main target symptoms of treatment, while the target symptoms of some children could be more prominent comorbid symptoms, such as hyperactivity, impulsiveness, obsessive compulsion, and so on.

For children with mild TD, medical education and psychological support could be offered first or only, and a watchful waiting period with regular follow-ups should be appropriate. The principle of treatment for moderate to severe TD is similarly to try non-pharmacological intervention first, and behavioral therapy could be combined with pharmacological treatment. Nevertheless, medical education and psychological support should be provided throughout the entire treatment course. Please refer to **Figure 1** for therapeutic order and steps.

Education and Support of Patients With TD and Their Families

Before to and at the same time of the active treatment of TD/TS, we would first recommend medical education and psychological support to the patients, as well as their parents, peers, and teachers in school and communities (24, 28, 79, 80), which could be in the forms of parent management training, parent-child interaction therapy, parent-school teacher interaction etc. It is essential to inform and educate patients and their parents that for most people with TD, the tics subside on their own by the end of adolescence. This fact about TD could lead to a much more conservative therapeutic need and approach.

Parent Management Training

Parents could be taught to record short videos of the children's symptoms at home and show them to the doctors at the clinics so that the doctors could have a better assessment of the condition. Parents could be encouraged to face the diagnosis of TD/TS with their children instead of being embarrassed and trying to deny or hide or finding "excuses" for the tics symptoms for their children. Parents could also be advised to reassure the children with TD/TS to interact confidently with their classmates and people around them so their social adaptability could be improved. They could also motivate the children to actively take part in physical and social activities, instead of over-protecting the children by keeping them inside and isolated from others, which is common in the Chinese culture, particularly in the current generation of one-child families in China (12). The parents could also be instructed to carefully observe with the children the conditions and factors that could provoke or increase the tic symptoms, and subsequently avoid such "risk factors".

The Chinese TD/TS Association, which is a non-governmental, non-profit organization, has built a platform for health education, patient-physician and patient-patient interaction, and information exchange among physicians, patients with TD, and their families. It is a very useful resource for information and support for patients with TD/TS and their families.

School and Educational Support

Parents could also communicate more often with school-teachers to help them better understand the medical condition so that the children with TD/TS could avoid being punished for "unexpected or uncontrolled movements" and could have reduced academic work-load to lower their stress level. School teachers could also help educating other students not to laugh at, isolate, and stigmatize the children with TD/TS.

We would also recommend special educational support for children with TD/TS, and particularly those with problems of learning, social adaptation, and self-esteem. We believe such special support could promote rehabilitation and help children return to healthy life. Most children with mild TD and good social adaptability can achieve effective results through psychological education and support only.

Cognitive Behavioral Therapy of TD

Cognitive behavioral therapy (CBT) and/or pharmacological interventions should be considered in addition to psychoeducation for patients with TD who have clear impairment associated with the tics. There is no clear consensus on what constitutes an indication to start treatment in TS. However, the European guidelines published in 2011 (15) recommends starting behavioral or pharmacological treatment for tics in the following situations: (1) subjective discomfort otherwise requires other treatment; (2) social impairment; (3) emotional difficulties; (4) functional disabilities. These situations mainly correspond to moderate to severe TD.

Behavioral therapy is an effective means to reduce tic symptoms and comorbidity, and to improve social function (79, 81, 82). Multiple behavioral interventions have been developed for the treatment of TD/TS and its associated comorbidities, including habitual reversal training (HRT), effective prevention of exposure, relaxation training, positive reinforcement, self-monitoring, regression exercise, to name a few (3, 79). The most commonly used one is comprehensive behavioral intervention for tics (CBIT) (3, 29), which trains patients to become aware of their tics and teaches them specific behavioral strategies to reduce tics. CBIT has been shown superior to supportive psychotherapy for children aged 10–17 years with TS and considered as first-line therapy when available, including those with comorbid OCD and ADHD (3, 27–29).

However, behavioral therapies are unlikely to be helpful in very young children (aged 9 years and younger), who have limited cognitive function to recognize and control pre-impulses that are the core of behavioral therapy; or in children with severe, untreated ADHD, who may have difficulties sustaining engagement in therapy. Furthermore, behavioral interventions are resource-intensive and require the presence of highly skilled clinicians, typically psychologists, occupational therapists, or specially trained physicians, and their significant time commitment (83). Due to the shortage of such trained professionals, as well as lack of experience and confidence among clinicians and parents, widespread implementation of behavioral interventions of TD still faces challenges in China (84). Currently, it is only available at certain pediatric mental health centers in some big cities' of China, e.g., Beijing, Shanghai, and Wuhan. Nevertheless, behavioral therapies are much safer than pharmacological medications and have been proven effective in older children with TD/TS (24, 26–28). Therefore, it has been highly recommended by the Western experts and guidelines, and it could be gradually introduced and implemented in some centers in China with a large volume of TD/TS patients and available resources.

Pharmacological Treatment of TD

For children with moderate to severe TD that affects daily life, school, and social activities, and when psychological education and behavior therapy are not effective or unavailable, pharmacological therapy is needed (28, 85). The patients and their parents need to understand at the beginning of pharmacological therapy that the outcome of the medication(s) is unlikely to be completely tic-free but rather to improve the control and reduction of tic severity. The currently available medications could reduce tics by over 60%, such as aripiprazole in 60.2% (86–88), tiapride in 76.0% (27, 59). In general, a two-tiered medication choice and multi-stage treatment course are recommended, with the use of first-line medications for milder tics and the use of second-line medications reserved for more difficult cases. Therapeutic agents should start with monotherapy at its lowest effective dosage and gradually increased as needed. It is inappropriate to change the medication(s) or to discontinue the medication(s) too early or abruptly.

Course of Pharmacological Treatment

It is highly recommended that the pharmacological treatment of TD will take a gradual process and divide into multiple stages with careful evaluation at each step (19, 20). The complete course of treatment usually takes 1–2 years. If symptoms reoccur or aggravate at any time during the course, then return to the previous step or resume the process from the beginning.

1. Acute treatment period: Actively control the symptoms and shorten the course of the illness. Starting from the minimum dose, slowly increase (1–2 weekly added) to target treatment dose. The course of treatment is dependent on the patient's response to the medication until satisfactory result is achieved.
2. Consolidating treatment period: Consolidate therapeutic effect, prevent relapse, and promote social function recovery. After the tic symptom is mostly under control, the same dosage should be continued for at least 1–3 months.
3. Maintenance treatment period: Prevent relapse, maintain good daily function, and improve the quality of life. After the consolidation period, if the condition is well-controlled, the treatment should remain for 6–12 months, and the maintenance dose is generally 1/2–2/3 of the maximum dose previously used.
4. Medication withdrawal period: After the maintenance treatment, if the symptom(s) are well under control, the medication can be gradually withdrawn; and the withdrawing period should be gradual and last at least 1–3 months.

Medication Options

Some recommended medications for the treatment of pediatric patients with TD in China are shown in **Table 2**, which are based on our clinical experience and availability of the drugs in China, including two proprietary polyherbal Chinese medicines that have been approved by the Chinese National Administration of Traditional Chinese Medicine (TCM), and are recommended as the first-line TCMs for pediatric patients with TD by the National Guideline of TCM for Pediatric Diagnosis and Treatment through a TD Expert Committee (22) (please also refer to the *Traditional Chinese Medicine Treatment of TD in China*). The choice of medication is often driven in part by the patient's comorbidity profile, and treatment sometimes needs to target multiple symptoms, for example, tics plus hyperactivity, or anxiety, or compulsion. Every patient need be carefully followed up and has periodic evaluation and check-up to assess medication efficacy, side effects, and the need for continued therapy.

Comorbidity Treatment

Comorbid With ADHD (TD+ADHD)

This is one of the most common clinical comorbidities (49). Alpha 2 receptor agonist, such as clonidine and atomoxetine hydrochloride, is the first-line treatment, which has the anti-tic function and improves attention (28, 98). Atomoxetine hydrochloride does not induce or aggravate tics, so it can also be applied to TD children with ADHD (99). Guanfacine is not available in China. There was also successful clinical experience in using methylphenidate for TD+ADHD treatment (21, 100). Central stimulant, mainly methylphenidate, is the second-line treatment of TD+ADHD in China. However, there is a potential risk of aggravating or inducing tics by psychostimulants (21). It is generally advocated that conventional doses of dopamine receptor blockers, such as tiapride, should be combined with low doses of psychostimulants, such as methylphenidate, 1/4~1/2 of the conventional dosage, to treat children with TD+ADHD (101). Such treatment can effectively control the symptoms of ADHD but has little effect on the tic symptoms of most children. Evidence from pharmacological studies conducted over the last decade supports the use of stimulants to prioritize the treatment of debilitating ADHD symptoms in patients with TD/TS.

Comorbid With OCD (TD+OCD)

Cognitive-behavioral therapy (CBT) with an exposure/response prevention (ERP) component has the strongest evidence-based

TABLE 2 | Recommended medications in the treatment of TD.

Recommendation References	Drug Name	Type	Mechanism of Action	Initial Dose	Therapeutic Dose [a]	Common Side Effects
First-line Med (20, 89, 90)	Tiapride	Antipsychotic, typical neuroleptic	D2 receptor blockade	50–100 mg/d	100–600 mg/d	Somnolence, gastrointestinal reactions
First-line Med (20, 23, 86, 88–91)	Aripiprazole	Antipsychotic, atypical neuroleptic	Partial agonist of dopaminergic (D2, D3, and D4 receptor) and serotonergic (5-HT1A and 5-HT2C) receptors	1.25–5.00 mg/d	2.50–20.00 mg/d	Somnolence, weight gain, gastrointestinal reactions
First-line Med (TD +ADHD) (20, 23, 92–94)	Clonidine [b]	Alpha agonist	α2 adrenergic receptor agonist	1.0 mg/w	1.0–2.0 mg/w	Somnolence, dry mouth, dizziness, headache, fatigue, occasional orthostatic hypotension, and bradycardia
First-line Med (22, 95)	Changma Xifeng Tables	TCM [c]	Unknown	0.53–1.59 g/d	1.59–4.77 g/d	No obvious adverse reaction
First-line Med (22)	Jiuwei Xifeng Granule	TCM	Unknown	6.0–12.0 g/d	12.0–24.0 g/d	No obvious adverse reaction
Second-line drug (20, 23, 89, 90)	Haloperidol	Antipsychotic, typical neuroleptic	D2 receptor blockade	0.25–1.00 mg/d	1.00–6.00 mg/d	Somnolence, extrapyramidal symptoms, increased appetite, and hepatic insufficiency
Second-line drug, off-label use (20, 23, 89, 90)	Risperidone	Antipsychotic, atypical neuroleptic	5-HT2 receptor antagonist at low doses andD2 antagonist at high doses	0.25–1.00 mg/d	1.00–4.00 mg/d	Weight gain and extrapyramidal response
Second-line drug, off-label use (20, 23, 96, 97)	Topiramate	Anticonvulsant	Enhanced GABA and reduced AMPA function	12.50–25.00 mg/d	25.00–100.00 mg/d	Weight loss and cognitive impairment, drowsiness, headache, and risk of renal stones

[a] The recommended dosage is based on age. Patients who are younger than 8 years of age use the minimum therapeutic dose to approximately 1/2 maximum therapeutic dose, such as tiapride (100–350 mg/d). For patients who are older than 8-year-old use the maximum therapeutic dose of 1/2 to maximum therapeutic dose, such as tiapride (350–600 mg/d).
[b] Transdermal patch. [C] TCM, Traditional Chinese Medicine.

effect and is considered to be the first-line treatment for TD +OCD, if available (27, 28). Selective serotonin reuptake inhibitors (SSRIs), such as sertraline, are the first-line pharmacological agents. SSRIs are the only class of medications that has primary efficacy for OCD (27, 28). SSRIs should start with a small dose and gradually increase. Tricyclic antidepressants, such as clomipramine, could be used as a second-line medication for TD+OCD, but with more side effects (59). Newer antidepressants can also be used to treat TD comorbid with OCD. The European clinical guidelines suggest to use risperidone as a first-line choice for TD+OCD (23). Dopamine receptor blockers, such as aripiprazole and risperidone, are often used in combination with SSRIs, such as sertraline, to treat TD with severe OCD symptoms (21, 102).

Comorbid With Other Behavioral Disorders

TD cases with other significant behavioral disorders, such as learning difficulties, sleep disorders, self-injurious behaviors, and conduct disorder, should be consulted with or referred to professionals in specialized education, psychological intervention, behavioral therapies, and sleep disorders (103). In some complicated severe cases, it is necessary to timely transfer the patients to advanced pediatric psychiatry and/or neuropsychological services for comprehensive assessment and treatment.

Refractory TD Treatment

When treatment outcome is not satisfactory as expected, some common scenarios should be investigated to first exclude false refractory TD, such as misdiagnosis, improper medication choice, insufficient dosage, intolerance of side effects, or poor medication compliance. For children with refractory TD/TS in China, it is recommended that such patients should be referred to pediatric psychiatry or multidisciplinary team for evaluation and management. Once the diagnosis of refractory TD is established, a comprehensive treatment plan could include combined medications, newer medications, non-pharmacological therapy, and proper treatment of comorbidities (3, 104).

Some newer medications were reported to be effective for the treatment of adult patients with refractory TD in the Western countries. These include new D1/D5 receptor antagonists (e.g., ecopipam), vesicular monoamine transporter inhibitors (e.g., tetrabenzazine), antagonist of the nicotinic acetylcholine receptors (e.g., mecamylamine), cannabinoids (e.g., tetrahydrocannabinol), glutamatergic blocker (e.g., riluzole), γ-aminobutyric acid (GABA), finasteride, and omega-3 etc (3, 28, 89, 96, 104). However, these newer medications are currently unavailable or not widely used in pediatric clinics in China.

Botulinum toxin injections for the treatment of adolescents and adults with localized and bothersome simple motor tics have been recommended in the Western countries when the benefits of treatment outweigh the risks (3, 27, 28, 96). Various neural regulation therapies have been reported, including repetitive transcranial magnetic stimulation (rTMS), cranial electrotherapy stimulation (CES), and EEG biofeedback. However, the effects of such treatments are non-conclusive, and sometimes controversial

(3, 105); therefore, they should be prescribed with caution and reservation. The beneficial effect of deep brain stimulation (DBS) has been more consistently reported in small scale clinical trials for refractory TS (3, 28, 96). But DBS belongs to invasive treatments, so it should only be considered for older children (above 12 years old) or adult refractory TS or special cases, such as the ones with severe self-injurious behaviors. It should also be under multidisciplinary assessments, follow stringent criteria, and ethically approved protocols (3, 26, 28, 78, 96, 105, 106).

Traditional Chinese Medicine Treatment of TD in China

Pharmacological treatments often have significant side-effects; while currently CBT is not easily assessable in most parts of China. Neither of the non-pharmacological and pharmacological treatments results in complete resolution of tic symptoms. Therefore, a variety of seemingly safer and easier complementary alternative medicine (CAM) have become available to the patients with TD and their caregivers (107).

Traditional Chinese Medicine (TCM), which mainly includes TCM medication and acupuncture that have been developed for the prevention and treatment of various diseases and refined by the Chinese people over thousands of years, is widely available and commonly used all over China. TCM medication could be prescribed as individualized formula, often in the form of liquid decoctions; or in pre-made dry decoctions or granules or tablets, based on some commonly used formula with specific medical ingredients for specific diagnoses or medical purposes (108). Notably, in recent years TCM has drawn increasing attention worldwide, and the ICD-11 will formally include a chapter on TCM classification for the first time in 2022 (109–111).

Based on the TCM diagnostic procedures, patients with TD could be further diagnosed and classified as different specific subtypes of TD, which are defined by different types of yin-yang imbalances of the functional entities of the body and mind (22). In China, TCM could be used to treat TD alone by specialized TCM physicians, often using individualized, freshly made daily liquid decoctions; or used by pediatric neurologists and psychiatrists in combination with Western Medicine, often prescribed as premade formulated granules or tablets (12).

There has been a standardized national guideline for the diagnosis and treatment of TD using TCM in China since 2012, and an updated version in 2019 (22). Some meta-analyses have supported the efficacy and safety of TCM alone and TCM plus Western Medicine in treating patients with TD/TS (112–116). For example, a randomized, placebo-controlled, double-blind clinical study investigated the short-term effectiveness and safety of one premade TCM medication, Ningdong (ND) Granule in pediatric subjects (aged 7–18 years) with TS, showed a 41.39% reduction in the total tic score, while the placebo group showed a 10.79% decrease (117). In another multicenter, double-blind, double-dummy, randomized, placebo-controlled trial, 603 patients with TS aged 5–18 years were randomly assigned to either treatment with placebo (n = 117), or tiapride (n = 123, 200–400 mg/d) or 5-Ling Granule (5-LGr), a proprietary polyherbal product (n = 363, 15.0–22.5 g/d), for 8 weeks; and the results showed that the clinical efficacy of 5-LGr was comparable to tiapride in reducing tics but its safety profile was better than tiapride (118). Changma Xifeng Tablet is another proprietary polyherbal Chinese medicine often used to treat patients with TD in China. In another multicenter, double-blind, double-dummy, randomized, parallel positive drug-controlled trial, patients with TS aged 4–18 years were randomly assigned to treatment with Changma Xifeng Tablets (n = 438) or tiapride (n = 110); and Changma Xifeng Tablet showed similar clinical efficacy as tiapride (86.59% vs. 82.73%) but with fewer side effects as compared with tiapride (0.00% vs. 5.45%) (95). Changma Xifeng Tablet has been approved as one of the first-line TCM for the treatment of pediatric patients with TD by the Chinese National Administration of Traditional Medicine (22). There has also been increasing modern medical research in China to understand the mechanisms of TCM. For example, another commonly used TCM decoction (Xiao-Er-An-Shen) for the treatment of TS in children in mainland China, its beneficial effect was shown to be associated with reversing abnormal changes of neurotransmitter levels and enhancing antioxidant status in an experimental mouse model of TS (119).

Similarly, acupuncture has been demonstrated to be an effective alternative therapy for TD/TS in China (114, 115). Two meta-analyses of seven and ten randomized clinical trials (564 participants and 703 participants, respectively) in China have shown that compared with Western medicine (e.g., haloperidol and risperidone), acupuncture seemed to be more effective in short-term to improve the YGTSS [MD −4.60, 95% CI −5.80 to −3.40) (114) or SMD -0.71, 95%Cl (-1.10, -0.33), Z=3.65, P = 0.0003) (115)]; and the response rate, compared to haloperidol or risperidone [(RR = 1.15, 95% CI (1.05,1.25), Z = 3.05, P = 0.002 (115) or RR = 1.19 (95% CI 1.08 to 1.31, Z = 3.42, P = 0.0006) (114)]. Acupuncture could also be used as an adjuvant therapy to enhance the effect of Western medicine in improving the YGTSS (MD −7.11, 95% CI −8.74 to −5.47) (114).

However, the total number and sample sizes of the reported RCTs on the TCM medications and acupuncture treatment for TD were still relatively small, compared to the RCTs of Western medicine. Therefore, high quality RCTs on TCM treatment of TD/TS remain scarce, and large-scale and well-designed RCTs with rigorous methods of TCM medication and acupuncture for TS are warranted (113).

PROGNOSIS AND MAIN DETERMINANTS OF QUALITY OF LIFE IN PATIENTS WITH TD

The overall prognosis of TD is relatively benign, and most children with TD can grow up to work and live as healthy adults. However, a small fraction of children with TD could carry prolonged tic symptoms and comorbidity into their adulthood that would compromise their quality of life and career.

Nearly half of the pediatric patients with TD would have complete remission in adolescence or adulthood, and about another 30% of them would have alleviated tics in adulthood;

up to 20% of the patients with TD would have deferred tics into adulthood or lifelong (43, 120). Only a small fraction (5–10%) of pediatric patients with TD not only experience tic worsening in adulthood but also develop the most severe and debilitating forms of TD, particularly those with comorbidities.

The prognosis of children with TD could be associated with certain risk factors, including a family history of mental or neurological disorders, childhood psychosocial stress, higher childhood tic severity score, smaller caudate volumes, and poor fine-motor control (120, 121). Since TD symptoms can be alleviated or relieved with age and brain development, the prognosis should be deferred until around 18 years old.

In the meantime, comorbid ADHD symptoms tend to decrease in only 20% of children during adolescence but later than tics. The strongest predictor of ADHD in early adulthood is ADHD severity in childhood. Furthermore, having a family history of ADHD or getting special education in childhood also significantly increases the risk of future ADHD. Studies have also shown that comorbid ADHD and OCD tend to persist, with ADHD symptoms (120) and OCD severity in childhood strongly predict OCD in early adulthood. Moreover, the presence of untreated comorbidities could also adversely affect the long-term outcome of patients with TD (120, 121).

TD is a chronic neuropsychiatric disorder that has a significant negative impact on the health-related quality of life (HR-QOL) of patients and their families, if not properly managed. A systematic review (122) and other studies (123–125) indicated that in patients with mild to moderate TS, HR-QOL relates primarily to co-morbidities of ADHD and OCB/OCD. ADHD with predominantly inattentive symptoms, rather than hyperactivity symptoms, was associated with lower QOL (123). However, young patients with severe tics associated with characteristic premonitory urges and a family history of TD appear to be at higher risk for poorer HR-QOL as adults (124). QOL profiles in children reflect more the impact of co-morbid attention-deficit and hyperactivity symptoms, which tend to improve with age, whereas adults' perception of QOL seems to be more strongly affected by the presence of depression and anxiety symptoms (122). Therefore, early interventions and effectively managing the comorbidities in pediatric patients, as well as proper treatment of depression, anxiety, and other comorbid symptoms in young adult patients with TD, will effectively improve their HR-QOL.

SUMMARY OF TD DIAGNOSIS AND TREATMENT

Clinical diagnosis of TD relies on detailed medical history, careful physical examination, and some auxiliary tests. Direct interview and exam of the children are essential to observe the tics, as well as the general behaviors and mental status, and to identify any additional concomitant sign(s) or symptom(s) to rule out any primary causative medical condition or for differential diagnoses. It needs to keep in mind that tic symptoms can be self-controlled for a short period, so it is easy to overlook and miss the diagnosis at the beginning. At the same time, TD can also be disguised by other prominent symptoms, particularly with comorbidities. Secondary TD, such as tic-like movements caused by rheumatic chorea, epilepsy, and other extrapyramidal disorders, should be excluded. Treatment should be considered at the individual level, gradually apply available non-pharmacological therapy, and pharmacological agents and other interventions with careful measurement of therapeutic effects, side effects, and overall outcomes. A simplified diagnosis and treatment roadmap is shown in **Figure 1**.

THE MEMBERSHIP OF THE CHINESE CHILD NEUROLOGY SOCIETY TIC DISORDERS CONSORTIUM (GROUPED BY THE GEOGRAPHIC LOCATIONS):

1. Beijing Anding Hospital Affiliated to Capital Medical University (Drs. Yan-Jie Qi, Yi Zheng), Beijing, China
2. Peking University First Hospital (Drs. Ying Han, Yu-Wu Jiang, Qin Lin, Zhi-Xian Yang), Beijing, China;
3. Peking University People's Hospital (Drs. Na Fu, Jiong Qin), Beijing, China;
4. Beijing Children's Hospital Affiliated to Capital Medical University (Drs. Fang Fang, Yong-Hua Cui), Beijing, China;
5. Children's Hospital Affiliated to Capital Institute of Pediatrics (Drs. Qian Chen, Li-Wen Wang, Jian Yang), Beijing, China;
6. People's Liberation Army General Hospital (Drs. Guang Yang, Li-Ping Zou), Beijing, China;
7. Pediatric Hospital Affiliated to Fudan University (Drs. Dao-Kai Sun, Yi Wang, Li-Fei Yu, Shui-Zhen Zhou), Shanghai, China;
8. Shanghai Children's Medical Center (Drs. Ji-Wen Wang, Zhi-Ping Wang), Shanghai, China;
9. Xinhua Hospital Affiliated to Shanghai Jiaotong Unniversity School of Medicine (Dr. Ling Li), Shanghai, China;
10. Children's Hospital Affiliated to Chongqing Medical University (Drs. Fang-Cheng Cai, Si-Qi Hong, Li Jiang), Chongqing, China;
11. Tianjin Children's Hospital (Dr. Yu-Qin Zhang), Tianjin, China;
12. Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology (Drs. Zhi-Sheng Liu, Qing Lu, Dan Sun), Wuhan, Hubei Province, China;
13. Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology (Dr. Yan Liu), Wuhan, Hubei Province, China;
14. Xiangya Hospital of Central South University (Drs. Jing Peng, Fei Yin), Changsha, Hunan Province, China;
15. Cangzhou People's Hospital of Hebei Province (Dr. Rong Wang), Cangzhou, Hebei Province, China;
16. Children's Hospital of Hebei Province (Dr. Rong-Fu Shi), Shijiazhuang, Hebei Province, China;
17. Xinxiang Medical College (Dr. Xue-Peng Guo), Xinxiang, Henan Province, China;

18. The Third Affiliated Hospital of Xinxiang Medical College (Dr. Jia-Qin Wang), Xinxiang, Henan Province, China;
19. People's Hospital of Henan Province (Dr. Li Gao), Zhengzhou, Henan Province, China;
20. The First Affiliated Hospital of Anhui Medical University (Drs. Jiu-Lai Tang, De Wu), Hefei, Anhui Province, China;
21. People's Hospital of Hainan Province (Dr. Li-Shuang Que), Haikou, Hainan Province, China;
22. Union Hospital Affiliated to Fujian Medical University (Drs. Yan-Hui Chen, Jun Hu), Fuzhou, Fujian Province, China;
23. The First Affiliated Hospital of Guangxi Medical University (Dr. Yun-Li Han), Nanning, Guangxi Province, China;
24. Guangzhou Medical Center for Women and Children (Drs. Jian-Ning Mai, Si-Da Yang), Guangzhou, Guangdong Province, China;
25. Shenzhen Children's Hospital (Drs. Bing-Rang Yang, Yan Hu, Jian-Xiang Liao), Shenzhen, Guangdong Province, China;
26. Haerbin Children's Hospital (Drs. Chun-Yu Wang, Wei Wang), Haerbin, Heilongjiang Province;
27. The First Affiliated Hospital of Jilin University (Drs. Dong Liang, Jian-Min Liang), Changchun, Jilin Province, China;
28. Children's Hospital of Jiangxi Province (Dr. Jian-Min Zhong), Nanchang, Jiangxi Province, China;
29. The Second Hospital Affiliated to Lanzhou University (Dr. Yong-Qian Chen), Lanzhou, Gansu Province, China;
30. General Hospital of Ningxia Medical University (Dr. Guang-Bo Bian), Yinchuan, Ningxia, China;
31. Qinghai Maternal and Child Health Care Hospital (Dr. Shou-Lei Wang), Xining, Qinghai Province, China;
32. Qilu Hospital of Shandong University (Drs. Bao-Min Li, Ruo-Peng Sun), Jinan, Shandong Province, China;
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All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00774/full#supplementary-material

REFERENCES

1. Kurlan R. *Handbook of Tourette"s Syndrome and Related Tic and Behavioral Disorders, 2nd Edition*. Maecel Dekker: New York, USA (2005).
2. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Fifth ed*. Washington, DC: American Psychiatric Association (2013).
3. Deeb W, Malaty IA, Mathews CA. Tourette disorder and other tic disorders. *Handb Clin Neurol* (2019) 165:123–53. doi: 10.1016/B978-0-444-64012-3.00008-3
4. Du JC, Chiu TF, Lee KM, Wu HL, Yang YC, Hsu SY, et al. Tourette syndrome in children: an updated review. *Pediatr Neonatol* (2010) 51(5):255–64. doi: 10.1016/S1875-9572(10)60050-2
5. Lebowitz ER, Motlagh MG, Katsovich L, King RA, Lombroso PJ, Grantz H, et al. Tourette syndrome in youth with and without obsessive compulsive disorder and attention deficit hyperactivity disorder. *Eur Child Adolesc Psychiatry* (2012) 21(8):451–7. doi: 10.1007/s00787-012-0278-5
6. Leckman JF. Tic disorders. *BMJ* (2012) 344:d7659. doi: 10.1136/bmj.d7659
7. Leckman JF, King RA, Bloch MH. Clinical Features of Tourette Syndrome and Tic Disorders. *J Obsessive Compuls Relat Disord* (2014) 3(4):372–9. doi: 10.1016/j.jocrd.2014.03.004
8. Liu ZS. *Tic Disorders in Children*. People's Medical Publishing House: Second Edition Beijing (2015).
9. Itard J M, Mémoire sur quelques fonctions involontaires des appareils de lalocomotion G. de la préhension et de la voix. *Arch General Méd* (1825) (8):385–407.

10. Gilles de la Tourette G. Etude sur une affection nerveuse caracterisee par de l'incoordination motrice accompagnee d'echolalie et de copralalie. *Arch Neurol* (1885) 9:158–200.
11. Muller-Vahl KR, Sambrani T, Jakubovski E. Tic disorders revisited: introduction of the term "tic spectrum disorders". *Eur Child Adolesc Psychiatry* (2019) 28(8):1129–35. doi: 10.1007/s00787-018-01272-7
12. Zheng Y, Zheng X. Current state and recent developments of child psychiatry in China. *Child Adolesc Psychiatry Ment Health* (2015) 9:10. doi: 10.1186/s13034-015-0040-0
13. Fernandez TV, State MW, Pittenger C. Tourette disorder and other tic disorders. *Handb Clin Neurol* (2018) 147:343–54. doi: 10.1016/B978-0-444-63233-3.00023-3
14. Knight T, Steeves T, Day L, Lowerison M, Jette N, Pringsheim T. Prevalence of tic disorders: a systematic review and meta-analysis. *Pediatr Neurol* (2012) 47(2):77–90. doi: 10.1016/j.pediatrneurol.2012.05.002
15. Roessner V, Rothenberger A, Rickards H, Hoekstra PJ. European clinical guidelines for Tourette syndrome and other tic disorders. *Eur Child Adolesc Psychiatry* (2011) 20(4):153–4. doi: 10.1007/s00787-011-0165-5
16. Sandor P, Carroll A. Canadian guidelines for the evidence-based treatment of tic disorders. *Can J Psychiatry* (2012) 57(3):131–2. doi: 10.1177/070674371205700301
17. Yang C, Zhang L, Zhu P, Zhu C, Guo Q. The prevalence of tic disorders for children in China: A systematic review and meta-analysis. *Med (Balt)* (2016) 95(30):e4354. doi: 10.1097/MD.0000000000004354
18. Jiang Y. Pediatric neurology in China: challenges and solutions. *Dev Med Child Neurol* (2018) 60(7):635. doi: 10.1111/dmcn.13786
19. Subspecialty Group of Neurology. the Society of Pediatrics Chinese Medical Association: [Diagnosis and treatment recommendations for tic disorders in children]. *Zhonghua Er Ke Za Zhi* (2013) 51(1):72–5. doi: 10.3760/cma.j.issn.0578-1310.2013.01.015
20. Chinese Child Neurology Society. Experts consensus of treatment and diagnosis of tic disorders in children (2017 practical version). *Chin J Appl Clin Pediatr* (2017) 32(15):1137–40. doi: 10.3760/cma.j.issn. 2095428x.2017.15.00
21. Cui YH. *Childhood Tic Disorders: Experts' Opinions*. Beijing, China: Chinese Medical Science and Technology Publishing House (2019).
22. Rong P, Ma R, Han X, Wu H. Guideline for TCM pediatrics clinical diagnosis and treatment - tic disorder (amendment). *J Pediatr TCM* (2019) 15(6):1–6. doi: 10.16840 /j.issn1673-4297.2019.06.01
23. Roessner V, Plessen KJ, Rothenberger A, Ludolph AG, Rizzo R, Skov L, et al. European clinical guidelines for Tourette syndrome and other tic disorders. Part II: pharmacological treatment. *Eur Child Adolesc Psychiatry* (2011) 20 (4):173–96. doi: 10.1007/s00787-011-0163-7
24. Verdellen C, van de Griendt J, Hartmann A, Murphy T, Group EG. European clinical guidelines for Tourette syndrome and other tic disorders. Part III: behavioural and psychosocial interventions. *Eur Child Adolesc Psychiatry* (2011) 20(4):197–207. doi: 10.1007/s00787-011-0167-3
25. Pringsheim T, Doja A, Gorman D, McKinlay D, Day L, Billinghurst L, et al. Canadian guidelines for the evidence-based treatment of tic disorders: pharmacotherapy. *Can J Psychiatry* (2012) 57(3):133–43. doi: 10.1177/070674371205700302
26. Steeves T, McKinlay BD, Gorman D, Billinghurst L, Day L, Carroll A, et al. Canadian guidelines for the evidence-based treatment of tic disorders: behavioural therapy, deep brain stimulation, and transcranial magnetic stimulation. *Can J Psychiatry* (2012) 57(3):144–51. doi: 10.1177/070674371205700303
27. Pringsheim T, Holler-Managan Y, Okun MS, Jankovic J, Piacentini J, Cavanna AE, et al. Comprehensive systematic review summary: Treatment of tics in people with Tourette syndrome and chronic tic disorders. *Neurology* (2019) 92(19):907–15. doi: 10.1212/WNL.0000000000007467
28. Pringsheim T, Okun MS, Muller-Vahl K, Martino D, Jankovic J, Cavanna AE, et al. Practice guideline recommendations summary: Treatment of tics in people with Tourette syndrome and chronic tic disorders. *Neurology* (2019) 92(19):896–906. doi: 10.1212/WNL.0000000000007466
29. Jankovic J, Kurlan R. Tourette syndrome: evolving concepts. *Mov Disord* (2011) 26(6):1149–56. doi: 10.1002/mds.23618
30. Ganos C. Tics and Tourette's: update on pathophysiology and tic control. *Curr Opin Neurol* (2016) 29(4):513–8. doi: 10.1097/WCO.0000000000000356
31. Singer HS. Tics and Tourette Syndrome. *Continuum (Minneap Minn)* (2019) 25(4):936–58. doi: 10.1212/CON.0000000000000752
32. Cath DC, Hedderly T, Ludolph AG, Stern JS, Murphy T, Hartmann A, et al. European clinical guidelines for Tourette syndrome and other tic disorders. Part I: assessment. *Eur Child Adolesc Psychiatry* (2011) 20(4):155–71. doi: 10.1007/s00787-011-0164-6
33. Baldan LC, Williams KA, Gallezot JD, Pogorelov V, Rapanelli M, Crowley M, et al. Histidine decarboxylase deficiency causes tourette syndrome: parallel findings in humans and mice. *Neuron* (2014) 81(1):77–90. doi: 10.1016/j.neuron.2013.10.052
34. Rapanelli M, Pittenger C. Histamine and histamine receptors in Tourette syndrome and other neuropsychiatric conditions. *Neuropharmacology* (2016) 106:85–90. doi: 10.1016/j.neuropharm.2015.08.019
35. Gadzicki D, Muller-Vahl KR, Heller D, Ossege S, Nothen MM, Hebebrand J, et al. Tourette syndrome is not caused by mutations in the central cannabinoid receptor (CNR1) gene. *Am J Med Genet B Neuropsychiatr Genet* (2004) 127B(1):97–103. doi: 10.1002/ajmg.b.20159
36. Muller-Vahl KR, Bindila L, Lutz B, Musshoff F, Skripuletz T, Baumgaertel C, et al. Cerebrospinal fluid endocannabinoid levels in Gilles de la Tourette syndrome. *Neuropsychopharmacology* (2020) 45(8):1323–9. doi: 10.1038/s41386-020-0671-6
37. Wang Z, Maia TV, Marsh R, Colibazzi T, Gerber A, Peterson BS. The neural circuits that generate tics in Tourette's syndrome. *Am J Psychiatry* (2011) 168(12):1326–37. doi: 10.1176/appi.ajp.2011.09111692
38. Robertson MM, Eapen V, Singer HS, Martino D, Scharf JM, Paschou P, et al. Gilles de la Tourette syndrome. *Nat Rev Dis Primers* (2017) 3:16097. doi: 10.1038/nrdp.2016.97
39. Qi Y, Zheng Y, Li Z, Liu Z, Xiong L. Genetic Studies of Tic Disorders and Tourette Syndrome. *Methods Mol Biol* (2019) 2011:547–71. doi: 10.1007/978-1-4939-9554-7_32
40. Yu D, Sul JH, Tsetsos F, Nawaz MS, Huang AY, Zelaya I, et al. Interrogating the Genetic Determinants of Tourette's Syndrome and Other Tic Disorders Through Genome-Wide Association Studies. *Am J Psychiatry* (2019) 176 (3):217–27. doi: 10.1176/appi.ajp.2018.18070857
41. Cross-Disorder Group of the Psychiatric Genomics Consortium. Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. *Cell* (2019) 179(7):1469–82 e11. doi: 10.1016/j.cell.2019.11.020
42. Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P. An international perspective on Tourette syndrome: selected findings from 3,500 individuals in 22 countries. *Dev Med Child Neurol* (2000) 42 (7):436–47. doi: 10.1017/s0012162200000839
43. Bloch MH, Leckman JF. Clinical course of Tourette syndrome. *J Psychosom Res* (2009) 67(6):497–501. doi: 10.1016/j.jpsychores.2009.09.002
44. Brandt VC, Beck C, Sajin V, Baaske MK, Baumer T, Beste C, et al. Temporal relationship between premonitory urges and tics in Gilles de la Tourette syndrome. *Cortex* (2016) 77:24–37. doi: 10.1016/j.cortex.2016.01.008
45. Cohen SC, Leckman JF, Bloch MH. Clinical assessment of Tourette syndrome and tic disorders. *Neurosci Biobehav Rev* (2013) 37(6):997–1007. doi: 10.1016/j.neubiorev.2012.11.013
46. Liu ZS. Diagnostic essentials for tics in children. *Chin J Pract Pediatr* (2012) 27(7):481–5.
47. Sambrani T, Jakubovski E, Muller-Vahl KR. New Insights into Clinical Characteristics of Gilles de la Tourette Syndrome: Findings in 1032 Patients from a Single German Center. *Front Neurosci* (2016) 10:415. doi: 10.3389/fnins.2016.00415
48. Ganos C, Ogrzal T, Schnitzler A, Munchau A. The pathophysiology of echopraxia/echolalia: relevance to Gilles de la Tourette syndrome. *Mov Disord* (2012) 27(10):1222–91. doi: 10.1002/mds.25103
49. Cavanna AE, Rickards H. The psychopathological spectrum of Gilles de la Tourette syndrome. *Neurosci Biobehav Rev* (2013) 37(6):1008–15. doi: 10.1016/j.neubiorev.2012.10.011
50. Byler DL, Chan L, Lehman E, Brown AD, Ahmad S, Berlin C. Tourette Syndrome: a general pediatrician's 35-year experience at a single center with follow-up in adulthood. *Clin Pediatr (Phila)* (2015) 54(2):138–44. doi: 10.1177/0009922814550396
51. Hirschtritt ME, Lee PC, Pauls DL, Dion Y, Grados MA, Illmann C, et al. Lifetime prevalence, age of risk, and genetic relationships of comorbid psychiatric disorders in Tourette syndrome. *JAMA Psychiatry* (2015) 72 (4):325–33. doi: 10.1001/jamapsychiatry.2014.2650

52. Modafferi S, Stornelli M, Chiarotti F, Cardona F, Bruni O. Sleep, anxiety and psychiatric symptoms in children with Tourette syndrome and tic disorders. *Eur J Paediatr Neurol* (2016) 20(5):696–703. doi: 10.1016/j.ejpn.2016.05.003
53. Hibberd C, Charman T, Bhatoa RS, Tekes S, Hedderly T, Gringras P, et al. Sleep difficulties in children with Tourette syndrome and chronic tic disorders: A systematic review of characteristics and associated factors. *Sleep* (2019) 43(6):zsz308. doi: 10.1093/sleep/zsz308
54. Szejko N, Jakubczyk A, Janik P. Prevalence and Clinical Correlates of Self-Harm Behaviors in Gilles de la Tourette Syndrome. *Front Psychiatry* (2019) 10:638. doi: 10.3389/fpsyt.2019.00638
55. Robertson MM, Cavanna AE, Eapen V. Gilles de la Tourette syndrome and disruptive behavior disorders: prevalence, associations, and explanation of the relationships. *J Neuropsychiatry Clin Neurosci* (2015) 27(1):33–41. doi: 10.1176/appi.neuropsych.13050112
56. Budman CL, Bruun RD, Park KS, Lesser M, Olson M. Explosive outbursts in children with Tourette's disorder. *J Am Acad Child Adolesc Psychiatry* (2000) 39(10):1270–6. doi: 10.1097/00004583-200010000-00014
57. Kurlan R. Clinical practice. Tourette's Syndrome. *N Engl J Med* (2010) 363 (24):2332–8. doi: 10.1056/NEJMcp1007805
58. Chen K, Budman CL, Diego Herrera L, Witkin JE, Weiss NT, Lowe TL, et al. Prevalence and clinical correlates of explosive outbursts in Tourette syndrome. *Psychiatry Res* (2013) 205(3):269–75. doi: 10.1016/j.psychres.2012.09.029
59. Liu ZS. Progress in diagnosis and treatment of Tourette syndrome comorbidities. *Chin J Child Health Care* (2010) 18(2):94–9.
60. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders IV-TR*. Fourth ed. Washington, DC: American Psychiatric Association (2000).
61. Chinese Society of Psychiatry. *The Chinese Classification and Diagnostic Criteria of Mental Disorders Version 3 (CCMD-3)*. Science and Technology Press: Jinan, Shandong (2001).
62. World Health Organization. *International classification of diseases for mortality and morbidity statistics*. (11th Revision). Geneva: World Health Organization. Available from: https://icd.who.int/browse11/l-m/en (2018).
63. Cui YH, Zheng Y, Liu HZ. Clinical features of refractory Tourette syndrome. *Shanghai Med Psychiatry* (2005) 17(1):13–7.
64. Gill CE, Kompoliti K. Clinical Features of Tourette Syndrome. *J Child Neurol* (2020) 35(2):166–74. doi: 10.1177/0883073819877335
65. Li HL, Sun JH, Du YS. Research advances in brain magnetic resonance imaging in children with tic disorders. *Chin J Pract Pediatr* (2011) 26(6):457–9.
66. Martino D, Hedderly T. Tics and stereotypies: A comparative clinical review. *Parkinsonism Relat Disord* (2019) 59:117–24. doi: 10.1016/j.parkreldis.2019.02.005
67. Liu F, Ye JP, Yao BZ, Wan AY, Wang JL. Meta -analysis of the Relationship between Tic Disorder and Streptococcal Infection in Children. *J Med Res* (2019) 48(3):124–30. doi: 10.11969/j.issn.1673-548X.2019.03.029
68. Cavanna AE, Schrag A, Morley D, Orth M, Robertson MM, Joyce E, et al. The Gilles de la Tourette syndrome-quality of life scale (GTS-QOL): development and validation. *Neurology* (2008) 71(18):1410–6. doi: 10.1212/01.wnl.0000327890.02893.61
69. Woods DW, Piacentini J, Himle MB, Chang S. Premonitory Urge for Tics Scale (PUTS): initial psychometric results and examination of the premonitory urge phenomenon in youths with Tic disorders. *J Dev Behav Pediatr* (2005) 26(6):397–403. doi: 10.1097/00004703-200512000-00001
70. Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry* (1989) 28(4):566–73. doi: 10.1097/00004583-198907000-00015
71. McGuire JF, Piacentini J, Storch EA, Murphy TK, Ricketts EJ, Woods DW, et al. A multicenter examination and strategic revisions of the Yale Global Tic Severity Scale. *Neurology* (2018) 90(19):e1711–e9. doi: 10.1212/WNL.0000000000005474
72. Sheehan DV, Sheehan KH, Shytle RD, Janavs J, Bannon Y, Rogers JE, et al. Reliability and validity of the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID). *J Clin Psychiatry* (2010) 71 (3):313–26. doi: 10.4088/JCP.09m05305whi
73. Liu YX, Liu J, Wang YF. Reliability and validity of Chinese version of the Mini International Neuropsychiatric Interview for Children and Adolescents (child version). *Chin Ment Health J* (2011) 25(1):8–13.
74. Oluwabusi OO, Parke S, Ambrosini PJ. Tourette syndrome associated with attention deficit hyperactivity disorder: The impact of tics and psychopharmacological treatment options. *World J Clin Pediatr* (2016) 5 (1):128–35. doi: 10.5409/wjcp.v5.i1.128
75. Marwitz L, Pringsheim T. Clinical Utility of Screening for Anxiety and Depression in Children with Tourette Syndrome. *J Can Acad Child Adolesc Psychiatry* (2018) 27(1):15–21.
76. Meier SM, Dalsgaard S, Mortensen PB, Leckman JF, Plessen KJ. Mortality risk in a nationwide cohort of individuals with tic disorders and with tourette syndrome. *Mov Disord* (2017) 32(4):605–9. doi: 10.1002/mds.26939
77. Cheung MY, Shahed J, Jankovic J. Malignant Tourette syndrome. *Mov Disord* (2007) 22(12):1743–50. doi: 10.1002/mds.21599
78. Muller-Vahl KR, Cath DC, Cavanna AE, Dehning S, Porta M, Robertson MM, et al. European clinical guidelines for Tourette syndrome and other tic disorders. Part IV: deep brain stimulation. *Eur Child Adolesc Psychiatry* (2011) 20(4):209–17. doi: 10.1007/s00787-011-0166-4
79. Lu DL, Su LY. Psychological assessment and psychotherapy of tic disorder in children. *Chin J Pract Pediatr* (2012) 27(7):494–9.
80. Nussey C, Pistrang N, Murphy T. How does psychoeducation help? A review of the effects of providing information about Tourette syndrome and attention-deficit/hyperactivity disorder. *Child Care Health Dev* (2013) 39 (5):617–27. doi: 10.1111/cch.12039
81. Rowe J, Yuen HK, Dure LS. Comprehensive behavioral intervention to improve occupational performance in children with Tourette disorder. *Am J Occup Ther* (2013) 67(2):194–200. doi: 10.5014/ajot.2013.007062
82. McGuire JF. Behavior Therapy for Youth With Tourette Disorder. *J Clin Psychol* (2016) 72(11):1191–9. doi: 10.1002/jclp.22398
83. Ganos C, Martino D, Pringsheim T. Tics in the Pediatric Population: Pragmatic Management. *Mov Disord Clin Pract* (2017) 4(2):160–72. doi: 10.1002/mdc3.12428
84. Shu QY, Feng JH. Treatment status of Tourette syndrome in children and adolescents. *J Clin Pediatr* (2019) 37(5):396–9. doi: 10.3969/j.issn.1000-3606.2019.05.018
85. Parraga HC, Harris KM, Parraga KL, Balen GM, Cruz C. An overview of the treatment of Tourette's disorder and tics. *J Child Adolesc Psychopharmacol* (2010) 20(4):249–62. doi: 10.1089/cap.2010.0027
86. Liu ZS, Chen YH, Zhong YQ, Zou LP, Wang H, Sun D, et al. A multicenter controlled study on aripiprazole treatment for children with Tourette syndrome in China. *Chin J Pediatr* (2011) 49(8):572–6. doi: 10.3760/cma.j.issn.0578-1310.2011.08.004
87. Yang CS, Huang H, Zhang LL, Zhu CR, Guo Q. Aripiprazole for the treatment of tic disorders in children: a systematic review and meta-analysis. *BMC Psychiatry* (2015) 15:179. doi: 10.1186/s12888-015-0504-z
88. Cai XN, He XL, Wu GF, Hu JS, Sun D, Liu ZS. Efficacy and safety of aripiprazole in the treatment of Tourette syndrome in children: a meta-analysis. *Chin J Appl Clin Pediatr* (2016) 31(18):1426–31. doi: 10.3760/cma.j.issn.2095.428x.18.01
89. Zheng Y. Drug therapy for tic disorders. *Chin J Pract Pediatr* (2012) 27 (7):491–4.
90. Yang C, Hao Z, Zhang LL, Zhu CR, Zhu P, Guo Q. Comparative Efficacy and Safety of Antipsychotic Drugs for Tic Disorders: A Systematic Review and Bayesian Network Meta-Analysis. *Pharmacopsychiatry* (2019) 52(1):7–15. doi: 10.1055/s-0043-124872
91. Lyon GJ, Samar S, Jummani R, Hirsch S, Spirgel A, Goldman R, et al. Aripiprazole in children and adolescents with Tourette's disorder: an open-label safety and tolerability study. *J Child Adolesc Psychopharmacol* (2009) 19 (6):623–33. doi: 10.1089/cap.2009.0035
92. Jiao F, Zhang X, Zhang X, Wang J. Clinical observation on treatment of Tourette syndrome in Chinese children by clonidine adhesive patch. *Eur J Paediatr Neurol* (2016) 20(1):80–4. doi: 10.1016/j.ejpn.2015.10.002
93. Wang S, Wei YZ, Yang J, Zhou Y, Zheng Y. Clonidine adhesive patch for the treatment of tic disorders: A systematic review and meta-analysis. *Eur J Paediatr Neurol* (2017) 21(4):614–20. doi: 10.1016/j.ejpn.2017.03.003
94. Guo JM, Shi XX, Yang SW, Qian QF, Huang Y, Xie YQ, et al. Efficacy of clonidine transdermal patch in treatment of moderate to severe tic in chindren disorders in children. *Chin J Contemp Pediatr* (2017) 19(7):786–9. doi: 10.7499/j.issn.1008-8830.2017.07.011
95. Hu SY, Ma R, Tian T, Zhong CL, Wei XW, Xiang XX, et al. Phase III clinical research of Changma Xifeng Tablets in treating multiple tics with liver wind

and sputum stirring internally. *Drugs Clin* (2014) 29(9):1044–9. doi: 10.7501/j.issn.1674-5515.2014.09.021
96. Pandey S, Dash D. Progress in Pharmacological and Surgical Management of Tourette Syndrome and Other Chronic Tic Disorders. *Neurologist* (2019) 24 (3):93–108. doi: 10.1097/NRL.0000000000000218
97. Yu L, Yan J, Wen F, Wang F, Liu J, Cui Y, et al. Revisiting the Efficacy and Tolerability of Topiramate for Tic Disorders: A Meta-Analysis. *J Child Adolesc Psychopharmacol* (2020) 30(5):316–25. doi: 10.1089/cap.2019.0161
98. Bloch MH, Panza KE, Landeros-Weisenberger A, Leckman JF. Meta-analysis: treatment of attention-deficit/hyperactivity disorder in children with comorbid tic disorders. *J Am Acad Child Adolesc Psychiatry* (2009) 48 (9):884–93. doi: 10.1097/CHI.0b013e3181b26e9f
99. Prasad S, Harpin V, Poole L, Zeitlin H, Jamdar S, Puvanendran K, et al. A multi-centre, randomised, open-label study of atomoxetine compared with standard current therapy in UK children and adolescents with attention-deficit/hyperactivity disorder (ADHD). *Curr Med Res Opin* (2007) 23 (2):379–94. doi: 10.1185/030079906X167309
100. Ogundele MO, Ayyash HF. Review of the evidence for the management of co-morbid Tic disorders in children and adolescents with attention deficit hyperactivity disorder. *World J Clin Pediatr* (2018) 7(1):36–42. doi: 10.5409/wjcp.v7.i1.36
101. Pliszka SR. Psychiatric comorbidities in children with attention deficit hyperactivity disorder: implications for management. *Paediatr Drugs* (2003) 5(11):741–50. doi: 10.2165/00148581-200305110-00003
102. Pringsheim T, Piacentini J. Tic-related obsessive-compulsive disorder. *J Psychiatry Neurosci* (2018) 43(6):431–2. doi: 10.1503/jpn.180086
103. Martino D, Ganos C, Pringsheim TM. Tourette Syndrome and Chronic Tic Disorders: The Clinical Spectrum Beyond Tics. *Int Rev Neurobiol* (2017) 134:1461–90. doi: 10.1016/bs.irn.2017.05.006
104. Kious BM, Jimenez-Shahed J, Shprecher DR. Treatment-refractory Tourette Syndrome. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 70:227–36. doi: 10.1016/j.pnpbp.2016.02.003
105. Ramirez-Zamora A, Giordano JJ, Gunduz A, Brown P, Sanchez JC, Foote KD, et al. Evolving Applications, Technological Challenges and Future Opportunities in Neuromodulation: Proceedings of the Fifth Annual Deep Brain Stimulation Think Tank. *Front Neurosci* (2017) 11:734:734. doi: 10.3389/fnins.2017.00734
106. Martinez-Ramirez D, Jimenez-Shahed J, Leckman JF, Porta M, Servello D, Meng FG, et al. Efficacy and Safety of Deep Brain Stimulation in Tourette Syndrome: The International Tourette Syndrome Deep Brain Stimulation Public Database and Registry. *JAMA Neurol* (2018) 75(3):353–9. doi: 10.1001/jamaneurol.2017.4317
107. Kumar A, Duda L, Mainali G, Asghar S, Byler D. A Comprehensive Review of Tourette Syndrome and Complementary Alternative Medicine. *Curr Dev Disord Rep* (2018) 5(2):95–100. doi: 10.1007/s40474-018-0137-2
108. Luan X, Zhang LJ, Li XQ, Rahman K, Zhang H, Chen HZ, et al. Compound-based Chinese medicine formula: From discovery to compatibility mechanism. *J Ethnopharmacol* (2020) 254:112687. doi: 10.1016/j.jep.2020.112687
109. Cyranoski D. Why Chinese medicine is heading for clinics around the world. *Nature* (2018) 561(7724):448–50. doi: 10.1038/d41586-018-06782-7
110. Lam WC, Lyu A, Bian Z. ICD-11: Impact on Traditional Chinese Medicine and World Healthcare Systems. *Pharmaceut Med* (2019) 33(5):373–7. doi: 10.1007/s40290-019-00295-y
111. Xu W, Zhu L, Zu L, Yan S, Zhang H, Dou D. Formulation and consideration of World Health Organization international classification of traditional medicine. *J Tradit Chin Med* (2020) 40(1):157–61.
112. Kim YH, Son CG, Ku BC, Lee HW, Lim HS, Lee MS. Herbal medicines for treating tic disorders: a systematic review of randomised controlled trials. *Chin Med* (2014) 9(1):6. doi: 10.1186/1749-8546-9-6
113. Yang C, Hao Z, Zhang LL, Guo Q. Efficacy and safety of acupuncture in children: an overview of systematic reviews. *Pediatr Res* (2015) 78(2):112–9. doi: 10.1038/pr.2015.91
114. Yu J, Ye Y, Liu J, Wang Y, Peng W, Liu Z. Acupuncture for Tourette Syndrome: A Systematic Review. *Evid Based Complement Alternat Med* (2016) 2016:1834646. doi: 10.1155/2016/1834646
115. Zhao RZ, Xin Y, Wang WX, Sun KX. Meta-analysis of clinical effect of acupuncture on tic disorder. *Shanghai J Acupuncture Moxibustion* (2020) 39: (244–252). doi: 10.13460/j.issn.1005-0957.2020.02.0244
116. Qi H, Liu R, Zheng W, Zhang L, Ungvari GS, Ng CH, et al. Efficacy and safety of traditional Chinese medicine for Tourette's syndrome: A meta-analysis of randomized controlled trials. *Asian J Psychiatr* (2020) 47:101853. doi: 10.1016/j.ajp.2019.101853
117. Zhao L, Li AY, Lv L, Liu FY, Qi FH. Traditional Chinese Medicine Ningdong Granule: the Beneficial Effects in Tourette's Disorder. *J Int Med Res* (2010) 38:169 – 75. doi: 10.1177/147323001003800119
118. Zheng Y, Zhang ZJ, Han XM, Ding Y, Chen YY, Wang XF, et al. A proprietary herbal medicine (5-Ling Granule) for Tourette syndrome: a randomized controlled trial. *J Child Psychol Psychiatry* (2016) 57(1):74–83. doi: 10.1111/jcpp.12432
119. Chen J, Leong PK, Leung HY, Chan WM, Li Z, Qiu J, et al. A Chinese Herbal Formulation, Xiao-Er-An-Shen Decoction, Attenuates Tourette Syndrome, Possibly by Reversing Abnormal Changes in Neurotransmitter Levels and Enhancing Antioxidant Status in Mouse Brain. *Front Pharmacol* (2019) 10:812. doi: 10.3389/fphar.2019.00812
120. Groth C, Skov L, Lange T, Debes NM. Predictors of the Clinical Course of Tourette Syndrome: A Longitudinal Study. *J Child Neurol* (2019) 34 (14):913–21. doi: 10.1177/0883073819867245
121. Hassan N, Cavanna AE. The prognosis of Tourette syndrome: implications for clinical practice. *Funct Neurol* (2012) 27(1):23–7.
122. Evans J, Seri S, Cavanna AE. The effects of Gilles de la Tourette syndrome and other chronic tic disorders on quality of life across the lifespan: a systematic review. *Eur Child Adolesc Psychiatry* (2016) 25(9):939–48. doi: 10.1007/s00787-016-0823-8
123. Bernard BA, Stebbins GT, Siegel S, Schultz TM, Hays C, Morrissey MJ, et al. Determinants of quality of life in children with Gilles de la Tourette syndrome. *Mov Disord* (2009) 24(7):1070–3. doi: 10.1002/mds.22487
124. Cavanna AE, David K, Orth M, Robertson MM. Predictors during childhood of future health-related quality of life in adults with Gilles de la Tourette syndrome. *Eur J Paediatr Neurol* (2012) 16(6):605–12. doi: 10.1016/j.ejpn.2012.02.004
125. Cavanna AE, Luoni C, Selvini C, Blangiardo R, Eddy CM, Silvestri PR, et al. Disease-specific quality of life in young patients with tourette syndrome. *Pediatr Neurol* (2013) 48(2):111–4. doi: 10.1016/j.pediatrneurol.2012.10.006

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A Rare Novel *CLCN2* Variation and Risk of Gilles de la Tourette Syndrome: Whole-Exome Sequencing in a Multiplex Family and a Follow-Up Study in a Chinese Population

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Rare inherited variations in multiplex families with Gilles de la Tourette syndrome (GTS) are suggested to play an important role in the genetic etiology of GTS. In order to explore the rare inherited variations with the risk of GTS, whole-exome sequencing (WES) was performed in a family with three affected patients with GTS. Among the five novel rare variations identified by WES, *CLCN2* G161S was presented in three patients, but not in four unaffected individuals, and thus co-segregated with GTS. A validation study was also performed in a cohort of Chinses Han population to further examine the identified rare variants. *CLCN2* G161S was genotyped in 207 sporadic patients with tic disorder including 111 patients with GTS and 489 healthy controls. Compared with that in controls [allele frequency (AF) = 0], *CLCN2* G161S had higher variant AF in patients with tic (AF = 0.00483) and in patients with GTS (0.00900), respectively. However, this variant was absent from the current 1000 Genome databases, and the variant AF is very low in the current public databases including ExAC (AF = 0.00001) and gnomAD (AF = 0.00003). Our results suggest that *CLCN2* G161S might play a major role in the genetic etiology of GTS, at least in a Chinese Han population.

Keywords: Gilles de la Tourette syndrome, whole-exome sequencing, CLCN2, rare variation, multiplex family

INTRODUCTION

Gilles de la Tourette syndrome (GTS), also known as Tourette's syndrome (TS) or Tourette's disorder, is a common, heritable neurological disorder manifested by chronic motor and vocal tics that persist for more than 1 year with childhood onset. The global prevalence of GTS ranges between 0.3 and 1% (1).

GTS is a complex disorder, and genetics plays a critical role in the pathogenesis of this disorder. From family studies, it has been determined that first-degree relatives

of affected individuals are at 5–15-fold increased risk of GTS compared to the general population (2). In twin studies, the heritability has been estimated at 70–80%, which is one of the highest heritability for a neuropsychiatric disorder (3). Association studies and linkage analyses of GTS conducted to date have led to identification of some risk genes relevant to neuronal outgrowth and neurotransmitter systems, such as genes encoding SLITRK1 (Slit and Trk-like 1), a member of a neuronal transmembrane protein family, serotonin receptors, and dopamine receptors, none of which have been consistently replicated (4). With the advent of the next-generation sequencing technology, rare variant studies using whole-exome sequencing (WES) might be useful for identifying susceptibility genes in complex neuropsychiatric diseases (5). A recent GTS study of *de novo* variation using WES found 25 *de novo* coding variants in 45 samples from 15 trios (6). A study examining rare inherited variants in multiplex families with GTS identified a rare nonsense mutation in *PNKD* that co-segregated with the phenotype of the disorder, which resulted in reduced expression of *PNKD* in neurons derived from individuals with GTS. Another GTS study of rare inherited variants identified three novel rare variations in *MRPL3*, *DNAJC13*, and *OFCC1* that segregated with chronic tic disorder phenotype in a three-generation pedigree with seven family members showing GTS symptoms (7).

In order to further investigate the potential rare variants with large effect sizes in patients with GTS, WES was performed on the individuals of a multiplex family with GTS. Furthermore, it is also critical to confirm whether the candidate risk variants in a multiplex family identified by WES are involved in the genetic etiology of the disease using independent samples. Therefore, a follow-up validation study was performed in a Chinese Han population.

MATERIALS AND METHODS

Participants

In the multiplex family (named family 1#, **Figure 1A**), the proband was a boy. He, his monozygotic twin brother, and his father were diagnosed with GTS (**Figure 1A**: IV-1, IV-2, and III-4). The proband's grandmother had been diagnosed with tic disorder (**Figure 1A**: II-1). The diagnosis of tic or GTS (as a subtype of tic disorder) was made by an experienced deputy chief psychiatrist according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, revision version (DSM-IV-TR) criteria made by the American Psychiatric Association (APA). The MINI-International Neuropsychiatric Interview (MINI), a semi-structured interview questionnaire, was conducted to meet the demand for a short but accurate structured psychiatric interview. The proband's mother, aunt, and uncles were confirmed as unaffected by an experienced psychiatrist using an unstructured interview (**Figure 1A**: III-5, III-1, III-2, and III-3). We were unable to obtain blood samples from several family members including the proband's grandmother and his grandmother's brother. Their current mental status was designated as unknown and needs to be confirmed through the formal interview, although the proband's father said his mother (the proband's grandmother) had only a few symptoms such as blinks and shrugs and the grandmother recalled that she often had loud throat vocalization. Rediagnosis of the proband's grandmother and diagnosis of the grandmother's brother were hindered by living in a remote rural location in China.

In the follow-up validation study, the cohort consisted of 207 patients with tic disorder (170 males and 37 females, mean age 10.6 [SD 3.6] years), including 111 patients with GTS (91 males and 20 females, mean age 11.4 [SD 3.2] years), and 489 healthy control individuals (296 males and 193 females, mean age 16.0 [SD 9.7] years) (**Table 1**). The patients and control groups were not sex or age matched. All patients with tics were subjected to psychiatric assessment. Specifically, patients were diagnosed according to DSM-IV-TR criteria by APA for tic disorder or GTS. Healthy controls were provided by the Women and Children's Hospital of Xiamen University and were assessed with a self-edited questionnaire in order to exclude psychiatric disorders and major organic diseases. Subjects were excluded if there were three generations of family members with mental illness.

This study was approved by the Ethics Committee of the Children's Hospital of Fudan University. Written informed consent was obtained from all participants and/or their families. All participants were of Han Chinese descent.

WES Study

The entire sequencing procedure was conducted. In brief, 3 μg of DNA extracted from peripheral blood samples was sheared through a Covaris M220 Ultrasonicator (Covaris, Woburn, MA, USA) to create 150–200-bp fragments. The adapter-ligated library was prepared with a SureSelectXT Library Prep Kit, and the capture library was constructed using a SureSelectXT Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA, USA) to enrich the coding exons and flanking intronic regions. Clusters were generated via isothermal bridge amplification and sequenced on the HiSeq 2000 system (Illumina, San Diego, CA, USA). Quality assessment of base calling and sequence reads was performed using the Illumina Sequence Control Software v4.0.4 with Real-Time Analysis. NextGENe® v2.4.1 software (SoftGenetics, LLC, State College, PA, USA) was used to align the sequence reads with the reference human genome (Human GRCh 37.3, http://hgdownload.soe.ucsc.edu/goldenPath/hg19/snp135Mask/). All acquired single-nucleotide variants (SNVs) and indels were saved in VCF file format and uploaded on Knowledge-Driven NGS Analysis-TGex™ (LifeMap Sciences, Inc., Alameda, CA, USA) for detailed filtering and interpretation.

To prioritize variations, we applied numerous filtering steps to variations in WES (**Table 2**). First, we filtered out variations with <20X coverage. Further, we filtered out variations whose population frequency was <0.1% in the gnomAD_EAS. Finally, the likely pathogenic (LP) and pathogenic (P) variations were selected according to the scoring criteria of the American College of Medical Genetics and Genomics (ACMG).

To assess co-segregation of prioritized variations, Sanger sequencing was performed for the individuals of the multiplex family including the proband's aunt and two uncles. PCR was carried out on a T100 Thermal Cycler (Bio-Rad, Inc. Hercules, CA, USA), and the resulting products were sequenced using the

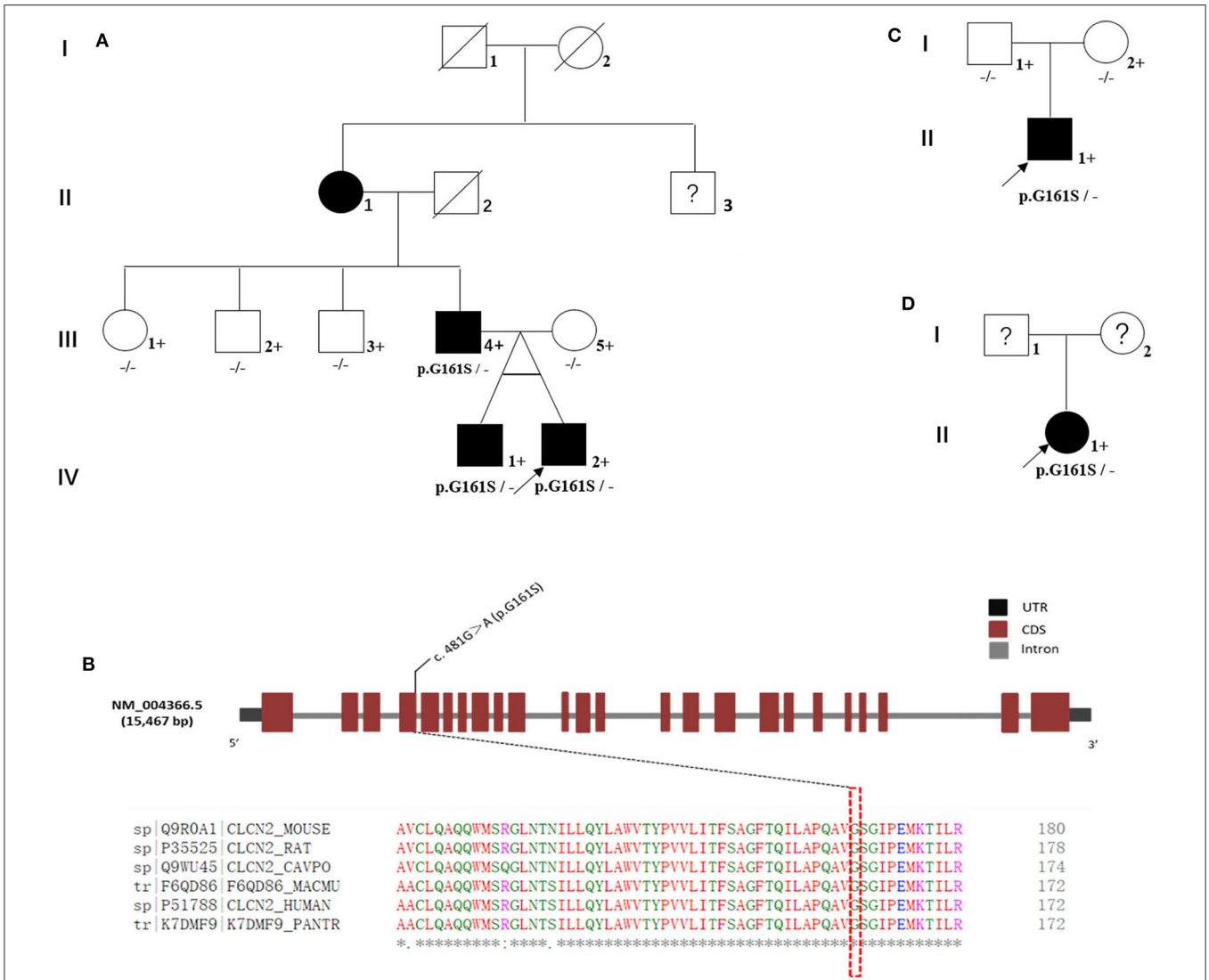

FIGURE 1 | Familial cohorts with Tourette syndrome. **(A)** Family 1#: a multiplex family with Tourette syndrome; three affected individuals were G161S heterozygous (III-4, IV-1, and IV-2). **(B)** *CLCN2* G161S is located in highly conserved regions in multiple species. **(C)** Family 2#: the biological parents of the proband carried the wild-type allele. *CLCN2* G161S was a *de novo* mutation in this family. **(D)** In family 3#, the biological parents of the proband could not be reviewed, and the DNA samples were unavailable. Whether *CLCN2* G161S was a *de novo* mutation in this family remained undetermined. Black-shaded symbols represent GTS-affected individual, unfilled symbols represent unaffected individuals, and question marks represent individuals with unknown disease status. Crosses represent individuals with available genomic DNA samples. Proband is marked with an arrow.

ABI 3130xl sequencer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with primers. The sequencing data were analyzed using Mutation Surveyor DNA Variant Analysis software v4.0 (SoftGenetics, LLC, State College, PA, USA). Primer sequences and detailed information of amplification conditions are available upon request.

Validation Study

To determine whether the rare splicing *CLCN2* variation prioritized in WES of the multiplex family contributed to the genetic etiology of GTS, *CLCN2* G161S was verified using an independent population comprising 207 sporadic patients with tic disorder and 489 healthy controls from the Women and Children's Hospital of Xiamen University. *CLCN2* G161S was genotyped using MALDI-TOF mass spectrometry (MS), as previously described (8). The *CLCN2* primers used were as follows: 5′-ACGTTGGATGATCACTTTCTCAGCCGGATTC-3′ (forward) and 5′-ACGTTGGATGATTCGATGCACCCATTTCAGG-3′ (reverse). To remove the remaining dNTPs in the PCRs, we performed shrimp alkaline phosphatase treatment followed by primer extension. The sequence for the extension primer was *CLCN2*_U: CCTGCCCCTCAGGCTGTC. The extension products were desalted for MS analysis, as previously described.

TABLE 1 | Demographics of participants and clinical evaluation of patients.

Characteristics	TD (*N* = 207)	GTS (*N* = 111)	HC (*N* = 489)
Age (years: mean ± SD)	10.6 (3.6)	11.4 (3.2)	16.0 (9.7)
Male gender (*n*, %)	170 (82.1)	91 (81.9)	296 (60.5)
YGTSS score			
Motor tic severity	14.2 (4.7)[a]	11.1 (7.5)[b]	/
Vocal tic severity	8.4 (7.0)[a]	10.2 (6.7)[b]	/
Total tic severity score	22.6 (9.5)[a]	25.1 (9.1)[b]	/
Functional impairment	24.1 (12.7)[a]	27.0 (13.5)[b]	/
Total YGTSS score	46.4 (19.3)[a]	51.8 (19.1)[b]	/

TD, tic disorder; GTS, Gilles de la Tourette Syndrome; HC, healthy controls; YGTSS, Yale Global Tic Severity Scale.
[a]*Data available for 147 TD subjects.*
[b]*Data available for 83 GTS subjects.*

TABLE 2 | Filtering steps to variations identified by WES for the multiplex GTS family.

Filtering criteria	Number of remaining variations
Total variations identified	30,534
Covered with ≥20 reads	23,658
Frequency in gnomAD_EAS ≤ 0.1%	1,764
LP/P according to the ACMG	5

WES, whole-exome sequencing; GTS, Gilles de la Tourette Syndrome; LP, likely pathogenic; P, pathogenic; ACMG, American College of Medical Genetics and Genomics.

Data acquisition from the SpectroCHIP was performed using Bruker Compact MALDI-TOF MS (Bruker Daltonics), and data analyses were carried out with the TyperAnalyzer application, version 4.0 (Sequenom). All primers were purchased from Integrated DNA Technologies. All other reagents were purchased from Sequenom.

Deviations from the Hardy–Weinberg equilibrium were tested using the χ^2 test for goodness of fit. Allelic association of variants was tested using Fisher's exact test. Allele frequencies (AFs) were estimated in all samples using the SHEsisPlus program [http://shesisplus.bio-x.cn/SHEsis.html (9)].

RESULTS

WES was performed for a multiplex family (family 1#) including the proband, his monozygotic twin brother, and the proband's father and mother and captured almost all regions (59.04 Mb) in each exome (**Supplementary Table 1**). The average read depth varied from 108.48X to 123.00X, and 94.10 to 96.00% of target regions were covered by 20 or more reads. A total of 30,534 sequence variations were called from WES in the multiplex family (**Table 2**). After several filtering steps, we prioritized four rare missense variations (*C2CD2L* R497W, *MSH4* E379K, *TULP4* S1451I, and *UTP4* R634W) and a rare splicing variation (*CLCN2* G161S). These variations were confirmed by Sanger sequencing. These five rare variants were predicted to be "probably damaging" by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant from Tolerant (SIFT: http://sift.bii.a-star.edu.sg/index.html). Among these five variants, *CLCN2* G161S was identified in three affected individuals (**Figure 1A**: III-4, IV-1, and IV-2), but not in one unaffected individual (**Figure 1A**: III-5; **Figure 1B**), which was verified by Sanger sequencing (**Supplementary Figure 1**). In contrast, the other four variations were present in both affected and unaffected individuals (**Table 3**). In order to investigate whether *CLCN2* G161S co-segregates with the phenotype of GTS, *CLCN2* G161S was examined in three unaffected relatives including the proband's aunt and two uncles by Sanger sequencing. The results showed that the proband's aunt and uncles carried the wild-type allele. Therefore, *CLCN2* was considered to be the most promising candidate gene for GTS.

Subsequently, a validation study was performed to assess the association of *CLCN2* G161S with tic disorder in an independent population. We genotyped *CLCN2* G161S in 489 healthy controls and 207 sporadic patients with tic disorders including 111 GTS patients. In sporadic patients, a heterozygous *CLCN2* G161S was identified in a male patient with GTS and attention deficit hyperactivity disorder (ADHD) and a female patient with GTS and obsessive–compulsive disorder (OCD), and the 489 controls all carried the wild-type allele (**Figure 2**). Compared with that in controls (AF = 0), *CLCN2* G161S had a higher variant AF in patients with tic (AF = 0.00483) and in patients with GTS (AF = 0.00900). No deviation from the Hardy–Weinberg equilibrium was found in genotype distribution of the variant. No significant association was detected between *CLCN2* G161S and tic disorder ($P = 0.029$, adjusted $P = 0.999$) and *CLCN2* G161S and GTS ($P = 0.003$, adjusted $P = 0.996$) after age and sex correction (**Table 4**).

In addition, in order to identify whether the *CLCN2* G161S carried by the two sporadic GTS patients was inherited from their parents, the proband's parents (family 2#) were genotyped using Sanger sequencing and were found to carry the wild-type allele (**Figure 1C**). Therefore, *CLCN2* G161S was a *de novo* mutation in family 2#. In family 3#, the biological parents of the proband could not be reviewed, their disease status was unknown, and DNA samples were unavailable (**Figure 1D**). Therefore, whether *CLCN2* G161S was a *de novo* mutation in family 3# remained undetermined.

The following are the clinical descriptions of the proband and his monozygotic twin brother with GTS in family 1#. The proband was a 12-year-old boy and was a younger monozygotic twin brother. He had many severe symptoms for more than 3 years including blinking, shaking head, shrugging, plucking up the abdomen, and shrinking the nose repeatedly, accompanied by repeated throat voice for more than 1 year. The total score of the Yale Global Tic Severity Scale (YGTSS) was 64, which indicated that the severity of his tics was high. Brain structure examination was performed using a 3.0-T magnetic resonance imaging (MRI) instrument, which showed a normal brain structure. The results of electroencephalogram (EEG) examination and biochemical and immunological examinations of peripheral blood were also normal (**Table 5**).

The elder monozygotic twin brother developed tic symptoms earlier than the proband, and his age of onset was 5 years

TABLE 3 | Rare variation prioritized by WES for the GTS multiplex family.

Chromosome	Position[a]	Gene	Protein	Allele[b]	PolyPhen-2/SIFT	Family member (available DNA sample)		Transmission
						Affected ($n = 3$)	Unaffected ($n = 4$)	
3	1.84E+08	*CLCN2*	G161S	C/T	Damaging	3	0	Father
11	1.19E+08	*C2CD2L*	R497W	C/T	Damaging	2	2	Mother
1	76,288,239	*MSH4*	E379K	G/A	Damaging	1	1	/
6	1.59E+08	*TULP4*	S1451I	G/T	Damaging	1	1	/
16	69,201,044	*UTP4*	R634W	C/T	Damaging	2	1	/

WES, whole-exome sequencing; GTS, Gilles de la Tourette syndrome; SIFT, Sorting Intolerant from Tolerant.
[a] *Position according to GRCh 37.*
[b] *Referenced/mutation allele.*

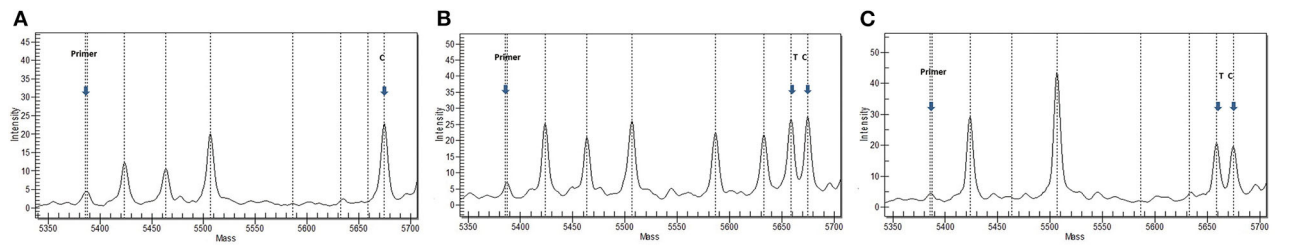

FIGURE 2 | A representative mass spectrum of the screening sample. **(A)** Wild-type mass spectrum for a patient with GTS. **(B)** Mutation mass spectrum for a patient with GTS. **(C)** Mutation mass spectrum for a patient with GTS. Primer, unextended primer; T, extension products for the mutant sequence; C, extension products for the wild-type sequence C.

old according to his mother's recollection. The first episode of symptoms included repeated blinking of the eyes. After 6 months of treatment, the symptoms improved. However, 2 years later, the blink symptoms recurred, and other tic symptoms also occurred intermittently, such as throat vocalization, sniffing, and sound from the nose. Although his age at onset was earlier than that of the proband, he still had a variety of tic symptoms, which were relatively less severe than that of the proband. The total score of YGTSS was 40, which indicated that his tic severity was moderate. His mother was more concerned about his younger brother's tics.

The proband of family 2# was an 11-year-old boy. He had a variety of twitching symptoms since the first grade of primary school, such as shrugging his shoulders, grinning, making voices in his throat, and liking to spit. In addition to twitching symptoms, he exhibited inattention symptoms in class and procrastination toward his homework. He was also diagnosed with ADHD. His total YGTSS score was 56, indicating a severe level (**Table 5**).

The proband of family 3# was a 12-year-old girl. She had a history of tics for more than 3 years. She twisted her neck, shook her limbs, blinked repeatedly, and smacked her lips back and forth to make a loud voice. Now she often blinks and shrugs repeatedly. The total YGTSS score was 40, indicating a moderate level. In addition, she had some OCD symptoms, such as writing repeatedly and modifying and counting numbers repeatedly although that was not what she wanted to do (**Table 5**).

DISCUSSION

In the present study, five rare variations were identified by WES in a multiplex family with GTS. Among these variations, only *CLCN2* G161S co-segregated with the phenotypes of GTS and was inherited from the proband's father. In the follow-up validation study, *CLCN2* G161S was identified in two sporadic patients with GTS. In family 2#, the unaffected parents of the proband carried the wild-type allele; therefore, *CLCN2* G161S was a *de novo* mutation in the family. In family 3#, whether *CLCN2* G161S was an inherited or a *de novo* mutation was unknown due to the unavailability of the sample of the proband's parents. In addition, our follow-up validation study showed that *CLCN2* G161S had a higher variant AF in sporadic patients with GTS (AF = 0.004) than in the controls (AF = 0). Interestingly, *CLCN2* G161S was absent from the current 1000 Genome databases, and the variant AF was very low in the current public databases including ExAC (AF = 0.00001) and gnomAD (AF = 0.00003). We screened the *CLCN2* gene for its relevance to neurodevelopmental disorders and found that *CLCN2* has been strongly implicated in neurological disorders.

Certain homozygous or heterozygous *CLCN2* mutations can cause two distinct phenotypes: leukoencephalopathy with ataxia (*CLCN2*; MIM#615651) and idiopathic generalized epilepsy (*CLCN2*; MIM#607628) including juvenile myoclonic epilepsy and juvenile absence epilepsy. For example, a homozygous 6-bp in-frame deletion (c.430_435del) was found in a woman

TABLE 4 | Genotyping of *CLCN2* G161S in the follow-up study.

Sample	Variation	Patient				Control				*P*	*P*[a]	OR	95% CI
		C/T	C/C	T/T	MAF	C/T	C/C	T/T	MAF				
Patients with tic ($n = 207$)	*CLCN2* G161S	2	205	0	0	0	489	0	0	0.03	1	-	-
Patients with GTS ($n = 111$)	*CLCN2* G161S	2	109	0	0.01	0	489	0	0	0	1	-	-

WES, whole-exome sequencing; MAF: mutant allele frequency; OR, odds ratio.
[a]*P-values are adjusted for age and sex.*

TABLE 5 | Clinical phenotypes of the five individuals heterozygous for *CLCN2* G161S.

Clinical phenotype	Family 1[#]			Family 2[#]	Family 3[#]
	Father	Younger monozygotic twin brother	Elder monozygotic twin brother	Proband	Proband
Transmission		Paternal	Paternal		
Sex	Male	Male	Male	Male	Female
Age	45Y	12Y3M	12Y3M	11Y4M	12Y
DSM-IV-TR diagnosis	GTS	GTS	GTS	GTS	GTS
Comorbidity (mental disorder)	No	No	No	ADHD	OCD
Illness duration	26Y	5Y	7Y	4Y	3Y
YGTSS score					
Motor tic severity	5	15	5	15	15
Vocal tic severity	6	19	15	11	5
Functional impairment	10	30	20	30	20
Total YGTSS scores	21	64	40	56	40
Past history	Nasal sinusitis	Hernia	No	No	Language and motor retardation
Brain MRI/EEG	Null	Normal	Normal	Normal	Normal
Laboratory tests					
Rheumatoid factor (RF, IU/ml)	Null	Null	Null	<8.88	<10.1
Antistreptolysin O (anti-O, IU/ml)	Null	<13.1	Null	<13.2	244↑
Erythrocyte sedimentation rate (ESR, mm/h)	Null	7	Null	27↑	7
Blood ceruloplasmin (g/L)	Null	0.223	Null	0.25	0.19↓

GTS, Gilles de la Tourette syndrome; ADHD, attention deficit/hyperactivity disorder; OCD, obsessive–compulsive disorders; YGTSS, Yale Global Tic Severity Scale; MRI, magnetic resonance imaging; EEG, electroencephalogram; Null, no information or unfinished; Y, years; M, months.

from North Africa, and a homozygous 6-bp in-frame deletion (c.430_435del) and heterozygous R235Q and R577Q were identified in a sib of Tunisian origin with juvenile myoclonic epilepsy and in two German sibs with idiopathic generalized epilepsy (10, 11). Interestingly, a recent study showed that *CLCN2* G161S, as a *de novo* mutation, was found in three patients with childhood absence epilepsy (12). In this study, we identified the heterozygous *CLCN2* G161S variant in a multiplex family with GTS (family 1[#]). In this family, the variant carried by monozygotic twin brothers was inherited from their father. In family 2[#], the *CLCN2* G161S variant, as a *de novo* mutation, was found in the patient but not his unaffected parents. Both patients with epilepsy and GTS had an early onset age. Therefore, we speculated that *CLCN2* G161S might cause a neurodevelopmental dysfunction underlying epilepsy and GTS. However, its functional implications remain to be clarified.

CLCN2 encodes chloride channel 2 (CLC-2), which is a type of permeable chloride channel that belongs to the family of *CLCN2* channel/transport proteins (13). *CLCN2* is expressed in glia precursors during development and is required for their differentiation into astrocytes and oligodendrocytes (14, 15). Mutations in *CLCN2* are responsible for leukoencephalopathy with ataxia (16). Several lines of evidence suggest that oligodendrocyte dysfunction and white matter disconnection are involved in the pathophysiology of GTS (17, 18). Compared to healthy control, fractional anisotropy decreases and radial diffusivity increases in deep white matter tracts in cortico-striato-thalamo-cortical circuit as well as superficial white matter in GTS children; furthermore, lower fractional anisotropy values and higher radial diffusivity values in white matter regions are correlated with more severe tics (19). Taking these findings into account, we speculate that *CLCN2* G161S might elicit abnormal

signal conduction by damaged myelin across different brain regions, which results in GTS development.

There were some limitations in our study. First, we prioritized the rare variations that were predicted to be "probably damaging" by PolyPhen-2 and SIFT. Thus, other rare variations might have been overlooked. Second, WES can detect exon variants but not promoter variants, which could contribute to GTS. Third, blood samples from the proband's grandmother were not available. As described by the proband's father, the proband's 82-year-old grandmother also showed symptoms of tic disorder; however, we did not obtain her peripheral blood sample for WES verification and needed face-to-face interviews to obtain more information supporting the GTS diagnosis or just tic disorder.

In conclusion, our study suggests that *CLCN2* G161S might play a major role in the genetic etiology of GTS, at least in the Chinese Han population.

DATA AVAILABILITY STATEMENT

According to national legislation/guidelines, specifically the Administrative Regulation of the People's Republic of China on Human Genetic Resources (https://www.gov.cn/zhengce/content/2019-06/10/content_5398829.html, https://english.www.gov.cn/policies/latest_releases/2019/06/10/content_281476708945462.html), no additional raw data is available at this time. Data of this project can be accessed after an approval application to the China National Genebank (CNGB, https://db.cngb.org/cnsa/). Please refer to https://db.cngb.org/, or email: CNGBdb@cngb.org for detailed application guidance. The accession code CNP0001372 should be included in the application.

ETHICS STATEMENT

Written informed consent has been obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JS, AY, and ZW contributed to the conception and design of the study. AY and ZW organized the database. AY and JS performed the statistical analysis. AY wrote the first draft of the manuscript. JS and ZW revised the manuscript. QD, WX, JH, and YZ were charged with the collection of clinical data and psychological assessment. All authors contributed to manuscript revision and read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.543911/full#supplementary-material

Supplementary Figure 1 | Confirmation of the missense mutation in family 1# by Sanger sequencing. III-5 is G/G; III-4, IV-1 and IV-2 are G/A heterozygote. The red dotted line shows the difference.

REFERENCES

1. Robertson MM. A personal 35 year perspective on Gilles de la Tourette syndrome: prevalence, phenomenology, comorbidities, and coexistent psychopathologies. *Lancet Psychiatry*. (2015) 2:68–87. doi: 10.1016/S2215-0366(14)00132-1
2. Scharf JM, Pauls DL. Genetics of Tic Disorders. In: Rimoin, DL, Connor, JM, Pyeritz RE, Korf BR, editors. *Emery and Rimoin's Principles and Practices of Medical Genetics*. 5th edn. Churchill Livingstone/Elsevier; Philadelphia (2007). p. 2737–54.
3. Mataix-Cols D, Isomura K, Pérez-Vigil A, Chang Z, Rück C, Larsson KJ, et al. Familial risks of tourette syndrome and chronic tic disorders a population-based cohort study. *JAMA Psychiatr.* (2015) 72:787–93. doi: 10.1001/jamapsychiatry.2015.0627
4. Qi Y, Zheng Y, Li Z, Liu Z, Xiong L. Genetic studies of tic disorders and tourette syndrome. *Methods Mol Biol.* (2019) 2011:547–71. doi: 10.1007/978-1-4939-9554-7_32
5. Egawa J, Hoya S, Watanabe Y, Nunokawa A, Shibuya, Ikeda M, et al. Rare UNC13B variations and risk of schizophrenia: whole-exome sequencing in a multiplex family and follow-up resequencing and a case-control study. *Am J Med Genet B Neuropsychiatr Genet*. (2016) 171:797–805. doi: 10.1002/ajmg.b.32444
6. Zhao X, Wang S, Hao J, Zhu P, Zhang X, Wu M. A whole-exome sequencing study of tourette disorder in a chinese population. *DNA Cell Biol*. (2020) 16:1–6. doi: 10.1089/dna.2019.4746
7. Sundaram SK, Huq AM, Sun Z, Bennett L, Wilson BJ, Behen ME, et al. Exome sequencing of a pedigree with tourette syndrome or chronic tic disorder. *Ann Neurol.* (2011) 69:901–4. doi: 10.1002/ana.22398
8. Luan J, Yuan J, Li X, Jin S, Yu L, Liao M, et al. Multiplex detection of 60 hepatitis B virus variants by MALDI-TOF mass spectrometry. *Clin Chem.* (2009) 55:1503–9. doi: 10.1373/clinchem.2009.124859
9. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. (2005) 15:97–8. doi: 10.1038/sj.cr.7290272

10. Saint-Martin C, Gauvain G, Teodorescu G, Gourfinkel-An I, Fedirko E, Weber YG, et al. Two novel CLCN2 mutations accelerating chloride channel deactivation are associated with idiopathic generalized epilepsy. *Hum Mutat*. (2009) 30:397–405. doi: 10.1002/humu.20876
11. Depienne C, Bugiani M, Dupuits C, Galanaud D, Touitou V, Postma N, et al. Brain white matter oedema due to ClC-2 chloride channel deficiency: an observational analytical study. *Lancet Neurol*. (2013) 12:659–68. doi: 10.1016/S1474-4422(13)70053-X
12. Xie H, Su W, Pei J, Zhang Y, Gao K, Li J, et al. *De novo* SCN1A, SCN8A, and CLCN2 mutations in childhood absence epilepsy. *Epilepsy Res*. (2019)154:55–61. doi: 10.1016/j.eplepsyres.2019.04.005
13. Wang H, Xu M, Kong Q, Sun P, Yan F, Tian W, et al. Research and progress on ClC2. *Mol Med Rep*. (2017) 16:11–22. doi: 10.3892/mmr.2017.6600
14. Makara JK, Rappert A, Matthias K, Steinhäuser C, Spät A, Kettenmann H. Astrocytes from mouse brain slices express ClC-2-mediated Cl- currents regulated during development and after injury. *Mol Cell Neurosci*. (2003) 23:521–30. doi: 10.1016/S1044-7431(03)00080-0
15. Sasieta HP, Zhu Q, Gaitán-Peñas H, Rios M, Estévez R, Morey M. Drosophila ClC-a is required in glia of the stem cell niche for proper neurogenesis and wiring of neural circuits. *Glia*. (2019) 67:2374–98. doi: 10.1002/glia.23691
16. Guo Z, Lu T, Peng L, Cheng H, Peng F, Li J, et al. CLCN2-related leukoencephalopathy: a case report and review of the literature. *BMC Neurol*. (2019) 19:156. doi: 10.1186/s12883-019-1390-7
17. Sigurdsson HP, Pépés SE, Jackson GM, Draper A, Morgan PS, Jackson SR. Alterations in the microstructure of white matter in children and adolescents with Tourette syndrome measured using tract-based spatial statistics and probabilistic tractography. *Cortex*. (2018) 104:75–89. doi: 10.1016/j.cortex.2018.04.004
18. Wen H, Liu Y, Wang J, Rekik I, Zhang J, Zhang Y, et al. Combining tract- and atlas-based analysis reveals microstructural abnormalities in early tourette syndrome children. *Hum Brain Mapp*. (2016) 37:1903–19. doi: 10.1002/hbm.23146

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Frequency-Specific Regional Homogeneity Alterations in Tourette Syndrome

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Tourette syndrome (TS) is a developmental neuropsychiatric disorder with onset during childhood. Because of its complex spectrum of phenotypes, the underlying pathophysiology of TS is still unclear. Resting-state functional magnetic resonance imaging demonstrated aberrant spontaneous neural synchronization in conventional frequency band (0.01–0.08 Hz) in TS. No published studies have reported abnormalities of local synchronization across different frequency bands. We estimated the alterations of local synchronization across five bands ranging from 0 to 0.25 Hz. Seventy-nine children with TS and 63 age-, sex-, and handedness-matched healthy children were recruited. Frequency-specific regional homogeneity (ReHo) and independent component analysis were used to identify functional alterations between TS and healthy children. TS patients showed significantly increased ReHo in the left precentral gyrus and decreased ReHo in the right operculum. Abnormal ReHo alterations of the superior frontal gyrus, superior parietal gyrus, anterior cingulate gyrus, putamen, superior temporal gyrus, and operculum were observed in different frequency bands. TS patients showed increased connectivity of the right superior frontal gyrus within the left executive control network. In addition, a significantly negative correlation was found between Yale Global Tic Severity Scale (YGTSS) vocal score and ReHo values of the right operculum in the highest frequency bands (0.198–0.25 Hz), while a significant positive correlation was found between YGTSS motor score and altered connectivity of the right superior frontal gyrus. The present study revealed frequency-specific abnormal alterations of ReHo in the whole brain and altered connectivity within the executive control network of TS children. Its neural importance and clinical practicability require further investigation.

Keywords: Tourette syndrome, resting-state functional MRI, regional homogeneity, frequency-specific, network

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INTRODUCTION

Tourette syndrome (TS) is a developmental neuropsychiatric disorder with an onset during childhood, defined by the co-occurrence of motor and phonic tics lasting at least 12 months. Tics typically show a waxing and waning pattern of severity, intensity, and frequency (1). The prevalence of TS for children in China is 1.7% (2). Previous studies showed that ∼90% of TS

children have comorbid conditions, including obsessive–compulsive disorder (OCD), attention-deficit hyperactivity disorder (ADHD), rage attacks, sleep disturbances, and depressive symptoms (3), which might have a negative impact on their quality of life (4). Moreover, the underlying pathophysiology of TS is controversial and poorly understood because of the complex spectrum of phenotypes involved in TS (5).

Advanced neuroimaging methods have been used to investigate the neural basis of TS over the last few decades. Many neuroimaging studies using structural magnetic resonance imaging (MRI), positron emission tomography (PET), and diffusion tensor imaging techniques have reported abnormalities in the basal ganglia and associated thalamic and frontal cortical regions (6), supporting a hypothesis of dysfunction in the cortico-striato-thalamo-cortical (CSTC) networks in TS (7). A recent meta-analysis of task-based neuroimaging studies showed that TS patients had abnormal activation in the prefrontal, anterior cingulate, motor preparation cortices, and sensory and temporo-parietal association cortices (8). A previous study using functional MRI (fMRI) showed abnormal functional connections in the executive control network, especially in the fronto-parietal network in TS patients (9). This suggested that the broadly distributed involvement of multiple cortical regions or brain networks, in addition to the CSTC network, might play a critical role in TS pathophysiology.

Because TS children cannot perform specified tasks well, there have been few task-related fMRI studies in TS children. Resting-state fMRI (RS-fMRI) has the advantage of measuring spontaneous neural activity such as regional homogeneity (ReHo) in the TS population (10, 11). A previous study of TS with a small sample size ($n = 21$) showed decreased ReHo in the right cerebellum that was positively correlated with disease duration (11); however, no cortical regions were identified with abnormal ReHo. That study only observed the local synchronization of spontaneous RS-fMRI signals at a low frequency band (0.01–0.08 Hz), and therefore information about other lower- or higher-frequency realms is unknown (12). In contrast, a broader band of 0.01–0.08 Hz, containing several mixed physiological alterations of potentially specific frequencies, may lead to negative or inexact results. Zuo et al. discovered that even in the conventional low-frequency band (<0.1 Hz), blood oxygenation level-dependent (BOLD) fluctuations were stronger at 0.01–0.027 Hz in the subcortical region and at 0.027–0.073 Hz in the cortical region (13). Frequency characteristic analysis demonstrated that ReHo oscillations lower than 0.02 Hz mainly occurred in the putamen and that higher-frequency oscillations mainly occurred in limbic areas (>0.08 Hz) (14). Thus, a frequency-specific approach may provide more information than that of the conventional band (<0.1 Hz) to help understand the local BOLD activity of the cortical regions in TS.

To discover frequency-specific functional alterations that might have an important role in the pathophysiology of TS, we used RS-fMRI in a frequency-specific manner (0–0.01 Hz, 0.01–0.027 Hz, 0.027–0.073 Hz, 0.073–0.198 Hz, and 0.198–0.25 Hz) to compare TS patients with healthy controls (HC) (13, 15). Independent component analysis (ICA) was used to detect connectivity differences within brain networks between TS and HC groups. In the current study, we included subjects with comorbid symptoms who are most typical of TS [as "pure" TS without any comorbidity occurs in only 10% of patients (3)] to obtain generalizable results for clinical practice.

TABLE 1 | Demographic variables and clinical characteristics.

Characteristics	TS ($n = 79$)	HC ($n = 63$)	*P*-value
Age (years)	9.56 ± 2.50	9.35 ± 2.19	0.599
Sex, male/female	74/5	53/10	0.066
Handedness, right/left	78/1	63/0	0.370
Duration (years)	2.28 ± 2.14	–	–
YGTSS (total score)	22.00 ± 7.58	–	–
YGTSS (motor score)	13.32 ± 3.49	–	–
YGTSS (vocal score)	8.67 ± 5.94	–	–
SNAP-IV	12.86 ± 7.64	–	–
CY-BOCS	0.52 ± 2.39	–	–
Drug information			
Tiapride	11	–	–
Topamax	5	–	–
Haloperidol	2	–	–
Combination drugs	2	–	–

Date are expressed as mean ± SD. YGTSS, Yale Global Tic Severity Scale; SNAP-IV, Swanson, Nolan, and Pelham IV Scale; CY-BOCS, Children's Yale–Brown Obsessive Compulsive Scale.

MATERIALS AND METHODS

Participants

Ninety-four children with TS were recruited from outpatient clinics of the Second Affiliated Hospital, Zhejiang University School of Medicine. All patients met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision (DSM-IV-TR) criteria. Sixty-three age-, sex-, and handedness-matched healthy children without any neurological or psychiatric disorders were recruited as HC. Participants were excluded if they had (i) any neurological (other than tics) or psychiatric diseases (other than ADHD and OCD), (ii) structural abnormalities on visual inspection of structural imaging, and (iii) head motion exceeding 3 mm in translation or 3° rotation in any direction. After head-motion control, fifteen patients were excluded. Seventy-nine TS patients were eligible to take part in the current study. All clinical evaluations were performed on the day of acquisition of MRI scans by an experienced pediatric neurologist (**Table 1**). The Yale Global Tic Severity Scale (YGTSS) was used to assess current tic severity. The Swanson, Nolan, and Pelham IV Scale (SNAP-IV) was used to assess ADHD symptoms, and the Children's Yale–Brown Obsessive Compulsive Scale (CY-BOCS) was used to quantify OCD symptoms. Twenty-five children with TS in this study were using psychoactive medication during the MRI scan. Eighteen children with TS were on monotherapy (Tiapride 11/18, Topamax 5/18, Haloperidol 2/18), while seven subjects were taking combination drug therapy. Informed written consent from parents and assent by children for TS and HC were

obtained before participation. The study was approved by the Medical Ethics Committee of the Center for Cognition and Brain Disorders, Hangzhou Normal University, China.

Image Acquisition

MRI images were acquired on a 3.0-Tesla MRI scanner (GE Discovery 750 MRI, General Electric, Milwaukee, WI, USA) at the Center for Cognition and Brain Disorders, Hangzhou Normal University. Foam pads were used to minimize head motion for all subjects. A gradient-recalled echo planar imaging sequence (repetition time = 2,000 ms, echo time = 30 ms, and flip angle = 90°) was used to obtain functional images. Forty-three axial slices (field of view = 220 × 220 mm, matrix = 64 × 64, slice thickness/gap = 3.2/0 mm, and 240 volumes) were acquired. Participants were instructed to rest with their eyes closed, not to think of anything in particular, and not to fall asleep. Three-dimensional T1-weighted images were obtained in the sagittal orientation using a magnetization-prepared rapid acquisition gradient-echo sequence (repetition time = 8.068 ms, echo time = 3.136 ms, flip angle = 8°, field of view = 250 × 250 mm, matrix = 256 × 256, slice thickness/gap = 1/0 mm, and 176 slices). After each scanning session, the responsiveness of the subjects was determined by asking whether they had fallen asleep during the scan.

Image Data Preprocessing

Statistical Parametric Map (SPM12, http://www.fil.ion.ucl.ac.uk/spm) software and the Data Processing & Analysis for Brain Imaging (DPABI) toolbox (http://rfmri.org/dpabi) were used for preprocessing (16). The first 10 image volumes of fMRI scans were discarded for scanner calibration and the subject's adaptation to the scanning noise. After slice time correction, functional images were spatially realigned to the first image of each session for head motion correction. Head movements assessed by the realignment parameters were tolerated up to ±3 mm and ±3°. Then, several nuisance variables including a linear trend, head-motion parameter (the Friston 24-parameter model) (17), white matter, cerebrospinal fluid, and global mean signals were regressed out (16). Individual T1 images were coregistered to functional images, then segmented (gray matter, white matter, and cerebrospinal fluid) and normalized to Montreal Neurological Institute (MNI) space. The transformation matrix obtained from T1 segmentation was applied to the functional images. The normalized functional images were resampled to 3 × 3 × 3 mm. Finally, we performed bandpass filtering (0.01–0.08 Hz, 0–0.01 Hz, 0.01–0.027 Hz, 0.027–0.073 Hz, 0.073–0.198 Hz, 0.198–0.25 Hz). ReHowas calculated (27 voxels) and ReHo maps from each frequency band for each individual were divided by the global mean ReHo value and then spatially smoothed with a Gaussian kernel (full width at half maximum = 6 mm). Ultimately, smReHo maps were used for statistical analysis.

For ICA, fMRI data were spatially smoothed with 6 mm full width half maximum Gaussian kernel after normalization. Subsequently, Group ICA of the fMRI Toolbox v3.0b (GIFT, http://icatb.sourceforge.net) was used to calculate spatially independent components (ICs). Dimension estimation of data from both groups was conducted using the minimum description length (MDL) criterion to determine the number of ICs (18). Then, individual maps in each group were conjoined and the temporal dimension of the convergent data was reduced via principal component analysis, followed by IC estimation. For all subjects, the spatial component maps were converted into z-score maps.

The number of ICs in the two groups (TS and HC) was 43. These components were selected based on the largest spatial correlation with a specific resting-state network template (http://findlab.stanford.edu/functional_ROIs.html) for further analysis. The brain networks included basal ganglia, sensorimotor, and the left and right executive control networks (one sample *t*-test, multiple-comparison correction based on Gaussian random field theory, single voxel $p < 0.001$, cluster level $p < 0.05$, **Figure 1**).

Statistical Analysis

Two-way repeated-measures analysis of variance (ANOVA) was performed to examine the effects of group and frequency band on the ReHo maps. Group (TS and HC) served as a between-subject factor and frequency band (0–0.01 Hz, 0.01–0.027 Hz, 0.027–0.073 Hz, 0.073–0.198 Hz, and 0.198–0.25 Hz) served as a within-subject factor. An F-map of the "frequency by group" interaction effect and an F-map of the group main effect were obtained. Multiple-comparison correction was conducted based on Gaussian random field (GRF) theory (single voxel $p < 0.001$, cluster level $p < 0.05$). The peak ReHo values of the clusters surviving from the group main effect were extracted and then entered SPSS software for two-sample *t*-tests in each frequency band

The peak ReHo values of TS patients were correlated against the clinical measurements, including YGTSS scores and SNAP-IV scores.

To explore connectivity differences between the two groups, two sample *t*-tests were performed within each network. Multiple-comparison correction was conducted based on GRF theory (single voxel $p < 0.001$, cluster level $p < 0.05$).

RESULTS

There were no significant differences in age ($p = 0.599$), sex ($p = 0.066$), and handedness ($p = 0.370$) between the two groups. The detailed demographic variables and clinical characteristics of participants are shown in **Table 1**.

Differences in Regional Homogeneity

ANOVA showed a significant main effect of group and a significant interaction effect (**Figure 2**, **Table 2**). For the main effect of group, the brain regions included the left precentral gyrus and the right operculum (frontal and temporal lobes). For the interaction effect, the brain regions included the right anterior cingulate gyrus (ACC)/supplementary motor cortex (SMA), right superior frontal gyrus, right Rolandic operculum, right putamen, right superior parietal gyrus, left superior temporal gyrus, and left white matter. Generally, in TS patients, the right superior frontal, and superior parietal gyrus showed an increased ReHo in lower bands and turned to normal in the higher bands, while the right

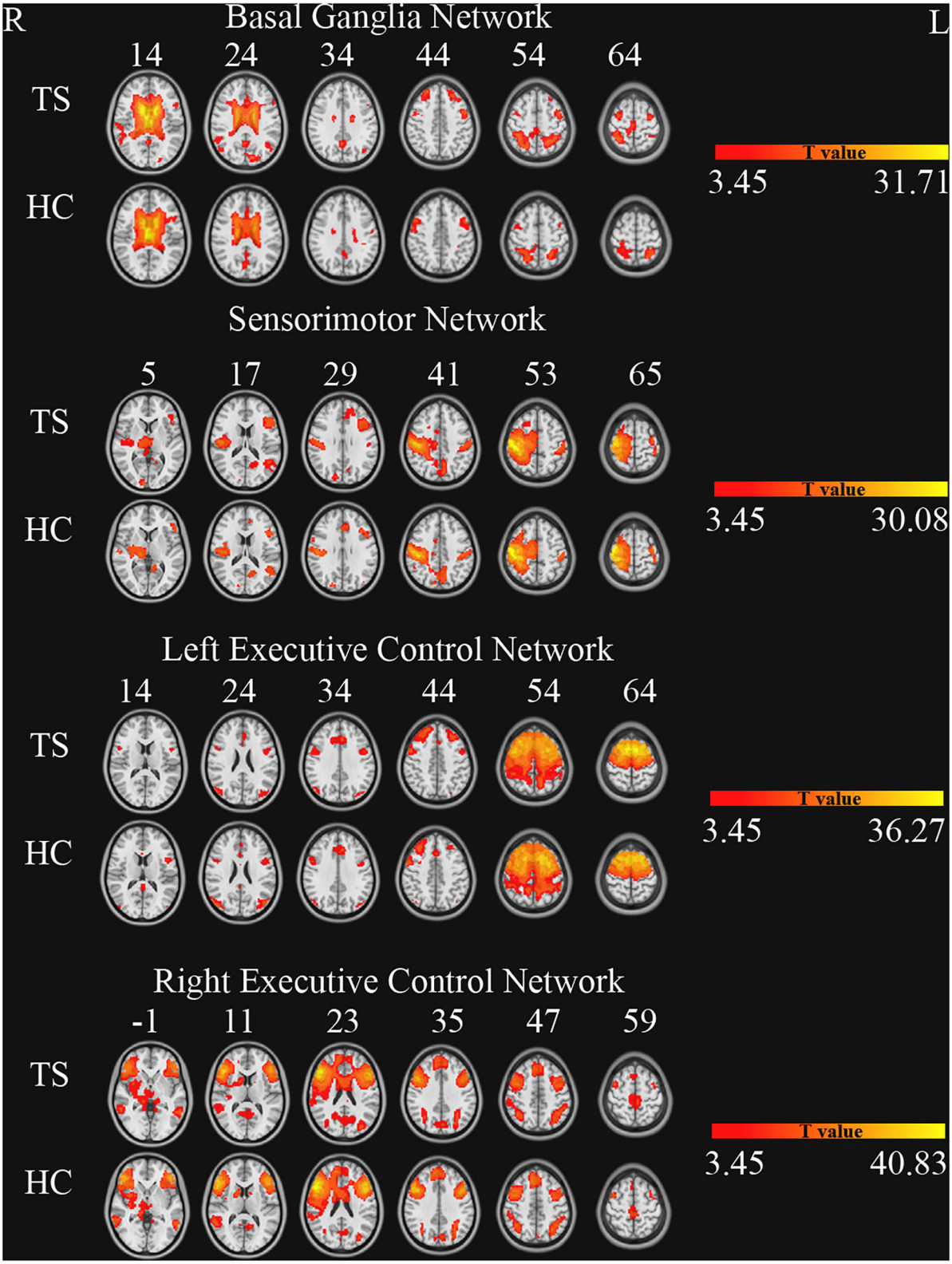

FIGURE 1 | Brain networks of independent component analysis for each group (one sample *t*-test, GRF correction, single voxel $p < 0.001$, cluster level $p < 0.05$).

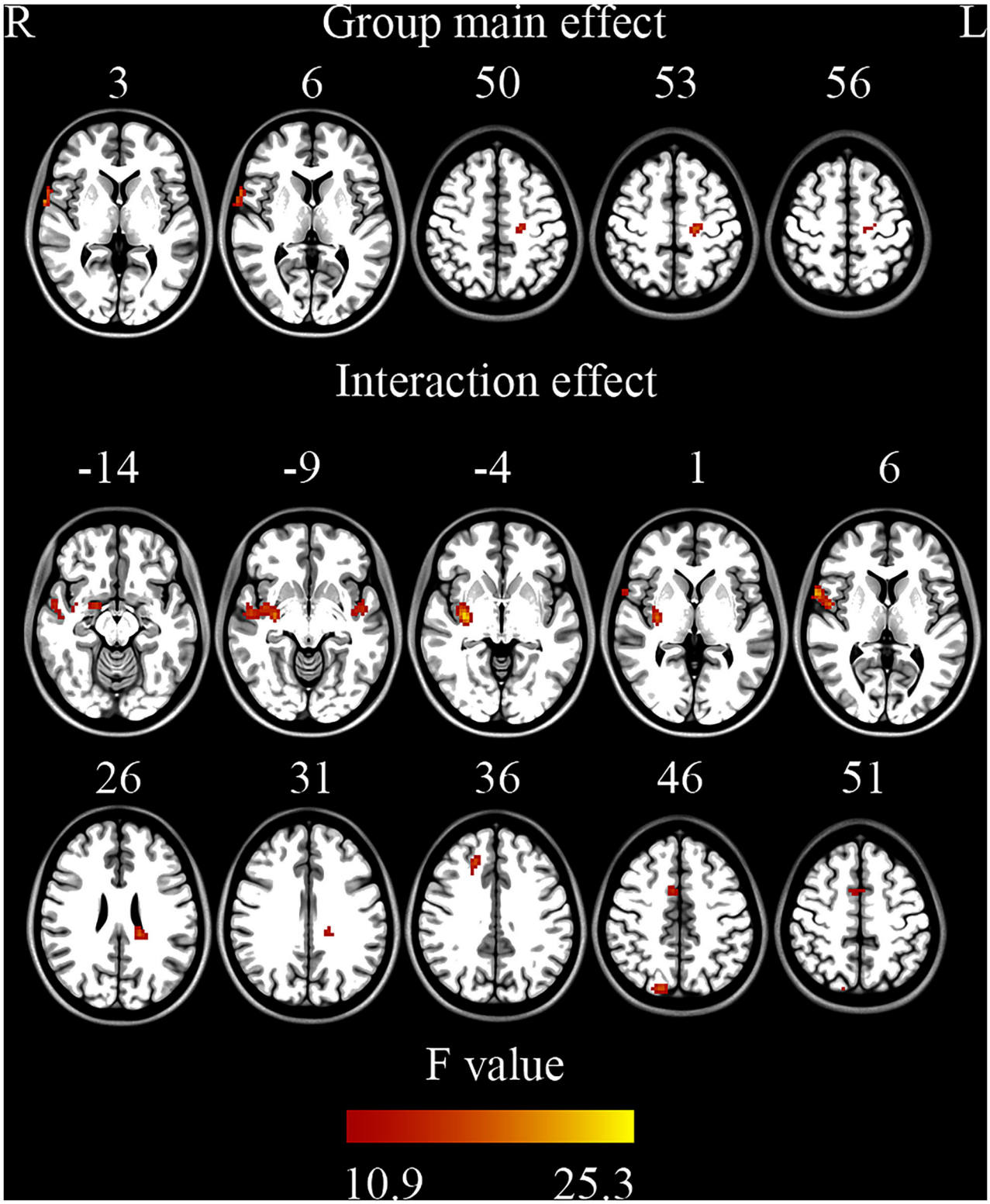

FIGURE 2 | Brain regions showing significant differences on ReHo (ANOVA) (GRF correction, single voxel $p < 0.001$, cluster level $p < 0.05$).

TABLE 2 | Brain regions showed significant differences (ANOVA).

Brian regions	Hemisphere	Brodmann's area	MNI coordinate (X, Y, Z)			Cluster size (mm^3)	*F*-value	*P*-value
Main effect of group								
Operculum (frontal and temporal lobe)	R	48	66	3	3	621	15.95	<0.05
Precentral gyrus	L	N/A	−18	−21	54	621	14.96	<0.05
Interaction effect of group × frequency								
Superior temporal gyrus/putamen	R	20	30	−12	−6	4,833	25.40	<0.05
Anterior cingulate gyrus/supplementary motor cortex	R	24	6	6	45	783	14.98	<0.05
Superior frontal gyrus	R	32	18	33	36	756	16.78	<0.05
Parietal lobe/precuneus	R	7	15	−81	48	702	17.81	<0.05
Rolandic operculum	R	48	63	9	6	2,511	21.58	<0.05
Superior temporal gyrus	L	48	−48	−6	−9	702	15.10	<0.05
White matter	L	N/A	−18	−33	27	999	17.89	<0.05

P-value, GRF correction, single voxel $p < 0.001$, cluster level $p < 0.05$.

Rolandic operculum and ACC/SMA showed a decreased ReHo in lower bands and turned to normal in higher bands. The right putamen and the left superior temporal gyrus showed a trend of decreased ReHo in lower frequency bands and increased ReHo in higher frequency bands (**Figure 3**, **Table 3**).

The two-sample *t*-tests showed significantly increased ReHo in full bands of the left precentral gyrus and significantly decreased ReHo in full bands of the right operculum (frontal and temporal lobes) (**Table 4**).

The ReHo value of the right operculum (frontal and temporal lobes) showed significant negative correlations with vocal scores of YGTSS in the highest frequency bands (0.198–0.25 Hz) (Bonferroni correction, $0.05/15 = 0.0033$; **Figure 4**, **Table 5**). No significant correlation was found between the ReHo value and any rating score in the left precentral gyrus.

Differences of Connectivity Within Networks

Compared with HC, TS patients showed increased connectivity of the right superior frontal gyrus (BA 6, MNI coordinates $X = 21$, $Y = 9$, $Z = 63$, $T = 4.132$, Cluster size = 945 mm^3) within the left executive control network (**Figure 5**). No significant group difference was found in the basal ganglia, sensorimotor, and right executive control network. The peak *Z*-value of the right superior frontal gyrus was extracted to perform correlation analysis against clinical assessment scores. Only the motor score of YGTSS showed a positive correlation with abnormal connectivity (**Figure 6**).

DISCUSSION

Reduced Synchronization Within the CSTC Circuit in TS

The present study found decreased ReHo in the right putamen and increased ReHo in the left precentral gyrus of TS children. The putamen, a key node of the basal ganglia, is involved in motor loop circuits (19). The precentral gyrus responds to visual input and codes information during movement preparation (20), which involves cognitive processing for movements such as motor preparation and execution (21). The putamen and precentral gyrus are both parts of the CSTC circuit.

Functional deficits in the CSTC circuit may contribute to the motor symptoms of TS (7). The putamen, the center of the motor portion of the CSTC in the genesis of tics, receives abundant projections from the motor and somatosensory cortices (22). Defects in the modulation of activity in the structures of the motor CSTC network may underlie the failure to inhibit unwanted impulses in patients with TS (7). Previous studies demonstrated the aberrant structure, metabolism, and activity of the putamen and primary motor cortices in TS patients (23–25). Microstructural changes in the white matter of the left precentral gyrus and putamen in unmedicated and pure TS, and the regional apparent diffusion coefficient values were positively correlated with tic severity (25). A proton magnetic resonance spectroscopy study of children with TS showed significant reductions of N-acetylaspartate and choline in the putamen and reduced N-acetylaspartate in the frontal cortical (24). TS children were shown to have increased spontaneous local brain activity in the putamen and bilateral thalamus during RS-fMRI scans (26). Two event-related functional neuroimaging studies support the idea that the primary motor cortex processes preparatory signals related to motor tic behavior in TS (23, 27). One study showed significant fMRI activity in the primary motor cortex before tic onset (23). Another study found that cortical structures, including the primary motor cortex, preceded subcortical activation prior to tic onset (27). Decreased ReHo in the putamen and increased ReHo in the precentral gyrus in the current study indicated reduced synchronization of spontaneous neural activity in the putamen might inhibit the output nuclei of the basal ganglia, which in turn excite the precentral cortex to produce tics.

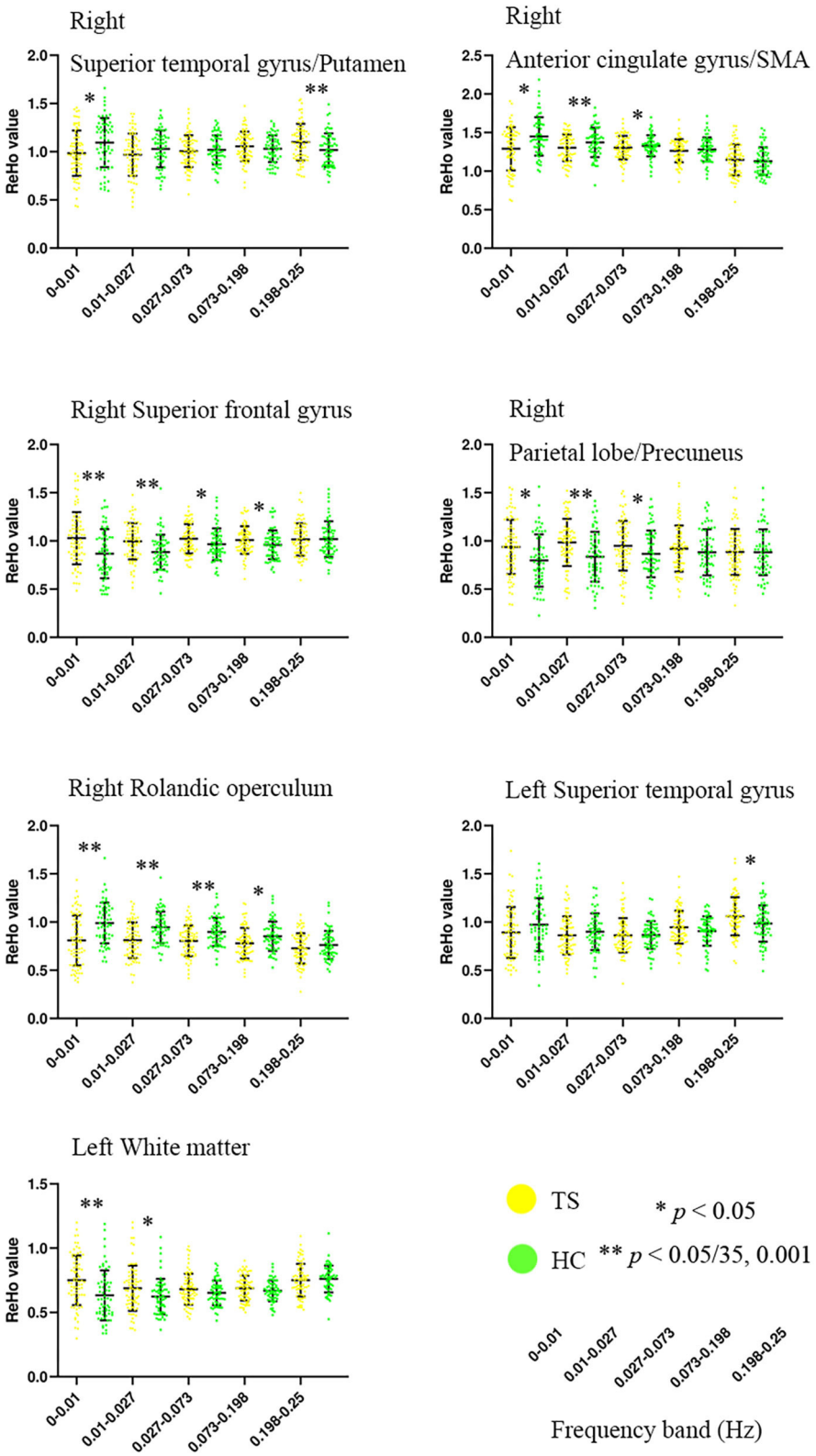

FIGURE 3 | Altered ReHo of brain regions in TS patients across five frequency bands ranging from 0 to 0.25 Hz. SMA, supplementary motor cortex. **Bonferroni correction, i.e., 0.05/35 = 0.001.

TABLE 3 | Details of simple effect for the brain regions in ANOVA (interaction effect of group × frequency).

Frequency band	TS (n = 79) mean ± SD	HC (n = 63) mean ± SD	T-value	P-value
Right superior temporal gyrus/putamen				
0–0.01 Hz	0.98 ± 0.24	1.09 ± 0.26	2.659	0.0088*
0.01–0.027 Hz	0.97 ± 0.22	1.03 ± 0.19	1.728	0.0862
0.027–0.073 Hz	1.01 ± 0.16	1.02 ± 0.15	0.472	0.6377
0.073–0.198 Hz	1.06 ± 0.15	1.03 ± 0.14	1.011	0.3139
0.198–0.25 Hz	1.10 ± 0.19	1.02 ± 0.17	2.681	0.0082*
Right ACC/SMA				
0–0.01 Hz	1.29 ± 0.28	1.45 ± 0.25	3.547	0.0005**
0.01–0.027 Hz	1.30 ± 0.17	1.37 ± 0.19	2.294	0.0233*
0.027–0.073 Hz	1.30 ± 0.15	1.33 ± 0.14	1.030	0.3049
0.073–0.198 Hz	1.26 ± 0.15	1.28 ± 0.16	0.645	0.5201
0.198–0.25 Hz	1.15 ± 0.20	1.13 ± 0.18	0.583	0.5609
Right superior frontal gyrus				
0–0.01 Hz	1.03 ± 0.27	0.87 ± 0.26	3.601	0.0004**
0.01–0.027 Hz	1.00 ± 0.19	0.88 ± 0.18	3.595	0.0004**
0.027–0.073 Hz	1.02 ± 0.15	0.97 ± 0.17	2.122	0.0356*
0.073–0.198 Hz	1.01 ± 0.14	0.96 ± 0.15	1.995	0.0479*
0.198–0.25 Hz	1.02 ± 0.17	1.02 ± 0.19	0.098	0.9224
Right parietal lobe/precuneus				
0–0.01 Hz	0.94 ± 0.28	0.80 ± 0.27	2.999	0.0032*
0.01–0.027 Hz	0.98 ± 0.24	0.84 ± 0.26	3.504	0.0006**
0.027–0.073 Hz	0.95 ± 0.26	0.87 ± 0.24	1.996	0.0479*
0.073–0.198 Hz	0.92 ± 0.24	0.88 ± 0.24	0.936	0.3510
0.198–0.25 Hz	0.89 ± 0.24	0.88 ± 0.24	0.072	0.9430
Right Rolandic operculum				
0–0.01 Hz	0.81 ± 0.26	0.99 ± 0.21	4.498	<0.0001**
0.01–0.027 Hz	0.81 ± 0.18	0.95 ± 0.16	4.475	< 0.0001**
0.027–0.073 Hz	0.80 ± 0.16	0.90 ± 0.15	3.607	0.0004**
0.073–0.198 Hz	0.78 ± 0.16	0.85 ± 0.15	2.762	0.0065*
0.198–0.25 Hz	0.73 ± 0.16	0.76 ± 0.15	1.348	0.1798
Left superior temporal gyrus				
0–0.01 Hz	0.89 ± 0.26	0.97 ± 0.27	1.767	0.0794
0.01–0.027 Hz	0.86 ± 0.20	0.90 ± 0.19	1.161	0.2477
0.027–0.073 Hz	0.86 ± 0.18	0.86 ± 0.14	0.126	0.8997
0.073–0.198 Hz	0.95 ± 0.17	0.91 ± 0.15	1.420	0.1577
0.198–0.25 Hz	1.06 ± 0.20	0.98 ± 0.19	2.308	0.0224*
Left white matter				
0–0.01 Hz	0.75 ± 0.19	0.63 ± 0.19	3.601	0.0004**
0.01–0.027 Hz	0.69 ± 0.18	0.62 ± 0.14	2.413	0.0171*
0.027–0.073 Hz	0.68 ± 0.12	0.65 ± 0.10	1.549	0.1238
0.073–0.198 Hz	0.69 ± 0.10	0.67 ± 0.08	1.345	0.1807
0.198–0.25 Hz	0.75 ± 0.13	0.76 ± 0.10	0.474	0.6365

ACC, anterior cingulate gyrus; SMA, supplementary motor cortex.
The peak regional homogeneity (ReHo) value is expressed as mean ± SD.
*$p < 0.05$; **$p < 0.05/35, 0.001$ (Bonferroni correction).*

Abnormal Synchronization Within Executive Control Networks in TS

Executive control networks are thought to include the dorsolateral prefrontal, anterior cingulate cortices, and the orbitofrontal cortex (28). Many studies provided evidence of possible executive deficits in TS during various executive tasks such as working memory, fluency, shifting, and especially inhibition (29). TS patients could not suppress unwanted movements, and mainly comorbid with ADHD (3). The present study found that ReHo was decreased in the frontal and temporal

TABLE 4 | Two sample *t*-tests for the peak ReHo value of the clusters showed significant differences in ANOVA (group main effect).

Frequency band	TS ($n = 79$)	HC ($n = 63$)	*T*-value	*P*-value
	Mean ± SD	Mean ± SD		
Left precentral gyrus				
0–0.01 Hz	0.86 ± 0.25	0.74 ± 0.22	3.521	0.0006**
0.01–0.027 Hz	0.87 ± 0.21	0.73 ± 0.18	4.292	0.0003**
0.027–0.073 Hz	0.87 ± 0.20	0.75 ± 0.14	4.137	0.00006**
0.073–0.198 Hz	0.95 ± 0.19	0.84 ± 0.16	3.590	0.0005**
0.198–0.25 Hz	1.03 ± 0.20	0.94 ± 0.17	2.817	0.006*
Right operculum (frontal and temporal lobe)				
0–0.01 Hz	0.60 ± 0.19	0.73 ± 0.22	−3.632	0.0004**
0.01–0.027 Hz	0.61 ± 0.16	0.71 ± 0.18	−3.469	0.0007**
0.027–0.073 Hz	0.59 ± 0.15	0.68 ± 0.16	−3.469	0.0007**
0.073–0.198 Hz	0.59 ± 0.14	0.66 ± 0.16	−3.040	0.003*
0.198–0.25 Hz	0.55 ± 0.12	0.61 ± 0.14	−2.469	0.015*

*$p < 0.05$; **Bonferroni correction, i.e., $p < 0.05/35, 0.001$.

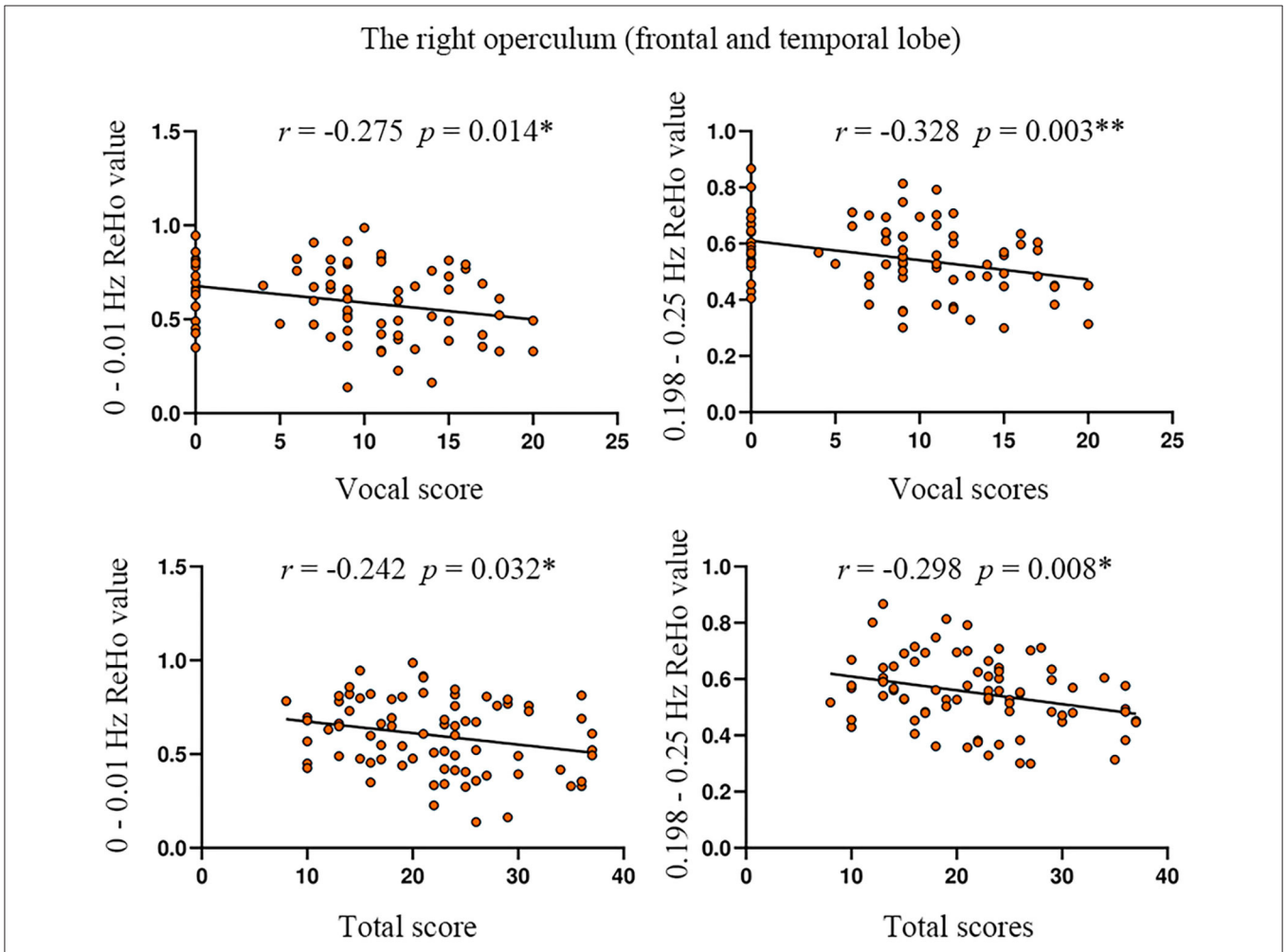

FIGURE 4 | The correlation between ReHo value and YGTSS scores in the right operculum. YGTSS, Yale Global Tic Severity Scale. The total score is the sum of motor score and vocal score. *$p < 0.05$; **Bonferroni correction, i.e., 0.05/15 = 0.0033.

TABLE 5 | The correlation between ReHo and the scores of YGTSS in the significantly different brain regions of ANOVA (group main effect).

Brain area	Frequency band	ReHo value (Mean ± SD)	YGTSS score (Mean ± SD)	*R*-value	*P*-value
Right operculum (frontal and temporal lobe)	0–0.01 Hz	0.60 ± 0.19	8.67 ± 5.94 (vocal score)	−0.275	0.014*
			22.00 ± 7.58 (total score)	−0.242	0.032*
	0.198–0.25 Hz	0.55 ± 0.12	8.67 ± 5.94 (vocal score)	−0.328	0.003**
			22.00 ± 7.58 (total score)	−0.298	0.008*

The total score is the sum of motor score and vocal score.
**p < 0.05; **Bonferroni correction, i.e., p < 0.05/15, 0.0033.*

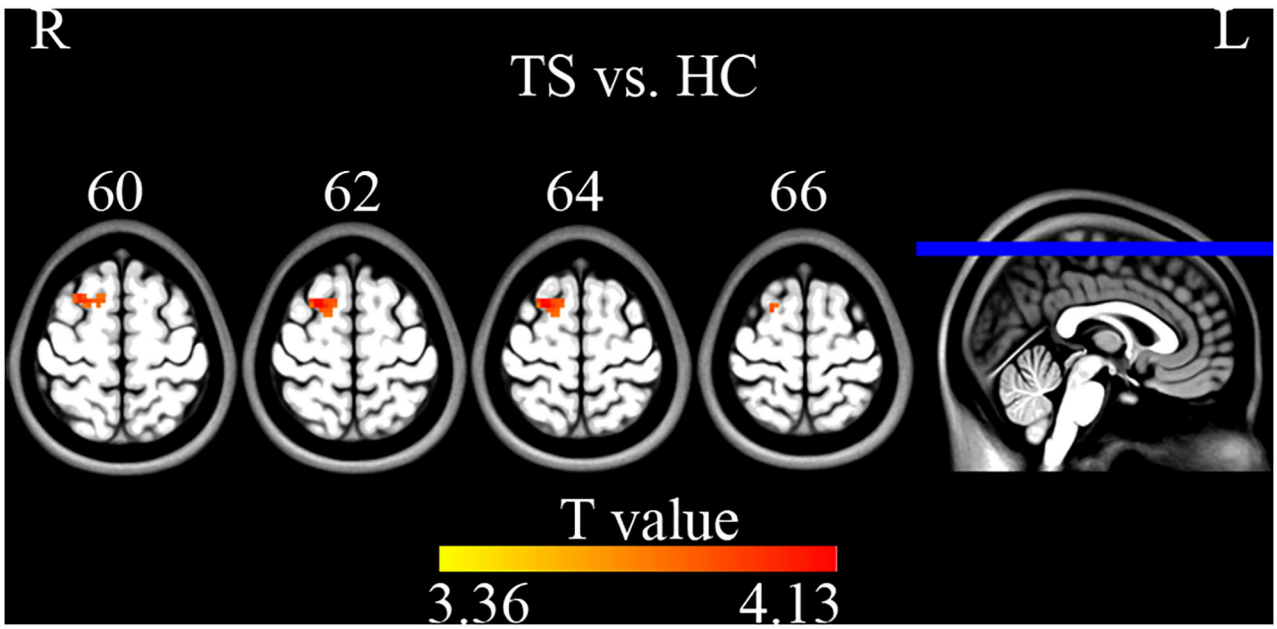

FIGURE 5 | Two sample *t*-tests on ICA maps (GRF correction, single voxel $p < 0.001$, cluster level $p < 0.05$). TS patients showed increased connectivity of the right superior frontal gyrus within the left executive control network.

operculum and ACC, but increased in the superior frontal and parietal cortexes. These regions were reported to be involved in two principal attention control networks. The cingulo-opercular network showed sustained activation across all tasks or nearly all tasks and was hypothesized to maintain task sets (30). The fronto-parietal network, which flexibly segregated or integrated in different aspects of control such as focused proactive control and episodic memory (31), has a crucial role in rapidly adaptive online control (32).

The present results showed reduced synchronization within the cingulo-opercular network (frontal operculum and ACC). Task-maintenance processes may be affected resulting in unwanted breakthroughs of normally suppressed behaviors, such as tics. However, the hyperactive frontal-parietal network (superior frontal and parietal cortexes) motivated initiate and adjust change in task control, again leading to unwanted movements and vocalizations (9). In previous fMRI studies of TS, activation of the operculum, ACC, insular, SMA, primary motor, and somatosensory cortex were found prior to tic onset (23), while decreased amplitude of low-frequency fluctuation (ALFF) and fractional ALFF in the ACC, frontal and parietal gyrus were detected during the resting state (26). In addition, fewer functional connections were found in the fronto-parietal and cingulo-opercular networks in TS children vs. adults and adolescents (9). Abnormalities of metabolism, anatomy, and function in the ACC, an important component of the cingulo-opercular network, have been reported in many previous TS studies (33–37). For instance, significantly decreased gray matter volumes were found in the ACC of TS patients (36). A single-photon emission tomography study reported the hypoperfusion

of the ACC during the resting state, which was related to the severity of tics (35), while another study found increased ACC activation during voluntary tic suppression (34, 37). A recent proton magnetic resonance spectroscopy study of TS patients also found that concentrations of glutamine, an important excitatory neurotransmitter, negatively correlated with tic severity scores in ACC (33). Furthermore, we observed a negative correlation between tic severity and ReHoin the operculum. The greater the reduction of synchronization in the operculum, the more serious the disease is thought to become. Above all, altered ReHo of the cingulo-opercular and fronto-parietal networks, especially in the operculum and ACC in the current study, suggested that these brain areas may have an important role in the progress of tic generation mainly by breaking task set maintenance and enhancing adaptive tasks.

In addition to local synchronization, the present study used ICA to detect connectivity differences within the basal ganglia, sensorimotor, and executive control networks. We found increased connectivity of the right superior frontal gyrus within the left executive control network. The executive control network shares major nodes with the frontal-parietal network, including the dorsolateral prefrontal cortex and posterior parietal cortex. It is involved in the control of higher-order cognitive neural functions such as attention, planning, decision-making, and working memory (38). Recent years transcranial magnetic stimulation (TMS) studies attempted to indirectly impact deep or remote brain areas via functional connectivity. Some researchers have successfully observed TMS-induced functional connectivity alterations by stimulating the superficial cortex (39, 40). Our previous work also found increased ReHo in cerebellum after high frequency TMS on precentral gyrus (41). In addition, there was a significant positive correlation between functional connectivity of the right superior frontal gyrus and YGTSS motor score. The right superior frontal gyrus might be a direct or indirect target of TMS for TS treatment. This also suggested that Tics may be aggravated by enhanced functional connectivity within the executive control network, supporting the putative role of the control network in the pathogenesis of TS.

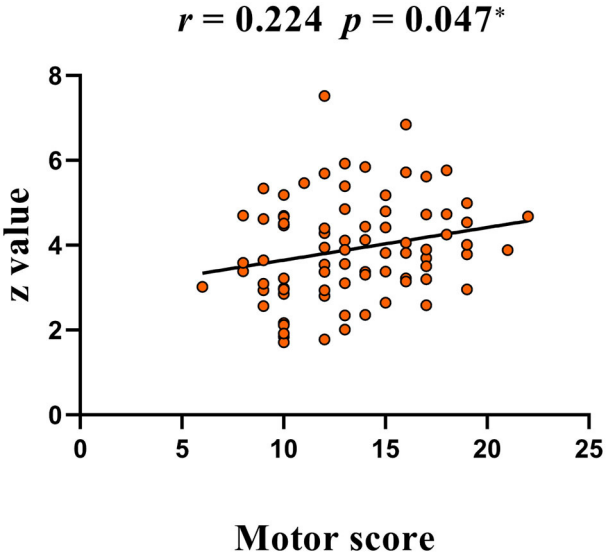

FIGURE 6 | A positive correlation between peak *Z*-value of the right superior frontal gyrus and YGTSS scores (motor score). YGTSS, Yale Global Tic Severity Scale.

Frequency-Specific Altered Synchronization in TS

To the best of our knowledge, this is the first study to examine abnormalities of regional synchronization in a relatively large and typical pediatric TS population using the frequency specificity of ReHo. Several studies demonstrated the frequency specificity of ReHo changes in neurologic and psychiatric disorders, such as paroxysmal kinesigenic dyskinesia (42), schizophrenia (43), and major depression (44). In the present study, significantly increased ReHoin the superior frontal gyrus and superior parietal gyrus of TS children indicated compensatory functions of the frontal–parietal network in the lower frequency bands. These findings are consistent with previous reports that the lower frequency band (0.01–0.027 Hz) had a higher BOLD fluctuation in the cortical regions (13). We also found decreased ReHo in the putamen, ACC, and superior temporal gyrus in the lower-frequency bands, which was normal or reversed in the higher frequency bands. Previous RS-fMRI studies indicated that the higher band (0.027–0.073 Hz) exhibited increased ALFF compared with the lower band (0.01–0.027 Hz) in the subcortical regions (13, 45). The interaction of frequency band × group on ReHo indicated a significant difference in frequency-specific alterations (**Figure 3**, **Table 3**). The results of the current study suggested that the local intrinsic brain activity of TS was sensitive to specific frequency bands. More frequency-specific investigations are needed in the future.

The significant negative correlation between ReHo and YGTSS scores was found only in the highest frequency bands (0.198–0.25 Hz) (**Figure 4**, **Table 5**), suggesting that the ultra-high frequency band might be of clinical importance. These findings indicate that abnormal ReHoin TS is frequency-dependent and might be missed when using routine frequency band. Although the nature of these frequency-specific alterations of local neuronal homogeneity is still unclear, different frequency bands should be considered when measuring the ReHo of TS children in future studies to further understand the pathology of TS.

CONCLUSION

In summary, the present RS-fMRI study revealed frequency-specific abnormal regional homogeneity and altered connectivity in children with TS. The regions of frequency-specific abnormalities included the frontal–parietal network, ACC, and putamen, as well as an altered connectivity region in the executive control network. These brain regions are involved in multiple cognitive dysfunctions characteristic of TS. All these aberrant regions might be direct treatment targets or indirect targets for brain stimulation, especially those regions with

frequency-specific abnormalities, which might be sensitive to specific stimulation frequencies for TMS. These targets should be tested and verified in TS populations in the future.

LIMITATIONS

This study had several limitations. The drugs used for TS treatment may affect brain function. The dose and duration of drug information were not recorded completely in the current study. The variability of the doses and types of drugs used makes it difficult to analyze the contribution of each drug on ReHo. Therefore, drug-naïve status and type of medication used should be taken into consideration in the future.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the Center for Cognition and Brain Disorders, Hangzhou Normal University, China. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Y-TL: design the study, statistical analysis, and writing of the first draft. X-LL: fMRI data processing and statistical analysis. YW: neurological evaluation and psychiatric evaluation. G-JJ: fMRI data acquisition. Y-FZ: revised the article and developed the research concept. JW: fMRI data processing and statistical analysis, revised the article, and developed the research concept. J-HF: research project conception and organization, neurological evaluation, revised the article, and developed the research concept. All authors: contributed to the article and approved the submitted version.

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REFERENCES

1. Swain JE, Scahill L, Lombroso PJ, King RA, Leckman JF. Tourette syndrome and tic disorders: a decade of progress. *J Am Acad Child Adolesc Psychiatry.* (2007) 46:947–68. doi: 10.1097/chi.0b013e318068fbcc
2. Yang C, Zhang L, Zhu P, Zhu C, Guo Q. The prevalence of tic disorders for children in China: a systematic review and meta-analysis. *Medicine.* (2016) 95:e4354. doi: 10.1097/MD.0000000000004354
3. Mol Debes NM, Hjalgrim H, Skov L. Validation of the presence of comorbidities in a Danish clinical cohort of children with tourette syndrome. *J Child Neurol.* (2008) 23:1017–27. doi: 10.1177/0883073808316370
4. Evans J, Seri S, Cavanna AE. The effects of gilles de la tourette syndrome and other chronic tic disorders on quality of life across the lifespan: a systematic review. *Eur Child Adolesc Psychiatry.* (2016) 25:939–48. doi: 10.1007/s00787-016-0823-8
5. Debes NM, Skov L, Hjalgrim H. [Tourette syndrome. Genetics, neuroanatomy and neurotransmitters]. *Ugeskr Laeger.* (2008) 170:2695–700.
6. Greene DJ, Schlaggar BL, Black KJ. Neuroimaging in tourette syndrome: research highlights from 2014–2015. *Curr Dev Disord Rep.* (2015) 2:300–8. doi: 10.1007/s40474-015-0062-6
7. Mink JW. Neurobiology of basal ganglia and tourette syndrome: basal ganglia circuits and thalamocortical outputs. *Adv Neurol.* (2006) 99:89–98.
8. Polyanska L, Critchley HD, Rae CL. Centrality of prefrontal and motor preparation cortices to Tourette syndrome revealed by meta-analysis of task-based neuroimaging studies. *Neuroimage Clin.* (2017) 16:257–67. doi: 10.1016/j.nicl.2017.08.004
9. Church JA, Fair DA, Dosenbach NU, Cohen AL, Miezin FM, Petersen SE, et al. Control networks in paediatric tourette syndrome show immature and anomalous patterns of functional connectivity. *Brain.* (2009) 132:225–38. doi: 10.1093/brain/awn223
10. Ganos C, Kahl U, Brandt V, Schunke O, Baumer T, Thomalla G, et al. The neural correlates of tic inhibition in gilles de la tourette syndrome. *Neuropsychologia.* (2014) 65:297–301. doi: 10.1016/j.neuropsychologia.2014.08.007
11. Liu Y, Wang J, Zhang J, Wen H, Zhang Y, Kang H, et al. Altered spontaneous brain activity in children with early Tourette syndrome: a resting-state fMRI study. *Sci Rep.* (2017) 7:4808. doi: 10.1038/s41598-017-04148-z
12. Wu CW, Gu H, Lu H, Stein EA, Chen JH, Yang Y. Frequency specificity of functional connectivity in brain networks. *Neuroimage.* (2008) 42:1047–55. doi: 10.1016/j.neuroimage.2008.05.035
13. Zuo XN, Di Martino A, Kelly C, Shehzad ZE, Gee DG, Klein DF, et al. The oscillating brain: complex and reliable. *Neuroimage.* (2010) 49:1432–45. doi: 10.1016/j.neuroimage.2009.09.037
14. Song X, Zhang Y, Liu Y. Frequency specificity of regional homogeneity in the resting-state human brain. *PLoS ONE.* (2014) 9:e86818. doi: 10.1371/journal.pone.0086818
15. Wang J, Zhang Z, Ji GJ, Xu Q, Huang Y, Wang Z, et al. Frequency-specific alterations of local synchronization in idiopathic generalized epilepsy. *Medicine.* (2015) 94:e1374. doi: 10.1097/MD.0000000000001374
16. Yan CG, Wang XD, Zuo XN, Zang YF. DPABI: data processing & analysis for (resting-state) brain imaging. *Neuroinformatics.* (2016) 14:339–51. doi: 10.1007/s12021-016-9299-4
17. Yan CG, Cheung B, Kelly C, Colcombe S, Craddock RC, Di Martino A, et al. A comprehensive assessment of regional variation in the impact of head micromovements on functional connectomics. *Neuroimage.* (2013) 76:183–201. doi: 10.1016/j.neuroimage.2013.03.004
18. Li YO, Adali T, Calhoun VD. Estimating the number of independent components for functional magnetic resonance imaging data. *Hum Brain Mapp.* (2007) 28:1251–66. doi: 10.1002/hbm.20359
19. Middleton FA, Strick PL. Basal ganglia and cerebellar loops: motor and cognitive circuits. *Brain Res Brain Res Rev.* (2000) 31:236–50. doi: 10.1016/S0165-0173(99)00040-5
20. Gallivan JP, McLean DA, Flanagan JR, Culham JC. Where one hand meets the other: limb-specific and action-dependent movement plans decoded from

preparatory signals in single human frontoparietal brain areas. *J Neurosci.* (2013) 33:1991–2008. doi: 10.1523/JNEUROSCI.0541-12.2013
21. Smith FW, Goodale MA. Decoding visual object categories in early somatosensory cortex. *Cereb Cortex.* (2015) 25:1020–31. doi: 10.1093/cercor/bht292
22. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci.* (1986) 9:357–81. doi: 10.1146/annurev.ne.09.030186.002041
23. Bohlhalter S, Goldfine A, Matteson S, Garraux G, Hanakawa T, Kansaku K, et al. Neural correlates of tic generation in tourette syndrome: an event-related functional MRI study. *Brain.* (2006) 129:2029–37. doi: 10.1093/brain/awl050
24. DeVito TJ, Drost DJ, Pavlosky W, Neufeld RW, Rajakumar N, McKinlay BD, et al. Brain magnetic resonance spectroscopy in tourette's disorder. *J Am Acad Child Adolesc Psychiatry.* (2005) 44:1301–8. doi: 10.1097/01.chi.0000181046.52078.f4
25. Muller-Vahl KR, Grosskreutz J, Prell T, Kaufmann J, Bodammer N, Peschel T. Tics are caused by alterations in prefrontal areas, thalamus and putamen, while changes in the cingulate gyrus reflect secondary compensatory mechanisms. *BMC Neurosci.* (2014) 15:6. doi: 10.1186/1471-2202-15-6
26. Cui Y, Jin Z, Chen X, He Y, Liang X, Zheng Y. Abnormal baseline brain activity in drug-naive patients with tourette syndrome: a resting-state fMRI study. *Front Hum Neurosci.* (2014) 7:913. doi: 10.3389/fnhum.2013.00913
27. Neuner I, Werner CJ, Arrubla J, Stocker T, Ehlen C, Wegener HP, et al. Imaging the where and when of tic generation and resting state networks in adult tourette patients. *Front Hum Neurosci.* (2014) 8:362. doi: 10.3389/fnhum.2014.00362
28. Zgaljardic DJ, Borod JC, Foldi NS, Mattis PJ, Gordon MF, Feigin A, et al. An examination of executive dysfunction associated with frontostriatal circuitry in Parkinson's disease. *J Clin Exp Neuropsychol.* (2006) 28:1127–44. doi: 10.1080/13803390500246910
29. Eddy CM, Rizzo R, Cavanna AE. Neuropsychological aspects of tourette syndrome: a review. *J Psychosom Res.* (2009) 67:503–13. doi: 10.1016/j.jpsychores.2009.08.001
30. Dosenbach NU, Visscher KM, Palmer ED, Miezin FM, Wenger KK, Kang HC, et al. A core system for the implementation of task sets. *Neuron.* (2006) 50:799–812. doi: 10.1016/j.neuron.2006.04.031
31. Ray KL, Ragland JD, MacDonald AW, Gold JM, Silverstein SM, Barch DM, et al. Dynamic reorganization of the frontal parietal network during cognitive control and episodic memory. *Cogn Affect Behav Neurosci.* (2020) 20:76–90. doi: 10.3758/s13415-019-00753-9
32. Dosenbach NU, Fair DA, Cohen AL, Schlaggar BL, Petersen SE. A dual-networks architecture of top-down control. *Trends Cogn Sci.* (2008) 12:99–105. doi: 10.1016/j.tics.2008.01.001
33. Fan S, Cath DC, van den Heuvel OA, van der Werf YD, Schols C, Veltman DJ, et al. Abnormalities in metabolite concentrations in tourette's disorder and obsessive-compulsive disorder-A proton magnetic resonance spectroscopy study. *Psychoneuroendocrinology.* (2017) 77:211–17. doi: 10.1016/j.psyneuen.2016.12.007
34. Kawohl W, Bruhl A, Krowatschek G, Ketteler D, Herwig U. Functional magnetic resonance imaging of tics and tic suppression in gilles de la tourette syndrome. *World J Biol Psychiatry.* (2009) 10:567–70. doi: 10.1080/15622970802118356
35. Moriarty J, Costa DC, Schmitz B, Trimble MR, Ell PJ, Robertson MM. Brain perfusion abnormalities in gilles de la tourette's syndrome. *Br J Psychiatry.* (1995) 167:249–54. doi: 10.1192/bjp.167.2.249
36. Muller-Vahl KR, Kaufmann J, Grosskreutz J, Dengler R, Emrich HM, Peschel T. Prefrontal and anterior cingulate cortex abnormalities in tourette syndrome: evidence from voxel-based morphometry and magnetization transfer imaging. *BMC Neurosci.* (2009) 10:47. doi: 10.1186/1471-2202-10-47
37. van der Salm SMA, van der Meer JN, Cath DC, Groot PFC, van der Werf YD, Brouwers E, et al. Distinctive tics suppression network in Gilles de la Tourette syndrome distinguished from suppression of natural urges using multimodal imaging. *Neuroimage Clin.* (2018) 20:783–92. doi: 10.1016/j.nicl.2018.09.014
38. Menon V. Large-scale brain networks and psychopathology: a unifying triple network model. *Trends Cogn Sci.* (2011) 15:483–506. doi: 10.1016/j.tics.2011.08.003
39. Eldaief MC, Halko MA, Buckner RL, Pascual-Leone A. Transcranial magnetic stimulation modulates the brain's intrinsic activity in a frequency-dependent manner. *Proc Natl Acad Sci USA.* (2011) 108:21229–34. doi: 10.1073/pnas.1113103109
40. Wang JX, Rogers LM, Gross EZ, Ryals AJ, Dokucu ME, Brandstatt KL, et al. Targeted enhancement of cortical-hippocampal brain networks and associative memory. *Science.* (2014) 345:1054–7. doi: 10.1126/science.1252900
41. Wang J, Deng XP, Wu YY, Li XL, Feng ZJ, Wang HX, et al. High-frequency rTMS of the motor cortex modulates cerebellar and widespread activity as revealed by SVM. *Front. Neurosci.* (2020) 14:186. doi: 10.3389/fnins.2020.00186
42. Liu ZR, Miao HH, Yu Y, Ding MP, Liao W. Frequency-specific local synchronization changes in paroxysmal kinesigenic dyskinesia. *Medicine.* (2016) 95:e3293. doi: 10.1097/MD.0000000000003293
43. Yu R, Hsieh MH, Wang HL, Liu CM, Liu CC, Hwang TJ, et al. Frequency dependent alterations in regional homogeneity of baseline brain activity in schizophrenia. *PLoS ONE.* (2013) 8:e57516. doi: 10.1371/journal.pone.0057516
44. Xue S, Wang X, Wang W, Liu J, Qiu J. Frequency-dependent alterations in regional homogeneity in major depression. *Behav Brain Res.* (2016) 306:13–9. doi: 10.1016/j.bbr.2016.03.012
45. Gao L, Bai L, Zhang Y, Dai XJ, Netra R, Min Y, et al. Frequency-dependent changes of local resting oscillations in sleep-deprived brain. *PLoS ONE.* (2015) 10:e0120323. doi: 10.1371/journal.pone.0120323

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Efficacy of Fecal Microbiota Transplantation for Children With Tourette Syndrome: A Preliminary Study

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Therapies for Tourette syndrome (TS) are insufficient, and novel therapies are needed. Fecal microbiota transplantation (FMT) has been a potential therapy for several neurological diseases. Here, we report a preliminary study to investigate the effects of FMT on patients with TS. Five patients with TS received a single administration of FMT via endoscopy. Tic symptoms were assessed by Yale Global Tic Severity Scale-Total Tic Score (YGTSS-TTS) and adverse effects were recorded at week 8 following FMT. Lipopolysaccharide (LPS) levels and 14 cytokines levels were measured. The microbiota profile in feces were analyzed by shotgun metagenomics. Four patients (4/5) responded positively to FMT (YGTSS-TTS reduction rate >25%) at week 8 with high safety. The levels of LPS and cytokines varied after FMT. FMT shifted the composition of the gut microbiota in patients close to that of the donor and continuously changed the abundance of *Bacteroides coprocola, Dialister succinatiphilus* and *Bacteroides vulgatus*. The restoration of *B.coprocola* was correlated with the improvement in tic symptoms (Spearman $R = -0.900$, $P = 0.037$). In conclusion, FMT was indicated a potential effective and safe alternative for patients with TS. However, larger clinical trials are needed to confirm the influence of microbiota in TS.

Trial Registration: chictr.org.cn Identifier: ChiCTR-IIR-17011871, URL: http://www.chictr.org.cn/showproj.aspx?proj=19941.

Keywords: tourette syndrome, fecal microbiota transplantation, shotgun metagenomics, lipopolysaccharide, cytokines

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INTRODUCTION

Tourette syndrome (TS) is a combination of persistent multiple motor tics and at least one kind of vocal tic lasting for more than 1 year in youths before reaching the age of 18 years old (1). The prevalence of TS is ∼0.8% worldwide and 1.7% in China (2, 3). Approximately 80–90% of patients with TS have common neuropsychiatric comorbidities, such as attention-deficit/hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), anxiety, depressive disorders, and autism spectrum disorders (ASDs) (4–6). Generally, genetic and environmental

factors play a substantial role in the onset of TS; however, the intrinsic etiologies are currently poorly understood. Environmental factors include pre- and perinatal factors (7), psychosocial stress (8), and abnormal innate and adaptive immune responses (9). In addition, increasing evidence indicates that infections and immune activation might be part of the pathogenesis of TS (10). Infections with group A streptococci (GAS) have been implicated in the development of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), which is a subtype of pediatric OCD and/or TS. One potential mechanism of PANDAS is that GAS induce inflammatory/immunological dysregulation (11). Abnormal activation of the Toll-like receptor (TLR) pathway induced by lipopolysaccharide (LPS) produced by gram-negative bacteria has been observed in TS patients. LPS has further been found to aggravate tic symptoms and increase inflammatory cytokine production in rat models of TS (12).

Behavioral therapy and pharmacotherapy for TS are conducted with an emphasis on the individual. Pharmacotherapy includes antipsychotic medications (haloperidol, tiapride, haloperidol, and risperidone and aripiprazole) and alpha agonists (clonidine and guanfacine) (6). However, a certain proportion of patients may fail to respond to pharmacological treatments alone or in combination, and the above medications produce some potential adverse effects, such as drug-induced movement disorders, metabolic and hormonal effects, and sedation (13). Deep brain stimulation (DBS) has been used extensively as an invasive neuromodulation method in patients with severe, medically refractory TS. However, uncertainties surrounding targeted anatomical selection, controversial age cut-offs for patient consideration for surgery and a high frequency of postoperative infections are drawbacks of DBS (13). The TS requires further research to clarify its pathogenesis, and alternative treatment options still need to be explored.

Previous studies have suggested that gut microbiota substantially influence the development of the brain and behavior through bidirectional communication via the microbiota-gut-brain axis (14). ASD and ADHD, which are neurological diseases and sometimes co-occur with TS, has been associated with altered gut microbial profiles: decreased Alistipes, Dialister, Veillonella and increased Collinsella, Dorea, and Lactobacillus abundances are found in ASD patients (15); decreased Faecalibacterium abundance has been found in ADHD patients (16).

There are several interventions for modulating gut microbiota to relieve behavioral abnormalities, including probiotics, antibiotics, diet, and especially fecal microbiota transplantation (FMT) (14). FMT, which reconstitutes the balance of patients gut microbiota with that of fecal microbiota from healthy donors, has been effectively used for treating recurrent Clostridium difficile infection (17) and has also been used to treat inflammatory bowel disease (18) and hepatic encephalopathy (19). Recent clinical trials have further shown that FMT could persist in alleviating the symptoms of ASD (20) and epilepsy (21) by reconstituting the recipient gut microbiota. In addition, increasing evidence shows that gut microbiota modulates different neurological diseases via different mechanisms of the microbiota-gut-brain axis. For example, a previous study showed that TLR-4-mediated inflammation triggers intestinal and/or brain inflammation, which further aggravates neurodegeneration in Parkinson's disease patients (22); the gut microbiome of patients with schizophrenia alters the glutamate-glutamine-GABA cycle and worsens schizophrenia-relevant behaviors (23); microbial reconstitution reverses the social and synaptic deficits of maternal high-fat diet (MHFD) offspring by correcting oxytocin levels and synaptic potentiation (LTP) in the ventral tegmental areas (VTAs) (24).

Considering that new alternatives are urgently required to relieve the symptoms of patients with TS who fail to respond to medications or DBS, and the changes in gut microbial populations are correlated with multiple neuropsychiatric diseases (20–23), it is worth exploring the effect of FMT in TS. Our research team previously focused on performing FMT to patients with ulcerative colitis (UC), and achieved high clinical response and safety (25). Then we conducted an FMT to one patient with TS and found that the tic symptoms assessed by the Yale Global Tic Severity Scale-Total Tic Score (YGTSS-TTS) were ameliorated notably by 8 weeks (26). Here, we performed FMT treatment on five patients with TS to assess its efficacy and safety; we further explored the alterations of fecal microbial composition and serum cytokines after FMT.

MATERIALS AND METHODS

Ethical Approval

This trial was approved by the Ethical Committee of Chinese People's Liberation Army General Hospital (S2015-110-02) and registered in the Chinese Clinical Trial Registry (ChiCTR-IIR-17011871). The patients and their legal representative and/or the patients voluntarily participated in the study and signed the informed consent form.

Patient Recruitment

Five patients meeting the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) and YGTSS-TTS (combined motor tic and vocal tic score) > 13 were included (27). Patients were eligible if they had been diagnosed for more than one year, had a persistent high level of tic severity, and had a relapse or were intolerant to regular medications for tics disorders. Patients continued to receive regular medication therapy for tics if the medication was stable for at least 3 weeks and no changes occurred over the 8-week trial. Enrolled patients were required to stop using antimicrobial drugs or probiotics for more than 1 month.

Donor Screening and FMT Procedure

A questionnaire about the family medical history and individual medical history, and laboratory blood and fecal examinations for pathogens were used for donor screening. Healthy fecal donor inclusion criteria were as follows: aged 5–30 years without sex limitation. Exclusion criteria included known infectious diseases, current gastrointestinal disease, and other systematic diseases, and use of medications causing dysbiosis. One hundred and thirty one volunteers were screened for eligibility, including

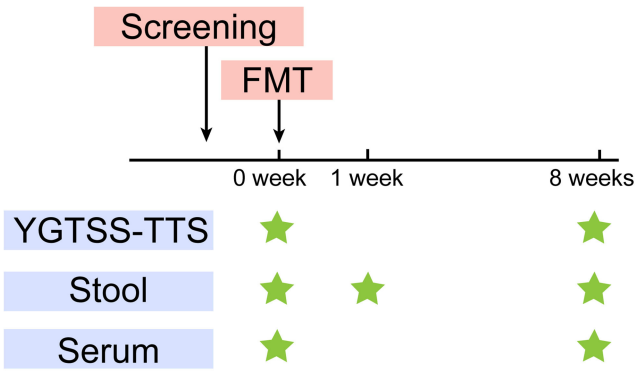

FIGURE 1 | Study design. After screening, FMT was performed at W0. YGTSS-TTS was assessed at baseline (W0) before FMT and W8. Stool samples were collected for metagenome sequencing and lipopolysaccharide (LPS) measurement at W0, W1, W8. Serum samples were collected for LPS and cytokines measurement at W0, W8.

59 males and 72 females, with an average age of 33.5 (range 3–67) years. Five people were enrolled as donors according the inclusion and exclusion criteria. In a separate study, we performed FMT to 62 patients with UC from the five donors included in the current study and compared the rate of clinical remission (defined as a total Mayo score (for UC activity) $\leq$ 2, combined with all Mayo subscores $\leq$ 1). We subsequently identified that the efficacy of FMT using the feces from a 14-year-old male was superior to those using other donors after comparison (data unpublished). Therefore, we selected the 14-year-old male donor for the five TS patients in the current study. We collected ∼120 g of fresh stool for every treatment. The stool was homogenized with 500 ml physiological saline (Kelidai, China) and then filtered to an ∼400 ml suspension (donor fecal liquid, DL). After intravenous anesthesia, FMT treatment was performed with 100 ml of fecal suspension delivered through a gastroscope into the duodenum and 300 ml delivered to the colon via a colonoscopy. The detailed process of donor screening and the FMT procedure were previously reported in Wang et al. (25).

Outcomes

The primary outcome was the YGTSS-TTS. The YGTSS is a multi-dimensional, clinician-rated scale assessing tic severity, including the Total Tic Score (TTS) (0–50) and the Overall Impairment Score (0–50). The YGTSS-TTS is the combination of the Total Motor Tic score and the Total Vocal Tic score, which are assessed separately from five dimensions: the number, frequency, intensity, complexity and inference. Each item is scored from 0 to 5. Clinical response is defined as a YGTSS-TTS score-reduction rate of > 25% (27). We assessed the tic severity at baseline (W0) and 8 weeks after treatment (W8) using YGTSS-TTS (**Figure 1**), which was conducted by an independent evaluator.

Sample Collection

The fecal samples were collected from the five TS patients at W0, W1 (1 week after FMT) and W8. The donor feces (DF) was collected for every treatment. We further collected the DL, which represented the microbial composition that was ultimately transplanted into the patients. We also collected serum samples from patients at W0 and W8 (**Figure 1**). All the samples were stored at −80°C.

Limulus Amoebocyte Lysate Assay and Cytokine Analysis

We detected fecal and serum LPS levels and the serum levels of 14 cytokines. Serum and fecal LPS levels were measured using a limulus amoebocyte lysate (LAL) assay (Xiamen Bioendo Technology Co., Ltd, Xiamen, China) following the manufacturer's protocol. In brief, serum was diluted 10-fold in pyrogen-free water and inactivated at 70°C for 10 min. One gram of fecal sample was dissolved in 10 ml of sterile PBS, vortexed gently, and centrifuged at 3,000 rpm for 15 min. The supernatant was filtered through a 0.45-μm filter and a 0.22-μm filter successively and inactivated at 90°C for 15 min (28). The cytokine analysis was conducted using a multiplexing bead immunoassay (AimPlex Biosciences, Inc., USA) following the manufacturer's protocol to measure the levels of 14 different cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17A, IL-17F, IL-22, TNFα, TNFβ, and IFN-γ. Briefly, 45 μl samples were incubated with antibody-coupled fluorescent beads, washed and incubated with Biotin-dAb and NR-dAb diluent. The beads were analyzed using a flow cytometer (BD Bioscience, San Diego, CA, USA). Standard curves were generated by FCAP Array 3.0 software (BD Bioscience, San Diego, CA, USA) to determine the concentration of unknown sample.

Shotgun Metagenomic Sequencing and Analysis

Shotgun metagenomic sequencing was conducted as previously reported (29). In brief, DNA in the fecal sample (200 mg) and DL (200 μl) was extracted using a QIAamp DNA Stool Mini kit (QIAGEN), and the concentration was gauged by a NanoDrop instrument. The DNA library construction was performed following the Illumina TruSeq DNA Sample Prep v2 Guide, and the libraries were sequenced using an Illumina HiSeq 4000 (10G per sample). After data quality control and host genome filtering, the Illumina short reads were *de novo* assembled using SOAP *de novo* software (V2.04), and the obtained Scaffolds were cut into contigs, which were further used for gene prediction. The microbial composition at different taxonomic levels was annotated by MEGAN software (version 5, http://ab.inf.uni-tuebingen.de/data/software/megan5/download/welcome.html) with matched genes. The abundance of a taxonomic group equalled the sum of the gene abundance annotated to the species. The genes were assigned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology/module group for functional annotation.

Statistical Analysis

Non-normally distributed continuous data were presented as median (range). LPS and cytokines levels were analyzed by Wilcoxon signed-rank test. The principal component analysis (PCA) was calculated by the unweighted UniFrac distance

metric and the analysis of similarities (ANOSIM) by the Vegan package in R (version 2.15.3). Microbiota differential abundance and function comparisons were performed using the linear discriminant analysis effect size (LEfSe) algorithm (http://huttenhower.sph.harvard.edu/galaxy), and an LDA score >3 was applied. Further pairwise comparisons of relative abundance among groups were analyzed via Metastats (http://metastats.cbcb.umd.edu/) (30). Spearman's correlation coefficient was calculated using R. $P < 0.05$ was considered statistically significant.

DATA AVAILABILITY

The metagenomic sequencing data are available in the BioProject database under research ID PRJNA628029 and the SRA accession number SUB7328137 (https://submit.ncbi.nlm.nih.gov/subs/sra/SUB7328137).

RESULTS

Patient Characteristics

Five boys with TS were enrolled for FMT treatment in this pilot study (**Table 1**). The average age of the patients was 8 years (range 7–10 years), and the body mass index (BMI) was 18.0 (range 13.2–26.3). The disease duration before FMT ranged from 1.5 to 4 years. All their anti-streptolysin O titres (ASOT) tests were negative. Patients 2,3, and 4 had TS combined with ADHD; patient 3 had variant asthma.

Efficacy and Safety of FMT in TS Patients

Table 2 show the YGTSS-TTS results for five patients. Four patients (4/5) achieved a clinical response at week 8 after FMT; patient 2 was the exception. The YGTSS-TTS of the remaining four patients decreased with a range of 7–35. The total tic symptoms of patient 3, who failed to adequately respond to medication, disappeared at week 8, and the vocal tic symptoms of patient 1 were also completely resolved. However, the vocal tic score of patient 2 increased from 13 to 17, which indicated a slight aggravation of symptoms. During the FMT process and follow-up period, no patients experienced any obvious adverse events, such as allergy, nausea, vomiting, diarrhea or abdominal pain. The body temperature of all five patients rose slightly 24 h after FMT treatment (**Table 3**).

Variations in LPS and Cytokine Levels Following FMT

We explored the changes in LPS and 14 cytokines in TS patients. There were no significant changes in the LPS concentration in the feces and serum or in the concentration of all the cytokines in the serum before and after FMT (**Table 4**). The LPS concentrations of patients 2 and 4 in stool and serum samples were dramatically higher than those of the donor at baseline and persistently decreased after FMT (**Figures 2A,B**). The concentration of serum IL-6 was reduced in patient 4 and patient 5 following FMT but elevated in the other three patients (**Figure 2C**). Levels of both IL-17F and IL-22 were decreased in patient 2 (**Figures 2D,E**).

Alterations in Patient Microbiota Composition Before and After FMT

We further investigated the microbiota composition and diversity in TS patients before and after FMT by metagenomics analysis. The number of non-redundant genes in TS patients prior to FMT was significantly decreased compared to that in the donor ($P = 0.073$, DF VS W0; $P = 0.043$, DL VS W0, analysis of variance (ANOVA), **Supplementary Figure 1**) and transiently increased following FMT treatment ($P = 0.053$, W0 VS W1, ANOVA, **Supplementary Figure 1**). Principal coordinates analyses (PCoA) calculated by the unweighted UniFrac distance metric revealed a clear cluster of patients with TS prior to FMT away from the donor (**Figure 3F**). The gut microbiota composition in TS patients 1, 2, 4, and 5 temporarily shifted close to the profile of the donor microbiota by W1 but moved away by W8 though remaining dissimilar to the composition prior to FMT and to that of the healthy donor (**Figures 3A,B,D,E**). However, there was no obvious change observed in patient 3 whose microbiota composition remained distinct from the donor (**Figure 3C**). The analysis of similarity (ANOSIM) showed a similar trend in microbial composition among the different time points (DF VS W0: R = 0.268, $P = 0.006$; DF VS W1: $R = 0.092$, $P = 0.161$; DF VS W8: $R = 0.212$, $P = 0.036$; W0 VS

TABLE 1 | The general information of the five patients.

Code	Sex	Age (years)	BMI	Duration (years)	ASOT	Comorbidities	Concomitant medication
P1	M	7	13.2	4	_	NR	Tiapride
P2	M	10	26.3	4	_	ADHD	Aripiprazole, trihexyphenidyl
P3	M	10	20.7	3	_	ADHD, Variant asthma	Aripiprazole, risperidone, trihexyphenidyl
P4	M	9	19.5	4	_	ADHD	Tiapride
P5	M	7	16.4	1.5	_	NR	Tiapride

The symptoms of tic disorders in patients persisted for more than 1 year, and concomitant medications were used during the treatment.
BMI, body mass index; ASOT, anti-streptolysin O titres; M, male; ADHD, attention-deficit/hyperactivity disorder; NR, no report.

TABLE 2 | YGTSS-TTS scores and reduction rate in the five patients.

	Motor tic score			Vocal tic score			Total tic score			
	W0	W8	Change	W0	W8	Change	W0	W8	Change	Reduction rate %
P1	20	7	−13	16	0	−16	36	7	−29	80.6
P2	14	14	0	13	17	4	27	31	4	−14.8
P3	18	0	−18	17	0	−17	35	0	−35	100
P4	11	9	−2	10	5	−5	21	14	−7	33.3
P5	24	8	−16	12	4	−8	36	12	−24	66.6

W0, Baseline; W8, Eight weeks after treatment.
DF, donor feces; DL, donor fecal liquid; W0, baseline of patients; W1, week 1 after FMT; W8, week 8 after FMT.

TABLE 3 | The adverse events after FMT.

	Temperature (°C)		Allergy	Nausea	Vomiting	Diarrhea	Abdominal pain
	Pre-FMT	Post-FMT					
P1	36.8	37.2	-	-	-	-	-
P2	36.1	37.1	-	-	-	-	-
P3	36.5	37.0	-	-	-	-	-
P4	37.2	37.2	-	-	-	-	-
P5	36.8	37.2	-	-	-	-	-

The body temperature of all five patients rose slightly 24 h after FMT treatment.

TABLE 4 | Fourteen cytokine levels in the serum before and after FMT.

	W0 (pg/ml), median(range)	W8 (pg/ml), median(range)	z	*P*
IL-1β	1.52 (1.30–2.27)	1.68 (0.79–2.41)	−0.674	0.500
IL-2	2.30 (0.84–4.91)	2.45 (0.90–43.43)	−0.405	0.686
IL-4	1.64 (0.41–3.77)	0.44 (0.10–1.16)	−1.753	0.080
IL-5	1.05 (0.51–3.91)	0.90 (0.80–1.66)	−0.542	0.588
IL-6	8.30 (5.95–13.53)	15.36 (2.59–16.92)	−1.214	0.225
IL-8	7.05 (2.19–34.00)	10.06 (3.85–57.18)	−0.405	0.686
IL-10	3.61 (2.66–4.66)	5.01 (3.24–28.80)	−1.753	0.080
IL-12P70	3.31 (2.61–4.00)	3.32 (3.08–3.83)	−0.135	0.893
IL-17A	0.95 (0.44–3.87)	1.17 (0.20–2.06)	−0.135	0.893
IL-17F	0.67 (0.38–3.42)	1.51 (1.15–2.26)	−0.674	0.500
IL-22	2.16 (0.39–3.56)	1.50 (0.46–4.71)	−0.135	0.893
TNFα	3.59 (3.08–4.30)	3.78 (2.81–4.36)	−0.944	0.345
TNFβ	1.84 (1.39–1.96)	1.94 (1.67–2.16)	−1.214	0.225
IFNγ	3.07 (2.68–4.12)	3.31 (2.25–5.13)	−0.135	0.893

W1: $R = 0.092$, $P = 0.235$; W0 VS W8: $R = -0.08$, $P = 0.714$; **Supplementary Figure 2**), indicating that FMT treatment transiently altered the gut microbiota composition of patients to that of the donor.

We further explored changes in the microbiota composition of TS patients following FMT. Four different phyla, Firmicutes (53%), Bacteroidetes (27%), Actinobacteria (4%) and Proteobacteria (4%), dominated the gut microbiota composition of the five TS patients (**Supplementary Figure 3**). We performed LEfSe analysis and Metastat analysis to investigate the significant differences between TS patients and the healthy donor. LEfSe analysis revealed that the genera Bifidobacterium, Collinella, Dorea and Catenibacterium were much lower in TS patients than in the donor, and the abundance of the above genera transiently increased to the level of the donor at W1 but decreased by W8 (**Supplementary Figure 4**). Furthermore, the abundance of species *Roseburia faecis, Bacteroides coprocola, Dialister succinatiphilus, Catenibacterium mitsuokai, Holdemanella biformis*, and *Allisonella histaminiformans* was significantly decreased in TS patients compared with the healthy donor (relative abundance of DF VS W0, 0.0153 VS 0.0032, $P = 0.0001$; 0.0064 VS 0.0021, $P = 0.0161$; 0.0070 VS 0.0003, $P = 1.59^{*}10^{-6}$; 0.0051 VS 0.0002, $P = 0.0009$; 0.0075 VS $4.8^{*}10^{-5}$, $P = 0.0004$; 0.0016 VS $2.8^{*}10^{-5}$, $P = 0.0002$, **Figure 4A**); however, the abundance of *Bacteroides vulgatus* was significantly increased (relative abundance of DF VS W8, 0.0027 VS 0.0089, $P = 0.0017$) (**Figure 4A**). Following FMT, *Bacteroides coprocola* abundance was restored in patients 3, 4, and 5 (**Figure 4B**), and the abundance of *Dialister succinatiphilus* was restored in patients 4 and 5 (**Figure 4C**). However, *Bacteroides vulgatus* abundance remained continuously reduced in all five patients (relative

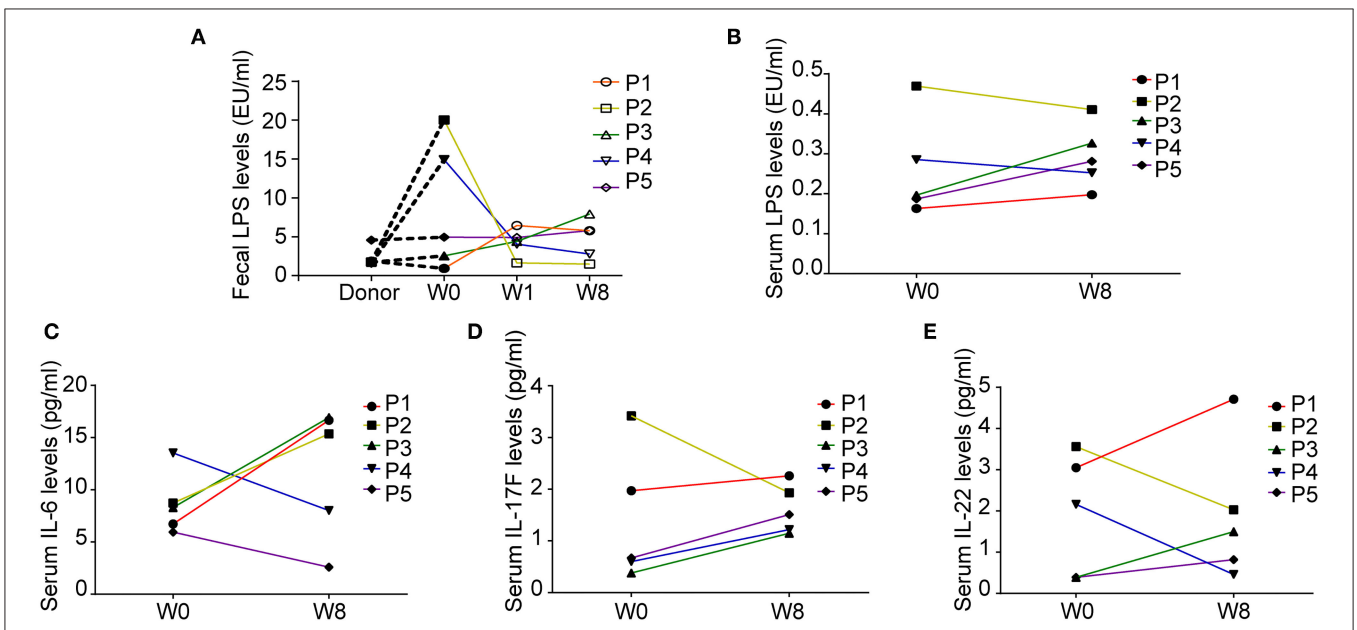

FIGURE 2 | Fecal and serum lipopolysaccharide (LPS) levels and serum cytokine levels in patients with Tourette Syndrome (TS) following FMT. **(A)** Fecal LPS levels of the donor and five patients with TS. **(B)** Serum LPS levels of five patients with TS before and after FMT. **(C)** Serum IL-6 levels. **(D)** Serum IL-17F levels. **(E)** Serum IL-22 levels.

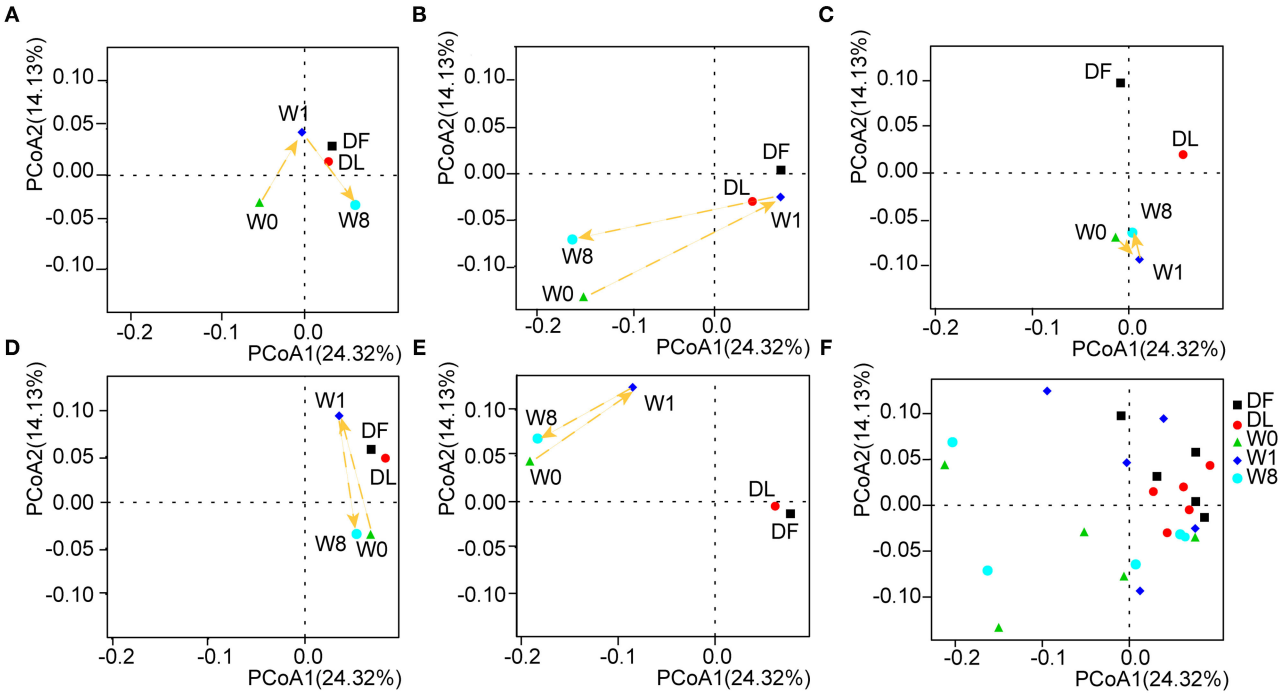

FIGURE 3 | The shift in gut microbiota in the five patients before and after FMT. Principal coordinates analyses (PCoA) was performed by the unweighted UniFrac distance metric using the whole data set and showed a shift in the gut microbiota of each patient separately **(A–E)** and in combination **(F)**.

abundance: W0 = 0.0089, W1 = 0.0038, W8 = 0.0040, P_{W0vsW1} = 0.0372, P_{W0vsW8} = 0.0380) (**Figure 4D**). Furthermore, the change in YGTSS-TTS showed a strong negative correlation with the abundance of *Bacteroides coprocola* (Spearman $R = -0.900$, $P = 0.037$).

Functional Transformation/Diversification Following FMT Treatment

We characterized the functional changes in the gut microbiota using the KEGG database to annotate the metagenomics data. Dramatic functional differences between TS patients and healthy donors and post-FMT changes in TS patients resembling the donor gut microbiota were found (**Figure 5A**). The genes predominantly related to the biosynthesis of amino acids, glycan biosynthesis, and metabolism were significantly different between TS patients (W0) and healthy donors (DF). Amino acid biosynthesis contributing to arginine biosynthesis, lysine biosynthesis, terpenoid backbone biosynthesis and peptidoglycan biosynthesis pathways were significantly enriched, whereas glycosphingolipid biosynthesis and sphingolipid metabolism pathways were depleted promptly after FMT treatment (W1); however, all KEGG KOs had shifted back to the primary state by W8 (**Figure 5B**).

DISCUSSION

Herein, we report the significant clinical efficacy and safety of FMT for treating patients with TS and assessed for changes in LPS, cytokine levels, and microbial composition following the modulation of gut microbiota through FMT.

FMT has been shown to have a certain therapeutic effect on several neuropsychiatric-related diseases, such as hepatic encephalopathy, ASD and epilepsy (19–21). Previously, we found that FMT dramatically ameliorated the tic severity in one case (26). In this study, five patients who inadequately responded to medication intervention received one single FMT treatment. As the FMT procedures were variable, we chose to combine gastroscopy and colonoscopy under intravenous anesthesia for FMT treatment. Four of these patients exhibited clinical responses, with the exception of patient 2, indicating the significant efficacy of FMT in TS patients. All five patients showed good tolerance to the FMT treatment, and no one experienced any obvious adverse effects.

In our study, no statistically significant changes of LPS or cytokines in the five patients were observed. However, previous studies have shown that the peripheral immune system in patients with TS might be skewed to a pro-inflammatory state. LPS has been reported to significantly aggravate stereotypical and autonomic activity in TS rat models with increased IL-1β, IL-6, and TNFα levels as well as high expression levels of TLR4 (31). Here, we found that the LPS level in feces in patients 2 and 4 at baseline was higher than that in the donor, whereas it persistently decreased after FMT (W1, W8) and was consistent with the changes in serum, indicating that FMT might have reduced the pro-inflammatory immune responses of the above two patients. Previous studies reported that the pro-inflammatory cytokines IL-1β, IL-6, IL-12, IL-17, and TNFα are significantly elevated in

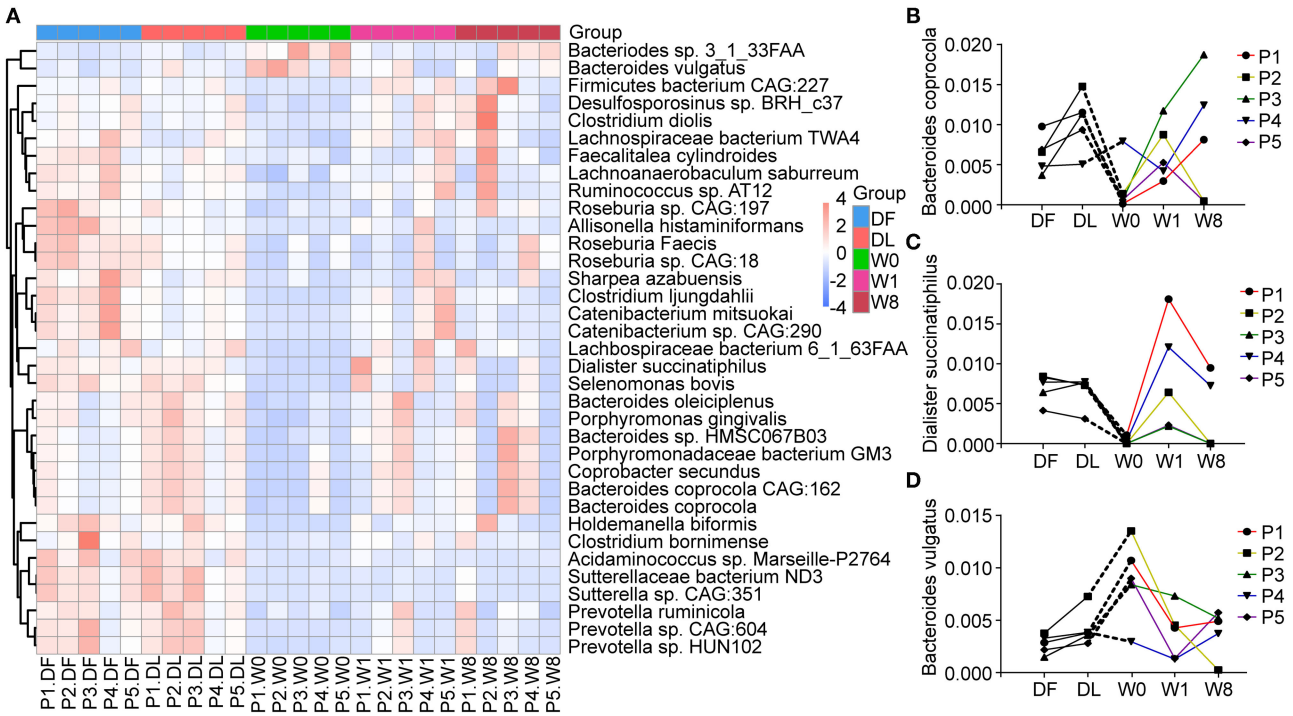

FIGURE 4 | Changes in microbial composition at the species levels after FMT generated with Metastats. **(A)** A heatmap showing the relative abundance of 35 species in different groups. **(B–D)** The dynamic changes in *Bacteroides coprocola, Dialister succinatiphilus,* and *Bacteroides vulgatus* in Tourette Syndrome (TS) patients.

patients with TS (12). The levels of IL-2, IL-12, and TNFα are positively correlated with the severity of tic symptoms (32).

In our study, the levels of IL-6 in patients 4 and 5, and IL-17F and IL-22 in patient 2 were down-regulated, suggesting a potential decrease in inflammation in these three patients. The impact of FMT treatment on the immune system cannot be ignored, while further studies with a larger sample size are needed to confirm the relevant alterations of LPS and cytokines after FMT.

Former literature focused on the composition and effect of gut microbiota in TS patients is rare. In our study, reduced gene numbers and a PCoA cluster separated from the donor cluster were observed at baseline, which is consistent with a previous study focusing on PANS/PANDAS showing a reduced operational taxonomic unit (OTU) number in the α-diversity analysis and a clear cluster apart from the healthy control cluster in the β-diversity analysis (33). Then, an obvious shift in the gut microbiota composition close to that of the donor microbiota was observed in four patients by the first week after FMT, indicating that the gut microbiota of the FMT recipients highly resembled that of the donor. However, the shift was transient, and the gut microbiota of the four patients deviated by week 8 to a state distinct from the baseline state. Interestingly, the tic symptoms of patients 1, 4, and 5 steadily improved by week 8, which is not consistent with the alterations in the overall microbiota shift. This phenomenon has also been observed in patients with other diseases treated with FMT, such as hepatic enteropathy (19) and refractory immune checkpoint inhibitor (ICI)-associated colitis (34). In addition, there is currently no uniform FMT frequency for the treatment of different diseases. Li et al. suggested that the interval should be <4 months for maintaining clinical efficacy in Crohn's disease after comprehensively assessing the changes in clinical symptoms, gut microbiota and metabolites (35).

In our study, changes in the abundance of three species remained stable at week 1 and week 8 after FMT: the relative abundance of *Bacteroides coprocola* and *Dialister succinatiphilus* was continuously increased in some of the patients, and *Bacteroides vulgatus* was absent in all patients. Moreover, *Bacteroides coprocola* abundance was negatively correlated with the improvement in tic symptoms. All the above results indicate that the three species have a potential influence on FMT treatment of TS. Previous studies reported that a special set of *B. coprocola* strains with a characteristic single nucleotide polymorphism (SNP) distribution was correlated with type 2 diabetes (T2D) (36). *D. succinatiphilus* participated in short-chain fatty acid (SCFA) generation via decarboxylating succinate to propionate (37). *B. vulgatus* is an opportunistic pathogen related to the increased incidence of T2D with increased inflammatory cytokines, specifically IL-6, and polycystic ovary syndrome by decreasing IL-22 secretion (38, 39), but negatively associated with atherosclerosis and LPS production (28). These findings suggest *B. vulgatus* plays an important role in different diseases through influencing the immune system. However, no obvious correlation between *B. vulgatus* and LPS/cytokines was

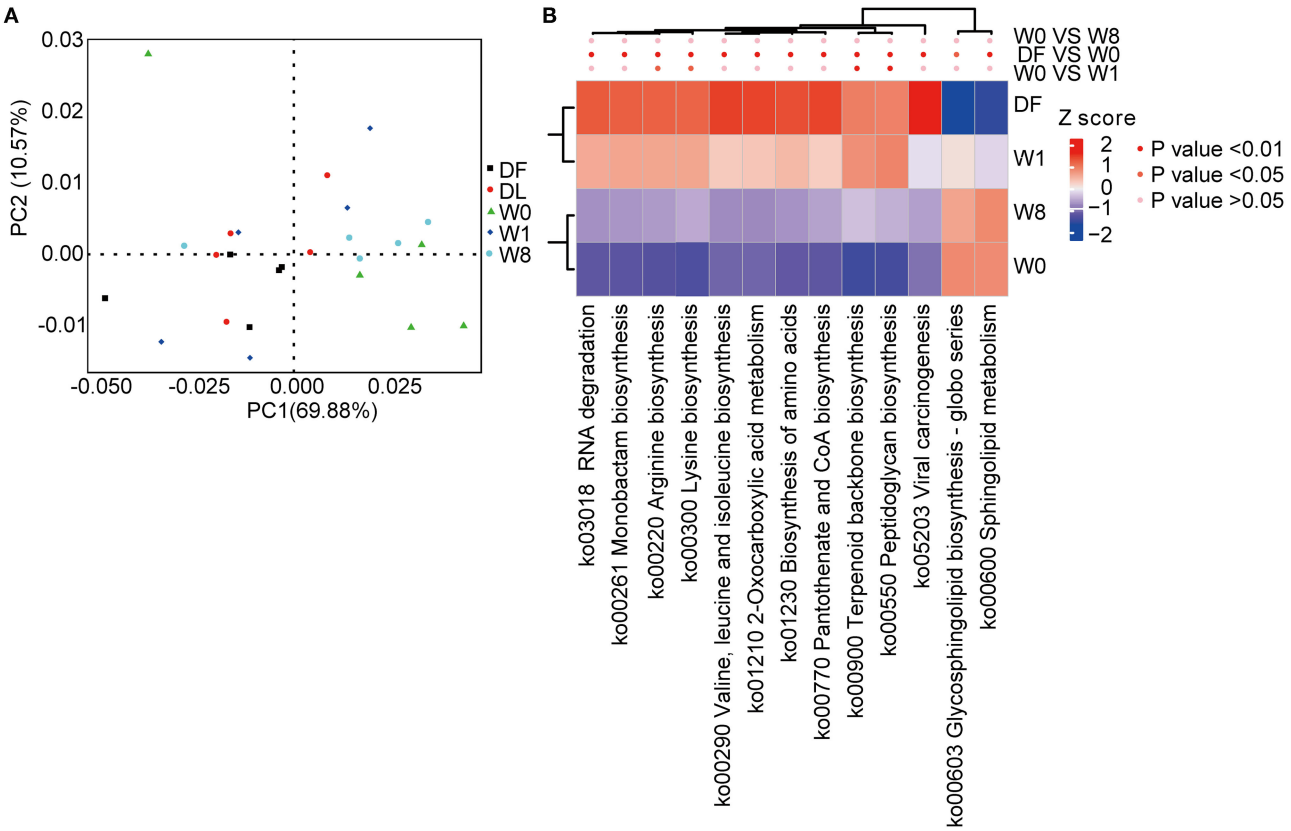

FIGURE 5 | Microbial functional differences after FMT assessed by metastats. **(A)** principal component analysis (PCA) showing the dynamic alterations in functional features. **(B)** A heatmap showing the significant differences in several Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

observed in our study, likely due to the limitation of trial design and the sample size enrolled.

It is critical to explore alterations in metabolites related to the intestinal microbial compositions in TS patients receiving FMT. We found that after FMT, a number of metabolic pathways showed fluctuations, such as significant changes in amino acid metabolism, including the biosynthesis of arginine, lysine, valine, leucine, and isoleucine. Previous studies have revealed that dopamine, GABA and glutamate, as neurotransmitters and the metabolites of amino acids, greatly influence tic pathophysiology (40); furthermore, GABA and glutamate antagonists have been suggested as treatment options for TS (41). However, the literature focusing on the microbiota and metabolites in the gut of patients with TS is scarce and more studies are urgently required.

This pilot study reported the efficacy and safety of FMT on five TS patients and the specific alterations of fecal microbial composition and serum cytokines following FMT. However, there are several limitations. Only a small number of patients was enrolled without a control group. The follow-up period is relatively short and long-term effect of FMT on TS cannot be estimated. Nevertheless, our study indicated the crucial role of gut microbiota in the pathogenesis of TS. Together, this study provides novel evidence that reconstitution of the gut microbiota through FMT might be a safe and effective alternative therapy for TS. A large-scale randomized, controlled clinical trial with a longer follow-up is needed to confirm the efficacy and safety.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI BioProject (accession: PRJNA628029).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Chinese PLA General Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

H-JZ and XL collected the samples, analyzed the data, and drafted the manuscript. Y-CY, L-HP, Y-CS, and J-FL performed the FMT treatments. GY, JW, and L-PZ enrolled the eligible patients. X-YS and L-YH conducted the YGTSS-TTS. FP and R-RR assisted with

analysis. Y-SY and L-PZ designed, funded, revised manuscript, and supervised the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.554441/full#supplementary-material

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, *5th Edn*. Washington, DC: American Psychiatric Publication (2013). doi: 10.1176/appi.books.9780890425596
2. Knight T, Steeves T, Day L, Lowerison M, Jette N, Pringsheim T. Prevalence of tic disorders: a systematic review and meta-analysis. *Pediatr Neurol*. (2012) 47:77–90. doi: 10.1016/j.pediatrneurol.2012.05.002
3. Yang C, Zhang L, Zhu P, Zhu C, Guo Q. The prevalence of tic disorders for children in China: a systematic review and meta-analysis. *Medicine*. (2016) 95: e4354. doi: 10.1097/MD.0000000000004354
4. Altman G, Staley JD, Wener P. Children with Tourette disorder: a follow-up study in adulthood. *J Nerv Ment Dis*. (2019) 197:305–310. doi: 10.1097/NMD.0b013e3181a206b1
5. Jalenques I, Auclair C, Morand D, Legrand G, Marcheix M, Ramanoel C, et al. Health-related quality of life, anxiety and depression in parents of adolescents with Gilles de la Tourette syndrome: a controlled study. *Eur Child Adolesc Psychiatr*. (2017) 26:603–17. doi: 10.1007/s00787-016-0923-5
6. Ludolph AG, Roessner V, Münchau A, Müller-Vahl K. Tourette syndrome and other tic disorders in childhood, adolescence and adulthood. *Dtsch Arztebl Int*. (2012) 109:821–288. doi: 10.3238/arztebl.2012.0821
7. Brander G, Rydell M, Kuja-Halkola R, Fernández de la Cruz L, Lichtenstein P, Serlachius E, Rück C, et al. Perinatal risk factors in Tourette's and chronic tic disorders: a total population sibling comparison study. *Mol Psychiatry*. (2018) 23:1189–97. doi: 10.1038/mp.2017.31
8. Buse J, Kirschbaum C, Leckman JF, Münchau A, Roessner V. The modulating role of stress in the onset and course of Tourette's syndrome: a review. *Behav Modif*. (2014) 38:184–216. doi: 10.1177/0145445514522056
9. Dalsgaard S, Waltoft BL, Leckman JF, Mortensen PB. Maternal history of autoimmune disease and later development of tourette syndrome in offspring. *J Am Acad Child Adolesc Psychiatr*. (2015) 54:495–501.e1. doi: 10.1016/j.jaac.2015.03.008
10. Krause DL, Müller N. The relationship between Tourette's Syndrome and infections. *Open Neurol J*. (2012) 6:124–8. doi: 10.2174/1874205X01206010124
11. Swedo SE, Seidlitz J, Kovacevic M, Latimer ME, Hommer R, Lougee L, et al. Clinical presentation of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections in research and community settings. *J Child Adolesc Psychopharmacol*. (2015) 25:26–30. doi: 10.1089/cap.2014.0073
12. Yeon SM, Lee JH, Kang D, Bae H, Lee KY, Jin S, et al. A cytokine study of pediatric Tourette's disorder without obsessive compulsive disorder. *Psychiatry Res*. (2017) 247:90–6. doi: 10.1016/j.psychres.2016.11.005
13. Martino D, Pringsheim TM. Tourette syndrome and other chronic tic disorders: an update on clinical management. *Expert Rev Neurother*. (2018) 18:125–37. doi: 10.1080/14737175.2018.1413938
14. Liang S, Wu X, Jin F. Gut-brain psychology: rethinking psychology from the microbiota-gut-brain axis. *Front Integr Neurosci*. (2018) 11:33. doi: 10.3389/fnint.2018.00033
15. Strati F, Cavalieri, Albanese D, De Felice C, Donati C, Hayek J, et al. New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome*. (2017) 5:24. doi: 10.1186/s40168-017-0242-1
16. Jiang HY, Zhou YY, Zhou GL, Li YC, Yuan J, Li XH, et al. Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. *Behav Brain Res*. (2018) 347:408–13. doi: 10.1016/j.bbr.2018.03.036
17. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol*. (2013) 108:478–98; quiz 499. doi: 10.1038/ajg.2013.4
18. Halkjær SI, Christensen AH, Lo BZS, Browne PD, Günther S, Hansen LH, et al. Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. *Gut*. (2018) 67:2107–15. doi: 10.1136/gutjnl-2018-316434
19. Bajaj JS, Kassam Z, Fagan A, Gavis EA, Liu E, Cox IJ, et al. Faecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. *Hepatology*. (2017) 66:1727–38. doi: 10.1002/hep.29306
20. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. (2017) 5:10. doi: 10.1186/s40168-016-0225-7
21. He Z, Cui BT, Zhang T, Li P, Long CY, Ji GZ, et al. Faecal microbiota transplantation cured epilepsy in a case with Crohn's disease: the first report. *World J Gastroenterol*. (2017) 23:3565–8. doi: 10.3748/wjg.v23.i19.3565
22. Perez-Pardo P, Dodiya HB, Engen PA, Forsyth CB, Huschens AM, Shaikh M, et al. Role of TLR4 in the gut-brain axis in Parkinson's disease: a translational study from men to mice. *Gut*. (2019) 68:829–43. doi: 10.1136/gutjnl-2018-316844
23. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Sci Adv*. (2019) 5: eaau8317. doi: 10.1126/sciadv.aau8317
24. Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell*. (2016) 165:1762–75. doi: 10.1016/j.cell.2016.06.001
25. Wang YZ, Ren RR, Sun G, Peng LH, Tian YP, Yang YS. Pilot study of cytokine changes evaluation after faecal microbiota transplantation in patients with ulcerative colitis. *Int Immunopharmacol*. (2020) 85:106661. doi: 10.1016/j.intimp.2020.106661
26. Zhao H, Shi Y, Luo X, Peng L, Yang Y, Zou L. The effect of faecal microbiota transplantation on a child with Tourette Syndrome. *Case Rep Med*. (2017) 2017:6165239. doi: 10.1155/2017/6165239
27. Jeon S, Walkup JT, Woods DW, Peterson A, Piacentini J, Wilhelm S, et al. Detecting a clinically meaningful change in tic severity in Tourette syndrome: a comparison of three methods. *Contemp Clin Trials*. (2013) 36:414–20. doi: 10.1016/j.cct.2013.08.012
28. Yoshida N, Emoto T, Yamashita T, Watanabe H, Hayashi T, Tabata T, et al. Bacteroides vulgatus and bacteroides dorei reduce gut microbial lipopolysaccharide production and inhibit atherosclerosis. *Circulation*. (2018) 138:2486–98. doi: 10.1161/CIRCULATIONAHA.118.033714
29. Shi YC, Guo H, Chen J, Sun G, Ren RR, Guo MZ, et al. Initial meconium microbiome in Chinese neonates delivered naturally or by cesarean section. *Sci Rep*. (2018) 8:3255. doi: 10.1038/s41598-018-21657-7
30. White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Comput Biol*. (2009) 5: e1000352. doi: 10.1371/journal.pcbi.1000352

31. Hongyan L, Zhenyang S, Chunyan W, Qingqing P. Lipopolysaccharide aggravated DOI-induced Tourette syndrome: elaboration for recurrence of Tourette syndrome. *Metab Brain Dis*. (2017) 32:1929–34. doi: 10.1007/s11011-017-0084-3
32. Leckman JF, Katsovich L, Kawikova I, Lin H, Zhang H, Krönig H, et al. Increased serum levels of interleukin-12 and tumor necrosis factor-alpha in Tourette's syndrome. *Biol Psychiatry*. (2005) 57:667–73. doi: 10.1016/j.biopsych.2004.12.004
33. Quagliariello A, Del Chierico F, Russo A, Reddel S, Conte G, Lopetuso LR, et al. Gut microbiota profiling and gut-brain crosstalk in children affected by pediatric acute-onset neuropsychiatric syndrome and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Front Microbiol*. (2018) 6; 9:675. doi: 10.3389/fmicb.2018.00675
34. Wang Y, Wiesnoski DH, Helmink BA, Gopalakrishnan V, Choi K, DuPont HL, et al. Faecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med*. (2018) 24:1804–1808. doi: 10.1038/s41591-018-0238-9
35. Li P, Zhang T, Xiao Y, Tian L, Cui B, Ji G, et al. Timing for the second faecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease. *Appl Microbiol Biotechnol*. (2019) 103:349–360. doi: 10.1007/s00253-018-9447-x
36. Chen Y, Li Z, Hu S, Zhang J, Wu J, Shao N, et al. Gut metagenomes of type 2 diabetic patients have characteristic single-nucleotide polymorphism distribution in Bacteroides coprocola. *Microbiome*. (2017) 5:15. doi: 10.1186/s40168-017-0232-3
37. Morotomi M, Nagai F, Sakon H, Tanaka R. Dialister succinatiphilus sp. nov. and Barnesiella intestinihominis sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol. (*2008) 58(Pt 12):2716–20. doi: 10.1099/ijs.0.2008/000810-0
38. Zafar H, Saier MH Jr. Comparative genomics of transport proteins in seven Bacteroides species. *PLoS ONE*. (2018) 13: e0208151. doi: 10.1371/journal.pone.0208151
39. Qi XY, Yun CY, Sun LL, Xia JL, Wu Q, Wang Y, et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat Med*. (2019) 25:1225–33. doi: 10.1038/s41591-019-0509-0
40. Singer HS. Habitual and goal-directed behaviours and Tourette syndrome. *Brain*. (2016) 139(Pt 2):312–6. doi: 10.1093/brain/awv378
41. Pogorelov V, Xu M, Smith HR, Buchanan GF, Pittenger C. Corticostriatal interactions in the generation of tic-like behaviors after local striatal disinhibition. *Exp Neurol*. (2015) 265:122–8. doi: 10.1016/j.expneurol.2015.01.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tourette Syndrome Risk Genes Regulate Mitochondrial Dynamics, Structure, and Function

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Gilles de la Tourette syndrome (GTS) is a neurodevelopmental disorder characterized by motor and vocal tics with an estimated prevalence of 1% in children and adolescents. GTS has high rates of inheritance with many rare mutations identified. Apart from the role of the neurexin trans-synaptic connexus (NTSC) little has been confirmed regarding the molecular basis of GTS. The NTSC pathway regulates neuronal circuitry development, synaptic connectivity and neurotransmission. In this study we integrate GTS mutations into mitochondrial pathways that also regulate neuronal circuitry development, synaptic connectivity and neurotransmission. Many deleterious mutations in GTS occur in genes with complementary and consecutive roles in mitochondrial dynamics, structure and function (MDSF) pathways. These genes include those involved in mitochondrial transport (*NDE1*, *DISC1, OPA1*), mitochondrial fusion (*OPA1*), fission (*ADCY2*, *DGKB, AMPK/PKA, RCAN1, PKC*), mitochondrial metabolic and bio-energetic optimization (*IMMP2L*, *MPV17, MRPL3, MRPL44*). This study is the first to develop and describe an integrated mitochondrial pathway in the pathogenesis of GTS. The evidence from this study and our earlier modeling of GTS molecular pathways provides compounding support for a GTS deficit in mitochondrial supply affecting neurotransmission.

Keywords: Tourette syndrome genes, Tourette syndrome cause, Tourette syndrome etiology, mitochondrial fission, mitochondrial supply

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INTRODUCTION

Gilles de la Tourette Syndrome (GTS) is a neurodevelopmental disorder with an estimated prevalence of 1% in children and adolescents (1). Neuroanatomical evidence suggests that GTS pathology is related to abnormal brain development and the physiological involvement of the cortico–striato–thalamo– cortical (CSTC) circuitry connecting the cortex, basal ganglia and thalamus (1). Clinical evidence further suggests the involvement of neurotransmitters such as dopamine, glutamate and γ-aminobutyric acid (GABA) (1). Epidemiological, phenomenological and genetic evidence demonstrate broad overlap between GTS and autism spectrum disorder (ASD) (2, 3) with both exhibiting high incidence in first-degree relatives, high monozygotic to dizygotic concordance (4), and with both conditions beginning during childhood with a high male preponderance. Furthermore, GTS and ASD share associated clinical features of compulsive behaviors, obsessions, involuntary movements (tics in GTS and stereotypies in ASD), poor speech control and echolalia common in both conditions (5). Attention deficit hyperactivity disorder (ADHD) is also present in both ASD and GTS (5, 6). GTS is over represented in ASD, with 5% having GTS and up to 40% experiencing tics (5). Similarly, the rate of autism in GTS exceeds

that expected by chance, with reports of ASD in around 22.8% of children and 8.7% in adults (7), subclinical autistic symptoms occurring in a third of GTS populations, and a further two-thirds showing social deficits relating to the autism spectrum (8). Pharmaco-therapeutic agents such as the α2-adrenergic agonists Clonidine and Guanfacine and the antipsychotics such as Risperidone and Aripiprazole are usually the first-line of therapy for moderate to severe GTS. However, side effects are particularly problematic during childhood years when the symptoms are most predominant and often affect compliance and hence there is a critical need for targeted therapeutic development based on a better understanding of the genetic etiology of the disorder.

GTS is one of the most heritable neuropsychiatric disorders of non-Mendelian inheritance, however, with the exception of the neurexin trans-synaptic connexus (NTSC) little is known regarding the molecular basis of GTS (9). One of the strongest mutation associations to date has been with *neurexin 1* and the genes encoding the NTSC which regulate neuronal circuitry development, synaptic connectivity and neurotransmission (2, 3, 10, 11). Members of the NTSC family of synaptic proteins bind across the synapse in different combinations to facilitate trans-synaptic cell-adhesion that helps establish and maintain neural circuits and neurotransmission within the brain. All major gene families of the NTSC (**Figure 1**) have been repeatedly mutated or otherwise associated with GTS and ASD (2, 11). Moreover, the number of mutations identified in and associated with the NTSC has continued to grow to such an extent that the NTSC now represents a collective mutation hot spot for GTS and ASD (2, 11, 13–22). Moreover, the NTSC model for GTS (**Figure 1**) provides a reliable starting point for further mutation pathway analysis into the mitochondrial regulation of neuronal circuitry development, synaptic connectivity and neurotransmission as it relates to mutations in GTS.

Neuronal Mitochondria: Up to 20% of the total energy consumed by humans at rest is attributable to brain activity despite a brain-to-body mass ratio of only 2% (33, 34). This high energy consumption by the human brain is largely attributed to requirements for synaptic transmission (33, 34). Neurons require particularly large amounts of energy for synaptic vesicle release and to power the ion pumps that restore ion gradients in the synapse following the ion influx associated with neuronal firing (34). These high energy demands are largely met by neuronal mitochondria, which also power other important neurodevelopmental processes including neurite outgrowth (35–40) which ultimately provides for optimal synaptic connections and neurotransmission. Mitochondria have additional roles in the neuron including calcium buffering, which is of particular importance in mitochondrial dynamics and neurotransmission (40). Although mitochondria are essential in almost every cell type, the extended branching structure and specialized function of neurons comes with unique demands over extended distances that render neurons especially sensitive to deficits in mitochondrial dynamics, structure and function. The sensitivity of neuronal development and function to mitochondrial deficiencies is corroborated by the strong association between the mutation of mitochondrial component molecules and neurological disorders (41), and there is increasing evidence that mitochondrial dynamics and dysfunction contribute to neuropsychiatric disorders including Schizophrenia (SCZ) and Bipolar Disorder (42–44).

The higher brain functions affected in neurodevelopmental and psychiatric disorders are thought to require precise spatiotemporal regulation of neuronal circuitry development. In this developmental process the relationship between neuronal outgrowth, synaptogenesis and synaptic transmission is widely appreciated. However, the requirements that these neuro developmental processes have on mitochondria is still emerging. In our pathway analysis, we outline the importance of mitochondrial dynamics, structure and function to neuronal outgrowth and development, synaptogenesis and neurotransmission as the basis for understanding the genetic etiology of GTS.

METHODS

Data Mining and Mutation Pathway Analysis: In this study we integrated the findings from published and unpublished (database) sources including ASD brain gene expression profiling, GTS risk-gene mouse modeling of behavior, genome wide linkage studies, genome wide association studies, chromosomal translocations and copy number variations (CNVs), gene set analysis, haplotype sharing, cell modeling, whole exome sequencing (WES), and whole genome sequencing (WGS) of rare and common deleterious mutations in GTS and ASD (see sources in **Table 1**). In the case where a CNV spanned multiple genes only one gene was selected for inclusion in our pathway analysis (**Table 2**). In these cases, first priority was given to genes that directly regulate those pathways already implicated in GTS (9) including neuronal development (e.g., *SLIT2*), synaptic connectivity (e.g., NTSC pathway genes), synaptic function (e.g., *SAP97*) and neurotransmission (**Figure 1**) (9). Only then was the second priority exercised to assign a pathway to those genes that encode mitochondrial proteins. Following this selection process all mutations, deletions and duplications of mitochondrial protein genes in GTS were included within the mutation pathway analysis thereby eliminating any bias or cherry picking in our development of the first contiguous mitochondrial pathways to GTS (**Figures 2–4**).

RESULTS AND DISCUSSION

Mitochondrial Dynamics: Mitochondrial Transport

To optimize the development and function of neuronal circuitry, mitochondria need to be located at the right place at the right time in sufficient numbers and to be functioning at optimum efficiency. Given that neurons are often hyper-extended with a complex network of axonal and dendritic branches, such optimisation requires the active bidirectional transport of mitochondria to their required destinations along microtubule tracks (39, 86). This may involve long-distance transport of mitochondria from the soma, where the majority of

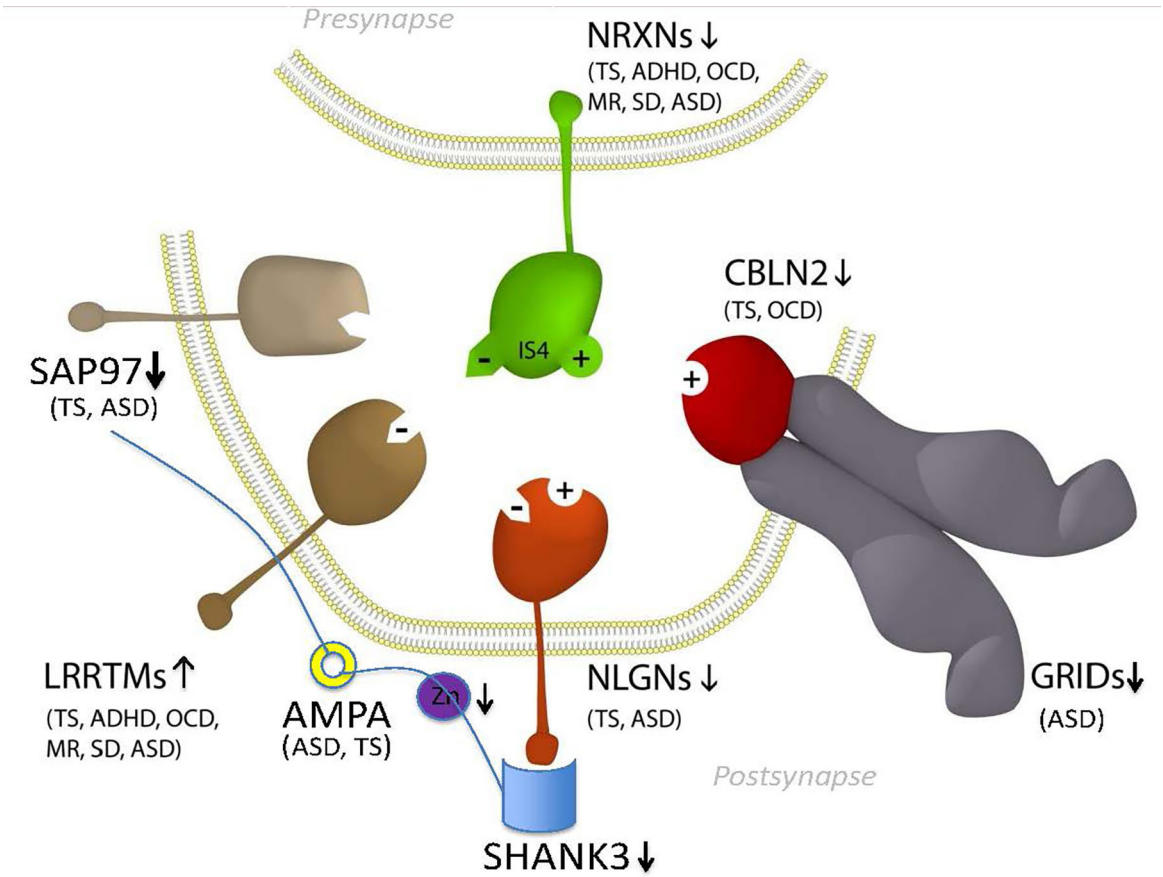

FIGURE 1 | Model NTSC pathway to GTS (2)—implicates the full complement of known neurexins (NRXNs) and their trans-synaptic cell-adhesion ligand gene families through multiple means of enquiry: neuroligins (NLGNs); leucine-rich repeat transmembrane proteins (LRRTMs); cerebellin precursors (CBLNs), Glutamate ionotropic receptor delta type subunit (GRIDS) and the synapse associated proteins (SAPs/DLGs) (2, 11, 12) in addition to the ProSAP proteins that interact in the NTSC postsynaptic signaling pathway (2, 11, 13–24). Presynaptic NRXNs 1-3 form competitive trans-synaptic complexes with postsynaptic ligands NLGNs, LRRTMs and CBLN-GRID complexes in the formation and/or maintenance of neuronal circuitry within the brain. Vertical arrows indicate putative pathogenic dose effects. NRXN1 isoforms with (+) and without (–) the 30 amino-acid insert at splice site 4 (IS4) indicate competitive binding of NRXN1 between its ligands. NRXN4/CNTNAP2 binds across the synapse with SAP97/DLG1 (12, 25). Comorbidities listed are those associated with the gene mutations and copy number variations (CNVs) in ASD and GTS (2, 11, 12, 26–32). Zinc represents one environmental factor important for mitochondrial protein import/processing and function that is also known to affect SHANK3 function (23, 24). Moreover the Zinc transporter ZnT7 has been recurrently linked with GTS in GWAS (2).

mitochondrial biogenesis occurs, to the pre- and post-synaptic termini where demands for mitochondrial homeostasis is highest (39, 86). Mitochondrial transport is also thought to optimize mitochondrial fission, fusion and function (39, 86).

Dynein and Kinesin Motor Proteins

Transport of mitochondria is regulated by the dynein and kinesin motor proteins through their interaction with the microtubule cytoskeleton of the cell (**Figure 2**). The kinesins (KIFs) mediate anterograde mitochondrial transport away from the soma of the neuron while dynein complexes regulate retrograde transport (87). *DNAH6, the dynein axonemal heavy chain* 6 gene implicated in mitochondrial depletion syndrome is mutated in GTS as are *DNAH5* and a number of kinesin genes including *KIF6* and *KIF7* (**Figure 2**) (58). Moreover, *DYNC1H1* which encodes the dynein 1 heavy chain mitochondrial transporter is mutated in ASD and *KIF1A* which encodes the Kinesin 1A mitochondrial transporter is disrupted and duplicated in ASD (18, 20).

KIFs form homo and heteromeric complexes in the regulation of organelle transport including but not limited to mitochondria (88). The importance of the motor proteins, and the microtubule network they travel on, in nervous system development and function is evidenced from their strong association with neurological phenotypes. Mutations in KIF5-family members give rise to a range of dominant negative phenotypes including deficits in mitochondrial transport, structure and function and reduced activity of the electron transport chain (ETC) (89, 90), axonal degeneration and aberrant synaptic transmission (**Figure 2**) (58, 91). GTS mutations have also been identified in genes that regulate tubulin and microtubule dynamics including: duplication of the *TUBB2A* and *TUBB2B* tubulin genes and the mutation of *TUBB3* (58); mutation of the *TTLL1*, *TTLL2,* and *TTLL5* tubulin ligase genes (58); recurrent duplication of the

TABLE 1 | Data sources.

Data type	Author	References
Genome wide linkage studies	Curtis 2004	(45)
	Zhang 2002	(46)
	Verkerk 2006	(12)
	Simonic 1998	(47)
	IMGSAC 1998	(48)
	Barret 1999	(49)
	Shao 2002	(50)
	Shellenberg 2006	(51)
	Maestrini 2010	(52)
	TSAIC 2007	(53)
Genome wide association studies (GWAS)	Suarez-Rama 2015	(54)
	Lintas 2009	(55)
	Philippi 2005	(56)
	Eicher 2015	(57)
Copy number variations (CNVs)	Wang 2018	(58)
	Lintas 2017	(20)
	Malhotra 2012	(59)
	Johnstone 2015	(60)
	Fernandez 2012	(61)
	McGrath 2014	(62)
	Sundaram 2010	(63)
	Clarke 2018	(11)
	Bertelsen 2014	(64)
	Elia 2010	(65)
	Jang 2019	(66)
	Huang 2017	(31)
Whole exome sequencing (WES)	Wang 2018	(58)
	Sundaram 2011	(67)
	Gauthier 2011	(30)
Whole genome sequencing (WGS)	RK CY 2017	(18)
	Turner 2016	(21)
	Leblond 2019	(22)
Gene set analysis	Wittkowski 2014	(68)
	Clarke 2012	(2)
	Wang 2011	(69)
	De Leeuw 2015	(70)
Haplotype sharing	Casey 2012	(71)
Karyotype, LOH Analysis and PCR	Clarke 2018	(11)
	Boghosian-Sell 1996	(72)
	Petek 2001	(73)
	Zhang 2015	(74)
	Patel 2011	(75)
	Robertson 2006	(76)
	Clarke 2009	(25)
	Fang 2017	(11)
ASD brain gene expression profiling	Tang 2013	(77)
	Anitha 2013	(78)
	Anitha 2012	(79)
	Voineagu 2012	(80)
	Schwede 2018	(81)
	Ji L 2012	(82)
	Lintas 2009	(55)
GTS gene mouse modeling and behavior	Shen 2019	(83)
	Lu B 2008	(84)
	Kreilaus 2019	(85)
	Shoen 2019	(23)
Cell modeling	Lam 2019	(27)

microtubule polymerization gene *KANK1* implicated in spastic paraplegia (58, 92); and mutation of the *CAMSAP1* gene that regulates microtubule dynamics and neurite outgrowth (**Table 2** and **Figure 2**) (58, 93).

Mitochondrial Transport Adaptor and Accessory Proteins

Mitochondrial transport provides for the site-specific requirements and function of the neuron (39, 86, 94–97) and it has been demonstrated that mitochondria directly regulate synaptic transmission (94). Furthermore, synapses with mitochondria can sustain repeated cycles of neurotransmitter release whereas the transport of mitochondria either in or out of the synapse dynamically modulates this synaptic strength (95–97). To halt the transport of mitochondria at a required destination such as the synapse requires a braking system. In this respect, synaptic firing renders synapses to be sites of high calcium influx. After synaptic firing the high (Ca^{2+}) acts to halt mitochondrial transport through the action of the Ca^{2+} sensitive GTPase of MIRO which is embedded within the outer mitochondrial membrane which then inactivates the molecular motor kinesin (**Figure 2**). To summarize, the precise location and relocation of mitochondria closely matches the site-specific requirements of the neuron including a rich supply of energy, in the form of ATP, to power the synaptic calcium ion pumps that expel calcium from the cell and for direct mitochondrial buffering of Ca^{2+} (98–100). Furthermore, mitochondria directly regulate the strength of synaptic transmission (94).

Motor proteins interact with mitochondria through adaptor and accessory proteins (98, 101) that determine the direction of mitochondrial transport (**Figure 2**). The mitochondrial transport adaptor proteins TRAK1 and TRAK2 link mitochondria to the motor proteins kinesin and dynein (102). TRAK1 and TRAK2 interact with the mitochondria through the Ca^{2+} sensitive GTPases MIRO1 and MIRO2 embedded within the outer mitochondrial membrane (**Figure 1**) (102–106). MIRO and TRAK work in concert with the transport accessory proteins DISC1 and NDE1 that determine the direction of mitochondrial movement (**Figure 2**). While no mutations in *MIRO* or *TRAK* have been identified (107) both *DISC1* and

TABLE 2 | GTS risk genes in mitochondrial dynamics, structure, and function.

Deleterious mutations
OPA1 (x2), MPV17, PDP1***, ME2, SLC1A3/EAAT1/GLAST, MRPL44 (x3), MRPL48, MRPL3 - Familial, PTCD3, GK2, DPP4, SLC25A26, SLC25A6, SLC52A2, ATP5B, AGK/TIMM22, ACOX3, DGAT2, UBE3A, BCKDHA, ENOSF1, ACOT12*
CNV duplications
SLC25A1(x3), SAP97- Familial duplicated mediated downregulation (14)
CNV deletions
IMMP2L (x12) (x9 in ASD), IMMP1L (x3 in ASD), GPD2 (x1 in ASD), RMRP, SLC25A1 and Txrnd2 (x3), TIMM13, NDUFA4 (Familial deletion), NDUFA13/ETC Complex I, SAP97—Duplication mediated downregulation (14)
Adjacent to deletion
ACOT12
Adjacent to duplication
MGME1 mitochondria DNA maintenance
***GTS linkage/association studies**
MPV17 Mutated and non parametric linkage analysis
PDP1 Mutated and Linked and Associated
SLC25A4 4q35Linkage region in Sib pairs
NDUFS3 D11S1377 in Africana families
SAP97 Parametric linkage in large Dutch pedigree
IMMP2L Autism linkage
Mitochondrial transport protein genes
NDE1 (x2 deletions), *DISC1* (x3 deletions), *DNAH6, DNAH5, and DYNC2H1*, *KIF6 and KLC2* have been mutated, *KIF7* deleted and *KIF16B* is adjacent to a GTS deletion breakpoint at 20p12.1
Mitochondrial dynamics regulators
OPA1 (x2), PRKAB2 (x 2 deletions + adjacent to deletion), *ADCY2 (x2), RCAN1 (duplicated), DGKB (x2 duplications), PLPP2 and PLPP4, PI4K2A and ITPR3* all mutated
Microtubule associated genes
TUBB2A and TUBB2B tubulin genes (duplicated), *TTLL1, TTLL2 and TTLL5* tubulin ligase genes mutated, *MICAL2 and MICAL3* microtubule regulators mutated, *KANK1* microtubule polymerization (x2 duplications), *CCT6A, BRPF1, SKA2, SPAST, KATNAL2, MARK2, TUBGCP5* and *CAMSAP1* which regulates microtubule dynamics and neurite outgrowth and *CAMD1* involved in microtubule stability and radial neuronal cell migration in the developing cerebral cortex lies immediately adjacent to the deletion of the Titin gene in GTS
Ubiquitin ligase genes
UBE3A (duplicated), UBE4A, DTX3, RNF41, RNF213 (x2), SH3RF3, SHPRH, WWP2, UBR4 and TRIM37 all mutated
Ubiquitin modifying genes
UBE4B ubiquination factor, *USP1 and USP47 and USP34* ubiquitin peptidases, *CYLD and BIRC6* all mutated
Cellular energy metabolism
HK2, ME1 (from GTS associated 33 metabolic enzyme gene set)

NDE1 display recurrent hemizygous deletion in GTS, ASD and SCZ (**Table 2**) (58–62). DISC1 is localized predominantly to mitochondria and in synapses, centrosomes, nuclei, endoplasmic reticulum and the Golgi (108, 109). DISC1 forms oligomers that interact with kinesin (110) and dynein (111), MIRO and TRAK, and the mitochondrial transport accessory proteins LIS1, GSK3β, NDE1 and its homolog NDEL1 (**Figure 2**) (112). DISC1 promotes anterograde and retrograde mitochondrial transport in a dose dependent manner in both axons and dendrites (112–114) possibly through blocking SNPH-mediated anchoring of mitochondria (115). DISC1 is also directly linked to anomalies in mitochondrial fission, fusion, structure and function (112–114).

NDE1 interacts with DISC1, TRAK1, NDEL1, LIS1, and with the dynein motor protein to promote retrograde axonal transport (**Figure 2**) (112). NDE1, and its close homolog NDEL1 form a complex with LIS1 that regulates neuronal proliferation, differentiation, and migration within the brain (116). NDE1 is a centrosomal protein with a crucial role in the growth of the cerebral cortex (116). Homozygous frame shift mutations in NDE1 are associated with extreme microlissencephaly (117, 118), whereas heterozygous deletions (LOH) in NDE1 are associated with GTS, ASD, and SCZ (62). Interestingly, the subcellular localization of NDE1, its protein-protein interactions and its regulation of retrograde mitochondrial transport, are all modulated through its phosphorylation by AMP-activated protein kinase A (AMPK/PKA) (**Figure 2**) (119). This regulatory association between NDE1 and PKA is note-worthy on many counts. Firstly, the gene encoding beta subunit 2 of PKA (PRKAB2) is recurrently deleted in GTS (**Table 2**) (58, 63, 120, 121) and PKA activity is greatly decreased in the frontal cortex of

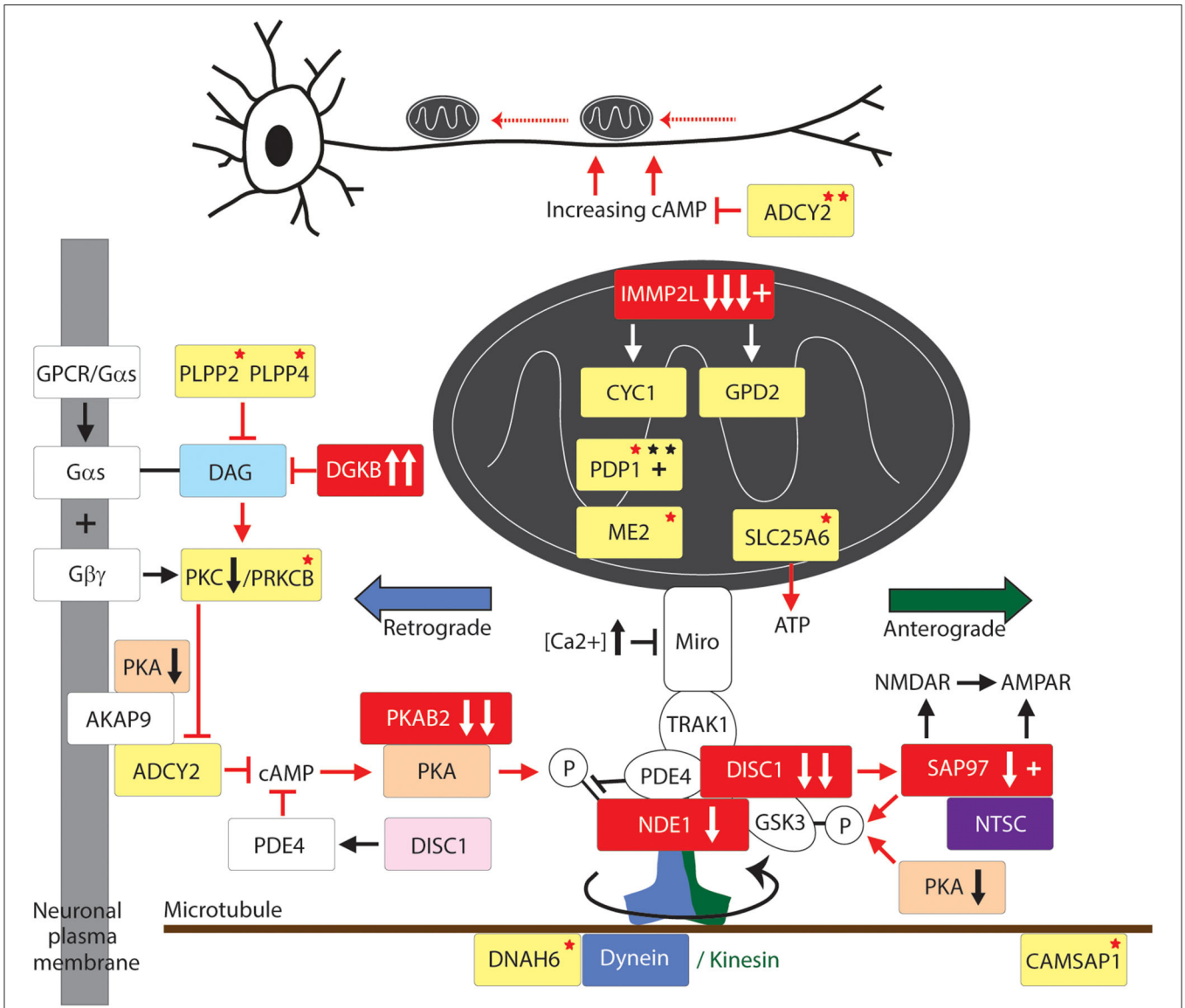

FIGURE 2 | Mitochondrial transport pathway to GTS. Red fill indicates CNV deletion (arrows down) or duplication (arrows up); Yellow fill indicates GTS associated gene; Red asterisk* indicates deleterious mutation; Black asterisk indicates another GTS genetic association; + indicates linkage; Red arrow indicates GTS affected pathway. Vertical arrows beside PKA and PKC indicate putative pathogenic dose effects.

subjects with regressive autism (122). Secondly, cAMP mediated PKA phosphorylation of NDE1 at threonine residue 131 regulates NDE1's all-important interactions with NDEL1 and LIS1 that are thought to activate dynein and facilitate its ability to move high-load cargo like mitochondria (123, 124). Thirdly, PKA's activation by rising cAMP levels provides a mitochondrial transport switch that can be activated on depletion of cellular ATP. Finally, DISC1 modulates the phosphorylation of NDE1 by PKA through its regulation of PDE4, a cAMP-hydrolyzing enzyme which creates a co-complex with DISC1 and NDE1 (**Figure 2**) and LIS1 and NDEL1 (112, 119, 125).

Mitochondria also localize to sites of neuronal branching. During development of neuronal circuitry the axons are guided to their target sites by extracellular guidance molecules like DSCAM, ROBO1, SLIT2 and SLIT3. *SLIT3* has been recurrently associated with GTS (2, 45, 46) as has *SLIT2* with ASD (2, 68, 69). Moreover, *DSCAM* and *ROBO1* have been recurrently mutated in ASD as have *DSCAM's* DNA regulatory elements (21, 22).

Neuronal growth cone navigation also relies on intracellular changes to microtubule and F-actin architecture downstream of these guidance cues (126), for example CAMSAP1 which was mentioned above in relation to its regulation of microtubule dynamics and neurite outgrowth (**Table 2** and **Figure 2**) (58, 93). Furthermore, AMPK/PKA regulates F-actin cytoskeletal dynamics (127). After extension to their target sites axons undergo local branching to establish the appropriate functional connections between pre- and postsynaptic neuronal termini. The intracellular mechanism that regulates this axonal branching

also involves PKA through its regulation of mitochondrial transport and recruitment to sites of future axon branching (128). Here, neuronal depolarization-induced rebalance of mitochondrial motility between anterograde and retrograde transport underlies the formation of axonal branches (128). Axon branching is formed in an ATP-depletion dependent manner through an increase in activated/phosphorylated PKA—a function which can be recapitulated by the pharmacological activation of PKA (128). Following neuronal depolarization there is an increase in anterograde transport of mitochondria into axons thus providing a mechanism for mitochondrial relocation and recruitment to sites of high energy demand that would appear to include sites of future branching. Moreover, the continued localization of mitochondria at branch points correlates with the longevity of axonal branches indicating a probable role for mitochondrial localization in the maintenance of axon branches (128). To summarize, a role for mitochondria in neuronal function and neurological disease has been established. Moreover, the role of mitochondrial transport in GTS is greatly strengthened by the interacting roles of the dynein motor protein, DISC1, NDE1, and PKA in mitochondrial transport and the complementary nature of their deleterious mutations in GTS and ASD (**Table 2**) (61–63, 112).

The master regulator glycogen synthase kinase 3β (GSK3β) is phospho-deactivated by another master regulator AMPK/PKA (129–131). This is important given that GSK3β associates with both DISC1 and TRAK1 in the regulation of mitochondrial transport (**Figure 2**) (112). In the synapse GSK3β is also deactivated by SAP97 downstream of DISC1 (132) (**Figure 2**). *SAP97* is linked to GTS (12) and downregulated in GTS and ASD (11) and forms part of the high-risk NTSC pathway to GTS (**Figure 1**) (2, 12). As such, SAP97 functions at the intersection of the NTSC, DISC1 and mitochondrial transport pathways to GTS (**Figures 1**, **2**) (2, 11, 12). GSK3β is also translocated into the mitochondria where it regulates mitochondrial homeostasis (112, 129, 133, 134). In the mitochondria GSK3β regulates the structure and function of the inner mitochondrial membrane (134). This is noteworthy given that the LOH and/or downregulation of *SAP97*, *DISC1,* and *PRKAB2* in GTS (2, 11, 12, 58, 61–63) are all consistent with stronger activation of GSK3β which is in turn consistent with the success of lithium chloride in the treatment of psychosis through its highly selective phospho-deactivation of GSK3 in both the cytosol and mitochondria (12, 134).

Mitochondrial Fusion and Fission

The constant optimisation of mitochondrial function requires mitochondria to undergo fusion and fission. Fusion of suboptimal mitochondria with healthy mitochondria creates larger healthier mitochondria where the damage is diluted (98, 135). Fission of mitochondria can rapidly increase the number of healthy mitochondria to allow for their wider distribution in the extended network of neuronal branches and boutons (98, 135). Fission can also help separate out damaged mitochondrial components for clearance by mitophagy (98, 102, 135). Conversely, if transport of mitochondria is retarded or otherwise defective mitochondria are less likely to merge thereby decreasing the clearance of damaged mitochondria and the overall health of the mitochondrial pool (39, 86).

Mitochondrial Fusion

Fusion of the outer mitochondrial membrane is coordinated by Mitofusins 1 and 2 (Mfn1/2) whereas fusion of the inner mitochondrial membrane is regulated by Optic Atrophy 1 (OPA1) (136, 137). This is most relevant as *OPA1* is recurrently mutated in GTS (**Table 2** and **Figure 3**) (58) and the levels of MFN1, MFN2, and OPA1 are decreased in the temporal lobe of the autistic brain, and there are deficits in MFN1 and MFN2 in Fragile X syndrome (**Figure 3**) (58, 77, 83). Mfn1/2 and OPA1 act through the formation of complexes both within and across the membranes (136, 137). Mfn2 coding mutations appear to inhibit mitochondrial fusion by forming a complex, in a dominant-negative fashion, with wild-type Mfn1 and Mfn2 (138). Mutations in Mfn2 cause Charcot Marie Tooth Disease Type 2A, a severe and early onset motor and sensory peripheral neuropathy with autosomal dominant inheritance (111, 139). These Mfn2 mutations promote mitochondrial fragmentation in dorsal root ganglion neurons and impair axonal mitochondrial transport which is suggestive of a link between mitochondrial fission/fusion equilibrium and mitochondrial transport (140). This link is supported further by the physical interaction between Mfn2 and the MIRO complex (141) and the finding that Purkinje-neuron-specific deletion of Mfn2 in mice (total knockout of Mfn2 is embryonic lethal) impairs mitochondrial fusion and the dendritic localization of mitochondria, dendrite development, degeneration of Purkinje neurons (142, 143). Similar to the situation in mice, Mfn2 loss-of-function in zebrafish reduces mitochondrial transport and depletes mitochondria from distal axons (144). Acting in a similar dominant-negative fashion to Mfn2, hypomorphic mutations in OPA1 cause dominant optic atrophy (DOA), the most common cause of hereditary blindness. Dominant optic atrophy is characterized by the early loss of retinal ganglion cells and degeneration of the optic nerve (145). Moreover, DOA patients often present with neurological disorders, including ataxia, myopathy, deafness and peripheral neuropathy, indicating an essential neurological role for OPA1 (146, 147). Like Mfn2, knockout of Opa1 in mice is embryonic lethal (141) whereas Opa1 LOH in mice recapitulates the DOA seen in patients, including early-onset degeneration of the optic nerve and vision loss (148). *In vitro* experiments indicate that Opa1 has a critical role in dendritogenesis and synaptogenesis. Knock-down of Opa1 in cultured rat cortical neurons promotes mitochondrial fragmentation, decreases expression of ETC components, mitochondrial DNA content, dendritic outgrowth and synapse formation (149). As such, the characterization of mitochondrial phenotypes in those GTS patients identified with recurrent *OPA1* mutations is eagerly anticipated (**Figure 3**) (58).

Mitochondrial Fission Regulatory Genes in GTS

The number and the complementary nature of the deleterious GTS mutations identified in mitochondrial fission pathways provide compelling evidence for mitochondrial fission deficiency

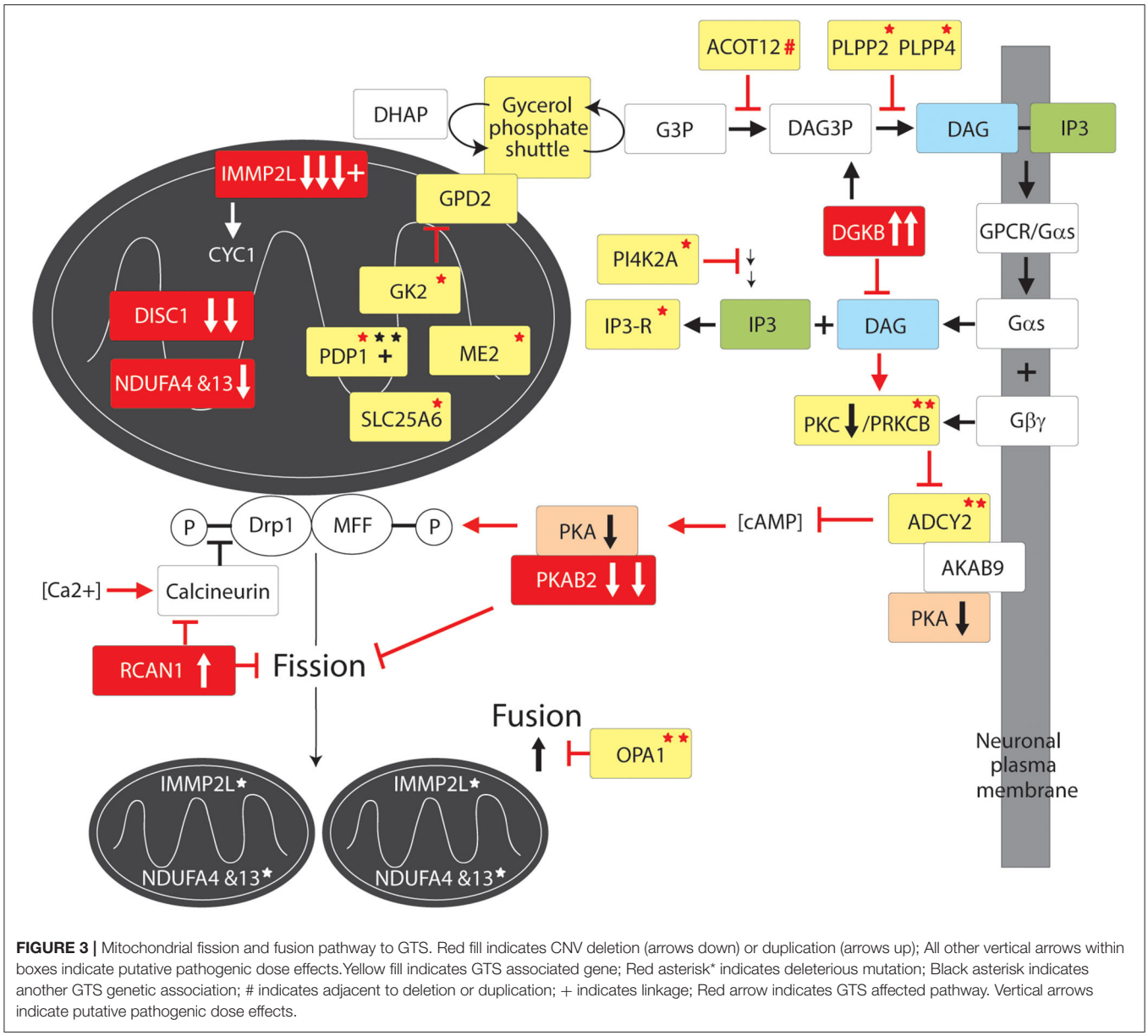

FIGURE 3 | Mitochondrial fission and fusion pathway to GTS. Red fill indicates CNV deletion (arrows down) or duplication (arrows up); All other vertical arrows within boxes indicate putative pathogenic dose effects.Yellow fill indicates GTS associated gene; Red asterisk* indicates deleterious mutation; Black asterisk indicates another GTS genetic association; # indicates adjacent to deletion or duplication; + indicates linkage; Red arrow indicates GTS affected pathway. Vertical arrows indicate putative pathogenic dose effects.

in the etiology of GTS. The fission of the mitochondrial membranes is dependent on Dynamin-related protein1 (Drp1) (150–160) which is downregulated in the brain of patients with ASD (78–81). Drp1 also regulates peroxisomal fission and proliferation (161). Primarily a cytosolic enzyme, Drp1 translocates to the outer mitochondrial membrane when dephospho-activated by the Ca^{2+}-activated phosphatase calcineurin/PPP3CA (**Figure 3**) (162, 163). Conversely, calcineurin is inhibited by RCAN1 which blocks Drp1 translocation to the mitochondria (164, 165) and hence it is of immense interest that *RCAN1* has been duplicated in GTS and *Calcineurin* is downregulated in the cerebral cortex of patients with ASD (**Figure 3**) (47, 63, 80, 81, 166). Calcinueurin activated Drp1 is then recruited to the outer mitochondrial membrane by phospho-activated mitochondrial fission factor (MFF). MFF is phospho-activated in neurones by PKA in response to increasing levels of cAMP (**Figure 3**) (167, 168). Activated Drp1 then assembles into spirals around the mitochondrion, which constrict and ultimately divide the organelle in two while dynamin-2 catalyzes the final membrane scission event (169). As mentioned earlier, the gene for beta subunit 2 of cAMP activated PKA (*PRKAB2*) is recurrently deleted in GTS (58, 63). Furthermore, cAMP levels in the brain are largely regulated by *ADCY2* (170), the adenylate cyclase gene recurrently mutated in GTS including the loss of an *ADCY2* intron/exon splice site in GTS (58). *ADCY2* is also mutated in bipolar disorder and a mutation in the ADCY2 binding site of the AKAP9 synaptic scaffolding protein has been found associated with SCZ (54, 170). This is fascinating since AKAP9 acts as a synaptic membrane anchor for ADCY2 and PKA which keeps this primary target of

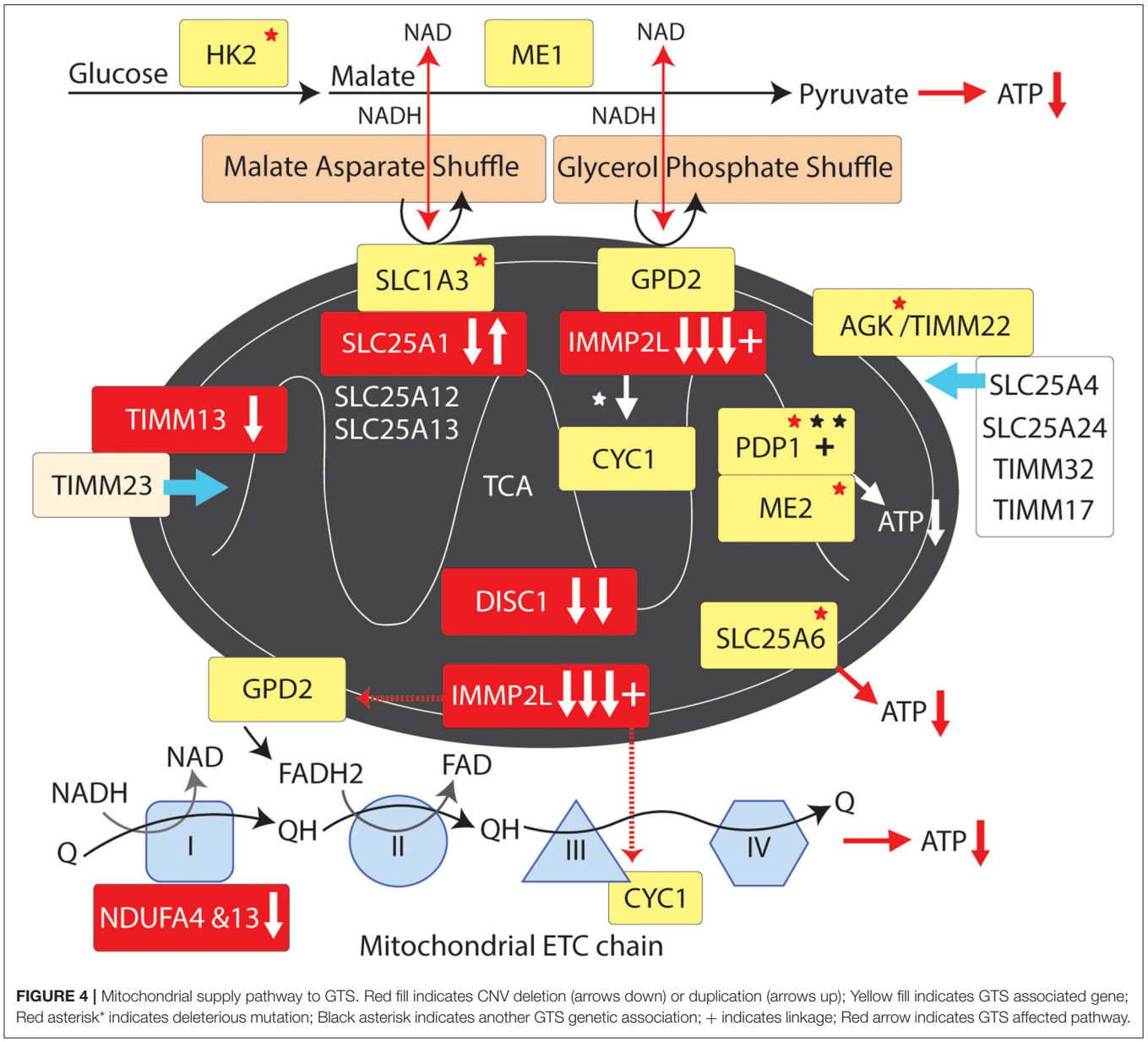

FIGURE 4 | Mitochondrial supply pathway to GTS. Red fill indicates CNV deletion (arrows down) or duplication (arrows up); Yellow fill indicates GTS associated gene; Red asterisk* indicates deleterious mutation; Black asterisk indicates another GTS genetic association; + indicates linkage; Red arrow indicates GTS affected pathway.

cAMP (PKA) in close proximity to the primary cAMP generator ADCY2 (170–173).

ADCY2 is activated by G-protein signaling through release of the alpha subunit of trimeric G protein (Gαs) (170, 174). Gαs is released when the G protein coupled receptor (GPCR) in the plasma membrane is bound by an extracellular regulatory molecule such as the neurotransmitter dopamine (**Figure 3**) (174, 175). When released, Gαs activates Phospholipase-C which in turn acts on phospholipid (phosphatidylinositol-triphosphate) within the plasma membrane cleaving off the inositol triphosphate (IP3) second messenger which frees yet another important second messenger diacylglycerol (DAG) (175). DAG activates Protein Kinase C (PKC), which in turn activates ADCY2 and other proteins (**Figure 3**) (175). Notably, the gene encoding PKC subunit B (PRKCB1) is mutated in GTS (**Figure 3**) (58) as well as being both linked and strongly associated with ASD, moreover, PKC activity is significantly reduced in the frontal cortex of subjects with regressive autism (55, 56, 82). *PRKCB1* is also associated with nominal autistic-like traits in the general population (176). Another compelling finding that links the PKCB/ADCY2 pathway to GTS is the recurrent duplication of the DAG kinase gene in GTS (DGKB) (58). DGKB terminates DAG-based signals by reducing DAG levels by converting DAG to diacylglycerol-3-phosphate (DAG3P) (**Figure 3**). Moreover, the phosphatases PLPP2 and PLPP4 which convert DAG3P back to DAG are both mutated in GTS (**Figure 3**) (58). It is also worthy of mention here that PI4K2A, an enzyme in the synthesis pathway

of phosphatidylinositol-triphosphate with the potential to limit the bioavailability of both the IP3 and DAG second messengers, is mutated in GTS. This together with a GTS mutation in the IP3 receptor ITPR3 (**Figure 3** and **Table 2**) (58) suggests the potential for an IP3 signaling affect in GTS notwithstanding ambiguity with regards to the calcium sensitivity of ACDY2 (170, 174, 175). To summarize, there is an impressive number of GTS gene mutations with the potential to limit the activation of Drp1, or the MFF-mediated recruitment of Drp1, for mitochondrial fission (**Figure 3**).

Optimisation of Mitochondrial Supply

Many of the genes mutated in GTS regulate mitochondrial function. Most notable is the *IMMP2L* gene commonly disrupted/deleted in GTS (64, 72–75, 84). *IMMP2L* encodes a mitochondrial peptidase (inner mitochondrial membrane peptidase-2-like protein) which processes other mitochondrial proteins within the inner mitochondrial membrane (**IMM**) (**Figure 4** and **Table 2**) (84). The *IMMP2L* association with GTS was first reported in a GTS family with a balanced *t* (7;18) (q22–q31; q22.3) translocation that disrupted the *IMMP2L* gene (72). More recently a Danish study reported 5′-end intragenic deletions in *IMMP2L* in seven out of a cohort of 188 GTS patients (3.7%) which was significantly higher than that of the control population (64). The IMMP2L gene has been repeatedly linked to ASD inheritance at the Autism 1 (AUTS1) locus (48–52). In addition, *IMMP2L* has demonstrated haplotype sharing in multiple ASD populations and deleterious exon deletions have been identified in ASD individuals and families (22, 52, 65, 66, 71, 74, 177) at significantly higher frequency than in control populations. Furthermore, reducing Immp2l dose in mice causes behavioral changes relevant to GTS behavioral deficits (85).

IMMP2L cleaves IMM signature signal peptides from a number of IMM proteins including cytochrome C1 (CYC1) and mitochondrial glycerol-3-phosphate dehydrogenase (GPD2) (84). CYC1 (oxidative phosphorylation complex 111 subunit 4) is a heme-containing subunit of the cytochrome complex of the electron transport chain (ETC). CYC1 has an important role in accepting electrons from the Rieske protein and transferring them to Cytochrome C in the respiratory chain. On the other hand GPD2 functions as part of the glycerol phosphate shuttle (**Figure 4**). GPD2, which is activated by IMMP2L, is located on the outer surface of the inner mitochondrial membrane where it catalyzes the interconversion of glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate. Interestingly, mitochondrial glycerol kinase (GK2) which generates G3P is also mutated in GTS (58). Together, GPD1 and GPD2 constitute the glycerol phosphate shuttle, which generates $FADH_2$ for the mitochondrial ETC and NAD+ for glycolysis in the cytosol. The coordinated action of GPD1 and GPD2 results in the transfer of two reducing equivalents from G3P to the mobile electron carrier ubiquinone (Coenzyme Q10) which in turn passes these electrons to CYC1 located downstream in the ETC (80, 81, 178–180).

A recurrent functional variant in the glutamate aspartate transporter GLAST/*SLC1A3* has been identified in GTS (181) (**Table 2**). SLC1A3 is also of interest as it imports glutamate from the cytosol into the mitochondrial matrix and exports aspartate from the matrix to the cytosol at varying levels in different cell types including astrocytes and neurons (178, 181). In addition to reducing glutamate signaling within the synapse the malate aspartate shuttle, like the glycerol phosphate shuttle, provides NADH to the ETC to generate ATP and NAD+ for another round of glycolysis (**Figure 4**) (178). In addition, the mitochondrial ADP/ATP exchange transporter SLC25A6 is mutated in GTS as is the mitochondrial ATPase ATP5B (**Table 2**) (58). SLC25A4 is a nuclear encoded protein located at the 4q35 GTS linkage locus (2, 46) (**Table 1**). SLC25A4 is transported into the mitochondria by the TIMM22 mitochondrial translocase complex inclusive of AGK, a vital component of TIMM22 involved in its assembly and function, and which is downregulated in the cerebral cortex of autism sufferers (80, 81). Importantly, *AGK* is mutated in GTS as is the *TIMM13* (**Table 2** and **Figure 4**) (58, 182, 183). TIMM13 facilitates translocation of the TIMM23 translocase into the IMM which in turn forms a translocase complex with TIMM17A/B, which itself is translocated into the IMM by TIMM22 (184). Importantly, TIMM17A/B facilitates the translocation of two additional glutamate/aspartate exchange transporters into the IMM, namely SLC25A12 and SLC25A13 (**Figure 4**) with the former being downregulated in the brain of patients with ASD (78, 79).

Mitochondrial Maintenance

The MPV17 channel protein (53, 58) that regulates the transmembrane potential of the IMM and mitochondrial DNA maintenance has been mutated in GTS (**Table 2**), moreover, the *MPV17* gene is located at the 2p23.2 non-parametric linkage locus identified in GTS (53) (**Table 2**). *MGME1* which also regulates mitochondrial DNA maintenance is located immediately adjacent to a genomic DNA duplication at 20p11 in GTS (**Table 2**) (58). *RMRP*, a gene which regulates mitochondrial RNA processing, is deleted in GTS as is the *PTCD3* gene which regulates translation in the mitochondria (**Table 2**). In addition, a number of mitochondrial ribosomal protein genes are mutated in GTS: *MRPL44* was found mutated in 3 unrelated GTS patients (**Table 2**) (58), a mutation in *MRPL3* was found segregating with GTS in a large affected family (67) and *MRPL48* was found mutated in another GTS patient (**Table 2**) (58). Together these findings are reminiscent of the *MRPL19* gene association with ASD, Dyslexia and Reading Disorder (57).

CONCLUSION

The mitochondrial pathways involved in GTS overlap and interact making it possible to trace these pathways to common endpoints in mitochondrial dynamics and supply. While it is unlikely that all of the genes cited in this study are causative in GTS, or that they all act alone in GTS etiology, we present convincing weight of evidence that mitochondria are implicated in GTS. Notwithstanding, the deleterious mutation of genes directly involved in mitochondrial dynamics and supply (**Figure 4**) have the potential to limit neurodevelopment and neurotransmission during periods of peak demand. The exact

mitochondrial mechanism implicated in GTS has not been identified as there is no evidence of mitochondrial mediated increases in ROS or ROS related neurodegeneration in GTS as is commonly the case in neurodegenerative disorders. A deficit in neuronal energy supply during development is one possible contributing factor in the etiology of GTS, however, the waning of tic severity in GTS over time appears more consistent with a deficit in neurotransmission possibly compensated for at later ages (185–189). We have no non-molecular evidence of a mitochondrial pathway to GTS at this time, notwithstanding, this is the 1st study to report a mitochondrial pathway to GTS and we are confident it will not be the last. The mitochondrial pathways identified in this study (**Figures 2–4**) have roles in neuronal circuitry development, synaptic connectivity and neurotransmission (**Figure 1**) (2, 11). The NTSC and DISC1 and mitochondrial pathways to GTS all intersect around the pivotal role of SAP97 in regulating synaptic signaling downstream of the NTSC and mitochondrial transport downstream of DISC1 thus providing compounding support for a GTS deficit in mitochondrial supply affecting neurotransmission.

DATA AVAILABILITY STATEMENT

The original contributions generated in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Robertson MM, Eapen V, Singer HS, Martino D, Scharf JM, Paschou P, et al. *Gilles de la Tourette syndrome. Nat Rev Dis Primers*. (2017) 3:16097. doi: 10.1038/nrdp.2016.97
2. Clarke RA, Lee S, Eapen V. Pathogenetic model for Tourette syndrome delineates overlap with related neurodevelopmental disorders including Autism. *Transl Psychiatry*. (2012) 2:e158. doi: 10.1038/tp.2012.75
3. State MW. The genetics of child psychiatric disorders: focus on autism and Tourette syndrome. *Neuron*. (2010) 68:254–69. doi: 10.1016/j.neuron.2010.10.004
4. O'Rourke JA, Scharf JM, Yu D, Pauls DL. The genetics of Tourette syndrome: a review. *J Psychosom Res*. (2009) 67:533–45. doi: 10.1016/j.jpsychores.2009.06.006
5. Burd L, Li Q, Kerbeshian J, Klug MG, Freeman RD. Tourette syndrome and comorbid pervasive developmental disorders. *J Child Neurol*. (2009) 24:170–5. doi: 10.1177/0883073808322666
6. Green JL, Rinehart N, Anderson V, Nicholson JM, Jongeling B, Sciberras E. Autism spectrum disorder symptoms in children with ADHD: a community-based study. *Res Dev Disabil*. (2015) 47:175–84. doi: 10.1016/j.ridd.2015.09.016
7. Darrow SM, Grados M, Sandor P, Hirschtritt ME, Illmann C, Osiecki L, et al. Autism spectrum symptoms in a Tourette's disorder sample. *J Am Acad Child Adolesc Psychiatry*. (2017) 56:610–7.e1. doi: 10.1016/j.jaac.2017.05.002
8. Kadesjo B, Gillberg C. Tourette's disorder: epidemiology and comorbidity in primary school children. *J Am Acad Child Adolesc Psychiatry*. (2000) 39:548–55. doi: 10.1097/00004583-200005000-00007
9. Eapen V, Robertson MM. Are there distinct subtypes in Tourette syndrome? Pure-Tourette syndrome versus Tourette syndrome-plus, and simple versus complex tics. *Neuropsychiatr Dis Treat*. (2015) 11:1431–6. doi: 10.2147/NDT.S72284
10. Clarke RA, Eapen V. Balance within the neurexin trans-synaptic connexus stabilizes behavioral control. *Front Hum Neurosci*. (2014) 8:52. doi: 10.3389/fnhum.2014.00052
11. Fang Z, Eapen V, Clarke RA. CTNNA3 discordant regulation of nested LRRTM3, implications for autism spectrum disorder and Tourette syndrome. *Meta Gene*. (2017) 11:43–8. doi: 10.1016/j.mgene.2016.11.003
12. Verkerk AJ, Cath DC, van der Linde HC, Both J, Heutink P, Breedveld G, et al. Genetic and clinical analysis of a large Dutch Gilles de la Tourette family. *Mol Psychiatry*. (2006) 11:954–64. doi: 10.1038/sj.mp.4001877
13. Griswold AJ, Ma D, Cukier HN, Nations LD, Schmidt MA, Chung RH, et al. Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. *Hum Mol Genet*. (2012) 21:3513–23. doi: 10.1093/hmg/dds164
14. Sundberg M, Tochitsky I, Buchholz DE, Winden K, Kujala V, Kapur K, et al. Purkinje cells derived from TSC patients display hypoexcitability and synaptic deficits associated with reduced FMRP levels and reversed by rapamycin. *Mol Psychiatry*. (2018) 23:2167–83. doi: 10.1038/s41380-018-0018-4
15. Gazzellone MJ, Zhou X, Lionel AC, Uddin M, Thiruvahindrapuram B, Liang S, et al. Copy number variation in Han Chinese individuals with autism spectrum disorder. *J Neurodev Disord*. (2014) 6:34. doi: 10.1186/1866-1955-6-34
16. He WZ, Liu WQ, Zhong XQ, Chen XL, Li SY, Zhang HM, et al. Analysis of de novo copy number variations in a family affected with autism spectrum disorders using high-resolution array-based comparative genomic hybridization. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. (2012) 29:266–9. doi: 10.3760/cma.j.issn.1003-9406.2012.03.004
17. Kalkan Z, Durasi IM, Sezerman U, Atasever-Arslan B. Potential of GRID2 receptor gene for preventing TNF-induced neurodegeneration in autism. *Neurosci Lett*. (2016) 620:62–9. doi: 10.1016/j.neulet.2016.03.043
18. RK CY, Merico D, Bookman M, Jennifer LH, Thiruvahindrapuram B, Patel RV, et al. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nat Neurosci*. (2017) 20:602–11. doi: 10.1038/nn.4524
19. Turner TN, Coe BP, Dickel DE, Hoekzema K, Nelson BJ, Zody MC, et al. Genomic patterns of *de novo* mutation in simplex autism. *Cell*. (2017) 171:710–22.e12. doi: 10.1016/j.cell.2017.08.047
20. Lintas C, Picinelli C, Piras IS, Sacco R, Brogna C, Persico AM. Copy number variation in 19 Italian multiplex families with autism spectrum disorder: importance of synaptic and neurite elongation genes. *Am J Med Genet B Neuropsychiatr Genet*. (2017) 174:547–56. doi: 10.1002/ajmg.b.32537
21. Turner TN, Hormozdiari F, Duyzend MH, McClymont SA, Hook PW, Iossifov I, et al. Genome sequencing of autism-affected families reveals disruption of putative noncoding regulatory DNA. *Am J Hum Genet*. (2016) 98:58–74. doi: 10.1016/j.ajhg.2015.11.023
22. Leblond CS, Cliquet F, Carton C, Huguet G, Mathieu A, Kergrohen T, et al. Both rare and common genetic variants contribute to autism in the Faroe Islands. *NPJ Genom Med*. (2019) 4:1. doi: 10.1038/s41525-018-0075-2
23. Schoen M, Asoglu H, Bauer HF, Muller HP, Abaei A, Sauer AK, et al. Shank3 Transgenic and prenatal zinc-deficient autism mouse models show convergent and individual alterations of brain structures in MRI. *Front Neural Circuits*. (2019) 13:6. doi: 10.3389/fncir.2019.00006
24. Napoli E, Ross-Inta C, Song G, Wong S, Hagerman R, Gane LW, et al. Premutation in the fragile X mental retardation 1 (FMR1) gene affects maternal Zn-milk and perinatal brain bioenergetics and scaffolding. *Front Neurosci*. (2016) 10:159. doi: 10.3389/fnins.2016.00159

25. Clarke RA, Fang ZM, Diwan AD, Gilbert DL. Tourette syndrome and klippel-feil anomaly in a child with chromosome 22q11 duplication. *Case Rep Med*. (2009) 2009:361518. doi: 10.1155/2009/361518
26. Krishnan V, Stoppel DC, Nong Y, Johnson MA, Nadler MJ, Ozkaynak E, et al. Autism gene Ube3a and seizures impair sociability by repressing VTA Cbln1. *Nature*. (2017) 543:507–12. doi: 10.1038/nature21678
27. Lam M, Moslem M, Bryois J, Pronk RJ, Uhlin E, Ellstrom ID, et al. Single cell analysis of autism patient with bi-allelic NRXN1-alpha deletion reveals skewed fate choice in neural progenitors and impaired neuronal functionality. *Exp Cell Res*. (2019) 383:111469. doi: 10.1016/j.yexcr.2019.06.014
28. Zhang P, Lu H, Peixoto RT, Pines MK, Ge Y, Oku S, et al. Heparan sulfate organizes neuronal synapses through neurexin partnerships. *Cell*. (2018) 174:1450–64.e23. doi: 10.1016/j.cell.2018.07.002
29. Kasem E, Kurihara T, Tabuchi K. Neurexins and neuropsychiatric disorders. *Neurosci Res*. (2018) 127:53–60. doi: 10.1016/j.neures.2017.10.012
30. Gauthier J, Siddiqui TJ, Huashan P, Yokomaku D, Hamdan FF, Champagne N, et al. Truncating mutations in NRXN2 and NRXN1 in autism spectrum disorders and schizophrenia. *Hum Genet*. (2011) 130:563–73. doi: 10.1007/s00439-011-0975-z
31. Huang AY, Yu D, Davis LK, Sul JH, Tsetsos F, Ramensky V, et al. Rare copy number variants in NRXN1 and CNTN6 increase risk for Tourette syndrome. *Neuron*. (2017) 94:1101–11.e7.
32. Roppongi RT, Karimi B, Siddiqui TJ. Role of LRRTMs in synapse development and plasticity. *Neurosci Res*. (2017) 116:18–28. doi: 10.1016/j.neures.2016.10.003
33. Harris JJ, Jolivet R, Attwell D. Synaptic energy use and supply. *Neuron*. (2012) 75:762–77. doi: 10.1016/j.neuron.2012.08.019
34. Raichle ME, Gusnard DA. Appraising the brain's energy budget. *Proc Natl Acad Sci USA*. (2002) 99:10237–9. doi: 10.1073/pnas.172399499
35. Bogan N, Cabot JB. Light and electron microscopic analyses of intraspinal axon collaterals of sympathetic preganglionic neurons. *Brain Res*. (1991) 541:241–51. doi: 10.1016/0006-8993(91)91024-U
36. Mutsaers SE, Carroll WM. Focal accumulation of intra-axonal mitochondria in demyelination of the cat optic nerve. *Acta Neuropathol*. (1998) 96:139–43. doi: 10.1007/s004010050873
37. Fabricius C, Berthold CH, Rydmark M. Axoplasmic organelles at nodes of Ranvier. II. Occurrence and distribution in large myelinated spinal cord axons of the adult cat. *J Neurocytol*. (1993) 22l:941–54. doi: 10.1007/BF01218352
38. Shepherd GM, Harris KM. Three-dimensional structure and composition of CA3–>CA1 axons in rat hippocampal slices: implications for presynaptic connectivity and compartmentalization. *J Neurosci*. (1998) 18:8300–10. doi: 10.1523/JNEUROSCI.18-20-08300.1998
39. Flippo KH, Strack S. An emerging role for mitochondrial dynamics in schizophrenia. *Schizophr Res*. (2017) 187:26–32. doi: 10.1016/j.schres.2017.05.003
40. Kimura T, Murakami F. Evidence that dendritic mitochondria negatively regulate dendritic branching in pyramidal neurons in the neocortex. *J Neurosci*. (2014) 34:6938–51. doi: 10.1523/JNEUROSCI.5095-13.2014
41. Chaturvedi RK, Flint Beal M. Mitochondrial diseases of the brain. *Free Radic Biol Med*. (2013) 63:1–29. doi: 10.1016/j.freeradbiomed.2013.03.018
42. Adzic M, Brkic Z, Bulajic S, Mitic M, Radojcic MB. Antidepressant action on mitochondrial dysfunction in psychiatric disorders. *Drug Dev Res*. (2016) 77:400–6. doi: 10.1002/ddr.21332
43. Bergman O, Ben-Shachar D. Mitochondrial oxidative phosphorylation system (OXPHOS) deficits in schizophrenia: possible interactions with cellular processes. *Can J Psychiatry*. (2016) 61:457–69. doi: 10.1177/0706743716648290
44. Machado AK, Pan AY, da Silva TM, Duong A, Andreazza AC. Upstream pathways controlling mitochondrial function in major psychosis: a focus on bipolar disorder. *Can J Psychiatry*. (2016) 61:446–56. doi: 10.1177/0706743716648297
45. Curtis D, Brett P, Dearlove AM, McQuillin A, Kalsi G, Robertson MM, et al. Genome scan of Tourette syndrome in a single large pedigree shows some support for linkage to regions of chromosomes 5, 10 and 13. *Psychiatr Genet*. (2004) 14:83–7. doi: 10.1097/01.ypg.0000107927.32051.f5
46. Zhang H, Leckman JF, Pauls DL, Tsai CP, Kidd KK, Campos MR, et al. Genomewide scan of hoarding in sib pairs in which both sibs have Gilles de la Tourette syndrome. *Am J Hum Genet*. (2002) 70:896–904. doi: 10.1086/339520
47. Simonic I, Gericke GS, Ott J, Weber JL. Identification of genetic markers associated with Gilles de la Tourette syndrome in an Afrikaner population. *Am J Hum Genet*. (1998) 63:839–46. doi: 10.1086/302002
48. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International molecular genetic study of autism consortium. *Hum Mol Genet*. (1998) 7:571–8. doi: 10.1093/hmg/7.3.571
49. Barrett S, Beck JC, Bernier R, Bisson E, Braun TA, Casavant TL, et al. An autosomal genomic screen for autism. Collaborative linkage study of autism. *Am J Med Genet*. (1999) 88:609–15. doi: 10.1002/(SICI)1096-8628(19991215)88:6<609::AID-AJMG7>3.0.CO;2-L
50. Shao Y, Wolpert CM, Raiford KL, Menold MM, Donnelly SL, Ravan SA, et al. Genomic screen and follow-up analysis for autistic disorder. *Am J Med Genet*. (2002) 114:99–105. doi: 10.1002/ajmg.10153
51. Schellenberg GD, Dawson G, Sung YJ, Estes A, Munson J, Rosenthal E, et al. Evidence for multiple loci from a genome scan of autism kindreds. *Mol Psychiatry*. (2006) 11:1049–60. doi: 10.1038/sj.mp.4001874
52. Maestrini E, Pagnamenta AT, Lamb JA, Bacchelli E, Sykes NH, Sousa I, et al. High-density SNP association study and copy number variation analysis of the AUTS1 and AUTS5 loci implicate the IMMP2L-DOCK4 gene region in autism susceptibility. *Mol Psychiatry*. (2010) 15:954–68. doi: 10.1038/mp.2009.34
53. Tourette Syndrome Association International Consortium for G. Genome scan for Tourette disorder in affected-sibling-pair and multigenerational families. *Am J Hum Genet*. (2007) 80:265–72. doi: 10.1086/511052
54. Suarez-Rama JJ, Arrojo M, Sobrino B, Amigo J, Brenlla J, Agra S, et al. Resequencing and association analysis of coding regions at twenty candidate genes suggest a role for rare risk variation at AKAP9 and protective variation at NRXN1 in schizophrenia susceptibility. *J Psychiatr Res*. (2015) 66, 67:38–44. doi: 10.1016/j.jpsychires.2015.04.013
55. Lintas C, Sacco R, Garbett K, Mirnics K, Militerni R, Bravaccio C, et al. Involvement of the PRKCB1 gene in autistic disorder: significant genetic association and reduced neocortical gene expression. *Mol Psychiatry*. (2009) 14:705–18. doi: 10.1038/mp.2008.21
56. Philippi A, Roschmann E, Tores F, Lindenbaum P, Benajou A, Germain-Leclerc L, et al. Haplotypes in the gene encoding protein kinase c-beta (PRKCB1) on chromosome 16 are associated with autism. *Mol Psychiatry*. (2005) 10:950–60. doi: 10.1038/sj.mp.4001704
57. Eicher JD, Gruen JR. Language impairment and dyslexia genes influence language skills in children with autism spectrum disorders. *Autism Res*. (2015) 8:229–34. doi: 10.1002/aur.1436
58. Wang S, Mandell JD, Kumar Y, Sun N, Morris MT, Arbelaez J, et al. *De novo* sequence and copy number variants are strongly associated with tourette disorder and implicate cell polarity in pathogenesis. *Cell Rep*. (2018) 25:3544. doi: 10.1016/j.celrep.2018.12.024
59. Malhotra D, Sebat J. CNVs: harbingers of a rare variant revolution in psychiatric genetics. *Cell*. (2012) 148:1223–41. doi: 10.1016/j.cell.2012.02.039
60. Johnstone M, Maclean A, Heyrman L, Lenaerts AS, Nordin A, Nilsson LG, et al. Copy number variations in DISC1 and DISC1-interacting partners in major mental illness. *Mol Neuropsychiatry*. (2015) 1:175–90. doi: 10.1159/000438788
61. Fernandez TV, Sanders SJ, Yurkiewicz IR, Ercan-Sencicek AG, Kim YS, Fishman DO, et al. Rare copy number variants in tourette syndrome disrupt genes in histaminergic pathways and overlap with autism. *Biol Psychiatry*. (2012) 71:392–402. doi: 10.1016/j.biopsych.2011.09.034
62. McGrath LM, Yu D, Marshall C, Davis LK, Thiruvahindrapuram B, Li B, et al. Copy number variation in obsessive-compulsive disorder and tourette syndrome: a cross-disorder study. *J Am Acad Child Adolesc Psychiatry*. (2014) 53:910–9. doi: 10.1016/j.jaac.2014.04.022
63. Sundaram SK, Huq AM, Wilson BJ, Chugani HT. Tourette syndrome is associated with recurrent exonic copy number variants. *Neurology*. (2010) 74:1583–90. doi: 10.1212/WNL.0b013e3181e0f147

64. Bertelsen B, Melchior L, Jensen LR, Groth C, Glenthoj B, Rizzo R, et al. Intragenic deletions affecting two alternative transcripts of the IMMP2L gene in patients with Tourette syndrome. *Eur J Hum Genet*. (2014) 22:1283–9. doi: 10.1038/ejhg.2014.24
65. Elia J, Gai X, Xie HM, Perin JC, Geiger E, Glessner JT, et al. Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. *Mol Psychiatry*. (2010) 15:637–46. doi: 10.1038/mp.2009.57
66. Jang W, Kim Y, Han E, Park J, Chae H, Kwon A, et al. Chromosomal microarray analysis as a first-tier clinical diagnostic test in patients with developmental delay/intellectual disability, autism spectrum disorders, and multiple congenital anomalies: a prospective multicenter study in Korea. *Ann Lab Med*. (2019) 39:299–310. doi: 10.3343/alm.2019.39.3.299
67. Sundaram SK, Huq AM, Sun Z, Yu W, Bennett L, Wilson BJ, et al. Exome sequencing of a pedigree with Tourette syndrome or chronic tic disorder. *Ann Neurol*. (2011) 69:901–4. doi: 10.1002/ana.22398
68. Wittkowski KM, Sonakya V, Bigio B, Tonn MK, Shic F, Ascano M, et al. A novel computational biostatistics approach implies impaired dephosphorylation of growth factor receptors as associated with severity of autism. *Transl Psychiatry*. (2014) 4:e354. doi: 10.1038/tp.2013.124
69. Wang L, Jia P, Wolfinger RD, Chen X, Zhao Z. Gene set analysis of genome-wide association studies: methodological issues and perspectives. *Genomics*. (2011) 98:1–8. doi: 10.1016/j.ygeno.2011.04.006
70. de Leeuw C, Goudriaan A, Smit AB, Yu D, Mathews CA, Scharf JM, et al. Involvement of astrocyte metabolic coupling in Tourette syndrome pathogenesis. *Eur J Hum Genet*. (2015) 23:1519–22. doi: 10.1038/ejhg.2015.22
71. Casey JP, Magalhaes T, Conroy JM, Regan R, Shah N, Anney R, et al. A novel approach of homozygous haplotype sharing identifies candidate genes in autism spectrum disorder. *Hum Genet*. (2012) 131:565–79. doi: 10.1007/s00439-011-1094-6
72. Boghosian-Sell L, Comings DE, Overhauser J. Tourette syndrome in a pedigree with a 7;18 translocation: identification of a YAC spanning the translocation breakpoint at 18q22.3. *Am J Hum Genet*. (1996) 59:999–1005.
73. Petek E, Windpassinger C, Vincent JB, Cheung J, Boright AP, Scherer SW, et al. Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome. *Am J Hum Genet*. (2001) 68:848–58. doi: 10.1086/319523
74. Zhang Y, Liu Y, Zarrei M, Tong W, Dong R, Wang Y, et al. Association of IMMP2L deletions with autism spectrum disorder: a trio family study and meta-analysis. *Am J Med Genet B Neuropsychiatr Genet*. (2018) 177:93–100. doi: 10.1002/ajmg.b.32608
75. Patel C, Cooper-Charles L, McMullan DJ, Walker JM, Davison V, Morton J. Translocation breakpoint at 7q31 associated with tics: further evidence for IMMP2L as a candidate gene for Tourette syndrome. *Eur J Hum Genet*. (2011) 19:634–9. doi: 10.1038/ejhg.2010.238
76. Robertson MM, Shelley BP, Dalwai S, Brewer C, Critchley HD. A patient with both Gilles de la Tourette's syndrome and chromosome 22q11 deletion syndrome: clue to the genetics of Gilles de la Tourette's syndrome? *J Psychosom Res*. (2006) 61:365–8. doi: 10.1016/j.jpsychores.2006.06.011
77. Tang G, Gutierrez Rios P, Kuo SH, Akman HO, Rosoklija G, Tanji K, et al. Mitochondrial abnormalities in temporal lobe of autistic brain. *Neurobiol Dis*. (2013) 54:349–61. doi: 10.1016/j.nbd.2013.01.006
78. Anitha A, Nakamura K, Thanseem I, Matsuzaki H, Miyachi T, Tsujii M, et al. Downregulation of the expression of mitochondrial electron transport complex genes in autism brains. *Brain Pathol*. (2013) 23:294–302. doi: 10.1111/bpa.12002
79. Anitha A, Nakamura K, Thanseem I, Yamada K, Iwayama Y, Toyota T, et al. Brain region-specific altered expression and association of mitochondria-related genes in autism. *Mol Autism*. (2012) 3:12. doi: 10.1186/2040-2392-3-12
80. Voineagu I. Gene expression studies in autism: moving from the genome to the transcriptome and beyond. *Neurobiol Dis*. (2012) 45:69–75. doi: 10.1016/j.nbd.2011.07.017
81. Schwede M, Nagpal S, Gandal MJ, Parikshak NN, Mirnics K, Geschwind DH, et al. Strong correlation of downregulated genes related to synaptic transmission and mitochondria in post-mortem autism cerebral cortex. *J Neurodev Disord*. (2018) 10:18. doi: 10.1186/s11689-018-9237-x
82. Ji L, Chauhan A, Chauhan V. Reduced activity of protein kinase C in the frontal cortex of subjects with regressive autism: relationship with developmental abnormalities. *Int J Biol Sci*. (2012) 8:1075–84. doi: 10.7150/ijbs.4742
83. Shen M, Wang F, Li M, Sah N, Stockton ME, Tidei JJ, et al. Reduced mitochondrial fusion and Huntingtin levels contribute to impaired dendritic maturation and behavioral deficits in Fmr1-mutant mice. *Nat Neurosci*. (2019) 22:386–400. doi: 10.1038/s41593-019-0338-y
84. Lu B, Poirier C, Gaspar T, Gratzke C, Harrison W, Busija D, et al. A mutation in the inner mitochondrial membrane peptidase 2-like gene (Immp2l) affects mitochondrial function and impairs fertility in mice. *Biol Reprod*. (2008) 78:601–10. doi: 10.1095/biolreprod.107.065987
85. Kreilaus F, Chesworth R, Eapen V, Clarke R, Karl T. First behavioural assessment of a novel Immp2l knockdown mouse model with relevance for Gilles de la Tourette syndrome and Autism spectrum disorder. *Behav Brain Res*. (2019) 374:112057. doi: 10.1016/j.bbr.2019.112057
86. Norkett R, Modi S, Kittler JT. Mitochondrial roles of the psychiatric disease risk factor DISC1. *Schizophr Res*. (2017) 187:47–54. doi: 10.1016/j.schres.2016.12.025
87. Hirokawa N, Niwa S, Tanaka Y. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron*. (2010) 68:610–38. doi: 10.1016/j.neuron.2010.09.039
88. Kanai Y, Okada Y, Tanaka Y, Harada A, Terada S, Hirokawa N. KIF5C, a novel neuronal kinesin enriched in motor neurons. *J Neurosci*. (2000) 20:6374–84. doi: 10.1523/JNEUROSCI.20-17-06374.2000
89. Iworima DG, Pasqualotto BA, Rintoul GL. Kif5 regulates mitochondrial movement, morphology, function and neuronal survival. *Mol Cell Neurosci*. (2016) 72:22–33. doi: 10.1016/j.mcn.2015.12.014
90. Duis J, Dean S, Applegate C, Harper A, Xiao R, He W, et al. KIF5A mutations cause an infantile onset phenotype including severe myoclonus with evidence of mitochondrial dysfunction. *Ann Neurol*. (2016) 80:633–7. doi: 10.1002/ana.24744
91. Campbell PD, Shen K, Sapio MR, Glenn TD, Talbot WS, Marlow FL. Unique function of Kinesin Kif5A in localization of mitochondria in axons. *J Neurosci*. (2014) 34:14717–32. doi: 10.1523/JNEUROSCI.2770-14.2014
92. Guo Q, Liao S, Zhu Z, Li Y, Li F, Xu C. Structural basis for the recognition of kinesin family member 21A (KIF21A) by the ankyrin domains of KANK1 and KANK2 proteins. *J Biol Chem*. (2018) 293:557–66. doi: 10.1074/jbc.M117.817494
93. King MD, Phillips GW, Bignone PA, Hayes NV, Pinder JC, Baines AJ. A conserved sequence in calmodulin regulated spectrin-associated protein 1 links its interaction with spectrin and calmodulin to neurite outgrowth. *J Neurochem*. (2014) 128:391–402. doi.org/10.1111/jnc.12462
94. Vos M, Lauwers E, Verstreken P. Synaptic mitochondria in synaptic transmission and organization of vesicle pools in health and disease. *Front Synaptic Neurosci*. (2010) 2:139. doi: 10.3389/fnsyn.2010.00139
95. Sun T, Qiao H, Pan PY, Chen Y, Sheng ZH. Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell Rep*. (2013) 4:413–9. doi: 10.1016/j.celrep.2013.06.040
96. Picard M, McEwen BS. Mitochondria impact brain function and cognition. *Proc Natl Acad Sci USA*. (2014) 111:7–8. doi: 10.1073/pnas.1321881111
97. Hara Y, Yuk F, Puri R, Janssen WG, Rapp PR, Morrison JH. Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. *Proc Natl Acad Sci USA*. (2014) 111:486–91. doi: 10.1073/pnas.1311310110
98. Schwarz TL. Mitochondrial trafficking in neurons. *Cold Spring Harb Perspect Biol*. (2013) 5. doi: 10.1101/cshperspect.a011304
99. Kang M, Akbarali HI. Denitration of L-type calcium channel. *FEBS Lett*. (2008) 582:3033–6. doi: 10.1016/j.febslet.2008.07.042
100. Cai Q, Sheng ZH. Moving or stopping mitochondria: miro as a traffic cop by sensing calcium. *Neuron*. (2009) 61:493–6. doi: 10.1016/j.neuron.2009.02.003
101. Saxton WM, Hollenbeck PJ. The axonal transport of mitochondria. *J Cell Sci*. (2012) 125:2095–104. doi: 10.1242/jcs.053850
102. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ*. (2013) 20:31–42. doi: 10.1038/cdd.2012.81

103. MacAskill AF, Brickley K, Stephenson FA, Kittler JT. GTPase dependent recruitment of Grif-1 by Miro1 regulates mitochondrial trafficking in hippocampal neurons. *Mol Cell Neurosci*. (2009) 40:301–12. doi: 10.1016/j.mcn.2008.10.016
104. van Spronsen M, Mikhaylova M, Lipka J, Schlager MA, van den Heuvel DJ, Kuijpers M, et al. TRAK/Milton motor-adaptor proteins steer mitochondrial trafficking to axons and dendrites. *Neuron*. (2013) 77:485–502. doi: 10.1016/j.neuron.2012.11.027
105. Loss O, Stephenson FA. Localization of the kinesin adaptor proteins trafficking kinesin proteins 1 and 2 in primary cultures of hippocampal pyramidal and cortical neurons. *J Neurosci Res*. (2015) 93:1056–66. doi: 10.1002/jnr.23549
106. Brickley K, Stephenson FA. Trafficking kinesin protein (TRAK)-mediated transport of mitochondria in axons of hippocampal neurons. *J Biol Chem*. (2011) 286:18079–92. doi: 10.1074/jbc.M111.236018
107. Nguyen TT, Oh SS, Weaver D, Lewandowska A, Maxfield D, Schuler MH, et al. Loss of Miro1-directed mitochondrial movement results in a novel murine model for neuron disease. *Proc Natl Acad Sci USA*. (2014) 111:E3631–40. doi: 10.1073/pnas.1402449111
108. Millar JK, James R, Christie S, Porteous DJ. Disrupted in schizophrenia 1 (DISC1): subcellular targeting and induction of ring mitochondria. *Mol Cell Neurosci*. (2005) 30:477–84. doi: 10.1016/j.mcn.2005.08.021
109. Park YU, Jeong J, Lee H, Mun JY, Kim JH, Lee JS, et al. Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. *Proc Natl Acad Sci USA*. (2010) 107:17785–90. doi: 10.1073/pnas.1004361107
110. Tsuboi D, Kuroda K, Tanaka M, Namba T, Iizuka Y, Taya S, et al. Disrupted-in-schizophrenia 1 regulates transport of ITPR1 mRNA for synaptic plasticity. *Nat Neurosci*. (2015) 18:698–707. doi: 10.1038/nn.3984
111. Kijima K, Numakura C, Izumino H, Umetsu K, Nezu A, Shiiki T, et al. Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. *Hum Genet*. (2005) 116:23–7. doi: 10.1007/s00439-004-1199-2
112. Ogawa F, Murphy LC, Malavasi EL, O'Sullivan ST, Torrance HS, Porteous DJ, et al. NDE1 and GSK3beta associate with TRAK1 and regulate axonal mitochondrial motility: identification of cyclic AMP as a novel modulator of axonal mitochondrial trafficking. *ACS Chem Neurosci*. (2016) 7:553–64. doi: 10.1021/acschemneuro.5b00255
113. Atkin TA, MacAskill AF, Brandon NJ, Kittler JT. Disrupted in Schizophrenia-1 regulates intracellular trafficking of mitochondria in neurons. *Mol Psychiatry*. (2011) 16:122–4. doi: 10.1038/mp.2010.110
114. Norkett R, Modi S, Birsa N, Atkin TA, Ivankovic D, Pathania M, et al. DISC1-dependent regulation of mitochondrial dynamics controls the morphogenesis of complex neuronal dendrites. *J Biol Chem*. (2016) 291:613–29. doi: 10.1074/jbc.M115.699447
115. Amiott EA, Lott P, Soto J, Kang PB, McCaffery JM, DiMauro S, et al. Mitochondrial fusion and function in Charcot-Marie-Tooth type 2A patient fibroblasts with mitofusin 2 mutations. *Exp Neurol*. (2008) 211:115–27. doi: 10.1016/j.expneurol.2008.01.010
116. Feng Y, Walsh CA. Protein-protein interactions, cytoskeletal regulation and neuronal migration. *Nat Rev Neurosci*. (2001) 2:408–16. doi: 10.1038/35077559
117. Feng Y, Walsh CA. Mitotic spindle regulation by Nde1 controls cerebral cortical size. *Neuron*. (2004) 44:279–93. doi: 10.1016/j.neuron.2004.09.023
118. Alkuraya FS, Cai X, Emery C, Mochida GH, Al-Dosari MS, Felie JM, et al. Human mutations in NDE1 cause extreme microcephaly with lissencephaly [corrected]. *Am J Hum Genet*. (2011) 88:536–47. doi: 10.1016/j.ajhg.2011.04.003
119. Bradshaw NJ, Ogawa F, Antolin-Fontes B, Chubb JE, Carlyle BC, Christie S, et al. DISC1, PDE4B, and NDE1 at the centrosome and synapse. *Biochem Biophys Res Commun*. (2008) 377:1091–6. doi: 10.1016/j.bbrc.2008.10.120
120. Dasgupta B, Milbrandt J. AMP-activated protein kinase phosphorylates retinoblastoma protein to control mammalian brain development. *Dev Cell*. (2009) 16:256–70. doi: 10.1016/j.devcel.2009.01.005
121. Williams T, Courchet J, Viollet B, Brenman JE, Polleux F. AMP-activated protein kinase (AMPK) activity is not required for neuronal development but regulates axogenesis during metabolic stress. *Proc Natl Acad Sci USA*. (2011) 108:5849–54. doi: 10.1073/pnas.1013660108
122. Ji L, Chauhan V, Flory MJ, Chauhan A. Brain region-specific decrease in the activity and expression of protein kinase A in the frontal cortex of regressive autism. *PLoS ONE*. (2011) 6:e23751. doi: 10.1371/journal.pone.0023751
123. Cianfrocco MA, DeSantis ME, Leschziner AE, Reck-Peterson SL. Mechanism and regulation of cytoplasmic dynein. *Annu Rev Cell Dev Biol*. (2015) 31:83–108. doi: 10.1146/annurev-cellbio-100814-125438
124. Reddy BJ, Mattson M, Wynne CL, Vadpey O, Durra A, Chapman D, et al. Load-induced enhancement of Dynein force production by LIS1-NudE *in vivo* and *in vitro*. *Nat Commun*. (2016) 7:12259. doi: 10.1038/ncomms12259
125. Bradshaw NJ, Soares DC, Carlyle BC, Ogawa F, Davidson-Smith H, Christie S, et al. PKA phosphorylation of NDE1 is DISC1/PDE4 dependent and modulates its interaction with LIS1 and NDEL1. *J Neurosci*. (2011) 31:9043–54. doi: 10.1523/JNEUROSCI.5410-10.2011
126. Slater PG, Cammarata GM, Samuelson AG, Magee A, Hu Y, Lowery LA. XMAP215 promotes microtubule-F-actin interactions to regulate growth cone microtubules during axon guidance. *J Cell Sci*. (2019) 132:1–14. doi: 10.1242/jcs.224311
127. Sears JC, Choi WJ, Broadie K. Fragile X Mental Retardation Protein positively regulates PKA anchor Rugose and PKA activity to control actin assembly in learning/memory circuitry. *Neurobiol Dis*. (2019) 127:53–64. doi: 10.1016/j.nbd.2019.02.004
128. Tao K, Matsuki N, Koyama R. AMP-activated protein kinase mediates activity-dependent axon branching by recruiting mitochondria to axon. *Dev Neurobiol*. (2014) 74:557–73. doi: 10.1002/dneu.22149
129. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther*. (2015) 148:114–31. doi: 10.1016/j.pharmthera.2014.11.016
130. Bijur GN, Jope RS. Glycogen synthase kinase-3 beta is highly activated in nuclei and mitochondria. *Neuroreport*. (2003) 14:2415–9. doi: 10.1097/00001756-200312190-00025
131. Nishihara M, Miura T, Miki T, Tanno M, Yano T, Naitoh K, et al. Modulation of the mitochondrial permeability transition pore complex in GSK-3beta-mediated myocardial protection. *J Mol Cell Cardiol*. (2007) 43:564–70. doi: 10.1016/j.yjmcc.2007.08.010
132. Boccitto M, Doshi S, Newton IP, Nathke I, Neve R, Dong F, et al. Opposing actions of the synapse-associated protein of 97-kDa molecular weight (SAP97) and Disrupted in Schizophrenia 1 (DISC1) on Wnt/beta-catenin signaling. *Neuroscience*. (2016) 326:22–30. doi: 10.1016/j.neuroscience.2016.03.048
133. Bijur GN, Jope RS. Rapid accumulation of Akt in mitochondria following phosphatidylinositol 3-kinase activation. *J Neurochem*. (2003) 87:1427–35. doi: 10.1046/j.1471-4159.2003.02113.x
134. King TD, Clodfelder-Miller B, Barksdale KA, Bijur GN. Unregulated mitochondrial GSK3beta activity results in NADH: ubiquinone oxidoreductase deficiency. *Neurotox Res*. (2008) 14:367–82. doi: 10.1007/BF03033861
135. Chen H, Chan DC. Mitochondrial dynamics–fusion, fission, movement, and mitophagy–in neurodegenerative diseases. *Hum Mol Genet*. (2009) 18:R169–76. doi: 10.1093/hmg/ddp326
136. Ishihara N, Otera H, Oka T, Mihara K. Regulation and physiologic functions of GTPases in mitochondrial fusion and fission in mammals. *Antioxid Redox Signal*. (2013) 19:389–99. doi: 10.1089/ars.2012.4830
137. Schrepfer E, Scorrano L. Mitofusins, from Mitochondria to Metabolism. *Mol Cell*. (2016) 61:683–94. doi: 10.1016/j.molcel.2016.02.022
138. Cartoni R, Martinou JC. Role of mitofusin 2 mutations in the physiopathology of Charcot-Marie-Tooth disease type 2A. *Exp Neurol*. (2009) 218:268–73. doi: 10.1016/j.expneurol.2009.05.003
139. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet*. (2004) 36:449–51. doi: 10.1038/ng1341
140. Baloh RH, Schmidt RE, Pestronk A, Milbrandt J. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-Tooth disease from mitofusin 2 mutations. *J Neurosci*. (2007) 27:422–30. doi: 10.1523/JNEUROSCI.4798-06.2007

141. Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH. Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. *J Neurosci*. (2010) 30:4232–40. doi: 10.1523/JNEUROSCI.6248-09.2010
142. Chen H, McCaffery JM, Chan DC. Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell*. (2007) 130:548–62. doi: 10.1016/j.cell.2007.06.026
143. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol*. (2003) 160:189–200. doi: 10.1083/jcb.200211046
144. Chapman AL, Bennett EJ, Ramesh TM, De Vos KJ, Grierson AJ. Axonal transport defects in a mitofusin 2 loss of function model of charcot-marie-tooth disease in Zebrafish. *PLoS ONE*. (2013) 8:e67276. doi: 10.1371/journal.pone.0067276
145. Lenaers G, Hamel C, Delettre C, Amati-Bonneau P, Procaccio V, Bonneau D, et al. Dominant optic atrophy. *Orphanet J Rare Dis*. (2012) 7:46. doi: 10.1186/1750-1172-7-46
146. Yu-Wai-Man P, Griffiths PG, Gorman GS, Lourenco CM, Wright AF, Auer-Grumbach M, et al. Multi-system neurological disease is common in patients with OPA1 mutations. *Brain*. (2010) 133:771–86. doi: 10.1093/brain/awq007
147. Chao de la Barca JM, Prunier-Mirebeau D, Amati-Bonneau P, Ferre M, Sarzi E, Bris C, et al. OPA1-related disorders: diversity of clinical expression, modes of inheritance and pathophysiology. *Neurobiol Dis*. (2016) 90:20–6. doi: 10.1016/j.nbd.2015.08.015
148. Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, et al. Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Hum Mol Genet*. (2007) 16:1307–18. doi: 10.1093/hmg/ddm079
149. Bertholet AM, Millet AM, Guillermin O, Daloyau M, Davezac N, Miquel MC, et al. OPA1 loss of function affects *in vitro* neuronal maturation. *Brain*. (2013) 136:1518–33. doi: 10.1093/brain/awt060
150. Otera H, Ishihara N, Mihara K. New insights into the function and regulation of mitochondrial fission. *Biochim Biophys Acta*. (2013) 1833:1256–68. doi: 10.1016/j.bbamcr.2013.02.002
151. Sheffer R, Douiev L, Edvardson S, Shaag A, Tamimi K, Soiferman D, et al. Postnatal microcephaly and pain insensitivity due to a *de novo* heterozygous DNM1L mutation causing impaired mitochondrial fission and function. *Am J Med Genet A*. (2016) 170:1603–7. doi: 10.1002/ajmg.a.37624
152. Waterham HR, Koster J, van Roermund CW, Mooyer PA, Wanders RJ, Leonard JV. A lethal defect of mitochondrial and peroxisomal fission. *N Engl J Med*. (2007) 356:1736–41. doi: 10.1056/NEJMoa064436
153. Fahrner JA, Liu R, Perry MS, Klein J, Chan DC. A novel *de novo* dominant negative mutation in DNM1L impairs mitochondrial fission and presents as childhood epileptic encephalopathy. *Am J Med Genet A*. (2016) 170:2002–11. doi: 10.1002/ajmg.a.37721
154. Nasca A, Legati A, Baruffini E, Nolli C, Moroni I, Ardissone A, et al. Biallelic mutations in DNM1L are associated with a slowly progressive infantile encephalopathy. *Hum Mutat*. (2016) 37:898–903. doi: 10.1002/humu.23033
155. Vanstone JR, Smith AM, McBride S, Naas T, Holcik M, Antoun G, et al. DNM1L-related mitochondrial fission defect presenting as refractory epilepsy. *Eur J Hum Genet*. (2016) 24:1084–8. doi: 10.1038/ejhg.2015.243
156. Yoon G, Malam Z, Paton T, Marshall CR, Hyatt E, Ivakine Z, et al. Lethal disorder of mitochondrial fission caused by mutations in DNM1L. *J Pediatr*. (2016) 171:313–6.e1-2. doi: 10.1016/j.jpeds.2015.12.060
157. Oettinghaus B, Schulz JM, Restelli LM, Licci M, Savoia C, Schmidt A, et al. Synaptic dysfunction, memory deficits and hippocampal atrophy due to ablation of mitochondrial fission in adult forebrain neurons. *Cell Death Differ*. (2016) 23:18–28. doi: 10.1038/cdd.2015.39
158. Shields LY, Kim H, Zhu L, Haddad D, Berthet A, Pathak D, et al. Dynamin-related protein 1 is required for normal mitochondrial bioenergetic and synaptic function in CA1 hippocampal neurons. *Cell Death Dis*. (2015) 6:e1725. doi: 10.1038/cddis.2015.94
159. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, et al. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol*. (2009) 11:958–66. doi: 10.1038/ncb1907
160. Wakabayashi J, Zhang Z, Wakabayashi N, Tamura Y, Fukaya M, Kensler TW, et al. The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. *J Cell Biol*. (2009) 186:805–16. doi: 10.1083/jcb.200903065
161. Koch A, Thiemann M, Grabenbauer M, Yoon Y, McNiven MA, Schrader M. Dynamin-like protein 1 is involved in peroxisomal fission. *J Biol Chem*. (2003) 278:8597–605. doi: 10.1074/jbc.M211761200
162. Chang CR, Blackstone C. Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *J Biol Chem*. (2007) 282:21583–7. doi: 10.1074/jbc.C700083200
163. Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep*. (2007) 8:939–44. doi: 10.1038/sj.embor.7401062
164. Wong H, Levenga J, Cain P, Rothermel B, Klann E, Hoeffer C. RCAN1 overexpression promotes age-dependent mitochondrial dysregulation related to neurodegeneration in Alzheimer's disease. *Acta Neuropathol*. (2015) 130:829–43. doi: 10.1007/s00401-015-1499-8
165. Parra V, Altamirano F, Hernandez-Fuentes CP, Tong D, Kyrychenko V, Rotter D, et al. Down syndrome critical region 1 gene, Rcan1, helps maintain a more fused mitochondrial network. *Circ Res*. (2018) 122:e20–33. doi: 10.1161/CIRCRESAHA.117.311522
166. Mizuguchi T, Nakashima M, Kato M, Okamoto N, Kurahashi H, Ekhilevitch N, et al. Loss-of-function and gain-of-function mutations in PPP3CA cause two distinct disorders. *Hum Mol Genet*. (2018) 27:1421–33. doi: 10.1093/hmg/ddy052
167. Toyama EQ, Herzig S, Courchet J, Lewis TL, Jr., Loson OC, Hellberg K, et al. Metabolism. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. *Science*. (2016) 351:275–81. doi: 10.1126/science.aab4138
168. Yu R, Liu T, Jin SB, Ning C, Lendahl U, Nister M, et al. MIEF1/2 function as adaptors to recruit Drp1 to mitochondria and regulate the association of Drp1 with Mff. *Sci Rep*. (2017) 7:880. doi: 10.1038/s41598-017-00853-x
169. Lee JE, Westrate LM, Wu H, Page C, Voeltz GK. Multiple dynamin family members collaborate to drive mitochondrial division. *Nature*. (2016) 540:139–43. doi: 10.1038/nature20555
170. Piggott LA, Bauman AL, Scott JD, Dessauer CW. The A-kinase anchoring protein Yotiao binds and regulates adenylyl cyclase in brain. *Proc Natl Acad Sci USA*. (2008) 105:13835–40. doi: 10.1073/pnas.0712100105
171. Sanderson JL, Scott JD, Dell'Acqua ML. Control of homeostatic synaptic plasticity by AKAP-anchored kinase and phosphatase regulation of Ca(2+)-Permeable AMPA receptors. *J Neurosci*. (2018) 38:2863–76. doi: 10.1523/JNEUROSCI.2362-17.2018
172. Murphy JG, Sanderson JL, Gorski JA, Scott JD, Catterall WA, Sather WA, et al. AKAP-anchored PKA maintains neuronal L-type calcium channel activity and NFAT transcriptional signaling. *Cell Rep*. (2014) 7:1577–88. doi: 10.1016/j.celrep.2014.04.027
173. Sanderson JL, Dell'Acqua ML. AKAP signaling complexes in regulation of excitatory synaptic plasticity. *Neuroscientist*. (2011) 17:321–36. doi: 10.1177/1073858410384740
174. Gilman AG. G proteins: transducers of receptor-generated signals. *Annu Rev Biochem*. (1987) 56:615–49. doi: 10.1146/annurev.bi.56.070187.003151
175. Glovaci I, Chapman CA. Activation of phosphatidylinositol-linked dopamine receptors induces a facilitation of glutamate-mediated synaptic transmission in the lateral entorhinal cortex. *PLoS ONE*. (2015) 10:e0131948. doi: 10.1371/journal.pone.0131948
176. Jones RM, Cadby G, Melton PE, Abraham LJ, Whitehouse AJ, Moses EK. Genome-wide association study of autistic-like traits in a general population study of young adults. *Front Hum Neurosci*. (2013) 7:658. doi: 10.3389/fnhum.2013.00658
177. Pagnamenta AT, Bacchelli E, de Jonge MV, Mirza G, Scerri TS, Minopoli F, et al. Characterization of a family with rare deletions in CNTNAP5 and DOCK4 suggests novel risk loci for autism and dyslexia. *Biol Psychiatry*. (2010) 68:320–8. doi: 10.1016/j.biopsych.2010.02.002
178. McKenna MC, Waagepetersen HS, Schousboe A, Sonnewald U. Neuronal and astrocytic shuttle mechanisms for cytosolic-mitochondrial transfer of reducing equivalents: current evidence and pharmacological tools. *Biochem Pharmacol*. (2006) 71:399–407. doi: 10.1016/j.bcp.2005.10.011

179. Sica V, Kroemer G. IMMP2L: a mitochondrial protease suppressing cellular senescence. *Cell Res*. (2018) 28:607–8. doi: 10.1038/s41422-018-0051-5
180. Yuan L, Zhai L, Qian L, Huang, Ding Y, Xiang H, et al. Switching off IMMP2L signaling drives senescence via simultaneous metabolic alteration and blockage of cell death. *Cell Res*. (2018) 28:625–43. doi: 10.1038/s41422-018-0043-5
181. Adamczyk A, Gause CD, Sattler R, Vidensky S, Rothstein JD, Singer H, et al. Genetic and functional studies of a missense variant in a glutamate transporter, SLC1A3, in Tourette syndrome. *Psychiatr Genet*. (2011) 21:90–7. doi: 10.1097/YPG.0b013e328341a307
182. Jackson TD, Palmer CS, Stojanovski D. Mitochondrial diseases caused by dysfunctional mitochondrial protein import. *Biochem Soc Trans*. (2018) 46:1225–38. doi: 10.1042/BST20180239
183. Martensson CU, Becker T. Acylglycerol Kinase: mitochondrial protein transport meets lipid biosynthesis. *Trends Cell Biol*. (2017) 27:700–2. doi: 10.1016/j.tcb.2017.08.006
184. Curran SP, Leuenberger D, Schmidt E, Koehler CM. The role of the Tim8p-Tim13p complex in a conserved import pathway for mitochondrial polytopic inner membrane proteins. *J Cell Biol*. (2002) 158:1017–27. doi: 10.1083/jcb.200205124
185. Kadenbach B. Regulation of mammalian 13-subunit cytochrome c oxidase and binding of other proteins: role of NDUFA4. *Trends Endocrinol Metab*. (2017) 28:761–70. doi: 10.1016/j.tem.2017.09.003
186. Simonic I, Nyholt DR, Gericke GS, Gordon D, Matsumoto N, Ledbetter DH, et al. Further evidence for linkage of Gilles de la Tourette syndrome (GTS) susceptibility loci on chromosomes 2p11, 8q22 and 11q23-24 in South African Afrikaners. *Am J Med Genet*. (2001) 105:163–7. doi: 10.1002/ajmg.1192
187. Gokhale A, Hartwig C, Freeman AAH, Bassell JL, Zlatic SA, Sapp Savas C, et al. Systems analysis of the 22q11.2 microdeletion syndrome converges on a mitochondrial interactome necessary for synapse function and behavior. *J Neurosci*. (2019) 39:3561–81. doi: 10.1523/JNEUROSCI.1983-18.2019
188. Knight S, Coon H, Johnson M, Leppert MF, Camp NJ, McMahon WM, et al. Linkage analysis of Tourette syndrome in a large Utah pedigree. *Am J Med Genet B Neuropsychiatr Genet*. (2010) 153B:656–62. doi: 10.1002/ajmg.b.31035
189. Matsumoto N, David DE, Johnson EW, Konecki D, Burmester JK, Ledbetter DH, et al. Breakpoint sequences of an 1;8 translocation in a family with Gilles de la Tourette syndrome. *Eur J Hum Genet*. (2000) 8:875–83. doi: 10.1038/sj.ejhg.5200549

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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