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Blood gene expression correlated with tic severity in medicated and unmedicated patients with Tourette Syndrome

Background: Tourette Syndrome (TS) has been linked to both genetic and environmental factors. Gene-expression studies provide valuable insight into the causes of TS; however, many studies of gene expression in TS do not account for the effects of medication. **Materials & methods:** To investigate the effects of medication on gene expression in TS patients, RNA was isolated from the peripheral blood of 20 medicated TS subjects (MED) and 23 unmedicated TS subjects (UNMED), and quantified using whole-genome Affymetrix microarrays. **Results:** D2 dopamine receptor expression correlated positively with tic severity in MED but not UNMED. $GABA_A$ receptor ε subunit expression negatively correlated with tic severity in UNMED but not MED. Phenylethanolamine *N*-methyltransferase expression positively correlated with tic severity in UNMED but not MED. **Conclusion:** Modulation of tics by TS medication is associated with changes in dopamine, norepinephrine and GABA pathways.

KEYWORDS: ***GABRE*** ▪ **gene expression** ▪ **microarray** ▪ ***NPAS4*** ▪ **phenylethanolamine *N*-methyltransferase** ▪ ***PNMT*** ▪ **Tourette Syndrome**

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Tourette Syndrome (TS) is a developmental neuropsychiatric disorder characterized by the presence of patterned movements or sounds performed involuntarily or in response to urges. Clinic-based TS cohorts show extremely high rates of obsessive compulsive behaviors and attention deficit hyperactivity disorder (ADHD) symptoms. Currently, there is no biological marker for this disorder and its pathophysiology is poorly understood. Despite high apparent heritability, sometimes in an autosomal dominant fashion with increased penetrance of tics in males and obsessive compulsive behaviors in females, no dominant gene effect has been identified. The current standard for diagnosis is based on the observed presence of tics, codified in Diagnostic and Statistical Manual, Fourth Edition, Text Revision (DSM IV-TR) [1] as:

- Multiple motor and one or more vocal tics
- Tics that occur daily or intermittently but must be present for at least 1 year
- Onset before 18 years of age

The severity and frequency of tics wax and wane, tend to peak in adolescence and often improve by the end of adolescence or early adulthood (for reviews, see [2–4]).

Our research group has used gene-expression profiling, through the quantification of RNA levels, in peripheral blood to glean insights into this complex disorder. Ideally, tic severity should be examined in relation to gene expression in brain tissue, but this is not feasible using the current technology. The advantage of studying blood gene expression is the ability to characterize the relationship between genes, environmental factors and disease symptoms in a noninvasive manner. As such, blood gene expression could provide accessible biomarkers for TS subgroups, prognosis, pathophysiology and treatment efficacy.

Our previous study comparing gene expression of TS subjects to controls found several interesting differences [5]. Notably, a subgroup of TS subjects overexpressed natural killer cell genes. However, one limitation of this patient sample was medication exposure; patients taking medication were grouped with patients that were medication-free. Patients with severe tics and comorbidities are often treated pharmacologically using antipsychotics and α2-adrenergic agonists, which act upon dopaminergic and adrenergic signaling pathways. We hypothesized that these treatments might influence gene expression and that the degree to which this occurrs might be associated with tic severity. Therefore, in this study, we evaluated the gene expression of medicated (MED) and unmedicated (UNMED) TS subjects in relation to medication status and symptom severity, specifically focusing on the genes involved in catecholamine signaling and metabolism.

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Materials & methods

Subject recruitment & assessment

Medicated subjects were recruited from the Tourette Syndrome Clinic at Cincinnati Children's Hospital Medical Center (Ohio, USA). UNMED subjects were recruited via the Tourette Syndrome Association, clinical referrals, local advertisements, physician referrals and through the University of California at Davis (CA, USA). UNMED subjects were excluded if their estimated IQ was less than 75, if they had taken medication to treat tics, or if they had serious neurological, psychiatric or medical conditions other than TS, comorbid ADHD or comorbid obsessive compulsive disorder (OCD). Most UNMED subjects also participated in a functional neuroimaging study of tic severity and cognitive control conducted by Bunge *et al.* [6]. UNMED subjects were medication naive as per parental reports, except for two subjects that had previously taken atomoxetine (Strattera™; Eli Lilly, IN, USA) to treat ADHD symptoms. One of these subjects ended medication approximately 1 month before participation in the study; the other stopped taking medication 40 h before participation. Behavioral measures and principal component analysis of gene-expression data indicate that these two subjects are not outliers.

Informed consent was obtained from each participant, parent or legal guardian. Recruitment, interview and sample collection proceeded according to established institutional review board protocols. TS diagnosis was based on Diagnostic and Statistical Manual of Mental Disorders criteria [1]. Tic severity was assessed via direct child and parent interview using the Yale Global Tic Severity Scale (YGTSS) [7].

Sample collection

A total of 15 ml of whole blood was collected from each subject via antecubital fossa venipuncture into six PAXgene Vacutainer tubes (Qiagen, Hilden, Germany). These tubes contain a solution that immediately lyses all of the cells in whole blood and stabilizes the RNA without measurable degradation. The RNA represents genes expressed in all white blood cells, immature red blood cells and immature platelets. Blood samples were frozen at -70°C for storage.

RNA isolation from whole blood

Total RNA was isolated using the PAXgene Blood RNA Kit (Qiagen) according to the manufacturer's protocol. RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Foster City, CA, USA) and quantified by fiberoptic spectrophotometry using the Nanodrop ND-1000 (Nanodrop Inc., Wilmington, DE, USA). Samples were excluded if purified RNA did not have both an A_{260}:A_{280} absorbance ratio greater than 2.0 and a 28s:18s rRNA ratio equal to or exceeding 1.8.

Gene expression measurement

Biotin-labeled cDNA was synthesized from 50 ng of RNA from each sample using the Ovation Whole Blood Solution (NuGEN Technologies, Inc., San Carlos, CA, USA) according to the manufacturer's protocol. cDNA was hybridized with probes on Affymetrix Human Genome U133 Plus 2.0 microarrays (Affymetrix Inc., CA, USA). Microarrays were washed and stained on a Fluidics Station 450 (Affymetrix) and were scanned on a GeneChip® Scanner 3000 (Affymetrix).

Data analysis

Scanned microarray images in the form of Affymetrix CEL files were imported into Partek Genomics Suite 6.4 (Partek Inc., St Louis, MO, USA) using Partek default settings (GC content adjustment, robust multichip average background correction, log base 2 normalization). An analysis of variance using the model 'expression = YGTSS' was performed with age, gender and scan date (i.e., microarray batch) as covariates of no interest.

Within Partek, the batch remover algorithm was used to remove the effects of age, gender and batch, and the resulting expression data were plotted against tic severity for each gene. However, because MED and UNMED microarrays were processed separately, it is not possible to completely remove the batch effect. Thus, MED and UNMED data cannot be compared directly. Accordingly, Pearson correlation coefficients were chosen as a within-group metric and calculated separately for MED and UNMED. For each gene, the correlation between gene expression and tic severity was measured, and the associated p-value of each correlation was calculated (with a null hypothesis of no correlation, or r: 0).

To test whether gene expression values and YGTSS were normally distributed, a one-sample Kolmogorov–Smirnov test was performed within Partek on each probeset and on the YGTSS. The null hypothesis was that the observed values were from a normal distribution. Of the UNMED data, 47 of

54,683 probesets were not normally distributed ($p < 0.05$), and of the MED data, 23 of 54,679 probesets were not normally distributed ($p < 0.05$). Given the null hypothesis, the probability of obtaining the observed YGTSS values was $p = 0.895$ for MED subjects and $p = 0.98$ for UNMED subjects. Thus, we cannot reject the null hypothesis that the observed values are from a normal distribution for almost all probesets and for the YGTSS at the 0.05 significance level, indicating that the default Partek import setting of log base 2 is an effective normalization for this dataset and that the observed YGTSS values are reasonably normally distributed.

To focus on the genes that are most likely to be related to tics, and to address the problem of multiple comparisons, only genes that were most strongly correlated with tic severity (see Supplementary Tables 1 & 2 at www.futuremedicine.com/doi/suppl/10.2217/pgs.10.160) and known to be expressed in either catecholamine-related pathways or neurotransmitter pathways were further analyzed. Catecholamine-related pathways were chosen because medications that act on these pathways are known to decrease tics in TS patients [8]. To create a list of neurotransmitter genes, we consulted the Kyoto Encyclopedia of Genes and Genomes [9] and all genes from the 'neuroactive ligand–receptor interaction' pathway were used. To create a list of catecholamine-related pathways, we consulted the Gene Ontology (GO) database [10], and all genes associated with GO terms including the keyword 'catecholamine' were used. In addition, to maintain gene symbol format compatibility, only genes from GO using Mouse Genome Informatics accession numbers were used. Combining these two lists, and removing 10 genes not represented on the microarrays, yielded a list of 286 genes (see supplementary Table 3 at www.futuremedicine.com/doi/suppl/10.2217/pgs.10.160).

Results

Subject demographics & medications

Subject demographics are summarized in Supplementary Table 4 (www.futuremedicine.com/doi/suppl/10.2217/pgs.10.160). The mean age of MED and UNMED subjects did not differ significantly (11.55 vs 10.69, $p < 0.20$). The mean tic severity of MED subjects was significantly less than that of UNMED subjects (13.4 vs 23.6, $p < 0.0001$). Both MED and UNMED groups were predominantly male (90 and 78%, respectively). Numbers of subjects taking each type of medication to treat tics and/or comorbid ADHD/OCD are also listed in Supplementary Table 4.

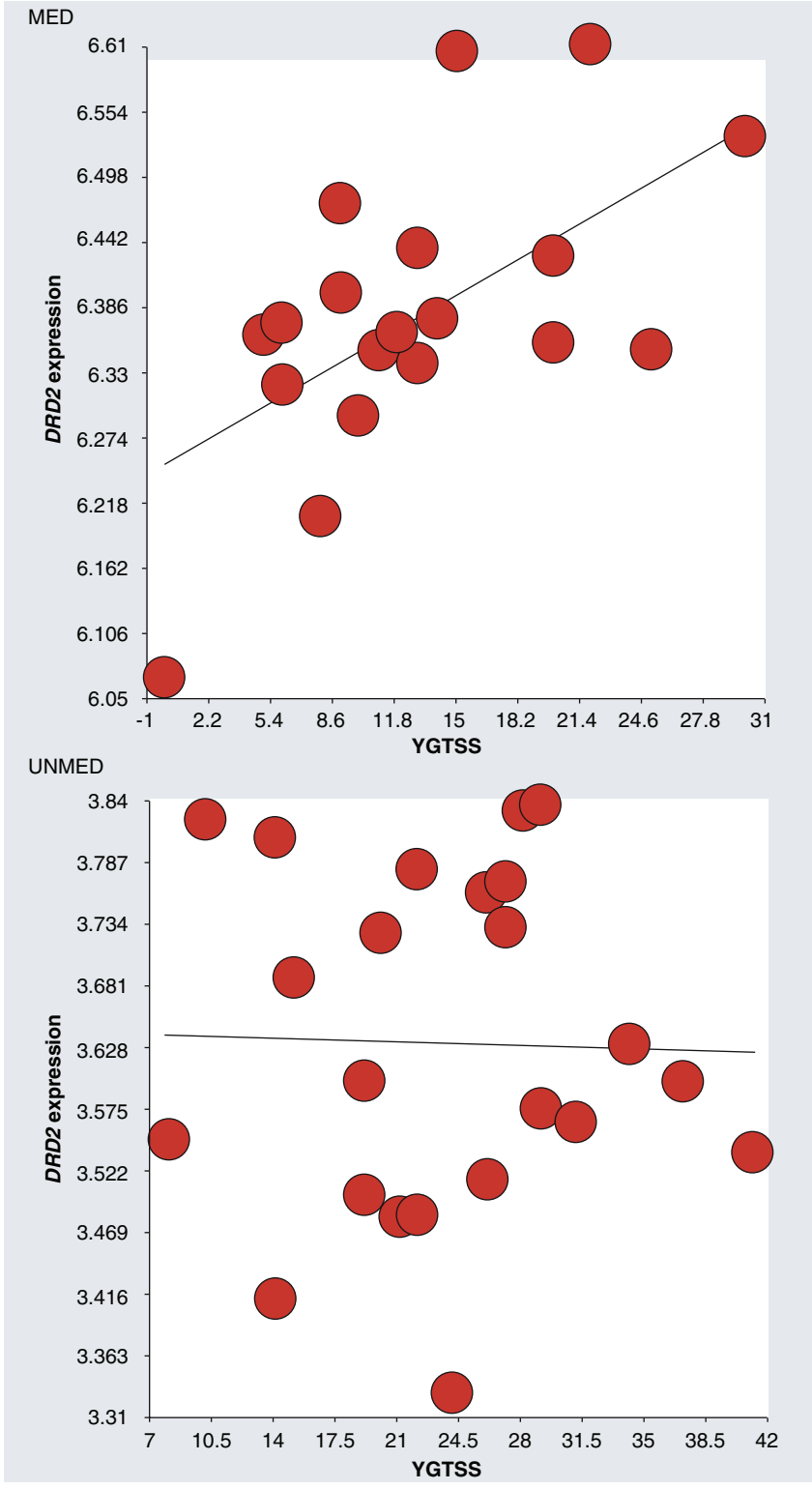

Figure 1. *DRD2* expression (y-axis) versus Yale Global Tic Severity Scale (x-axis) for medicated and unmedicated Tourette Syndrome subjects.
MED: r = +0.58; UNMED: r = -0.028.
DRD2: D2 dopamine receptor; MED: Medicated; UNMED: Unmedicated; YGTSS: Yale Global Tic Severity Scale.

Table 1. Correlation coefficients for gene expression versus tic severity for selected genes.

Symbol	Probeset ID	Gene title	MED r	UNMED r
DRD2	206590_x_at	Dopamine receptor D2	0.58†	-0.028
GABRE	204537_s_at	$GABA_A$ receptor ε	0.097	-0.71†
PNMT	206793_at	Phenylethanolamine *N*-methyltransferase	-0.24	0.52‡
NPAS4	1554299_at	Neuronal PAS domain protein 4	0.51‡	0.45‡

†$p \leq 0.001$.
‡$p \leq 0.05$.
MED r: Correlation coefficient for medicated subjects; UNMED r: Correlation coefficient for unmedicated subjects.

Gene-expression correlations with tic severity, stratified by medication status

Genes that correlated with tic severity ($p < 0.05$) in MED are listed in Supplementary Table 1. Similarly, genes that correlated with tic severity ($p < 0.05$) in UNMED are listed in Supplementary Table 2. 116 genes were shared between the two lists. Of these 116 genes, 53 were correlated in the same direction in MED and UNMED, and 63 genes were correlated in opposite directions in MED and UNMED.

Catecholamine & neurotransmitter-related genes correlated with tic severity

Filtering the list of genes correlated with tic severity using the list of catecholamine or neurotransmitter-related genes yielded 26 genes in UNMED and 22 genes in MED subjects (see online supplementary Table 5 at www.futuremedicine.com/doi/suppl/10.2217/pgs.10.160). D2 dopamine receptor (*DRD2*) gene expression had significant positive correlation with tic severity (YGTSS) in MED but not UNMED subjects (Figure 1 & Table 1). By contrast, phenylethanolamine-*N*-methyltransferase (*PNMT*) expression had significant positive correlation with tic severity in UNMED but not MED subjects (Figure 2 & Table 1). $GABA_A$ receptor ε subunit (*GABRE*) expression had significant negative correlation with tic severity in UNMED but not MED subjects (Figure 3 & Table 1). Trend lines were fitted using linear regression and are shown in Figures 1, 2 & 3. Correlation coefficients and significance levels for these genes are summarized in Table 1.

Discussion

Expression of *PNMT* and *GABRE* both correlate with tic severity in UNMED but not MED subjects. These correlations would not have been found if MED and UNMED subjects had been analyzed as a single group. Previous studies examining only MED subjects or examining all TS subjects as a single group regardless of medication status may have overlooked these genes. Thus, it is important for gene-expression studies of TS to account for the effects of medication. This may be true for other diseases and medications as well.

Dopaminergic pathways implicated in this study

The observed correlation between *DRD2* expression and tic severity in subjects taking medication (Figure 1), supports the theory that dopamine neurotransmission is involved in tic production. Both typical and atypical antipsychotics block dopamine receptors, which induce compensatory increases in DRD2 mRNA and protein expression [11]. We postulate that a correlation is observed in MED subjects because this induced expression occurs in varying amounts, resulting in some subjects overcoming their DRD2 blockade to a greater degree than others. Thus, in MED subjects, greater *DRD2* expression leads to greater activation of the direct pathway or greater inhibition of the indirect pathway, both of which result in greater tic severity. UNMED subjects, on the other hand, may not exhibit an association between *DRD2* expression and tic severity because DRD2 mRNA levels at the time of blood draw do not necessarily reflect the number of active, pre-existing D2 dopamine receptors in the substantia nigra. It might be possible to clarify this issue by combining our study methodology with D2 receptor radioligand PET studies.

Decreased *GABRE* expression correlates with increased tic severity in UNMED subjects (Figure 3). GABRE is the ε subunit of the $GABA_A$ receptor; $GABA_A$ receptors are pentamers, with the most common configuration comprising two α subunits, two β subunits, and one γ subunit [12]. The ε subunit can be incorporated into the receptor instead of the γ subunit, resulting in increased sensitivity to GABA and increased spontaneous channel opening [13,14]. Thus, one would expect decreased expression of *GABRE* to result in decreased $GABA_A$ receptor activation

in areas where *GABRE* is expressed, such as in dopaminergic neurons in the substantia nigra pars compacta (SNc) [15]. We speculate that our results may signal a decrease in *GABRE* expression from normal levels in these neurons. The consequent reduction in inhibitory input would yield increased dopamine release into striatum, increasing tic severity.

Adrenergic pathways implicated in this study

Expression of *PNMT* correlates with tic severity in UNMED but not MED subjects (Figure 2). PNMT is an enzyme that synthesizes epinephrine from norepinephrine, and is expressed in medullary projections to the locus ceruleus (LC) of rats and monkeys [15,16]. By modulating LC activity, *PNMT* expression could indirectly affect SNc activity via excitatory noradrenergic LC projections to the dopaminergic neurons of the SNc. Although LC projections to the SNc are relatively sparse compared with LC projections to the ventral tegmental area, some still exist [17]. Therefore, higher expression of *PNMT*, which we found to be correlated with greater tic severity, could result in increased norepinephrine synthesis in projections to the LC, causing greater activation of the LC and, in turn, greater activation of the SNc. This would lead to an increase in motor output, such as tics.

Expression of *GABRE* also correlates with tic severity in UNMED but not MED subjects. *GABRE* is expressed in the LC of rats and monkeys [18,19]. Decreased expression of *GABRE*, which correlates with greater tic severity, would decrease GABAergic inhibition of LC and result in greater overall LC activation, possibly influencing tic severity. Alternatively, changes in *PNMT* and *GABRE* expression could also lead to tics without LC involvement, via direct projections from the medulla to the SNc [17,20]. Increased *PNMT* expression in these neurons would lead to greater epinephrine synthesis, causing greater activation of SNc and increased dopamine release. Decreased expression of GABRE receptors localized to these PNMT-rich neurons [15] would also cause them to be more active, leading to increased SNc activation and tic exacerbation.

Furthermore, since the expression of *PNMT* and *GABRE* correlate with tic severity in UNMED but not MED subjects, the relationship between expression of these two genes and tic severity may be affected by current medication. As such, PNMT and GABRE may be partly responsible for the beneficial effects of medication, and could represent novel targets for specific pharmaceutical therapy in the treatment of TS and tic disorders, particularly to avoid Parkinsonian side effects and tardive dyskinesia

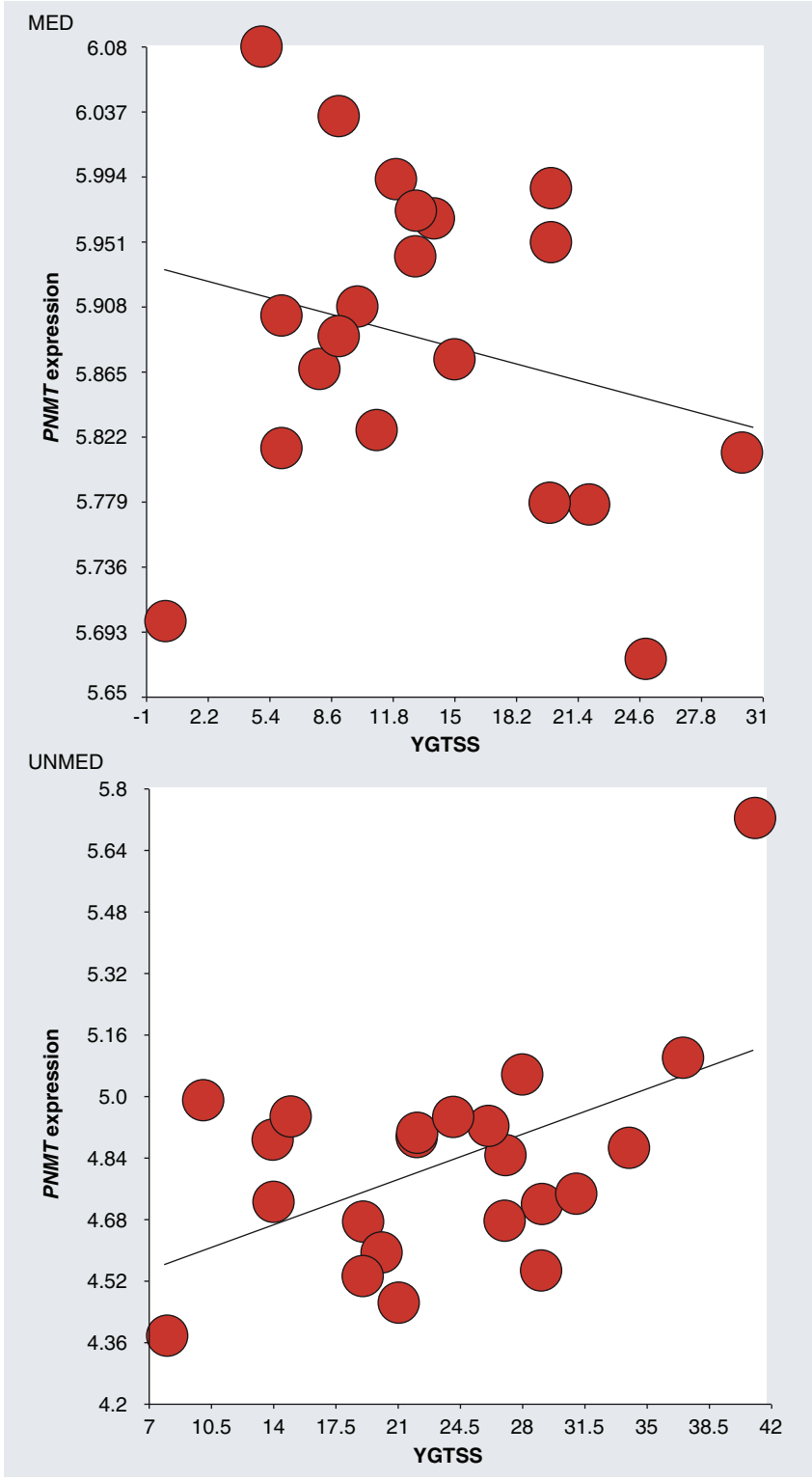

Figure 2. *PNMT* expression (y-axis) versus Yale Global Tic Severity Scale (x-axis) for medicated and unmedicated Tourette Syndrome subjects.
MED: r = -0.24; UNMED: r = +0.52.
MED: Medicated; PNMT: Phenylethanolamine-*N*-methyltransferase; UNMED: Unmedicated; YGTSS: Yale Global Tic Severity Scale.

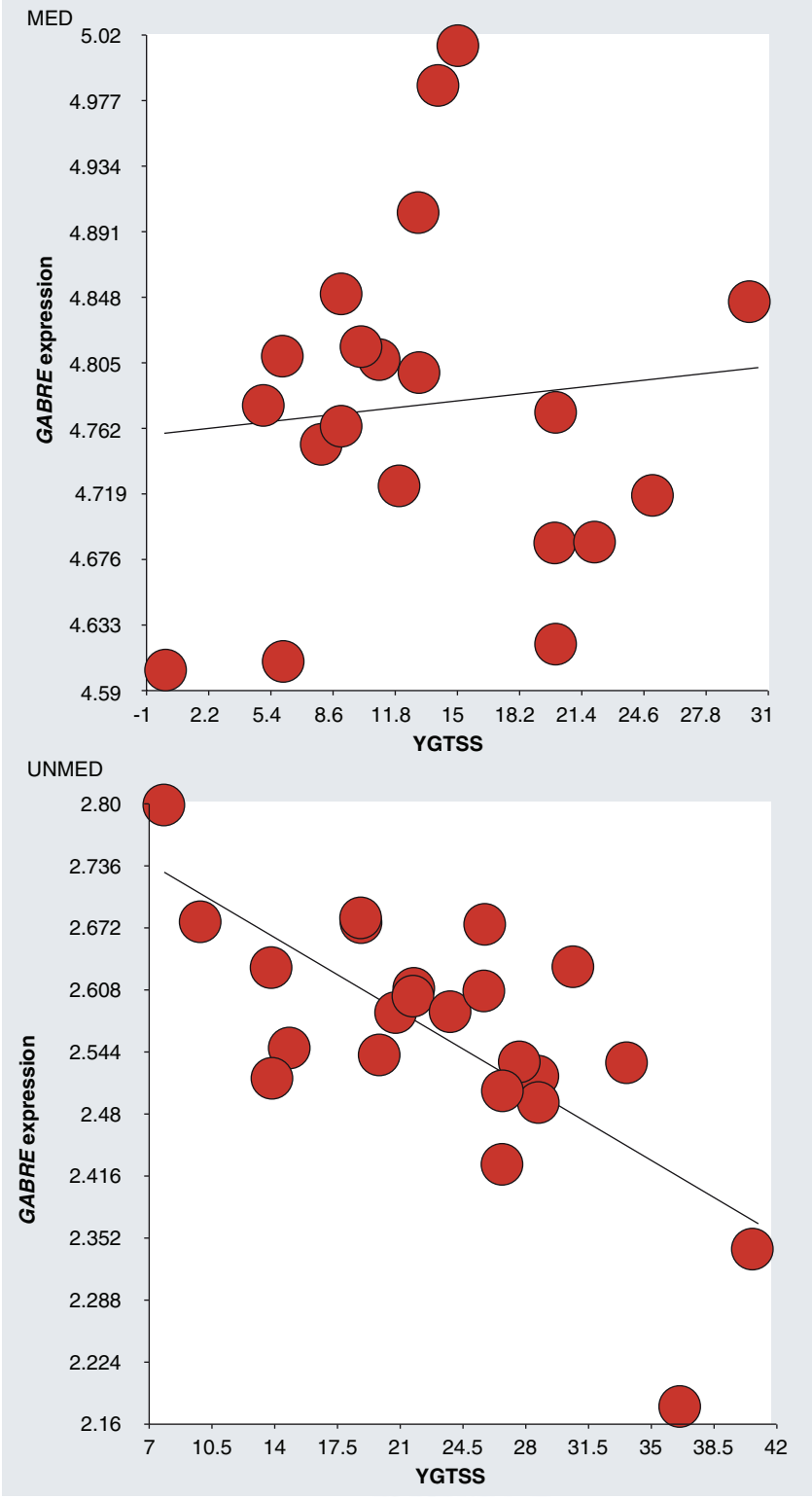

Figure 3. *GABRE* expression (y-axis) versus Yale Global Tic Severity Scale (x-axis) for medicated and unmedicated Tourette Syndrome subjects.
MED: r = +0.097; UNMED: r = -0.71.
GABRE: $GABA_A$ receptor ε subunit; MED: Medicated; UNMED: Unmedicated; YGTSS: Yale Global Tic Severity Scale.

associated with typical antipsychotics [21] and abnormalities in glucose metabolism associated with atypical antipsychotics [22].

Neuronal PAS domain protein 4 is positively correlated with tic severity in both MED & UNMED

Neuronal PAS domain protein 4 (NPAS4) was positively correlated with tic severity in both MED and UNMED subjects, with a correlation coefficient of r: 0.51 ($p < 0.02$) in MED subjects, and r: 0.45 ($p < 0.03$) in UNMED subjects (Table 1 & Figure 4). Although *NPAS4* was not included in the list of catecholamine or neurotransmitter-related genes, the fact that its expression has significant positive correlation with tic severity in both MED and UNMED groups is notable. No genes from the list of catecholamine or neurotransmitter-related genes were found to be significantly correlated with MED and UNMED subjects in the same direction.

Neuronal PAS domain protein 4, a transcription factor, regulates the expression of several activity-dependent genes, which determine the number of GABA-releasing synapses that form on excitatory neurons [23]. GABAergic mechanisms were implicated in TS in a study that found decreased numbers of parvalbumin-positive, GABAergic interneurons in the caudate and putamen of subjects with TS but increased numbers of GABAergic neurons in globus pallidus pars interna [24]. Our findings point to altered *NPAS4* expression as one possible explanation for these changes in numbers of GABAergic neurons in TS. In addition, *NPAS4* was positively correlated with tic severity regardless of whether subjects were taking medications or not, indicating that these medications seemed not to affect the relationship between *NPAS4* expression and tic severity. However, NPAS4 may not be a good candidate for pharmaceutical therapy, given that its regulatory effects on the formation of GABA-releasing synapses most likely occur early in development, before TS diagnosis is possible.

Other genes implicated in this study

Three catecholamine or neurotransmitter-related genes were significantly correlated with tic severity in both MED and UNMED groups: *GABRB3*, *GRIN1* and *SSTR5*. Interestingly, all three of these genes were correlated in opposite directions in MED and UNMED groups: *GABRB3* and *GRIN1* were positively correlated with tic severity in MED subjects and negatively correlated in UNMED subjects, while *SSTR5* was negatively correlated with tic severity in MED and positively correlated in UNMED subjects. No catecholamine or neurotransmitter-related genes were significantly correlated with tic severity in the same direction in both MED and UNMED subjects.

GABRB3 is linked to Angelman syndrome [25] and ADHD [26]. GRIN1 is associated with Parkinson's disease, as well as other brain disorders [27]. SSTR5 regulates the proliferation of many cell types [28]. The observation that these genes are correlated with tic severity in opposite directions in MED and UNMED, indicates that medications affect the relationship between their expression and tic severity. However, precisely how this occurs remains to be elucidated.

Conclusion

The predominant theory of TS pathophysiology, and other movement disorders, posits an imbalance between the excitatory direct pathway and the inhibitory indirect pathway from the striatum through the basal ganglia [29]. An overactive direct pathway or an underactive indirect pathway would result in increased psychomotor activity. The SNc provides dopaminergic input to the striatum, regulating both the direct pathway through *DRD1* and the indirect pathway through *DRD2*. Based on the findings in this study, we propose that altered expression of *GABRE* and *PNMT* may play a role in increased activation of the SNc in TS. Specifically, increased *PNMT* expression in adrenergic projections from the medulla to the LC would result in greater input to the LC, while decreased expression of *GABRE* in the LC would result in greater sensitivity to this input. As a result, the LC would become more activated, providing greater noradrenergic input to the SNc. This would lead to the hypothesized imbalance between the direct and indirect pathways, manifesting as an increase in motor actions, such as tics.

Future perspective

To further validate the expression values of genes in this study, quantitative real-time PCR (qRT-PCR) can be performed using probes for genes of interest. The drawbacks of qRT-PCR are that it is expensive, time-consuming, low-throughput and genes of interest must be selected *a priori* (by contrast, microarray studies assess all genes simultaneously). In addition, one recent paper has shown that appropriate preprocessing techniques can increase the correlation between microarray and qRT-PCR results [30].

Gene expression and tic severity were measured at only one time point per subject in this study. Future studies could assess whether tics vary in severity in proportion to gene-expression levels over time. Moreover, different types of tics might be associated with different changes in gene expression.

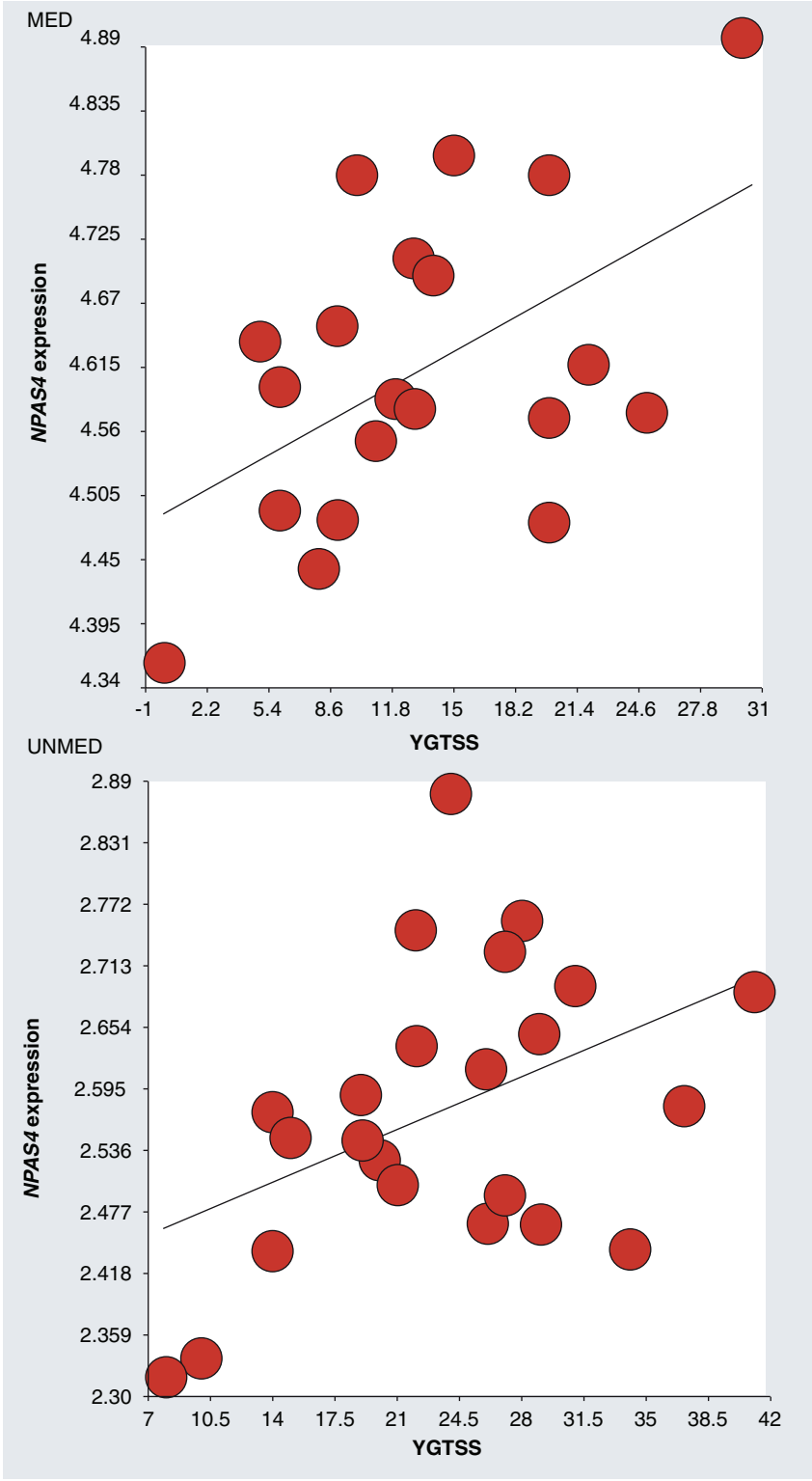

Figure 4. *NPAS4* expression (y-axis) versus Yale Global Tic Severity Scale (x-axis) for medicated and unmedicated Tourette Syndrome subjects.
MED: r = +0.51; UNMED: r = +0.45.
MED: Medicated; NPAS4: Neuronal PAS domain protein 4; UNMED: Unmedicated; YGTSS: Yale Global Tic Severity Scale.

Patients in the MED group were taking medications of different types. However, further dividing the MED group into subgroups

based on medication type resulted in sample sizes too small for meaningful analysis. Future studies interested in the effects of specific medications could restrict participants to those using medications of interest.

Similarly, comorbidities commonly associated with TS – namely ADHD and OCD – were present in the patient population but were not explicitly investigated due to sample size. Future studies could investigate the effects of medication on ADHD and OCD in isolation to determine whether the gene-expression correlations seen here are unique to TS or if they also correlate with measures of ADHD or OCD severity (Conners' Rating Scale and Children's Yale–Brown Obsessive–Compulsive Scale, respectively). In addition, age and gender were selected as covariates of no interest and further studies could examine these factors explicitly.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Tourette Syndrome (TS) is a movement disorder most likely caused by both genetic and environmental factors.
- Gene expression measures the regulation of genes in response to the environment.
- A better understanding of how gene expression is affected by TS medications could identify new drug targets and contribute to our understanding of TS.

Materials & methods

- To assess tic severity, the Yale Global Tic Severity Scale was used in a behavioral assessment of 20 medicated (MED) TS subjects and 23 unmedicated (UNMED) TS subjects.
- To measure gene expression, RNA from all subjects was purified, reverse transcribed and hybridized to Affymetrix microarrays.
- Gene-expression data was analyzed using GeneSpring® and Partek® software.

Results

- D2 dopamine receptor expression correlated positively with tic severity in MED but not UNMED subjects.
- $GABA_A$ receptor ε subunit expression negatively correlated with tic severity in UNMED but not MED subjects.
- Phenylethanolamine *N*-methyltransferase expression positively correlated with tic severity in UNMED but not MED subjects.

Conclusion

- Modulation of tics by TS medication is associated with changes in dopamine, norepinephrine and GABA pathways.

Bibliography

1 American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders: (Revised 4th Edition)*. Washington, DC, USA, 111–114 (2000).

2 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* 46, 947–968 (2007).

3 Müller N: Tourette's syndrome: clinical features, pathophysiology, and therapeutic approaches. *Dialogues Clin. Neurosci.* 9, 161–171 (2007).

4 Singer HS: Tourette's syndrome: from behaviour to biology. *Lancet Neurol.* 4, 149–159 (2005).

5 Lit L, Gilbert DL, Walker W, Sharp FRA: Subgroup of Tourette's patients overexpress specific natural killer cell genes in blood: a preliminary report. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 144B, 958–963 (2007).

6 Baym CL, Corbett BA, Wright SB, Bunge SA: Neural correlates of tic severity and cognitive control in children with Tourette syndrome. *Brain* 131, 165–179 (2008).

7 Leckman JF, Riddle MA, Hardin MT *et al.*: The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J. Am. Acad. Child Adolesc. Psychiatry* 28, 566–573 (1989).

8 Srour M, Lespérance P, Richer F, Chouinard S: Psychopharmacology of tic disorders. *J. Can. Acad. Child Adolesc. Psychiatry* 17, 150–159 (2008).

9 Ogata H, Susumu G, Kazushige S *et al.*: KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic. Acids Res.* 27, 29–34 (1999).

10 Blake JA, Harris MA: The Gene Ontology project: structured vocabularies for molecular biology and their application to genome and expression analysis. *Curr. Protoc. Bioinformatics* Chapter 7, Unit 7.2 (2002).

11 Sands S, Dickerson D, Morris S, Chronwall B: Dopamine D2 receptor stimulation alters G-protein expression in rat pituitary intermediate lobe melanotropes. *Endocrine* 6, 325–333 (1997).

12 Dunn SM, Bateson AN, Martin IL: Molecular neurobiology of the GABAA receptor. *Int. Rev. Neurobiol.* 36, 51–96 (1994).

13 Wagner DA, Goldschen-Ohm MP, Hales TG, Jones MV: Kinetics and spontaneous open probability conferred by the {ε} subunit of the $GABA_A$ receptor. *J. Neurosci.* 25, 10462–10468 (2005).

14 Ranna M, Sinkkonen ST, Möykkynen T, Uusi-Oukari M, Korpi ER: Impact of ε and θ subunits on pharmacological properties of α3β1 GABAA receptors expressed in Xenopus oocytes. *BMC Pharmacol.* 6, 1 (2006).

15 Carlton SM, Honda CN, Denoroy L: Distribution of phenylethanolamine *N*-methyltransferase cell bodies, axons, and terminals in monkey brainstem: an immunohistochemical mapping study. *J. Comp. Neurol.* 287, 273–285 (1989).

16 Milner TA, Abate C, Reis DJ, Pickel VM: Ultrastructural localization of phenylethanolamine *N*-methyltransferase-like immunoreactivity in the rat locus coeruleus. *Brain Res.* 478, 1–15 (1989).

17 Mejías-Aponte CA, Drouin C, Aston-Jones G: Adrenergic and noradrenergic innervation of the midbrain ventral tegmental area and retrorubral field: prominent inputs from medullary homeostatic centers. *J. Neurosci.* 29, 3613–3626 (2009).

18 Moragues N, Ciofi P, Tramu G, Garret M: Localisation of GABA(A) receptor ε-subunit in cholinergic and aminergic neurones and evidence for codistribution with the θ-subunit in rat brain. *Neuroscience* 111, 657–669 (2002).

19 Sinkkonen ST, Hanna MC, Kirkness EF, Korpi ER: $GABA_A$ receptor ε and θ subunits display unusual structural variation between species and are enriched in the rat locus ceruleus. *J. Neurosci.* 20, 3588–3595 (2000).

20 Nagatsu I, Ikemoto K, Takeuchi T *et al.*: Phenylethanolamine-*N*-methyltransferase – immunoreactive nerve terminals afferent to the mouse substantia nigra. *Neuroscience Letters* 245, 41–44 (1998).

21 Keck PE, McElroy SL, Strakowski SM, Soutullo CA: Antipsychotics in the treatment of mood disorders and risk of tardive dyskinesia. *J. Clin. Psychiatry* 61(Suppl. 4), 33–38 (2000).

22 Elias AN, Hofflich H: Abnormalities in glucose metabolism in patients with schizophrenia treated with atypical antipsychotic medications. *Am. J. Med.* 121, 98–104 (2008).

23 Lin Y, Bloodgood BL, Hauser JL *et al.*: Activity-dependent regulation of inhibitory synapse development by NPAS4. *Nature* 455, 1198–1204 (2008).

24 Kalanithi PSA Zheng W, Kataoka Y *et al.*: Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Tourette syndrome. *Proc. Natl. Acad. Sci.* USA 102, 13307–13312 (2005).

25 Dan B, Boyd SG: Angelman syndrome reviewed from a neurophysiological perspective. The UBE3A-GABRB3 hypothesis. *Neuropediatrics* 34, 169–176 (2003).

26 Comings DE: Clinical and molecular genetics of ADHD and Tourette syndrome. Two related polygenic disorders. *Ann. N. Y. Acad.* Sci 931, 50–83 (2001).

27 Wu S, Wang WF, Shyu HY *et al.*: Association analysis of *GRIN1* and *GRIN2B* polymorphisms and Parkinson's disease in a hospital-based case–control study. *Neurosci. Lett.* 478, 61–65 (2010).

28 Cordelier P, Estève JP, Najib S *et al.*: Regulation of neuronal nitric-oxide synthase activity by somatostatin analogs following SST5 somatostatin receptor activation. *J. Biol. Chem.* 281, 19156–19171 (2006).

29 Kulisevsky J, Litvan I, Berthier ML *et al.*: Neuropsychiatric assessment of Gilles de la Tourette patients: comparative study with other hyperkinetic and hypokinetic movement disorders. *Mov. Disord.* 16, 1098–1104 (2001).

30 Mieczkowski J, Tyburczy M, Dabrowski M, Pokarowski P: Probe set filtering increases correlation between Affymetrix GeneChip and qRT-PCR expression measurements. *BMC Bioinformatics* 11, 104 (2010).