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Age-related gene expression in Tourette syndrome

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Abstract

Because infection and immune responses have been implicated in the pathogenesis of Tourette syndrome (TS), we hypothesized that children with TS would have altered gene expression in blood compared to controls. In addition, because TS symptoms in childhood vary with age, we tested whether gene expression changes that occur with age in TS differ from normal control children. Whole blood was obtained from 30 children and adolescents with TS and 28 healthy children and adolescents matched for age, race, and gender. Gene expression (RNA) was assessed using whole genome Affymetrix microarrays. Age was analyzed as a continuous covariate and also stratified into three groups: 5–9 (common age for tic onset), 10–12 (when tics often peak), and 13–16 (tics may begin to wane). No global differences were found between TS and controls. However, expression of many genes and multiple pathways differed between TS and controls within each age group (5–9, 10–12, and 13–16), including genes involved in the immune-synapse, and proteasome- and ubiquitin-mediated proteolysis pathways. Notably, across age strata, expression of interferon response, viral processing, natural killer and cytotoxic T-lymphocyte cell genes differed. Our findings suggest age-related interferon, immune and protein degradation gene expression differences between TS and controls.

Keywords: Tourette syndrome; Tics; Viral infections; PANDAS; Gene expression

1. Introduction

Tourette syndrome (TS), characterized by motor and vocal tics present for greater than one year, often shows distinct age effects, with symptom onset between ages 5 and 9, most severe tics between 10 and 12, and waning tics in early adulthood (Leckman et al., 1998). This suggests complex age-related changes in TS pathogenesis.

Growing numbers of studies suggest immune abnormalities associated with TS (see review (Hoekstra et al., 2004)). These include autoimmune responses, altered cytokine profiles (see reviews (Dale, 2003; Hoekstra et al., 2004; Martino and Giovannoni, 2005)) and correlations of inflammatory mediators with symptom exacerbation (Leckman et al., 2005). Age-related changes in tic severity (Leckman et al., 1998) suggest immune-associated gene expression in TS might vary with age if the immune system is involved in the disease process.

Gene expression analysis of whole blood using microarrays allows assessment of genome-wide expression changes and pathway dysregulation (Sharp et al., 2006), and might be useful for identifying immune-mediated and genetic mechanisms underlying Tourette syndrome (Tang et al., 2005). In a previous pilot study of children and adults with TS compared to age-matched controls, using two types of statistical analyses, we assessed whole blood gene expression and found altered expression of immune-related genes primarily associated with natural killer cells (NK), the major cell type involved in the rapid, innate immune response to viral and other infections (Lit et al., 2007; Tang et al., 2005). Previous studies were limited by (1) use of older gene expression technology, (2) small

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sample size, and (3) use of a healthy control group that was not specifically recruited to match for age, sex, and race.

The present study is larger, includes carefully matched cases and controls, and uses Affymetrix U133-Plus 2 expression microarrays. This allows for a more generalizable and valid case–control comparison. In addition, we could begin to assess whether changes in blood gene expression might vary with age in TS. This is critical because if immune abnormalities contribute to symptoms in some TS patients, this might be more or less evident at different stages of this developmental disorder.

2. Methods

2.1. Participants

Twenty-eight healthy subjects (normal controls, NC) (21 male, mean age 11.4 years and 7 female, mean 11.4 years) and 30 subjects with Tourette syndrome (TS) (23 male, mean age 11.4 years and 7 female, mean 11.7 years) were enrolled in this study (Table 1). All subjects were between ages 5 through 16 at the time of study and were not febrile or reported any acute illness at the time of the blood draw. TS subjects were recruited from the Tourette Syndrome Clinic at Cincinnati Children's Hospital Medical Center. Diagnoses of TS, as well as of attention-deficit hyperactivity disorder (ADHD) (15/30) and OCD (16/30) were based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria (APA, 2000). Each TS subject's symptoms were rated using direct child and parent interview using the Yale Global Tic Severity Scale (YGTSS) (Leckman et al., 1989), the DuPaul ADHD Rating Scale (ADHDRS) (DuPaul et al., 1998), and the Child Yale–Brown OCD Scale (CYBOCS) (Scahill et al., 1997). The healthy controls were recruited (by DLG) from schools in the Cincinnati area that were demographically similar to those from which the children with TS originated. For each TS case, a NC matched for age within 2 years, gender, and race was recruited. Children with any neurological, psychiatric, or developmental diagnosis made by a physician were excluded, based on parent interview. Blood from these healthy controls was obtained and processed using identical methods to those used for the TS subjects. The protocols were approved by the institutional review boards at the Cincinnati Children's Hospital Medical Center and the University of California at Davis. Informed consent was obtained from the parent or legal guardian of each participant.

Table 1
Demographics and diagnoses

Variable	Tourette's (TS)	Controls (NC)
Males (%)	23 (77%)	21 (75%)
Age group 5–9 (%)	6 (20%)	7 (25%)
Age group 10–12 (%)	13 (43%)	11 (39%)
Age group 13–16 (%)	11 (37%)	10 (36%)
Diagnoses (%)		
TS + OCD + ADHD	10 (33%)	N/A
TS + ADHD	5 (17%)	N/A
TS + OCD	6 (20%)	N/A
TS	9 (30%)	N/A
Medications used (%)		
SSRI	9 (30%)	N/A
A2A	8 (27%)	N/A
NLEP	7 (23%)	N/A
STIM	4 (13%)	N/A
ADHDRx	6 (20%)	N/A
Yale Global Tic Severity score mean (range)	14 (0–30)	N/A
ADHD Rating Scale mean (range)	21.3 (0–52)	N/A
Children's Yale–Brown Obsessive–Compulsive Scale mean (range)	6.4 (0–30)	N/A
Totals	30 (100%)	28 (100%)

TS: Tourette's syndrome; ADHD: attention deficit hyperactivity disorder; OCD: obsessive–compulsive disorder; SSRI: selective serotonin reuptake inhibitor; A2A: alpha-2 adrenergic agonists; NLEP: neuroleptics; STIM: stimulants; ADHDRx: ADHD medications other.

2.2. Sample processing and array hybridization

Fifteen milliliters of blood was collected via antecubital fossa venipuncture into six PAXgene vacutainer tubes (PreAnalytiX, Germany), which immediately stabilize RNA and thus reduce RNA degradation. Tubes were frozen at −70 °C until the total RNA was isolated using the Paxgene blood RNA kit (PreAnalytiX, Germany) according to the manufacturer's instructions.

Sample labeling, hybridization to chips, and image scanning were performed using standard Affymetrix protocols (Affymetrix Expression Analysis Technical Manual). Gene expression was assessed on the human U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, CA), a single oligonucleotide microarray that surveys over 54,000 transcript sequences (probes). Samples were processed in four separate batches.

2.3. Probe-level data analysis

Raw expression values (probe level data) were imported into GENESPRING 7.2 software (Agilent Technologies, Palo Alto, CA) and processed using GC-RMA (Bolstad et al., 2003; Wu et al., 2004), followed by a three-step normalization (data transformation, per chip normalization, and per gene normalization) (Genespring, 2004). Probes with an expression value greater than 1 for at least one sample were analyzed (39,634 probes) (Fig. 1). Statistical analyses, principal components analysis, and unsupervised clustering analyses were performed with Partek Genomics Suite 6.3 beta (Partek Inc., St. Louis, MO) and GENESPRING 7.2.

The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://niaid.abcc.ncifcrf.gov/) was used to determine co-regulation of Kyoto Encyclopedia of Genes and Genomes (KEGG) gene pathways (Dennis et al., 2003; Hosack et al., 2003). DAVID calculates a modified Fisher Exact test score using the Expression Analysis