ORIGINAL ARTICLE

Preliminary Findings of Antistreptococcal Antibody Titers and Basal Ganglia Volumes in Tic, Obsessive-compulsive, and Attention-Deficit/ Hyperactivity Disorders

Bradley S. Peterson, MD; James F. Leckman, MD; Daniel Tucker, MD; Lawrence Scahill, MSN, PhD; Lawrence Staib, PhD; Heping Zhang, PhD; Robert King, MD; Donald J. Cohen, MD; John C. Gore, PhD; Paul Lombroso, MD

Background: Previous studies have provided preliminary serological evidence supporting the theory that symptoms of tic disorders or obsessive-compulsive disorder (OCD) may be sequelae of prior streptococcal infection. It is unclear, however, whether previously reported associations with streptococcal infection were obscured by the presence of diagnostic comorbidities. It is also unknown whether streptococcal infection is associated in vivo with anatomical alterations of the brain structures that have been implicated in the pathophysiology of these disorders.

Methods: Antistreptococcal antibody titers were measured in 105 people diagnosed as having CTD, OCD, or attention-deficit/hyperactivity disorder (ADHD) and in 37 community controls without a disorder. Subjects were unselected with regard to their history of streptococcal exposure. Basal ganglia volumes were measured in 113 of these subjects (79 patients and 34 controls).

Results: A *DSM-IV* diagnosis of ADHD was associated significantly with titers of 2 distinct antistreptococcal antibodies, antistreptolysin O and anti–deoxyribonuclease B. These associations remained significant after controlling for the effects of CTD and OCD comorbidity. No significant association was seen between antibody titers and a diagnosis of either CTD or OCD. When basal ganglia volumes were included in these analyses, the relationships between antibody titers and basal ganglia volumes were significantly different in OCD and ADHD subjects compared with other diagnostic groups. Higher antibody titers in these subjects were associated with larger volumes of the putamen and globus pallidus nuclei.

Conclusions: These findings suggest that the prior reports of an association between antistreptococcal antibodies and either CTD or OCD may have been confounded by the presence of ADHD. They also support the hypothesis that in susceptible persons who have ADHD or OCD, chronic or recurrent streptococcal infections are associated with structural alterations in basal ganglia nuclei.

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From the Yale Child Study Center (Drs Peterson, Leckman, Tucker, Scahill, King, and Cohen), and the Departments of Diagnostic Radiology (Drs Peterson, Staib, and Gore) and Epidemiology and Public Health (Dr Zhang), Yale School of Medicine, New Haven, Conn.

IMMUNE-BASED THEORIES of the causes of Tourette syndrome (TS) and obsessive-compulsive disorder (OCD) derive from the observed similarities between their symptoms and those of Sydenham chorea, a sequela of rheumatic fever. Rheumatic fever is thought to be an immunological illness in which infection with group A β-hemolytic streptococcus (GABHS) produces GABHS antibodies that cross-react with and presumably compromise neural proteins in susceptible persons.[1] The first controlled investigation of the association of GABHS and tic disorders that we are aware of studied 88 children who had attention-deficit/hyperactivity disorder (ADHD), conduct disorder, or a learning disability.[2] Group A β-hemolytic streptococcus and anticaudate antibody titers were elevated in the subset of 45 children who also had either tics or chorea. Another study of 41 children with TS and 39 controls reported elevated antiputamen antibody titers in TS, although elevated GABHS antibodies were not detected.[3] In a longitudinal study of 50 children, GABHS infection preceded 45 (31%) of 144 exacerbations of tic disorders or OCD symptoms.[4] Finally, patients with TS or OCD were reported to have a peripheral trait marker for rheumatic fever susceptibility more frequently than did the controls.[5,6] This protein marker is found on a subset of B cells and is recognized by a monoclonal antibody designated D8/17.

These preliminary studies, while intriguing, share several limitations. First, the extent to which the study subjects

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SUBJECTS AND METHODS

SUBJECT RECRUITMENT AND CHARACTERIZATION

Subjects were recruited in the context of a multidisciplinary study of childhood neuropsychiatric disorders. Subjects with CTD and OCD were recruited from the Tic and Obsessive-Compulsive Disorders Specialty Clinic at the Yale Child Study Center, New Haven, Conn. Subjects with a primary diagnosis of combined-type ADHD were recruited either through the Yale outpatient clinic or through a local support group. They were recruited without knowledge or regard for their history of GABHS infection. Controls (n = 37) were recruited from randomly selected names on a telemarketing list of families in the local community. Introductory letters were sent and then followed by recruitment and screening telephone calls. Controls were group matched with the patients by age, sex, socioeconomic status, and handedness. Approximately 10% of eligible families who were contacted from the telemarketing list ultimately participated. Written informed consent was obtained from all participants.

Subjects were aged 7 to 55 years and were predominantly right-handed.[9] Subjects with CTD, OCD, or ADHD (n = 105) had to meet *DSM-IV* criteria for 1 or more of these diagnoses.[10] Exclusionary criteria included a movement disorder other than CTD, a major psychiatric disorder that antedated the onset of CTD, OCD, or ADHD, past seizures or a history of head trauma with loss of consciousness, ongoing substance abuse or previous substance dependence, or an IQ below 80. Although we attempted to recruit subjects who were not taking any medications, 45 patients were taking medication at the time of the study. These medications included stimulants (n = 17), haloperidol or pimozide (n = 9), risperidone (n = 2), clonidine or guanfacine (n = 11), selective serotonin reuptake inhibitors (n = 10), or clomipramine (n = 5).

Neuropsychiatric diagnoses were established through clinical evaluation and adminstration of the Schedule for Tourette and Other Behavioral Syndromes[11] (**Table 1**). This is a structured interview that has been used extensively in TS family studies. It includes the Kiddie Schedule for Affective Disorders and Schizophrenia, Epidemiologic Version (K-SADS-PL)[12,13] and more detailed sections on TS and OCD.

The current severity of tic and OCD symptoms were quantified with the Yale Global Tic Severity Scale[14] and the adult or child version of the Yale-Brown Obsessive Compulsive Scale.[15,16] The Child Behavior Checklist[17] and the Conners Parent and Teacher Rating Scales[18] were obtained for subjects younger than 19 years. Socioeconomic status was measured with Hollingshead's Four-Factor Index of Social Status.[19]

ANTIBODY DETERMINATIONS

Whole blood was collected with 21-gauge butterfly needles into a 10-mL red-top vacuum tube that was free of preservatives. The blood was allowed to clot at room temperature for 30 to 40 minutes and was then centrifuged at 2000 rpm, 4°C, for 10 minutes. Serum samples were aliquoted and frozen at −80°C until batch-processed. The antibody assays for antistreptolysin O (ASO) and anti–deoxyribonuclease B (anti–DNAse B) have been described previously.[20] The cutoff for elevated titers, preestablished from prior studies, was a dilution of 1:240 or more. Using this criterion, 9 (24%) of the control subjects and 44 (42%) of the patients had elevated anti–DNAse B titers ($\chi^2 = 2.1$, $P = .15$), and 7 (19%) of the controls and 32 (30%) of the patients had elevated ASO titers ($\chi^2 = 0.68$, $P = .41$).

MAGNETIC RESONANCE IMAGING SCANNING AND BASAL GANGLIA VOLUMES

Magnetic resonance imaging (MRI) scans were taken of 113 of the participants (34 controls and 79 patients) using the same scanner (Signa 1.5-T scanner; General Electric, Milwaukee, Wis). Subjects not included in the MRI analyses include those whose scan had a motion artifact severe enough to warrant exclusion, those who had braces, those with previous head trauma or substance abuses, and those who refused to participate in the scanning protocol.

Head positioning was standardized using canthomeatal landmarks. In 91 subjects, a sagittal 3-dimensional volume spoiled gradient recall sequence was obtained (repetition time, 24 milliseconds; echo time, 5 milliseconds; 45° flip; 256 × 192 matrix; field of view, 30 cm; 2 excitations; slice thickness, 1.2 mm; 124 contiguous slices; scan time, 19.6 minutes). In the remaining 22 subjects, coronal spoiled gradient recall images were acquired (repetition time, 24; echo time, 8 milliseconds; 45° flip; 256 × 192 matrix; field of view, 36 cm; 0.75 excitations; slice thickness, 1.4 mm; no skip; 124 slices; scan time, 7.4 minutes). This coronal sequence, which we used initially, occasionally does not cover the entire anterior-posterior extent of the head in large adult subjects. We therefore changed the scan orientation to the sagittal plane to avoid this problem—hence the different pulse sequences. The proportion of subjects in whom the sagittal or coronal series were acquired did not differ between patient and normal control groups ($\chi^2 = 1.14$, $P = .23$). In 10 subjects who were scanned with both series, the within-subject correlation of basal ganglia volumes on the 2 series was excellent (intraclass $r > 0.95$). Volumetric data from these 2 series were pooled for statistical analyses.

Morphometric analyses were performed on Sun Ultra 1 workstations (Sun Microsystems Inc, Palo Alto, Calif) using ANALYZE 7.5 software (Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn) while blind to subject characteristics and hemisphere (images were randomly flipped in the transverse plane prior to region definition). Extracerebral tissues were removed using an isointensity contour function that thresholded cortical gray matter from overlying cerebrospinal fluid. Connecting dura and fat were removed manually on each slice in sagittal,

Continued on next page

were preselected for having had a recent, recurrent, or chronic infection with GABHS is unclear. Therefore, the reported associations of GABHS antibodies with particular diagnostic groups could have resulted from biased ascertainment. Second, precisely which diagnoses are associated most strongly with GABHS antibodies is unknown. Chronic tic disorder (CTD), OCD, and ADHD, for instance, frequently occur together.[7] Some

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coronal, and axial views. The brainstem was transected at the pontomedullary junction.

Basal ganglia were defined manually by 1 of 2 operators. Images were first cropped to the surrounding cortex and then enlarged 8-fold to minimize tracing error. Tissue contrast was enhanced through optimization of the pixel intensity histogram. Initial tracings of caudate, putamen, and globus pallidus nuclei in the axial plane were confirmed in orthogonal views. If corrections were made in these views, their accuracy had to be corroborated in the orthogonal imaging planes. The head of the caudate was distinguished from the nucleus accumbens in the coronal plane by the straight line connecting the inferiormost points of the internal capsule and lateral ventricles. Claustrum was excluded from the putamen's volume. An expert in these procedures (B.S.P.) reviewed all tracings for accuracy. The interrater reliability of the measurements was assessed on 10 scans at 4 equally spaced points in the study; intraclass correlation coeffecients were greater than 0.95 for the caudate and putamen and greater than 0.90 for the globus pallidus.

Each scan was rated blindly on a 6-point ordinal scale for the severity of motion artifact, ranging from a score of 0 (no motion artifact) to 5 (severe). Scores of 4 or 5 mandated exclusion from further analyses (8 patients and 5 controls). The severity of motion artifact in the retained scans did not differ between the normal control and patient groups ($\chi^2_3 = 1.79$, $P = .62$).

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS software version 8.0 (Statistical Product and Service Solutions Inc, Chicago, Ill). The primary null hypothesis was that mean antistreptococcal antibody levels would not differ between diagnostic groups. The secondary null hypothesis was that antibody titers would not predict basal ganglia volumes within diagnostic groups. The primary hypothesis was tested using an unbalanced multivariate analysis of covariance (MANCOVA) in which anti–DNAse B and ASO titers were entered as dependent variables. The secondary hypothesis was also tested in a MANCOVA, with the 6 basal ganglia subregions entered as the dependent variables. In testing both hypotheses, 3 dummy variables for the 4 diagnoses (CTD, OCD, ADHD, and normal controls) were entered as main effects, and age and sex were entered as continuous and dichotomous covariates, respectively. Antibody titers and cerebral volumes were entered as additional continuous covariates in the basal ganglia analyses. In both MANCOVAs, the effects of 2-way and 3-way interactions were assessed to be certain that they were not obscuring the analyses of main effects. If significant, they were included in the final model along with the main effects. Probability values were Bonferroni-adjusted to account for multiple comparisons. Appropriateness of the models was assessed in analyses of residuals.[21,22] Finally, dichotomized titers were entered as dependent variables in logistic regression equations to supplement testing of the primary hypothesis—specifically, to assess whether particular diagnoses were associated with "clinically meaningful" elevations in antibody titers.

Assessment of Possible Confounds

Because lower socioeconomic status and seasonality have been described as risk factors for exposure to GABHS and other infectious agents,[23,24] these variables were initially included as covariates in hypothesis testing. The months of greatest risk for GABHS in New England are October through March. A dichotomous seasonality variable was created to reflect whether blood was drawn during this window of elevated risk. Neither socioeconomic status (Pillai trace = 0.007; $F_{2,131} = 0.49$; $P = .62$) nor season (Pillai trace = 0.004; $F_{2,131} = 0.25$; $P = .78$) approached statistical significance in predicting the dependent variables, however, and they did not appreciably alter the multivariate analyses when included as either main effects or interactions with diagnosis. They were therefore not retained in the final models, so as not to introduce an excessive number of parameters to the analyses. Additionally, given recent reports that neuroleptic medications may increase basal ganglia volumes,[25,26] we repeated the basal ganglia analyses while excluding the 11 subjects who were taking typical or atypical neuroleptic medications. Finally, we tested the significance of the associations of diagnoses with antibody titers using nonparametric statistics (the Mann-Whitney test) to ensure that deviations of antibody titer from the normal distribution were not unduly influencing the parametric analyses.

In all hypothesis testing, we assessed the appropriateness of pooling data from children and adults. The interaction of each independent variable with the dichotomized variable "age group" (children $<$18 years and adults $\geq$18 years) was tested for its effects on the dependent variable (antibody titer or basal ganglia volume). In no instance did these interactions significantly predict the dependent variables, indicating that pooling of child and adult data was appropriate. We included age as a continuous covariate in the MANCOVAs as an added precaution against its confounding effect. However, to ensure the validity of the findings within a pediatric population, we also verified any positive findings by analyzing a subset of the data among subjects who were younger than 18 years.

Exploratory Analyses

The relationships between antibody levels and clinical variables were assessed using multiple linear regression. Clinical variables included the severity of tic and OCD symptoms, the Child Behavior Checklist, and the Conners Parent and Teacher Rating Scales scores. Covariates included age and sex. Analyses for tic and OCD symptom severity included only those subjects with those respective lifetime diagnoses. To assess whether GABHS exposure may have contributed to the asymmetries reported in these disorders,[27-30] basal ganglia asymmetry indices, calculated as 100 $(\text{Volume}_{\text{Right}} - \text{Volume}_{\text{Left}})/(\text{Volume}_{\text{Right}} + \text{Volume}_{\text{Left}})$ were entered in a MANCOVA as dependent variables.

reports emphasize the association of GABHS with movement disorders[2] and some emphasize the association with OCD.[8] However, to our knowledge, no studies have explicitly examined whether the presence of ADHD or other comorbidities may have confounded the observed associations. Third, the studies have not presented evidence that the antibodies and GABHS infection are associated with in vivo neuroanatomical abnormalities.

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Table 1. Diagnostic Classification*

	Diagnosis	Children No.	Children Age, y, Mean (SD)	Adults No.	Adults Age, y, Mean (SD)	Males	Females
Titers (n = 142)	Normal controls	20	12.4 (6.5)	17	27.4 (9.2)	21	16
	All patients	82	11.9 (2.8)	23	31.6 (11.6)	73	32
	CTD†	53	12.0 (3.0)	20	33.5 (11.3)	56	17
	OCD†	27	12.2 (3.6)	13	29.9 (10.3)	30	10
	ADHD†	41	11.5 (2.7)	6	32.0 (13.4)	37	10
Titers and basal ganglia volumes (n = 113)	Normal controls	17	10.3 (1.7)	17	27.4 (9.2)	19	15
	All patients	59	11.6 (1.9)	20	34.1 (11.0)	54	25
	CTD‡	33	11.6 (1.8)	18	35.0 (10.7)	37	14
	OCD‡	15	12.2 (2.3)	10	29.1 (9.1)	19	6
	ADHD‡	27	11.5 (2.1)	4	35.5 (12.6)	25	6

*Patient diagnoses are not mutually exclusive (see Table 3). CTD indicates chronic tic disorder; OCD, obsessive-compulsive disorder; and ADHD, attention-deficit/hyperactivity disorder.

†Thirty-one had pure CTD (no ADHD or OCD); 8 had pure OCD (no CTD or ADHD); and 17 had pure ADHD (no CTD or OCD).

‡Twenty-four had pure CTD (no ADHD or OCD); 6 had pure OCD (no CTD or ADHD); and 13 had pure ADHD (no CTD or OCD).

Table 2. Diagnoses Associated With Antibody Titers*

Independent Variable	Type 3 Sum of Squares	Marginal Mean Difference	SE	95% Confidence Interval	F	P†
			Antideoxyribonuclease B			
Age	$1\,696\,676.9_1$	NA	NA	NA	7.459	.007
Sex	$840\,403.3_1$	NA	NA	NA	3.69	.06
CTD	$62\,854.4_1$	44.30	84.27	−122.96 to 210.96	0.28	.60
OCD	$163\,452.4_1$	−82.15	96.90	−273.79 to 109.49	0.72	.40
ADHD	$1\,088\,638.9_1$	198.25	90.62	19.04 to 377.45	4.79	.03
Error	$30\,935\,444.1_{136}$	NA	NA	NA	NA	NA
			Antistreptolysin-O Titers			
Age	$18\,472.81_1$	NA	NA	NA	0.55	.46
Sex	$17\,543.7_1$	NA	NA	NA	0.52	.47
CTD	$12\,336.57_1$	−19.63	32.35	−83.61 to 44.35	0.37	.54
OCD	$12\,948.87_1$	23.12	37.20	−50.44 to 96.68	0.39	.53
ADHD	$159\,820.75_1$	75.96	34.78	7.17 to 144.75	4.77	.03
Error	$4\,558\,762.5_{136}$	NA	NA	NA	NA	NA

*NA indicates not applicable; CTD, chronic tic disorder; OCD, obsessive-compulsive disorder; ADHD, attention-deficit/hyperactivity disorder. Antideoxyribonuclease B and antistreptolysin O titers are the dependent variables and CTD, OCD, and ADHD are the main effects in the repeated-measures multivariate analyses of variance. The 2-way and 3-way interactions between independent variables were not significant and therefore were not included in the final model. Overall, $R^2 = 0.15$. The results of the post hoc univariate analyses are shown here. The marginal mean difference is calculated in the multivariate analysis of variance and represents the difference between the marginal mean titer for the relevant diagnostic group (ie, adjusted for covariates) and the marginal mean for subjects in all other diagnostic groups. Lighter shading indicates $P < .05$.

†P values are Bonferroni-adjusted.

The aims of this study were 2-fold. First, we wanted to clarify the relationship between antibody titers and the diagnoses of CTD, OCD, or ADHD in subjects who were not preselected for having had evidence of recent streptococcal infection. Second, we hoped to define the particular patterns of comorbidity and profiles of basal ganglia volumes that were associated with elevated GABHS antibody titers. In the first set of analyses, we tested the null hypothesis that elevated titers of GABHS antibodies were unrelated to diagnostic status. After rejection of the null hypothesis, we assessed the degree to which antibody titers, in combination with diagnostic status and appropriate covariates, were able to predict basal ganglia volumes.

RESULTS

PRIMARY HYPOTHESIS

After correction for multiple comparisons and while covarying for comorbid diagnoses, ADHD significantly predicted anti–DNAse B and ASO titers in both multivariate (Pillai trace = 0.05; $F_{2,135} = 3.15$; $P = .04$) and post hoc univariate analyses (**Table 2**), prompting rejection of our primary null hypothesis. Interactions of the dependent variables were not statistically significant and so were not included in the final MANCOVA. Identical results were obtained when analyzing data from the children alone, when using dichotomized antibody titers (nor-

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Table 3. Descriptive Statistics in Unique Diagnostic Groups*

	ADHD (n = 17)	CTD (n = 31)	OCD (n = 8)	ADHD and CTD (n = 17)	ADHD and OCD (n = 7)	CTD and OCD (n = 19)
			Antistreptolysin			
ADHD	**276.5 (265.6)**	156.8 (54.9), .005	168.1 (86.3), .05	76.5 (62.4), .22	96.5 (101.0), .34	87.5 (60.7), .15
CTD		**119.7 (119.1)**	11.3 (81.1), .89	−80.3 (54.9), .15	−60.3 (96.6), .53	−69.3 (52.9), .19
OCD			**108.3 (184.2)**	−91.7 (86.3), .29	−71.7 (117.4), .54	−80.6 (85.2), .35
ADHD and CTD				**200.0 (201.8)**	20.0 (101.0), .84	11.1 (60.7), .86
ADHD and OCD					**180.0 (168.1)**	−8.9 (100.0), .93
CTD and OCD						**188.9 (230.3)**
ADHD, CTD, and OCD						
Normal controls						
			Antideoxyribonuclease B			
ADHD	**423.3 (456.6)**	201.4 (148.5), .18	303.3 (233.6), .20	−168.5 (168.7), .32	213.3 (273.4), .44	226.9 (164.2), .17
CTD		**221.9 (322.5)**	101.9 (219.4), .64	−369.8 (148.5), .01	11.9 (261.4), .96	25.6 (143.3), .86
OCD			**120.0 (107.3)**	−471.8 (233.6), .04	−90.0 (317.6), .78	−76.3 (230.4), .74
ADHD and CTD				**591.8 (788.8)**	381.8 (273.4), .16	395.4 (164.2), .02
ADHD and OCD					**210.0 (204.9)**	13.7 (270.6), .96
CTD and OCD						**196.3 (299.4)**
ADHD, CTD, and OCD						
Normal controls						

ADHD indicates attention-deficit/hyperactivity disorder; CTD, chronic tic disorder; and OCD, obsessive-compulsive disorder. Lighter shading indicates cells in which P<.05. The means (SDs) of the raw antibody titer data for every possible unique diagnosis are in bold along the diagonal. The numbers of subjects in each nonoverlapping diagnostic group are listed in the top rows under the diagnostic headings. Off the diagonal are all the possible post hoc comparisons between diagnostic groupings, where the mean (SE) differences and P values are presented. These unique diagnostic groupings were entered into a 1-way analysis of variance as 8 levels within a "diagnosis" factor. The effect of diagnosis was significant for antideoxyribonuclease B titers (sum of squares = 3 669 670; $F_{7,134}$ = 2.17; P = .04) and it demonstrated a trend for antistreptolysin titers (sum of squares = 395 983; $F_{7,134}$ = 1.71; P = .11). When all subjects with ADHD were compared with subjects without ADHD (ie, normal controls, pure CTD, and pure OCD), the analyses of variance for these raw data demonstrated significantly higher values for both antistreptolysin O and antideoxyribonuclease B titers in the subjects with ADHD (antistreptolysin means, 237.7 vs 144.7; sum of squares = 258 880; $F_{1,140}$ = 7.93; P = .006; antideoxyribonuclease B means, 515.7 vs 229.8; sum of squares = 2 450 831; $F_{1,140}$ = 10.2; P = .002). These post hoc analyses support the findings of the multivariate analysis of covariance that antistreptococcal antibody titers are higher in subjects with ADHD.

mal or elevated) in logistic regression, and when using continuous antibody levels in nonparametric tests (data not shown).

In **Table 3**, we present the mean and SDs of the raw data for antibody titers in all unique, nonoverlapping diagnostic groups. These groups included all possible combinations of diagnostic comorbidity. The post hoc comparisons of antibody titers across diagnostic groups were statistically significant when ADHD was one of the terms of the comparison, and this was true whether the ADHD was "pure" or whether it was ADHD that occurred in combination with CTD or OCD. In every significant comparison, titers in the ADHD subjects were higher than in the comparison group. In these multivariate analyses, CTD was significantly associated with smaller putamen nuclei bilaterally.

SECONDARY HYPOTHESIS

After correction for multiple comparisons, the MANCOVA demonstrated significant associations between titers, diagnoses, and basal ganglia volumes, refuting our secondary null hypothesis (**Table 4**). Significant associations were seen, however, only for analyses involving ASO titers, not anti–DNAse B titers. While controlling for primary diagnosis, demographics, cerebral volume, and nonspecific effects of antibody titers, the interaction of ASO titer with ADHD predicted volumes of the left putamen and right globus pallidus, and the interaction of ASO titers with OCD predicted left globus pallidus volumes. The higher-order interaction of ASO titer with OCD and ADHD significantly predicted left putamen and right globus pallidus volumes. In all significant titer-by-diagnosis interactions, increasing titers in subjects who had the relevant diagnosis were associated with larger regional volumes (**Figure**). Similar results were obtained when using dichotomized titers in logistic regression analyses. Exclusion of the 11 subjects taking neuroleptic medications actually increased the significance of the multivariate association of the ASO-by-ADHD interaction with basal ganglia volumes (Pillai trace = 0.17; $F_{6,83}$ = 2.8; $P<.02$). Exclusion of adult subjects did not affect the marginal means of the antibody-by-diagnosis interactions that predicted basal ganglia volumes, although statistical significance was reduced to trend levels (eg, for the ASO-by-ADHD association with putamen volumes, all $P<.07$) owing to the reduced statistical power with fewer subjects.

EXPLORATORY ANALYSES

No significant correlations were noted between antibody titers and the severity of current tic or OCD symptoms. Standardized subscale scores on the Child Behavior Checklist did not correlate significantly with antibody titers, although the hyperactivity subscale of the Conners Parent and Teacher Rating Scales positively correlated with anti–DNAse B titers in children and adolescents ($\beta = .25$; $t = 2.0$; $P<.05$). Finally, when controlling for covariates and comorbid diagnoses, the interaction of anti–DNAse B titers with CTD correlated at a trend level of significance with the asymmetry of the caudate

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ADHD, CTD, and OCD (n = 6)	Normal Controls (n = 37)
−3.5 (92.5), .97	128.1 (52.1), .01
−160.3 (87.6), .07	−28.7 (42.8), .50
−171.7 (110.1), .12	−40.0 (79.2), .61
−80.0 (92.5), .39	51.6 (52.1), .32
−100.0 (121.9), .41	31.6 (95.1), .74
−91.1 (91.4), .32	40.6 (50.1), .42
280.0 (126.5)	131.6 (85.9), .12
	148.4 (150.5)
−392.7 (250.3), .12	157.7 (140.9), .26
−594.1 (237.1), .01	−43.6 (115.9), .71
−696.0 (297.9), .02	−145.6 (214.4), .50
−224.2 (250.3), .37	326.2 (140.9), .02
−606.0 (330.0), .07	−55.6 (257.2), .83
−619.7 (247.3), .01	−69.3 (135.5), .61
816.0 (1039.2)	550.4 (232.5), .02
	265.6 (485.7)

(sum of scales = 22.9; $F_{1,112}$ = 3.6; P = .06) and globus pallidus nuclei (sum of squares = 134.2; $F_{1,112}$ = 3.2; P = .08), with a greater rightward asymmetry present in the CTD group.

COMMENT

The first finding of this study is the association of ADHD with antistreptococcal antibody titers. Titers were not associated with either CTD or OCD, suggesting that the previous findings of elevated antistreptococcal and antineuronal antibodies in tic disorders may have been influenced by the presence of ADHD comorbidity in the clinical populations studied.[2,3] Reexamining the antistreptococcal and antineuronal antibody levels in these previous data sets for the effects of a comorbid ADHD diagnosis may prove informative.

Some features of the prior studies that reported an association of GABHS with CTD or OCD may also support the association of GABHS with ADHD observed here. The initial report of higher GABHS and antineuronal antibody titers in movement disorders, for example, studied children who were referred primarily for evaluation of ADHD and related behavioral disorders.[2] The association of GABHS titers with tics and chorea may therefore have been driven by comorbid ADHD in those children. Similarly, ADHD symptoms seemed to worsen along with tic and OCD symptoms after GABHS infection in the initial longitudinal study of putative GABHS-induced tics and OCD.[4] These changes in ADHD symptoms, unfortunately, were not assessed systematically. Finally, clinical and neuropsychological descriptions of patients who have OCD associated with Sydenham chorea have reported inattention, distractibility, impulsivity, and hyperactivity, particularly during periods of acute symptom exacerbation.[31-33]

Although the observed associations of ADHD with antibody titers are consistent with theories of an immune-mediated pathogenesis of complex neuropsychiatric disorders, we must emphasize that we have not demonstrated a causal relationship between titers and neuropsychiatric diagnosis. Even if the relationship were causal, the direction of that causation would need to be established. Hyperactivity and impulsivity could, for instance, predispose a person to higher rates of GABHS exposure and infection, rather than being a consequence of infection. Consistent with this possibility is that the onset of ADHD was long before the exposure to GABHS that we detected here as elevated antibody titers. It is also possible, on the other hand, that autoimmune theories are correct and that infection can produce these complex neuropsychiatric syndromes. Then the association of current titers with a remote onset of ADHD could be

Table 4. Antibody and Diagnosis Associations With Basal Ganglia Volumes*

Independent Variable	Basal Ganglia		Type 3 Sum of Squares	F†	P‡
Age	Caudate	R	185 622.2	0.70	.41
		L	329 784.1	1.16	.28
	Putamen	R	403 527.5	1.82	.18
		L	1 949 333.2	9.24	.003
	GP	R	68 399.0	1.80	.18
		L	1426.6	0.04	.85
Sex	Caudate	R	26 436.7	0.10	.75
		L	20 262.5	0.07	.79
	Putamen	R	41 336.3	0.19	.67
		L	3410.7	0.02	.90
	GP	R	75 676.4	2.0	.16
		L	3287.1	0.08	.77
Cerebral volume	Caudate	R	9 399 861.6	35.47	.0001
		L	9 539 401.1	33.61	.0001
	Putamen	R	14 425 018.5	65.15	.0001
		L	11 771 290.4	55.82	.0001
	GP	R	2 509 899.4	66.13	.0001
		L	2 017 826.0	50.34	.0001
CTD	Caudate	R	447 291.1	1.69	.20
		L	740 785.4	2.61	.11
	Putamen	R	2 122 069.4	9.58	.003§
		L	795 020.5	3.78	.05§
	GP	R	16 303.4	0.43	.51
		L	52 985.6	1.32	.25
OCD	Caudate	R	2751.9	0.01	.92
		L	7.9	.000	.99
	Putamen	R	154 842.7	0.70	.41
		L	34 663.3	0.16	.69
	GP	R	43 768.0	1.15	.28
		L	73 499.1	1.83	.18
ADHD	Caudate	R	2851.2	0.01	.92
		L	125.9	.000	.98
	Putamen	R	37 475.8	0.17	.68
		L	56 711.5	0.27	.61
	GP	R	179 236.2	4.72	.03‖
		L	103 525.9	2.58	.11

(continued)

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Table 4. Antibody and Diagnosis Associations With Basal Ganglia Volumes (cont)*

Independent Variable	Basal Ganglia		Type 3 Sum of Squares	F†	P‡
ASO titer	Caudate	R	77 976.8	0.29	.59
		L	163 312.5	0.57	.45
	Putamen	R	719 418.1	3.25	.07
		L	162 392.0	0.77	.38
	GP	R	124 003.6	3.27	.07
		L	311 963.2	7.78	.006
ASO × CTD	Caudate	R	14 529.1	0.05	.81
		L	888.4	0.003	.96
	Putamen	R	479 307.9	2.16	.14
		L	564 780.3	2.68	.10
	GP	R	2594.2	0.07	.79
		L	6767.6	0.16	.69
ASO × OCD	Caudate	R	17 949.4	0.07	.79
		L	31 020.8	0.11	.74
	Putamen	R	554 597.5	2.51	.12
		L	180 705.5	0.86	.36
	GP	R	47 453.5	1.25	.27
		L	271 584.3	6.78	.01
ASO × ADHD	Caudate	R	19 359.1	0.07	.79
		L	44 599.2	0.16	.69
	Putamen	R	382 533.3	1.73	.19
		L	1 284 129.5	6.10	.01
	GP	R	320 157.8	8.44	.005
		L	121 207.3	3.02	.08
ASO × ADHD × OCD	Caudate	R	24 437.7	0.09	.76
		L	7602.8	0.03	.87
	Putamen	R	713 789.6	3.22	.07
		L	1 223 781.8	5.80	.01
	GP	R	272 664.1	7.18	.009
		L	98 375.9	2.45	.12
Error	Caudate	R	26 502 840.2	. . .	. . .
		L	28 386 288.6	. . .	. . .
	Putamen	R	22 140 347.0	. . .	. . .
		L	21 087 448.1	. . .	. . .
	GP	R	3 795 193.9	. . .	. . .
		L	4 008 318.0	. . .	. . .

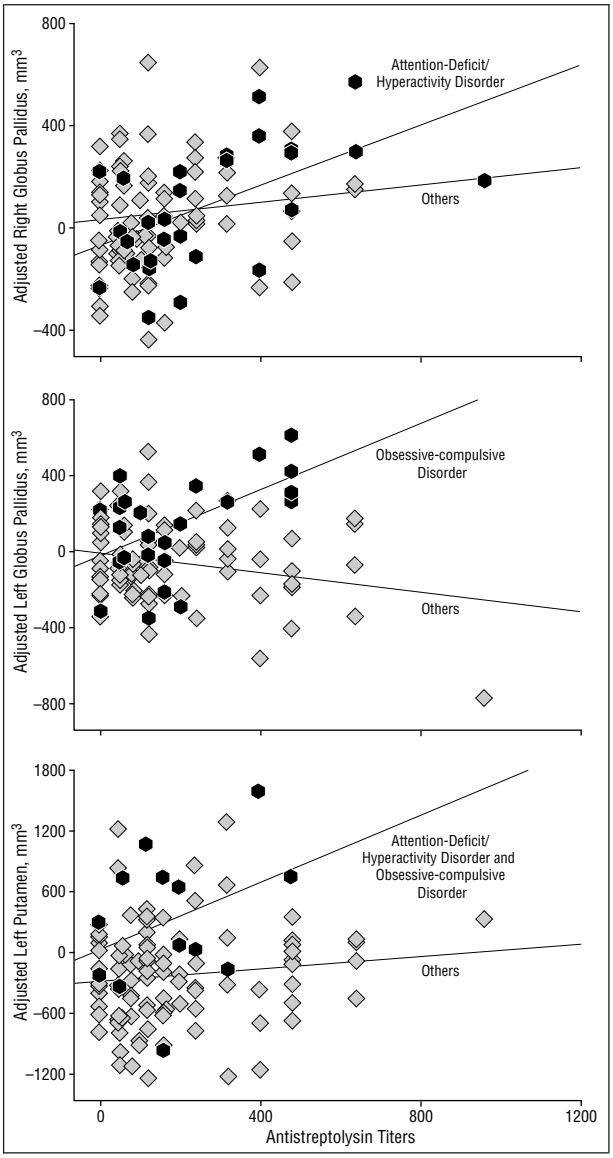
*Multivariate analysis of variance assessing the strength of the association of diagnosis-by-antibody interactions with basal ganglia volumes. R indicates right; L, left; GP, globus pallidus; ×, statistical interaction of adjacent terms; CTD, chronic tic disorder; OCD, obsessive-compulsive disorder; ADHD, attention-deficit/hyperactivity disorder; ASO, antistreptolysin O, and ellipses, error terms. Lighter shading indicates $P<.05$. Overall, $R^2 = 0.34$.

†df = 1 for all except error, where df = 100.

‡P values are Bonferroni-adjusted.

§The estimated marginal mean of the right and left putamen is smaller in subjects with CTD.

‖The estimated marginal mean of the right globus pallidus is smaller in subjects with ADHD.



Association of titers, diagnoses, and basal ganglia volumes. The interaction of antistreptolysin O titers with attention-deficit/hyperactivity disorder and obsessive-compulsive disorder diagnoses are presented graphically. Basal ganglia volumes are adjusted for the effects of all independent variables in the multivariate analysis of covariance (see Table 4) and hence the residuals of the volumes after the adjustment can be positive or negative. These volume residuals are plotted against the raw antistreptolysin O values for each of the relevant diagnostic groups. Titers are plotted in dark circles for the noted diagnostic group and in lighter diamonds for all other subjects.

explained as a consequence of a series of remote GABHS infections. The current association of ADHD with GABHS titers might reflect an underlying, lifelong biological predisposition to GABHS infection in a subset of individuals who have ADHD.

The second finding in this study is that in subjects who had ADHD, OCD, or both disorders, higher antibody titers predicted larger putamen and globus pallidus nuclei. These basal ganglia findings could suggest that the combination of ADHD and GABHS infection entails for some patients a further association with OCD and larger basal ganglia volumes. Considered in the context of an immune-based theory of pathogenesis, this further association would presumably depend on the host's susceptibility to autoimmune disorders. We hypothesize that in the presence of this host susceptibility, GABHS infection in patients with ADHD induces immune responses that damage and enlarge the host's basal ganglia, which is measurable with MRI. This basal ganglia injury subsequently supports the emergence of additional OCD and ADHD symptoms.

The associations of antibody titers with basal ganglia volumes were seen for ASO, but not anti–DNAse B, antibodies. Because ASO titers do not remain elevated as long as anti–DNAse B after acute GABHS infection,[20] elevated ASO titers are regarded as a marker of more re-

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cent infection than are those for anti–DNAse B. The specificity of the association of ASO titers with basal ganglia volumes may therefore suggest that these hypothesized GABHS-induced changes in basal ganglia morphology may be most evident soon after infection, presumably because basal ganglia swelling is relatively transient.

The associations of diagnosis, GABHS infection, and basal ganglia enlargement are similar to those seen in Sydenham chorea, in which the cause is more clearly established as autoimmune in nature by virtue of its association with rheumatic fever. Antibodies generated against GABHS are seen in the serum of patients with Sydenham chorea more frequently than in the serum of control subjects.[34] In some patients with Sydenham chorea, GABHS antibodies have been noted to cross-react strongly with antigens present in tissue from the caudate and subthalamic nuclei of a cadaver.[35] Finally, basal ganglia abnormalities have been reported in MRI studies of patients who have an acute onset of Sydenham chorea. These abnormalities include increased signal intensity on T2-weighted images in the caudate, putamen, and globus pallidus nuclei, which presumably represents edema as a consequence of an autoimmune-induced vasculitis within the basal ganglia.[36,37] This edema is thought to produce the larger caudate, putamen, and globus pallidus volumes seen in patients with Sydenham chorea compared with control subjects.[38]

Despite these consistencies with findings in other immune-based neuropsychiatric disorders, the findings reported here should be regarded as preliminary. First, the noted associations do not prove causation. Second, the statistically significant associations were driven by elevated titers in a the relatively small number of subjects and therefore must be replicated. Third and most important, the postulated chain of causality leading from an ADHD phenotype to increased rates of GABHS exposure, to OCD symptom exacerbation, and finally to enlarged basal ganglia requires support from longitudinal studies. Tracking chemical spectra and tissue relaxation times within the basal ganglia in those studies[39] could help to monitor the autoimmune vasculitis and edema that are hypothesized to follow GABHS infection in those studies. Given the preliminary nature of these findings and the risks associated with antibiotics (including the emergence of penicillin-resistant organisms),[40,41] we caution against antimicrobial therapies in the treatment of ADHD or OCD unless their use is supported by culture-documented GABHS pharyngitis, scarlet fever, or possibly by the presence of an acute otitis media or sinusitis that is presumed to be induced by GABHS.[42]

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Reprints: Bradley S. Peterson, MD, Yale Child Study Center, 230 S Frontage Rd, New Haven, CT 06520 (e-mail: bradley.peterson@yale.edu or paul.lombroso@yale.edu).

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