Developmental Trajectories of Brain Volume Abnormalities in Children and Adolescents With Attention-Deficit/Hyperactivity Disorder

F. Xavier Castellanos, MD
Patti P. Lee, MD
Wendy Sharp, MSW
Neal O. Jeffries, PhD
Deanna K. Greenstein, PhD
Liv S. Clasen, PhD
Jonathan D. Blumenthal, MA
Regina S. James, MD
Christen L. Ebens, BA
James M. Walter, MA
Alex Zijdenbos, PhD
Alan C. Evans, PhD
Jay N. Giedd, MD
Judith L. Rapoport, MD

Context Various anatomic brain abnormalities have been reported for attention-deficit/hyperactivity disorder (ADHD), with varying methods, small samples, cross-sectional designs, and without accounting for stimulant drug exposure.

Objective To compare regional brain volumes at initial scan and their change over time in medicated and previously unmedicated male and female patients with ADHD and healthy controls.

Design, Setting, and Participants Case-control study conducted from 1991-2001 at the National Institute of Mental Health, Bethesda, Md, of 152 children and adolescents with ADHD (age range, 5-18 years) and 139 age- and sex-matched controls (age range, 4.5-19 years) recruited from the local community, who contributed 544 anatomic magnetic resonance images.

Main Outcome Measures Using completely automated methods, initial volumes and prospective age-related changes of total cerebrum, cerebellum, gray and white matter for the 4 major lobes, and caudate nucleus of the brain were compared in patients and controls.

Results On initial scan, patients with ADHD had significantly smaller brain volumes in all regions, even after adjustment for significant covariates. This global difference was reflected in smaller total cerebral volumes (–3.2%, adjusted $F_{1,280}=8.30$, $P=.004$) and in significantly smaller cerebellar volumes (–3.5%, adjusted $F_{1,280}=12.29$, $P=.001$). Compared with controls, previously unmedicated children with ADHD demonstrated significantly smaller total cerebral volumes (overall $F_{2,288}=6.65$; all pairwise comparisons Bonferroni corrected, –5.8%; $P=.002$) and cerebellar volumes (–6.2%, $F_{2,288}=8.97$, $P<.001$). Unmedicated children with ADHD also exhibited strikingly smaller total white matter volumes ($F_{2,288}=11.65$) compared with controls (–10.7%, $P<.001$) and with medicated children with ADHD (–8.9%, $P<.001$). Volumetric abnormalities persisted with age in total and regional cerebral measures ($P=.002$) and in the cerebellum ($P=.003$). Caudate nucleus volumes were initially abnormal for patients with ADHD ($P=.05$), but diagnostic differences disappeared as caudate volumes decreased for patients and controls during adolescence. Results were comparable for male and female patients on all measures. Frontal and temporal gray matter, caudate, and cerebellar volumes correlated significantly with parent- and clinician-rated severity measures within the ADHD sample (Pearson coefficients between –0.16 and –0.26; all P values were $<.05$).

Conclusions Developmental trajectories for all structures, except caudate, remain roughly parallel for patients and controls during childhood and adolescence, suggesting that genetic and/or early environmental influences on brain development in ADHD are fixed, nonprogressive, and unrelated to stimulant treatment.

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ATTENTION-DEFICIT/HYPERactivity disorder (ADHD), the most common childhood psychiatric disorder, is thought to reflect subtle abnormalities in central nervous system functioning.[1] For this reason, ADHD is being studied increasingly with a variety of brain imaging techniques throughout the life span. Magnetic resonance imaging (MRI) is particularly suitable for the study of pediatric patients, providing high-resolution images without ionizing radiation. Previous MRI neuroimaging studies, most with small samples, have reported smaller anatomic areas and/or volumes in patients with ADHD in regions of the cor-

See also Patient Page.

Author Affiliations are listed at the end of this article.
Corresponding Author and Reprints: F. Xavier Castellanos, MD, New York University Child Study Center, 577 First Ave, New York, NY 10016 (e-mail: francisco.castellanos@med.nyu.edu).

pus callosum,[2-6] smaller volumes and/or hypoactivation of prefrontal brain,[7-11] basal ganglia,[8,9,12-16] and cerebellum.[16-18] However, a recent study noted inconsistencies in the ADHD neuroimaging literature and concluded that specific abnormalities have not yet been convincingly demonstrated.[19]

Although we previously conducted anatomic studies in male (n=112)[20] and female (n=100)[16] patients with ADHD and controls, we were unable to rigorously contrast or combine the 2 sets of findings because the original measurement techniques used were no longer available. Moreover, we have continued to recruit new patients, including a sizable number of patients who had never been previously exposed to psychotropic medications.

The present study was designed to examine brain anatomy using the same automated measures from cross-sectional scans of a large sample of male and female patients with ADHD, determine the effect of prior stimulant drug exposure on anatomic abnormalities in ADHD, and examine brain regional longitudinal growth trajectories in patients and controls.

We hypothesized that patients with ADHD would have smaller brain regional volumes, particularly in caudate nucleus,[8,13] cerebellum,[16-18] and frontal lobe[8,9]; previously unmedicated children and adolescents with ADHD would demonstrate similar brain abnormalities as medicated patients[16]; and caudate anatomic abnormalities would diminish with age. Examination of age-related changes in other brain regions was exploratory.

METHODS

Patients

A total of 89 male (mean initial age, 10.5 years; range, 5.1-18.4) and 63 female (mean initial age, 9.4; range, 5.3-16.0) children and adolescents with *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*[21])–defined ADHD were recruited from the surrounding community. Inclusion criteria were: hyperactive, inattentive, and impulsive behaviors that were impairing in at least 2 settings and a Conners' Teacher Hyperactivity rating greater than 2 SD above age- and sex-specific means.[22,23] The *DSM-IV* diagnosis of ADHD was based on the Parent Diagnostic Interview for Children and Adolescents,[24] Conners' Teacher Rating Scales,[22,23] and the Teacher Report Form.[25] A clinical psychologist administered the Wechsler Intelligence Scale for Children–Revised[26] to 110 patients with ADHD and the Wechsler Intelligence Scale for Children–III[27] to 41 patients (1 was too young to be tested). Exclusion criteria were a full-scale IQ of less than 80, evidence of medical or neurological disorders on examination or by clinical history, Tourette disorder, or any other axis I psychiatric disorder requiring treatment with medication at study entry.

A total of 56 unrelated healthy female (mean initial age, 10.0 years; range, 5.2-16.1) and 83 male (mean initial age, 10.9; range, 4.5-19.0) controls were recruited from the community via the National Institutes of Health Normal Volunteer Office and outreach to local schools. Screening included an initial telephone interview, parent and teacher rating scales,[25] in-person assessment including physical and neurological examinations including handedness,[28] and clinical history obtained by a child and adolescent psychiatrist (J.N.G.). Vocabulary and block design subtests from the Wechsler Intelligence Scale for Children–Revised (n=80), Wechsler Intelligence Scale for Children–III (n=23), Wechsler Abbreviated Scale of Intelligence[29] (n=20), Wechsler Preschool and Primary Scale of Intelligence[30] (n=10), and Wechsler Adult Intelligence Scale–Revised[31] (n=1) were obtained. Five controls were not tested but were within the healthy range by reported academic history. Approximately 4 candidates were screened for every 1 accepted,[32] with the most common exclusions being positive family psychiatric history and possible psychiatric diagnosis based on teacher report.

This study was conducted at the Child Psychiatry Branch of the National Institute of Mental Health in Bethesda, Md, between 1991 and 2001. The institutional review board approved the research protocol, and written informed consent and assent to participate in a study of brain development were obtained from parents and children, respectively, at study entry and at each subsequent MRI examination. Healthy volunteers and patients not currently participating in treatment studies were paid to participate.

Behavioral Measures

Primary symptom severity measures were those that remained constant across the study decade using the Attention Problems Factors from the Child Behavior Checklist and Teacher Report Form[25] and the Clinical Global Impressions scale for Severity of Illness.[33] Medication status was obtained from parental history.

MRI Acquisition

All patients and controls were studied on the same 1.5-T General Electric Signa scanner (Milwaukee, Wis). T1-weighted images with contiguous 1.5-mm slices in the axial plane and 2.0-mm slices in the coronal plane were obtained using 3-dimensional spoiled gradient recalled echo in the steady state. Imaging parameters were echo time of 5 ms, repetition time of 24 ms, flip angle of 45°, acquisition matrix of 256×192, number of excitations equals 1, and 24 cm field of view. Head placement was standardized as previously described.[16]

Image Analysis

T2-weighted images were obtained for evaluation by a clinical neuroradiologist. All raters were blind to demographic characteristics. Quantification of MRI images was performed via a 3-part fully automated image analysis process that determines the volumes of gray and white matter compartments in frontal, temporal, parietal, and occipital lobes as well as basal ganglia and cerebellum with excellent test-retest reliability as described elsewhere in detail.[34-36]

Visual inspection of each scan revealed that 544 of 594 total scans (92%)

were processed successfully; 50 were excluded because of classification and segmentation errors due to motion. Failure rate was significantly higher (χ^2_1 with Yates correction = 4.08, $P = .04$) in 34 of 317 patients (11%) than in 16 of 277 controls (6%). All remaining scans from patients with ADHD (283 scans) were used. The comparison group was selected from a pool of healthy controls after excluding siblings in order not to violate the statistical assumption of independence. The remaining 139 potential controls (ie, no more than 1 per family within the age range of our patients) were selected by the data manager (L.S.C.) to best match each target patient for sex, age, and longitudinal intervals, prior to morphometric analyses. Whenever precise matching on all parameters was not possible, patients and controls were matched on average-age across their own scans. Because we were unable to match all patients and controls 1-to-1, we made every effort to maintain proportional scan-densities across the entire age-range of the 152 patients.

Statistical Analyses

Demographic and clinical measures were compared by 2-way analyses of variance (testing main effects of diagnoses and sex and their interaction) or 2-sample *t* tests for continuous measures, and with χ^2 or Fisher exact test for nominal measures. Analyses of variance of the 10 regional brain measures and 3 summary measures obtained at initial scan (n = 291 independent participants) were initially performed with diagnoses and sex as between-participant factors. Because we did not obtain full-scale IQ scores from controls, Wechsler vocabulary standard score was used, as it is the single best predictor of full-scale IQ.[27] To account for between-group differences in vocabulary, height, weight, handedness, and medication status, analyses of covariance were performed with these potential covariates. Nonsignificant covariates were deleted from the final models. Pearson correlations were computed for symptom severity measures and brain volumes in the patient sample.

To examine the influence of medications more closely we compared patients with ADHD who were never previously treated with psychotropic medications (unmedicated ADHD), medicated patients (medicated ADHD), and controls. The unmedicated ADHD patients were significantly younger than the medicated ADHD and controls; thus, we confirmed findings in age-matched subgroups (n = 128). All pairwise comparisons were conducted with Bonferroni corrections.

Finally, longitudinal analytic methods[37,38] were used to examine growth patterns of caudate, cerebellum, total cerebrum, and the white and gray components of the 4 major lobes. The initial full longitudinal growth model was expressed as a cubic:

$$\text{Size} = \text{Intercept} + \beta_1 \times (\text{Age} - \text{Mean Age}) + \beta_2 \times (\text{Age} - \text{Mean Age})^2 + \beta_3 \times (\text{Age} - \text{Mean Age})^3 + \epsilon$$

The model parameters (intercept and β coefficients) were initially allowed to reflect interactions between sex and diagnostic group. To account for within-person correlations, intercepts were treated as normally distributed random effects that varied by individual, while β coefficients for age, age-squared, and age-cubed terms were modeled as fixed effects. The full cubic model was compared with simpler quadratic, linear, and constant models with interactions. Once the order of the model was established, testing was performed to determine whether an additive model could replace the interactions between sex and diagnostic group for the height and shape parameters of the curves. With respect to shape of the curves, there were neither significant sex differences nor sex by diagnosis interactions for any structure. Consequently, final models allowed for sex and diagnosis effects in the height parameters (intercept) of the curves and included only diagnostic differences in shape parameters.

Hypothesis tests and model selection were based on F statistics. We included data from individuals who had only a single scan (about 40% of both groups), because single scans provide additional information about between-participant variation and overall curve shape. These methods have been useful for combining cross-sectional and longitudinal anatomic MRI data.[39-41] Statistical power exceeded 80% at $P = .05$ for all brain measures. Minimally detectable adjusted differences ranged from 2.7% (caudate and cerebellum) to 5% for occipital gray matter, and averaged 3% for cortical volumes. Statistical analyses were performed using SPSS version 10.0 (SPSS Inc, Chicago, Ill), except for the mixed-model random regression analyses, which were performed with SAS version 8.02 (SAS Institute Inc, Cary, NC), and the power analyses, which were conducted with PASS 2000 (NCSS Statistical Software, Kaysville, Utah). Two-tailed significance levels were defined as $P \leq .05$.

RESULTS

Participants

Final study participants consisting of 152 children and adolescents with ADHD and 139 controls were each successfully scanned up to 4 times over a decade. As TABLE 1 shows, there were several group differences between male and female patients (females were younger, shorter, and weighed less), and between patients and controls. Patients were shorter and weighed less, had lower vocabulary standard scores, and a lower percentage of individuals were strongly right-handed (scoring 10 or more of 12 items). Sex and diagnosis did not interact significantly for any demographic measure. Female and male patients with ADHD were comparable on vocabulary, handedness, parent and teacher attention problem scores, and prevalence of learning disorders.[42] Physician's Clinical Global Impressions ratings reflected significantly greater severity in females, who also had a higher percentage of combined-type ADHD, mood disorder (history of major depression and/or dysthymia) and lower prevalence of conduct disorder and tic disorder not otherwise specified. At the time of the first scan, 103 patients (68%)

Table 1. Demographic and Diagnostic Characteristics of 152 Patients With ADHD and 139 Control Patients*

Characteristic	Patients With ADHD		Controls		*P* Value for Female vs Male†	*P* Value for Patients With ADHD vs Controls
	Female (n = 63)	Male (n = 89)	Female (n = 56)	Male (n = 83)		
Age at initial scan, mean (SD), y	9.4 (2.6)	10.5 (3.1)	10.0 (2.6)	10.9 (3.5)	.007	.13
Height, mean (SD), cm	134.9 (15.0)	141.7 (18.0)	140.2 (16.0)	147.3 (20.3)	.001	.01
Weight, mean (SD), kg	33.0 (12.2)	36.9 (14.4)	35.8 (12.5)	42.0 (16.5)	.003	.02
Birth weight, mean (SD), g	3264 (573) [n = 54]	3449 (606) [n = 66]	3396 (414) [n = 33]	3584 (544) [n = 48]	.02	.10
Scores, mean (SD)						
Vocabulary standard score	11.6 (3.1)	11.9 (3.0)	12.5 (3.1)	12.6 (3.0)	.61	.02
Clinical Global Impression	4.6 (1.0)	4.3 (0.9)	NA	NA	.04	NA
CBCL attention problems T-score	74.6 (7.7)	70.8 (9.6)	NA	NA	.01	NA
TRF attention problems T-score	68.2 (8.7)	68.9 (9.3)	NA	NA	.66	NA
Strongly right-handed, No. (%)	52 (82)	73 (82)	52 (93)	76 (93)	.85	.01‡
Prior stimulant treatment, No. (%)	41 (65)	62 (70)	NA	NA	.55	NA
DSM-IV diagnosed disorders, No. (%)						
ADHD, combined type	63 (100)	83 (93)			.04	
Oppositional defiant	26 (41)	30 (34)			.34	
Conduct	1 (2)	10 (11)			.02	
Learning	7 (11)	9 (10)			.84	
Mood	6 (9)	1 (1)			.01	
Anxiety	7 (11)	6 (7)			.34	
Tic, not otherwise specified	1 (2)	10 (11)			.02	

*ADHD indicates attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist (rated by parents); TRF, Teacher Report Form; *DSM-IV*, *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*; and NA, not applicable.
†None of the sex by diagnosis interactions on 2-way analysis of variance approached statistical significance. Frequencies compared with χ^2 or Fisher exact tests.
‡Frequency compared with χ^2 test.

were being treated with psychostimulants.

The 49 patients with ADHD (22 females) who were successfully scanned before ever being treated with psychotropic medications (unmedicated ADHD) were significantly younger than the medicated patients (medicated ADHD) and controls (TABLE 2). Unmedicated patients with ADHD were rated as comparable in severity by parents, but as significantly less severely affected by physicians and teachers. They also tended to score higher on the vocabulary IQ subtest, but not significantly (P=.06).

Sixty-one patients (40%) were scanned once, 61 (40%) twice, 20 (13%) 3 times, and 10 (7%) 4 times. Fifty-two controls (37%) were scanned once, 55 (40%) twice, 29 (21%) 3 times, and 3 (2%) 4 times. Mean ages at each scan did not differ significantly between diagnostic groups (at first scan, $F_{1,289}$=2.28, P=.13; at second scan, $F_{1,176}$=0.08, P=.78; at third scan, $F_{1,60}$=0.02, P=.89; at fourth scan, $F_{1,11}$=1.06, P=.32). Female participants (mean, 9.7 years [SD, 2.6]) were significantly younger than male participants (mean, 10.7 years [SD, 3.3]; P=.006), regardless of diagnosis. Mean intervals between scans did not differ significantly between diagnostic groups (mean for patients with ADHD, 2.6 years [1.1]; mean for controls, 2.4 years [1.0]; T_{229}=1.60; P=.11).

Table 2. Comparison Between Previously Unmedicated and Medicated Patients With ADHD*

	Mean (SD)		
	Unmedicated Patients With ADHD (n = 49)	Medicated Patients With ADHD (n = 103)†	*P* Value‡
Age at initial scan, y	8.3 (2.6)	10.9 (2.7)	.001
Vocabulary standard score	12.5 (3.3)	11.5 (2.9)	.06
Clinical Global Impression	4.2 (1.0)	4.6 (0.9)	.02
CBCL attention problems T-score	71.7 (9.7)	72.9 (8.6)	.47
TRF attention problems T-score	66.2 (9.1)	69.9 (8.7)	.02

*ADHD indicates attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; and TRF, Teacher Report Form.
†All previously and/or currently treated with stimulant medications.
‡Using 2-sample *t* test.

Analyses of Initial Scans

TABLE 3 contains the unadjusted means (SDs) of the 291 initial cross-sectional scans by diagnosis as well as the means (SEs) adjusted for all significant covariates. Three summary measures were obtained for the cerebrum, defined by excluding cerebellum, brainstem, and cerebrospinal fluid.

As expected,[43,44] all measures were significantly smaller in female partici-

pants ($F_{1, 287}$ ranged from 10.65 for parietal gray matter to 98.61 for cerebellum; $P<.001$), but sex did not interact significantly with diagnosis for any brain anatomy measure. Accordingly, mean values for sex and corresponding statistics are not presented here (they can be found at http://intramural.nimh.nih.gov/research/chp/index2.html). A significant main effect of diagnosis was found between patients with ADHD and controls for all measures with small-to-medium effect sizes ranging from 0.30 to 0.46, which remained significant or were somewhat enhanced (eg, adjusted effect size for temporal white matter = 0.64) when adjusted for the significant covariates of vocabulary, height, or medication status. When we adjusted for the significant group differences in total cerebral volume, the only brain region that remained significantly smaller in ADHD was the cerebellum (d = .27; 95% confidence interval [CI], 0.03-0.50; $F_{1,287}$ = 4.97; P = .03).

Effects of Prior Drug Treatment

TABLE 4 displays the contrasts between 3 nonoverlapping groups consisting of 49 unmedicated patients with ADHD, 103 medicated patients with ADHD, and 139 healthy controls. Unmedicated patients with ADHD did not differ significantly from medicated patients with ADHD on any gray matter measures, or in caudate or cerebellum. By contrast, unmedicated patients with ADHD had strikingly smaller white matter volumes ($F_{2,288}$ = 11.65) compared with controls (–10.7%, $P<.001$) and with medicated children with ADHD (–8.9%; $P<.001$; all pairwise comparisons Bonferroni corrected). Unmedicated patients with ADHD had smaller cerebellar volumes (–6.2%, $P<.001$), smaller temporal gray (–4.6%, P = .02), and smaller

Table 3. Initial Regional Brain Volumes, Unadjusted, and Adjusted Analyses for Patients With ADHD and Controls*

	Mean (SD)						
	Patients With ADHD (n = 152)	Controls (n = 139)	F Statistic	P Value	Difference Between the Means, %	Effect Size (95% Confidence Interval)	Covariates in Model†
			Unadjusted Analysis‡				
Total cerebral volume	1059.4 (117.5)	1104.5 (111.3)	12.55	<.001	–4.1	0.39 (0.16-0.63)	
Total gray matter	700.9 (77.3)	727.9 (74.3)	9.27	.003	–3.7	0.36 (0.12-0.59)	
Total white matter	358.5 (53.5)	376.6 (49.8)	10.42	.001	–4.8	0.35 (0.11-0.59)	
Frontal gray matter	217.3 (24.9)	225.2 (22.5)	8.05	.005	–3.5	0.33 (0.10-0.57)	
Parietal gray matter	116.6 (13.0)	122.0 (12.9)	11.42	.001	–4.4	0.41 (0.17-0.65)	
Temporal gray matter	174.0 (18.5)	181.6 (18.2)	13.02	<.001	–4.2	0.42 (0.18-0.66)	
Occipital gray matter	62.5 (9.6)	66.5 (10.5)	11.54	.001	–6.1	0.40 (0.16-0.64)	
Frontal white matter	135.8 (21.4)	141.9 (18.5)	8.31	.004	–4.3	0.30 (0.07-0.54)	
Parietal white matter	70.6 (10.4)	74.9 (9.8)	13.94	<.001	–5.7	0.42 (0.18-0.66)	
Temporal white matter	74.4 (11.0)	77.6 (10.6)	7.26	.01	–4.2	0.30 (0.06-0.54)	
Occipital white matter	30.3 (5.5)	32.2 (5.9)	8.68	.003	–6.0	0.34 (0.10-0.57)	
Caudate	10.4 (1.1)	10.8 (1.0)	10.59	.001	–3.7	0.37 (0.13-0.61)	
Cerebellum	124.1 (12.3)	129.8 (12.7)	17.44	<.001	–4.4	0.46 (0.22-0.70)	
			Adjusted Analysis§				
	Mean (SE)						
Total cerebral volume	1055.54 (8.17)	1090.06 (8.69)	8.30	.004	–3.2	0.34 (0.10-0.58)	V
Total gray matter	699.81 (5.70)	719.72 (6.07)	5.67	.02	–2.8	0.28 (0.04-0.52)	V, M, H
Total white matter	351.92 (4.31)	375.50 (4.64)	10.04	.002	–6.3	0.45 (0.20-0.69)	V, M, H
Frontal gray matter	216.87 (1.83)	222.94 (1.94)	5.13	.02	–2.7	0.27 (0.03-0.51)	V
Parietal gray matter	116.75 (1.03)	120.81 (1.10)	7.18	.008	–3.4	0.32 (0.08-0.56)	V
Temporal gray matter	173.56 (1.37)	179.51 (1.46)	8.75	.003	–3.3	0.35 (0.11-0.59)	V
Occipital gray matter	62.49 (0.80)	65.72 (0.85)	7.67	.006	–4.9	0.33 (0.09-0.57)	V
Frontal white matter	132.97 (1.68)	141.89 (1.80)	9.52	.002	–6.3	0.44 (0.19-0.68)	V, M, H
Parietal white matter	69.51 (0.90)	74.77 (0.97)	11.37	.001	–7.0	0.48 (0.23-0.72)	V, M, H
Temporal white matter	71.65 (0.92)	78.89 (1.00)	21.59	<.001	–9.2	0.64 (0.39-0.88)	V, M
Occipital white matter	30.03 (0.42)	31.57 (0.45)	6.13	.01	–4.9	0.30 (0.06-0.53)	V
Caudate	10.32 (0.08)	10.63 (0.09)	6.99	.009	–2.9	0.32 (0.08-0.56)	V
Cerebellum	123.58 (0.87)	128.07 (0.93)	12.29	.001	–3.5	0.42 (0.18-0.66)	V

*ADHD indicates attention-deficit/hyperactivity disorder.
†Potential covariates tested were vocabulary (V), medication status (M), height (H), weight, and handedness. Only significant covariates were included in final models, as indicated.
‡Two-way analysis of variance (diagnoses, sex); main effect of sex, $F_{1,287}$ >10 for all measures; no sex by diagnosis interactions approached significance.
§Two-way analysis of covariance; main effect of sex, $F_{1,277\text{-}284}$ >9 for all measures; no sex by diagnosis interactions approached significance.

total cerebral volumes (–5.8%, $P = .002$) compared with controls. Differences between unmedicated patients with ADHD and controls in frontal (–3.8%) and parietal gray matter (–4.1%) would also have been significant if not corrected for multiple comparisons. Medicated patients with ADHD did not differ significantly from controls on any white matter measure. Robust differences from controls remained for all gray matter measures (ranging from –3.4% to –6.6%), caudate (–4.3%), cerebellum (–3.6%), and the summary measures of total cerebral volume (–3.3%) and total gray volume (–3.9%).

Because the unmedicated patients with ADHD were significantly younger than the other 2 subgroups, and white matter increases with increasing age throughout the age range,[45] we performed secondary analyses restricted to an age-matched subset of 128 participants (consisting of 24 unmedicated patients with ADHD, 50 medicated patients with ADHD, and 54 controls [61 females]). All measures remained essentially unchanged.

Relationship to Clinical Measures

We examined correlations between the 10 regional measures and behavioral ratings. Within the patient group, smaller volumes were significantly correlated in the expected direction with greater symptom severity. Frontal and temporal gray matter, caudate, and cerebellar volumes were significantly and negatively correlated with physician's Clinical Global Impressions rating (n = 139, Pearson coefficients ranged between –0.16 for frontal gray and –0.26 for cerebellum, all $P < .05$). The same 4 regions were also significantly and negatively correlated with parent-rated child behavior checklist attention problems with Pearson coefficients between –0.16 and –0.22 (all $P < .05$). Correlations were largely unaffected when adjusted for age.

Wechsler vocabulary standard score was significantly and positively correlated with all anatomic volumes in patients with ADHD (n = 151; r ranged from 0.19-0.35; all $P < .02$), and in frontal and occipital gray and white matter and cerebellar volumes in controls (n = 134; r ranged from 0.18-0.24; all $P < .02$). Although the magnitude of the correlations was greater in patients than in controls, none of the coefficients differed significantly from each other, and all regional volumes correlated significantly with the vocabulary score when the 2 groups were combined (n = 285; eg, for total cerebral volume, $r = 0.31$; $P < .001$).

Analyses of Initial and Follow-up Scans

Sixty percent of all participants had at least 2 scans (n = 178), including 62 (21%), who had at least 3 scans and 13 (4%), who had 4 scans obtained at 2- to 3-year intervals. Data from all 544 resulting scans were used to derive longitudinal growth curves for patients and controls of both sexes. The age range for male participants extended between 4.6 and 19.0 years, while female participants ranged between 5.2 and 16.3 years, reflecting our initial focus on males with ADHD.[20]

Predicted longitudinal growth curve parameters did not differ significantly between male and female participants except for the height of each curve (intercept) at the corresponding age midpoint, which were significantly higher for males for all measures, regardless of diagnosis (empirical $P < .001$, derived from F statistics confirmed with permutation tests with 1000 iterations). There were no significant interactions between sex and diagnosis for any developmental growth patterns (intercepts or curve parameters $\beta_{1\text{-}3}$). FIGURE 1 shows the predicted developmental growth curves along with 95% CIs for each group's average total cerebral volume. Developmental curves

Table 4. Unadjusted Brain Volumes for Unmedicated and Medicated Patients With ADHD and Controls*

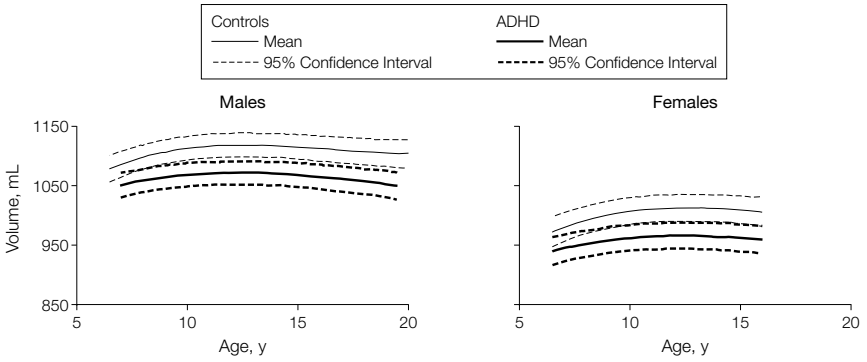
	Mean (SD)							
	Patients With ADHD					*P* Values (Bonferroni Comparison)		
	Unmedicated (n = 49)	Medicated (n = 103)	Controls (n = 139)	F Statistic†	*P* Values†	Unmedicated vs Medicated	Unmedicated vs Controls	Medicated vs Controls
Total cerebral volume	1040.4 (98.9)	1068.4 (124.9)	1104.5 (111.3)	6.65	.001	.58	.002	.03
Total gray matter	704.2 (70.0)	699.3 (80.8)	727.9 (74.3)	4.67	.01	>.99	.21	.01
Total white matter	336.2 (41.9)	369.1 (55.3)	376.6 (49.8)	11.65	<.001	<.001	<.001	.50
Frontal gray matter	216.6 (20.7)	217.6 (26.8)	225.2 (22.5)	4.06	.02	>.99	.10	.05
Parietal gray matter	117.0 (11.4)	116.4 (13.7)	122.0 (12.9)	6.22	.002	>.99	.08	.006
Temporal gray matter	173.2 (15.6)	174.4 (19.7)	181.6 (18.2)	6.32	.002	>.99	.02	.006
Occipital gray matter	63.2 (9.5)	62.1 (9.7)	66.5 (10.5)	6.05	.003	>.99	.17	.003
Frontal white matter	127.1 (16.6)	140.0 (22.2)	141.9 (18.5)	10.59	<.001	<.001	<.001	.84
Parietal white matter	66.5 (7.8)	72.6 (10.8)	74.9 (9.8)	12.86	<.001	.003	<.001	.14
Temporal white matter	69.7 (8.5)	76.6 (11.3)	77.6 (10.6)	10.54	<.001	<.001	<.001	>.99
Occipital white matter	28.6 (4.6)	31.1 (5.8)	32.2 (5.9)	7.39	.001	.06	<.001	.27
Caudate	10.50 (1.07)	10.29 (1.16)	10.75 (0.98)	5.69	.004	.52	.54	.002
Cerebellum	121.8 (11.7)	125.1 (12.4)	129.8 (12.7)	8.97	<.001	.47	<.001	.005

*ADHD indicates attention-deficit/hyperactivity disorder.
†Two-way analysis of variance (group [medicated vs unmedicated vs control] by sex); *df* (2, 288) for all regions. No sex by diagnoses interactions approached significance.

were significantly higher in controls than in patients with ADHD for total cerebral volume and for all other brain measures. Diagnostic differences in curve height remained significant after adjusting for vocabulary standard score (total cerebral volume, $P=.002$). There were no significant differences in curve shape between patients and controls, except for caudate. After adjustment for diagnostic differences in total cerebral volume, only caudate ($P=.02$) and cerebellum ($P=.003$) remained significantly smaller in patients with ADHD.

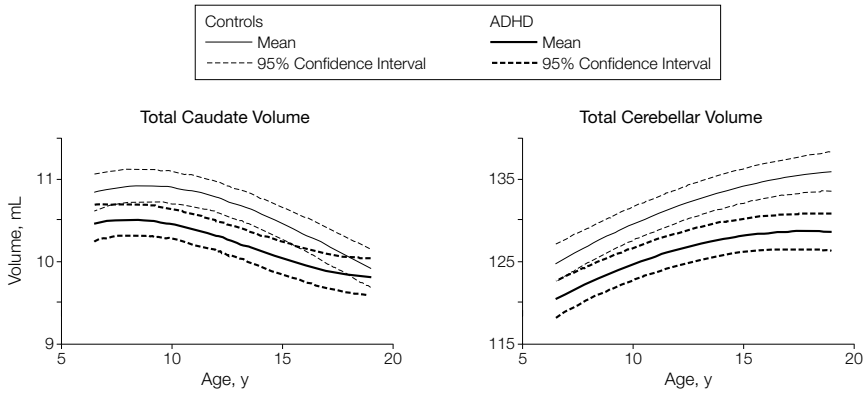
FIGURE 2 depicts unadjusted predicted growth curves for caudate nucleus and cerebellum. Caudate was the only region in which the developmental trajectories did not remain statistically parallel for patients and controls (adjusted, $P=.05$). These differences in shape represent a normalization of caudate volume for patients by midadolescence. By contrast, diagnostic differences in cerebellar curves continue throughout our age range (unadjusted, $P<.001$; adjusted, $P=.003$), with a nonsignificant tendency toward a greater difference in late adolescence (unadjusted, $P=.10$). The general absence of diagnostic differences in curve shapes indicates that developmental curves for patients with ADHD, although significantly lower, were essentially parallel to curves for healthy controls, with the exception of the caudate nucleus.

Figure 1. Predicted Unadjusted Longitudinal Growth Curves for Total Cerebral Volumes for Patients With ADHD and Controls



ADHD indicates attention-deficit/hyperactivity disorder. Curvature cubic, quadratic, and linear coefficients did not differ significantly between male and female patients, and sex did not interact significantly with diagnosis. Although all data were used in analyses, graphs of developmental curves are restricted to the central 90% of each sample's age distribution because fitted polynomial curves may be heavily influenced by outliers at the age range extremes.

Figure 2. Predicted Unadjusted Longitudinal Growth Curves for Total Caudate and Cerebellar Volume for Patients With ADHD vs Controls



ADHD indicates attention-deficit/hyperactivity disorder. Data beyond 16 years are for male patients only, because data from female patients did not exist beyond 16 years (effects ascribable to sex were assumed to be the same between ages 16-19 years as for ages 5-16 years, warranted as a single value to select the differences in intercepts [curve heights] for any case).

COMMENT

Fully automated measures of brain cortical and subcortical volumes from the initial scans of 291 male and female patients show that the cerebrum as a whole and the cerebellum are smaller in children and adolescents with predominantly combined-type ADHD. Rather than reflecting a selective frontal-striatal effect, volumes were decreased to a comparable extent in all 4 lobes and were statistically more prominent only in the cerebellum. Our findings were not ascribable to differences in cognitive level, height, age, weight, or handedness and were not related to comorbid diagnoses (data not shown).

This is the first neuroimaging study to our knowledge to include a substantial number (n=49) of previously unmedicated children and adolescents with ADHD. We attempted to recruit children with equivalent severity of ADHD symptoms by using identical diagnostic and symptom severity criteria. Unmedicated patients with ADHD did not differ from medicated children with ADHD on parent-rated attention problems, but they had significantly lower teacher and physician ratings, and higher vocabulary standard scores. These differences should have minimized anatomic brain differences between unmedicated patients with ADHD and controls. In fact, findings were generally as striking for the unmedicated patients with ADHD as for those who were being treated with medications, and were more pronounced for white matter volumes. Thus, our analyses show that decreased brain volumes in ADHD in both white and gray matter compartments

are not due to drug treatment. Conversely, we have no evidence that stimulant drugs cause abnormal brain development.[46]

Patients with ADHD had developmental trajectories for nearly all brain regions that paralleled growth curves for controls but on a lower track. The one exception, foreshadowed by an earlier cross-sectional study,[13] was the caudate nucleus, for which differences between patients and controls became negligible by midadolescence. As the caudate nucleus reaches its maximum volume around 10 years, the potential relationship between normalization of caudate volume in ADHD and decreased ratings of hyperactivity/impulsivity in children with ADHD,[47] as well as in quantitative measures of movement in normative samples,[48] should be addressed in future studies.

Longitudinal follow-up of functional outcome is continuing; hence, we cannot report definitively on the relationship between continuing anatomic deviance or normalization vs outcome. Preliminarily, global functional outcome in 64 patients with ADHD (20 females) evaluated 4 years after initial scan does not suggest any significant relationships between continuing anatomic deviance and clinical follow-up status.

We did not find evidence of a primarily frontal abnormality in ADHD. Instead, we found the smallest diagnostic effect sizes in frontal lobes. However, these results cannot be interpreted as definitive evidence against the frontal-striatal hypothesis of ADHD pathogenesis, because our units of analysis, while highly reliable, were too large. These methods have been useful in detecting age-, sex-, and diagnosis-specific differences in growth curves,[39-41,49] and their application to ADHD was warranted. Alternate approaches, such as unbiased pixel-based analyses,[50] may be needed to detect more localized anatomic abnormalities in regions such as cingulate, orbitalfrontal, or dorsolateral prefrontal cortex in patients with ADHD.[51] However, these methods may also require even larger or more closely matched contrast groups (eg, twin or sibling controls) given the mostly modest effect sizes and substantial between-subject variations in brain anatomy.[52]

Limitations of this study include the use of referred samples for patients and highly screened controls that may not be optimally representative. We recruited female patients with ADHD who were comparable in severity with our previous samples of males,[53] but in so doing may have selected females who are atypical of most community and clinical samples. We lost significantly more scans from children with ADHD because of excessive motion, but again, this bias should have removed the most symptomatic patients.

In conclusion, ADHD is associated with about a 3% (adjusted; 4% unadjusted) decrease in volume throughout the brain. Intriguingly, this decrease is most marked in white matter of unmedicated patients. Furthermore, with the exception of caudate nucleus, longitudinal growth curves are roughly parallel, suggesting that the fundamental developmental processes active during late childhood and adolescence are essentially healthy in ADHD, and that neuropsychiatric symptoms appear to reflect fixed earlier neurobiological insults or abnormalities. Future studies should focus on younger patients being enrolled into controlled treatment studies while in preschool and on the development of improved quantitative measures of brain anatomy and of the component endophenotypes of ADHD.[54] Finally, despite the importance of these findings, anatomic MRI studies remain appropriate only for research, as they cannot yet contribute to the diagnostic assessment of ADHD.

Author Affiliations: Child Psychiatry Branch, National Institute of Mental Health (Drs Castellanos, Lee, Greenstein, Clasen, James, Giedd, and Rapoport, Mss Sharp and Ebens, and Messrs Blumenthal and Walter), Biostatistics Branch, National Institute of Neurological Disorders and Stroke (Dr Jeffries), National Institutes of Health, Bethesda, Md; and Montreal Neurological Institute, McGill University, Montreal, Quebec (Drs Zijdenbos and Evans). Dr Castellanos is now with the New York University Child Study Center, New York, NY.

Author Contributions: *Study concept and design:* Castellanos, Evans, Giedd, Rapoport.
Acquisition of data: Castellanos, Sharp, Clasen, James, Ebens,Walter, Evans, Giedd.
Analysis and interpretation of data: Castellanos, Lee, Jeffries, Greenstein, Blumenthal, Walter, Zijdenbos, Evans, Giedd.
Drafting of the manuscript: Castellanos, Lee, Jeffries, James, Giedd, Rapoport.
Critical revision of the manuscript for important intellectual content: Castellanos, Lee, Sharp, Jeffries, Greenstein, Clasen, Blumenthal, Ebens,Walter, Zijdenbos, Evans, Giedd.
Statistical expertise: Jeffries, Greenstein, Walter, Evans.
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REFERENCES

1. Tannock R. Attention deficit hyperactivity disorder: advances in cognitive, neurobiological, and genetic research. *J Child Psychol Psychiatry*. 1998;39:65-99.
2. Hynd GW, Semrud-Clikeman M, Lorys AR, et al. Corpus callosum morphology in attention-deficit hyperactivity disorder: morphometric analysis of MRI. *J Learn Disabil*. 1991;24:141-146.
3. Giedd JN, Castellanos FX, Casey BJ, et al. Quantitative morphology of the corpus callosum in attention deficit hyperactivity disorder. *Am J Psychiatry*. 1994;151:665-669.
4. Semrud-Clikeman M, Filipek PA, Biederman J, et al. Attention-deficit hyperactivity disorder: magnetic resonance imaging morphometric analysis of the corpus callosum. *J Am Acad Child Adolesc Psychiatry*. 1994;33:875-881.
5. Baumgardner TL, Singer HS, Denckla MB, et al. Corpus callosum morphology in children with Tourette syndrome and attention deficit hyperactivity disorder. *Neurology*. 1996;47:477-482.
6. Lyoo IK, Noam GG, Lee CK, et al. The corpus callosum and lateral ventricles in children with attention-deficit hyperactivity disorder: a brain magnetic resonance imaging study. *Biol Psychiatry*. 1996;40:1060-1063.
7. Hynd GW, Semrud-Clikeman M, Lorys AR, et al. Brain morphology in developmental dyslexia and attention deficit disorder/hyperactivity. *Arch Neurol*. 1990;47:919-926.
8. Filipek PA, Semrud-Clikeman M, Steingard RJ, et al. Volumetric MRI analysis comparing attention-deficit hyperactivity disorder and normal controls. *Neurology*. 1997;48:589-601.
9. Casey BJ, Castellanos FX, Giedd JN, et al. Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry*. 1997;36:374-383.
10. Rubia K, Overmeyer S, Taylor E, et al. Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. *Am J Psychiatry*. 1999;156:891-896.
11. Bush G, Frazier JA, Rauch SL, et al. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. *Biol Psychiatry*. 1999;45:1542-1552.
12. Hynd GW, Hern KL, Novey ES, et al. Attention

deficit hyperactivity disorder and asymmetry of the caudate nucleus. *J Child Neurol.* 1993;8:339-347.
13. Castellanos FX, Giedd JN, Eckburg P, et al. Quantitative morphology of the caudate nucleus in attention deficit hyperactivity disorder. *Am J Psychiatry.* 1994;151:1791-1796.
14. Aylward EH, Reiss AL, Reader MJ, et al. Basal ganglia volumes in children with attention-deficit hyperactivity disorder. *J Child Neurol.* 1996;11:112-115.
15. Teicher MH, Anderson CM, Polcari A, et al. Functional deficits in basal ganglia of children with attention-deficit/hyperactivity disorder shown with functional magnetic resonance imaging relaxometry. *Nat Med.* 2000;6:470-473.
16. Castellanos FX, Giedd JN, Berquin PC, et al. Quantitative brain magnetic resonance imaging in girls with attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry.* 2001;58:289-295.
17. Berquin PC, Giedd JN, Jacobsen LK, et al. The cerebellum in attention-deficit/hyperactivity disorder: a morphometric study. *Neurology.* 1998;50:1087-1093.
18. Mostofsky SH, Reiss AL, Lockhart P, Denckla MB. Evaluation of cerebellar size in attention-deficit hyperactivity disorder. *J Child Neurol.* 1998;13:434-439.
19. Baumeister AA, Hawkins MF. Incoherence of neuroimaging studies of attention deficit/hyperactivity disorder. *Clin Neuropharmacol.* 2001;24:2-10.
20. Castellanos FX, Giedd JN, Marsh WL, et al. Quantitative brain magnetic resonance imaging in attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry.* 1996;53:607-616.
21. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.* Washington, DC: American Psychiatric Association; 1994.
22. Werry JS, Sprague RL, Cohen MN. Conners' Teacher Rating Scale for use in drug studies with children: an empirical study. *J Abnorm Child Psychol.* 1975;3:217-229.
23. Conners CK. *Conners' Rating Scales-Revised User's Manual.* North Tonawanda, NY: Multi-Health Systems Inc; 1997.
24. Reich W. Diagnostic Interview for Children and Adolescents (DICA). *J Am Acad Child Adolesc Psychiatry.* 2000;39:59-66.
25. Achenbach TM, Ruffle TM. The Child Behavior Checklist and related forms for assessing behavioral/emotional problems and competencies. *Pediatr Rev.* 2000;21:265-271.
26. Wechsler D. *Manual for the Wechsler Intelligence Scale for Children-Revised.* New York, NY: Psychological Corp; 1974.
27. Wechsler D. *Wechsler Intelligence Scale for Children.* 3rd ed. San Antonio, Tex: Psychological Corp; 1991.
28. Denckla MB. Revised physical and neurological examination for subtle signs. *Psychopharmacol Bull.* 1985;21:773-800.
29. Wechsler D. *Wechsler Abbreviated Scale of Intelligence (WASI).* San Antonio, Tex: Psychological Corp; 1999.
30. Wechsler D. *Manual for the Wechsler Preschool and Primary Scale of Intelligence.* New York, NY: Psychological Corp; 1967.
31. Wechsler D. *WAIS-R Manual: Wechsler Adult Intelligence Scale-Revised.* New York, NY: Psychological Corp; 1981.
32. Kruesi MJP, Lenane MC, Hibbs ED, Major J. Normal controls and biological reference values in child psychiatry: defining normal. *J Am Acad Child Adolesc Psychiatry.* 1990;29:449-452.
33. Clinical Global Impressions. *Psychopharmacol Bull.* 1985;21:839-843.
34. Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. *J Comput Assist Tomogr.* 1994;18:192-205.
35. Collins DL, Holmes CJ, Peters TM, Evans AC. Automatic 3-D model-based neuroanatomical segmentation. *Hum Brain Mapp.* 1995;3:190-208.
36. Paus T, Zijdenbos A, Worsley K, et al. Structural maturation of neural pathways in children and adolescents: in vivo study. *Science.* 1999;283:1908-1911.
37. Diggle PJ. *The Analysis of Longitudinal Data*. New York, NY: Oxford University Press; 1994.
38. Hand DJ, Crowder MJ. *Practical Longitudinal Data Analysis*. Boca Raton, Fla: CRC Press; 1996.
39. Giedd JN, Jeffries NO, Blumenthal J, et al. Childhood-onset schizophrenia: progressive brain changes during adolescence. *Biol Psychiatry.* 1999;46:892-898.
40. Giedd JN, Blumenthal J, Jeffries NO, et al. Brain development during childhood and adolescence: a longitudinal MRI study. *Nat Neurosci.* 1999;2:861-863.
41. Giedd JN, Blumenthal J, Jeffries NO, et al. Development of the human corpus callosum during childhood and adolescence: a longitudinal MRI study. *Prog Neuropsychopharmacol Biol Psychiatry.* 1999;23:571-588.
42. Reynolds CR. Critical measurement issues in learning disabilities. *J Spec Educ.* 1984;18:451-476.
43. Good CD, Johnsrude I, Ashburner J, et al. Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage.* 2001;14:685-700.
44. Nopoulos P, Flaum M, O'Leary D, Andreasen NC. Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. *Psychiatry Res.* 2000;98:1-13.
45. Paus T, Collins DL, Evans AC, et al. Maturation of white matter in the human brain: a review of magnetic resonance studies. *Brain Res Bull.* 2001;54:255-266.
46. Breggin PR. *Talking Back to Ritalin: What Doctors Aren't Telling You About Stimulants for Children.* Monroe, Me: Common Courage Press; 1998.
47. Hart EL, Lahey BB, Loeber R, Appelgate B, Frick PJ. Developmental change in attention-deficit/hyperactive disorder in boys: a four year longitudinal study. *J Abnorm Child Psychol.* 1995;23:729-749.
48. Eaton WO, McKeen NA, Campbell DW. The waxing and waning of movement: implications for psychological development. *Dev Rev.* 2001;21:205-223.
49. Rapoport JL, Giedd JN, Blumenthal J, et al. Progressive cortical change during adolescence in childhood-onset schizophrenia: a longitudinal magnetic resonance imaging study. *Arch Gen Psychiatry.* 1999;56:649-654.
50. Bullmore ET, Suckling J, Overmeyer S, et al. Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Trans Med Imaging.* 1999;18:32-42.
51. Overmeyer S, Bullmore ET, Suckling J, et al. Distributed grey and white matter deficits in hyperkinetic disorder: MRI evidence for anatomical abnormality in an attentional network. *Psychol Med.* 2001;31:1425-1435.
52. Lange N, Giedd JN, Castellanos FX, et al. Variability of human brain structure size: ages 4 to 20. *Psychiatry Res.* 1997;74:1-12.
53. Sharp WS, Walter JM, Marsh WL, et al. ADHD in girls: clinical comparability of a research sample. *J Am Acad Child Adolesc Psychiatry.* 1999;38:40-47.
54. Castellanos FX, Tannock R. Neuroscience of attention-deficit hyperactivity disorder: the search for endophenotypes. *Nat Rev Neurosci*. 2002;3:617-628.