ORIGINAL ARTICLE

Polymorphisms of the Dopamine D_4 Receptor, Clinical Outcome, and Cortical Structure in Attention-Deficit/Hyperactivity Disorder

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Context: Attention-deficit/hyperactivity disorder (ADHD) is one of the most heritable neuropsychiatric disorders, and a polymorphism within the dopamine D_4 receptor (*DRD4*) gene has been frequently implicated in its pathogenesis.

Objective: To examine the effects of the 7-repeat microsatellite in the *DRD4* gene on clinical outcome and cortical development in ADHD. We drew comparisons with a single nucleotide polymorphism in the dopamine D_1 receptor (*DRD1*) gene, which was associated with ADHD within our cohort, and a polymorphism within the dopamine transporter (*DAT1*) gene, reported to have additive effects with the *DRD4* 7-repeat allele.

Design: Longitudinal cohort study.

Setting: National Institutes of Health, Bethesda, Maryland.

Participants: One hundred five children (with 222 neuroanatomical magnetic resonance images) with ADHD (mean age at entry, 10.1 years) and 103 healthy controls (total of 220 magnetic resonance images). Sixty-seven subjects with ADHD (64%) had follow-up clinical evaluations (mean follow-up, 6 years).

Main Outcome Measures: Cortical thickness across the cerebrum and presence of *DSM-IV*–defined ADHD at follow-up.

Results: Possession of the *DRD4* 7-repeat allele was associated with a thinner right orbitofrontal/inferior prefrontal and posterior parietal cortex. This overlapped with regions that were generally thinner in subjects with ADHD compared with controls. Participants with ADHD carrying the *DRD4* 7-repeat allele had a better clinical outcome and a distinct trajectory of cortical development. This group showed normalization of the right parietal cortical region, a pattern that we have previously linked with better clinical outcome. By contrast, there were no significant effects of the *DRD1* or *DAT1* polymorphisms on clinical outcome or cortical development.

Conclusions: The *DRD4* 7-repeat allele, which is widely associated with a diagnosis of ADHD, and in our cohort with better clinical outcome, is associated with cortical thinning in regions important in attentional control. This regional thinning is most apparent in childhood and largely resolves during adolescence.

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ATTENTION-DEFICIT/HYPERactivity disorder (ADHD) is among the most heritable of neuropsychiatric disorders.[1] In keeping with a presumed polygenic disorder, several candidate genes have been proposed in its pathogenesis. One of the most consistently replicated polymorphisms associated with the disorder is the 7-repeat form of the 48–base pair (bp) variable number tandem repeat (VNTR) in exon 3 of the dopamine D_4 receptor (*DRD4*) gene, with meta-analysis estimating a pooled odds ratio for case-control studies of 1.45 and for family-based studies, 1.16.[1] We recently also replicated this association, finding a significantly increased frequency of the 7-repeat allele in 169 children with ADHD (23%) compared with 265 healthy controls (17%).[2] Previous studies have suggested that carriers of the risk allele may also have a unique neuropsychological,[3-6] clinical,[7-13] and pharmacological[14] profile, although there remains considerable debate over the exact nature of this phenotype.

The *DRD4* 7-repeat allele has also been linked with clinical outcome, albeit with divergent results. Recently, we ascertained the clinical status of 67 subjects from our cohort (mean [SD] age, 15 [3] years) at a mean follow-up of 6 years. We found that those with at least 1 copy of the *DRD4* 7-repeat allele were significantly less likely ($P=.01$) to retain the diagnosis of

Table 1. Demographic and Clinical Details of Groups of Those With ADHD and Healthy Controls Stratified by Carrier Status for the 7-Repeat Allele of the *DRD4* Gene

	ADHD 7-Repeat Allele Carrier (n=43)	ADHD Noncarrier (n=62)	Healthy 7-Repeat Allele Carrier (n=35)	Healthy Noncarrier (n=68)	Test Statistic	*P* Value
Demographic						
Sex, No.					χ^2=3.9	.28
M	23	27	17	41		
F	20	35	18	27		
Estimated IQ, mean (SD)	113 (15)	106 (15)	116 (13)	116 (13)	$F_{3,199}$=5.9	.001
Right handed, No. (%)	36 (84)	54 (87)	31 (88)	64 (94)	χ^2=3.26	.35
Race, No. (%)					White vs other, χ^2_3=2.3	.52
White, non-Hispanic	31 (72)	48 (77)	28 (80)	57 (84)		
Black	5 (12)	9 (14)	4 (11)	7 (10)		
White, Hispanic	5 (12)	4 (7)	0	1 (1.5)		
Asian	0	0	1 (3)	2 (3)		
Other	2 (4)	1 (2)	2 (6)	1 (1.5)		
Scanning details, total No. at each wave						
1 Scan	43	62	35	68		
2 Scans	25	45	22	44		
≥3 Scans	25	22	14	37		
Age at each scan, y, mean (SD)						
Time 1	10.2 (2.7)	10.1 (2.9)	10.3 (2.8)	10 (2.9)	$F_{3,204}$=0.14	.94
Time 2	13.6 (3.7)	12.8 (3.1)	12.7 (3.1)	12.2 (3.5)	$F_{3,132}$=0.89	.45
Time 3	16.6 (3.9)	15.4 (2.9)	14.6 (2.6)	14.3 (3.4)	$F_{3,94}$=2.4	.07
Outcome						
Final CGAS score, mean (SD)	64 (11)	59 (9)			t_{65}=1.7	.09
DSM-IV criteria at follow-up, No. (%)					Proportion retaining diagnosis of combined-type ADHD vs other, χ^2_1=6.1	.01
Combined type	6 (21)	20 (51)				
Inattentive subtype	15 (54)	7 (18)				
Hyperactive subtype	2 (7)	4 (10)				
In full remission	5 (18)	8 (21)				
Baseline characteristics						
CGAS score, mean (SD)	48 (6)	45 (6)			t_{88}=1.5	.13
CBCL Attention Problems T score, mean (SD)	74 (9)	72 (8)			t_{94}=0.65	.50
CBCL Externalizing Problems T score, mean (SD)	66 (9)	68 (10)			t_{94}=0.89	.38
TRF Attention Problems T score, mean (SD)	71 (11)	68 (9)			t_{88}=1.16	.25
Conners Teacher Hyperactivity Factor score, mean (SD)	1.4 (0.6)	1.7 (0.6)			t_{86}=2.1	.04
Conners Parent Hyperactivity Factor score, mean (SD)	2.2 (0.8)	2.4 (0.7)			t_{61}=1.0	.33
Family history of ADHD, No./total No. (%)	24/41 (59)	43/56 (77)			χ^2_1=3.7	.06
Comorbidity, No.						
Oppositional defiant disorder	19	23				
Conduct disorder	3	5				
Learning disorder	7	6				
Mood disorder	1	2				
Anxiety disorder	4	7				
Tic disorder, NOS	5	4				
Medication, No./total No. (%)						
Stimulants at baseline	32/43 (74)	50/62 (81)			χ^2_1=0.45	.48
Stimulants at follow-up	20/29 (69)	32/41 (62)			χ^2_1=0.74	.39

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; CGAS, Children's Global Assessment Scale; *DRD4*, dopamine D_4 receptor; NOS, not otherwise specified.

combined-type ADHD. Six of 28 subjects (21%) with the risk allele had combined-type ADHD at follow-up compared with 20 of 39 subjects with ADHD (51%) without the risk allele (**Table 1** and **Table 2**). However, others have found either no relationship between the outcome and the *DRD4* 7-repeat allele, or worse outcome.[6,10]

In addition to behavioral phenotyping, brain structure may be a particularly informative intermediate phenotype that lies closer to the biology of ADHD. In one of the few studies at this level, Durston and colleagues[15] found that unaffected siblings of probands with ADHD who were homozygous for the most common *DRD4* allele (the 4-repeat) had smaller prefrontal gray matter volumes than those with other variants. In an earlier report, we failed to detect any relationship between lobar volumes and the *DRD4* 7-repeat allele but noted our find-

Table 2. Additive Genetic Effects for *DRD4* and Either the *DAT1* Risk or *DRD1* Risk Genotype on IQ and Clinical Outcome

	DRD4 and *DAT1*: No. of Risk Genotypes					*DRD4* and *DRD1*: No. of Risk Genotypes				
	0	1	2	Test Statistic	*P* Value	0	1	2	Test Statistic	*P* Value
Clinical outcome[a]										
ADHD	9/19 (47)	14/31 (45)	1/12 (8)	χ^2_2=5.8	.05	6/10 (60)	12/36 (33)	5/17 (29)	χ^2_2=2.9	.23
IQ[b]										
ADHD	110 (14)	109 (10)	108 (12)	β=−0.86; SE=2.4; *t*=0.31	.72	103 (17)	109 (14)	113 (16)	β=5.0; SE=2.0; *t*=2.2	.03
Healthy controls	115 (16)	118 (13)	120 (14)	β=2.4; SE=2.4; *t*=0.99	.33	116 (14)	115 (14)	120 (11)	β=2.0; SE=2.0; *t*=1.0	.31

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; *DAT1*, dopamine transporter; *DRD1*, dopamine D_1 receptor; *DRD4*, dopamine D_4 receptor; SE, standard error.

[a] For clinical outcome, the difference is given as number/total number (percentage) of those with each genotype combination who retained the diagnosis of combined-type ADHD at follow-up.

[b] For IQ, presented as mean (SD), the significance of the relationship between total number of risk genotypes and IQ was determined through linear regression.

ing was preliminary given the small sample size of 41 affected probands.[16] A limitation of most previous studies is that they have not genotyped healthy controls. Thus, it is impossible to tell whether the 7-repeat allele acts in a similar manner in healthy controls or whether the allele has diagnostically specific effects.

We include a comparison with the neuroanatomical effects of a biallelic single nucleotide polymorphism in the dopamine D_1 (*DRD1*) receptor gene (a C/T variant in the 5′ untranslated region of the gene), the only other polymorphism associated with the diagnosis of ADHD in our cohort.[17] However, unlike the *DRD4* 7-repeat allele, as we will show, the *DRD1* polymorphism showed no association with clinical outcome in our cohort and there is no evidence of an associated phenotype.[18,19]

The 10-repeat allele of a VNTR within the dopamine receptor gene (*DAT1*) has also been linked with ADHD[1] and recently reported to have additive effects with the *DRD4* 7-repeat allele in determining lower IQ and poor outcome in ADHD.[6] We sought to replicate these findings and to explore possible cortical effects of the *DAT1* polymorphism, a priori unlikely given the paucity of cortical expression of the *DAT1* gene.[20]

In this neuroanatomical study, we used a measure of cortical thickness estimated at more than 40 000 cortical points, which is fully automated, reliable, and valid.[21,22] By applying this measure to our cohort, we previously identified a significantly thinner medial and superior prefrontal and orbitofrontal cortex in children with ADHD, regions important for attentional control.[23] In the current study, we hypothesized that the *DRD4* 7-repeat allele would be associated with a similar pattern of cortical change in view of the accumulating evidence of an associated cognitive and behavioral phenotype. We also recently reported that normalization of the thickness of the right parietal cortex characterizes subjects with ADHD with a better clinical outcome.[23] In the current study, we further predicted that subjects with ADHD with the *DRD4* 7-repeat allele would show normalization of the right parietal cortex, in keeping with their better clinical outcome. Inclusion of the effects of polymorphisms in the *DRD1* and *DAT1* genes allowed us both to test for additive genetic effects and to assess the specificity of the findings for the *DRD4* 7-repeat allele. In particular, we did not expect the distinctive pattern of right parietal cortical normalization to emerge with risk alleles that are not associated with clinical outcome in our cohort.

METHODS

SUBJECTS

One hundred five children with *DSM-IV*–defined ADHD with both neuroanatomical magnetic resonance images (MRIs) and DNA were included. Diagnosis was based on the Parent Diagnostic Interview for Children and Adolescents,[24] the Conners Teacher Rating Scales,[25] and the Teacher Report Form.[25] Exclusion criteria were IQ lower than 80 and evidence of medical or neurological disorders. All had combined-type ADHD at baseline. Data were available on the diagnostic status at a mean (SD) follow-up of 6 (3) years on 67 subjects (64%).

Unrelated healthy controls were also recruited from the same community using local media and school contacts (data available on request). Each subject completed the Childhood Behavior Checklist as a screening tool and then underwent a structured diagnostic interview by a child psychiatrist to rule out any psychiatric or neurological diagnoses.[26] Genotyping details are available at http://intramural.nimh.nih.gov/chp/AGP_ADHD_genetics2007.

The institutional review board of the National Institutes of Health approved the research protocol, and written informed consent and assent to participate in the study were obtained from the parents and children, respectively.

MRI ACQUISITION AND IMAGE ANALYSIS

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 on a 1.5-T General Electric Signa scanner (Milwaukee, Wisconsin) (echo time of 5 milliseconds, repetition time of 24 milliseconds, flip angle of 45°, acquisition matrix of 256×192, number of signals acquired, 1, and 24-cm field of view). The native MRIs were registered into standardized stereotaxic space using a linear transformation and corrected for nonuniformity artifacts.[27] The registered and corrected volumes were segmented into white matter, gray matter, cerebrospinal fluid, and background using an advanced neural net classifier.[28] A surface de-

formation algorithm was applied, which first fits the white matter surface, then expands outward to find the gray matter–cerebrospinal fluid intersection, defining a known relationship between each vertex of the white matter surface and its gray matter surface counterpart; cortical thickness can thus be defined as the distance between these linked vertices (a total of 40 962 such vertices are calculated).[29] The white and gray matter surfaces were resampled into native space by inverting the initial stereotaxic transformation. Cortical thickness was then computed in native space. To improve the ability to detect population changes, each subject's cortical thickness map was blurred using a 30-mm surface-based blurring kernel.[22]

STATISTICAL ANALYSIS

Demographic and clinical characteristics between groups at baseline were examined using 2-sample *t* tests or analysis of variance for continuous variables and χ^2 tests of independence for categorical variables.

In neuroanatomical analyses, we examined for both main effects and interactions between diagnostic group (ADHD vs healthy controls) and genotype (comparing all those with the risk genotype against those without the risk genotype). Mixed-model regression was used because it permits the inclusion of multiple measurements per person at different ages and irregular intervals between measurements, thereby increasing statistical power.[30] Initial analyses estimated group differences across the cortex and included a random-effect modeling within-person dependence and controlled for age. Thus, in the model for the group comparisons, the *i*th individual's *j*th cortical thickness at a given vertex was modeled as:

$$\text{Thickness}_{ij} = \text{Intercept} + d_i + \beta_{\text{group}}\,(\text{Diagnosis} = \text{Group}) + \beta_1(\text{Age}) + e_{ij}$$

where d_i is a random effect, the intercept and β terms are fixed effects, and e_{ij} represents the residual error. Results are reported both at an unadjusted $P = .05$ and following correction for multiple comparisons, which was made using the false discovery rate procedure with $q = 0.05$.[31,32] A false discovery rate threshold was determined for the statistical model using all *P* values pooled across all effects included in the model. Analyses were repeated, entering variables that differed significantly between groups as covariates.

Initial longitudinal analyses estimated the full quadratic model at each vertex but because the squared age term did not contribute significantly to the model across the cortex, a linear model, which included a term modeling the interaction of group and time, was used to fit the trajectories. Group differences in the slope of the trajectory of growth were determined by the significance of the term for the interaction of age and group. In line with the second hypothesis, we conducted planned pairwise contrasts between the ADHD *DRD4* 7-repeat carriers and healthy controls (both combined and split by *DRD4* genotype). Similar contrasts were made between the *DRD1* carriers of the risk allele, the *DAT1* 10-repeat allele homozygotes, and healthy controls.

Statistics at every cortical point were visualized through projection onto a standard brain template. Such visualization showed distinct clusters of cortical points that differed significantly between the groups. Follow-up analyses examined the average cortical thickness for each of these clusters. Graphs illustrating the developmental trajectories of clusters were generated using fixed-effects parameter estimates.

Risk genotypes were defined as follows: for the *DRD4* gene, possession of at least 1 copy of the 7-repeat allele; for *DAT1*, homozygosity of the 10-repeat allele; and for *DRD1*, possession of at least 1 copy of the C allele. We tested for additive effects of risk genotypes using likelihood-ratio tests at each cortical point to assess if the addition of the *DRD1* or the *DAT1* genotype significantly improved on a model that included only diagnosis and the *DRD4* genotype.

RESULTS

GENOTYPING

The most common *DRD4* allele was the 4-repeat (64% in subjects with ADHD and 72% in healthy controls), followed by the 7-repeat (23% in subjects with ADHD and 17% in controls) and the 2-repeat (10% in subjects with ADHD and 4% in controls). Other variants were rare. Overall, 45% of the subjects with ADHD and 34% of the healthy control group were designated at risk on the basis of possession of at least 1 *DRD4* 7-repeat allele. The most common *DAT1* allele was the 10-repeat (71% in both the subjects with ADHD and healthy controls), followed by the 9-repeat (27% in subjects with ADHD and 28% in controls), with other variants at less than 2% in both groups. Overall, 48.4% of the subjects with ADHD and 49.2% of the healthy control group were *DAT1* 10-repeat allele homozygotes. These frequencies are in line with those reported elsewhere.[6,33] For the single nucleotide polymorphism in the *DRD1* gene, the C allele was more common in those with ADHD (42% compared with 32% in the healthy controls). Overall, 62% of the subjects with ADHD were designated at risk by virtue of possessing at least 1 copy of the risk allele, compared with 52% of the healthy controls. Genotypes were in Hardy-Weinberg equilibrium.

DEMOGRAPHIC, CLINICAL, AND NEUROPSYCHOLOGICAL CHARACTERISTICS

The *DRD4* groups were similar in demographic characteristics. Those with the 7-repeat allele had lower teacher ratings of hyperactivity at baseline but did not differ in parental ratings of hyperactivity, nor in any measures of inattentive symptom scores, other baseline clinical variables, or treatment histories. There was a trend to a stronger family history of ADHD in those without the risk allele. Subjects with ADHD without the 7-repeat allele had a significantly lower IQ than all other groups (Table 1). Additionally, a significantly smaller proportion of ADHD *DRD4* 7-repeat allele carriers still had combined-type ADHD at follow-up (21%) than noncarriers (51%). There was a trend to a higher Children's Global Assessment Scale score (indicating better overall function) at follow-up in the ADHD 7-repeat allele carriers.

The ADHD group did not differ significantly by *DRD1* risk genotype on clinical or neuropsychological measures, including clinical outcome (data available at http://intramural.nimh.nih.gov/chp/AGP_ADHD_genetics2007. Subjects with ADHD with the *DAT1* risk genotype had a lower mean (SD) IQ at 105 (15) than all other groups (ADHD nonrisk mean [SD] IQ, 114 [16]; healthy controls with risk genotype mean [SD] IQ, 119 [13]; and healthy controls without risk genotype mean [SD] IQ, 116 [16]. The difference was significant ($F_{3,155} = 6.7$; $P < .001$), and subjects with ADHD with the *DAT1* risk genotype had a lower IQ than all other groups

($P<.05$). There was no significant effect of the *DAT1* risk genotype on clinical outcome (data available at http://intramural.nimh.nih.gov/chp/AGP_ADHD_genetics2007.

ADDITIVE GENE EFFECTS

To assess for additive effects of risk genotype, children received a score of 0 if they carried no risk genotypes, 1 if they had 1 risk genotype, and so on. A greater number of *DRD4* and *DAT1* risk genotypes was significantly associated with a better clinical outcome, although this association was driven mainly by the better outcome of the *DRD4* 7-repeat allele group (Table 2). There was no additive effect of the *DRD4* and *DRD1* risk genotypes on outcome. There was, however, an additive effect of the *DRD4* and *DRD1* risk genotypes in IQ, with an increasing total number of risk genotypes associated with a higher IQ (again attributable mainly to the *DRD4* group). This was not found in IQ for the combined effect of risk genotypes for *DRD4*. There was no association between total number of all 3 risk genotypes with either clinical outcome or IQ. A similar pattern of results was found when analyses were conducted using the total number of risk alleles (rather than risk genotype) (data available at http://intramural.nimh.nih.gov/chp/AGP_ADHD_genetics2007).

NEUROANATOMY

There was a significant main effect of diagnosis, with the ADHD group having a thinner cortex in the right, orbitofrontal, superior/medial prefrontal, and posterior parietal cortices (**Figure 1**A). There was also a significant main effect of the *DRD4* genotype, comparing all those (both subjects with ADHD and healthy controls) with the 7-repeat allele and those without the allele in very similar cortical regions (Figure 1B). The diagnostic main effect survived adjustment for multiple comparisons, whereas the genotypic main effect did not. There were no significant interactions between diagnostic group and the *DRD4* risk genotype.

As a result of these overlapping main effects and their order (cortex thinner in subjects with ADHD than healthy controls and thinner in *DRD4* 7-repeat allele carriers than noncarriers), there was a significant stepwise increase in cortical thickness in the right orbitofrontal/inferior frontal ($\beta=0.1$; SE=0.03; $t=3.8$; $P<.001$) and right parieto-occipital regions ($\beta=0.09$; SE=0.02; $t=3.9$; $P<.001$) (**Figure 2**). The ADHD 7-repeat allele carriers had the thinnest cortex, followed by the ADHD noncarriers, then the healthy 7-repeat allele carriers, and, finally, the healthy noncarriers. The regional differences between the ADHD *DRD4* 7-repeat allele carriers and healthy noncarriers were highly significant and survived adjustment for multiple comparisons (**Figure 3**).

Analyses were repeated including only non-Hispanic white individuals and the same pattern of significant results held (details available at http://intramural.nimh.nih.gov/chp/AGP_ADHD_genetics2007). Adjusting for age, sex, and IQ as covariates accentuated the differences between the ADHD 7-repeat allele carriers and healthy noncarriers. The pattern of significant results held when those with comorbid conduct disorder were excluded.

By contrast, the *DAT1* and *DRD1* risk genotypes were associated with very limited significant cortical thinning (Figure 1C and D). The overlap between the main effects for these risk genotypes and the main effect for diagnosis was minimal.

LONGITUDINAL ANALYSES

We predicted that ADHD *DRD4* 7-repeat allele carriers would converge to the developmental trajectory of the healthy controls. This prediction was confirmed: the ADHD 7-repeat allele carriers had a significantly different trajectory from the healthy controls in a region of the right supramarginal/angular gyrus ($t=2.6$; $P=.009$) and right inferior frontal/lateral orbitofrontal cortex ($t=2.3$; $P=.02$) (**Figure 4**). The ADHD noncarriers did not differ from healthy controls. When the healthy controls were further divided on the basis of genotype, the greatest trajectory difference was seen between healthy noncarriers and the ADHD 7-repeat allele carriers: for the right angular gyral region, $t=2.5$; $P=.01$, and for the right lateral orbitofrontal region, $t=2.6$; $P=.009$.

As a result of the trajectory differences, the group differences varied with age, estimated from the regression lines. In the right orbitofrontal regions, the ADHD carriers started at age 7 years from a significantly thinner cortex relative to healthy noncarriers ($t=-4$; $P<.001$), healthy carriers ($t=-1.8$; $P=.07$), and ADHD noncarriers ($t=-2.0$; $P=.05$) (**Figure 5**). However, group differences resolved by age 17 years (all pairwise contrasts, $P>.10$). Similarly, in the posterior region, the cortex was significantly thinner in ADHD carriers at age 7 years relative to healthy noncarriers ($t_{227}=-3.4$; $P=.001$), healthy carriers ($t=2$; $P=.05$), and ADHD noncarriers ($t=2.5$; $P=.01$). Again, no significant group differences were apparent by age 17 years (all $P>.10$).

By contrast, carriers of the risk *DRD1* polymorphism did not differ in trajectory at any cortical point from the healthy controls (either combined or split into carriers and noncarriers) nor from subjects with ADHD without the risk allele. Likewise, subjects with ADHD homozygous for the *DAT1* 10-repeat allele did not differ in trajectories from their ADHD counterparts who were not homozygous for the 10-repeat allele, nor from healthy controls. Likelihood ratio tests demonstrated that the addition of neither the *DAT1* nor *DRD1* genotype improved on a model that included just diagnosis and the *DRD4* genotype.

COMMENT

We found that a polymorphism of the 48-bp VNTR in exon 3 of the *DRD4* gene was associated with differences in cortical thickness in the right orbitofrontal and posterior parieto-occipital cortex, regions important in the control of attention.[34,35] Similar regions were found to be thinner in those with ADHD compared with healthy controls. As a result of the overlapping main effects of genotype and diagnosis, there was a stepwise increment in cortical thickness in these regions, with subjects with ADHD with the *DRD4* 7-repeat allele having the thinnest cortex, followed by subjects with ADHD lacking the

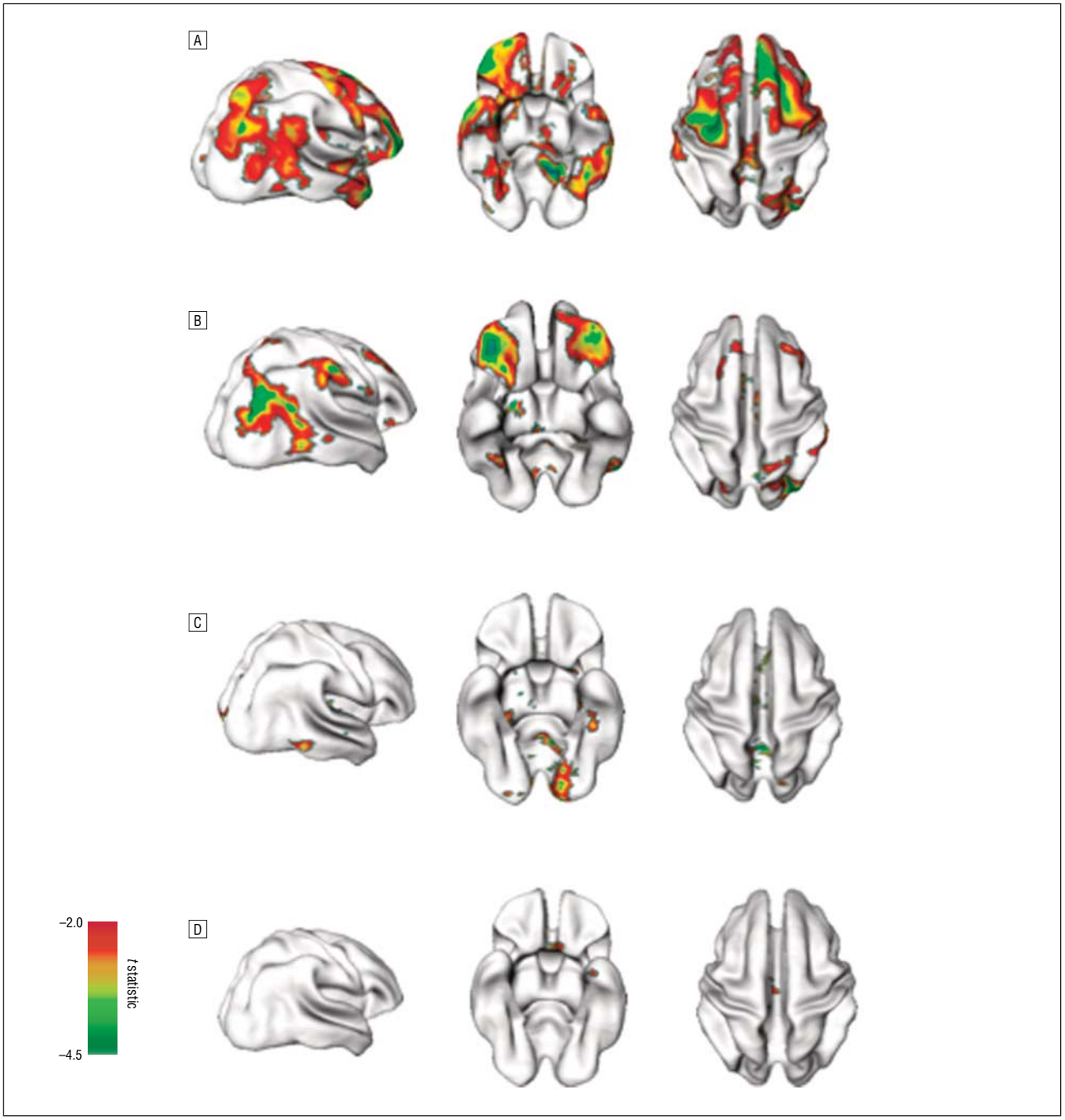

Figure 1. Overlapping main effects of diagnosis and genotype. In each panel, there is (from left to right) a right lateral, inferior, and superior view of a brain template. A, Regions where the attention-deficit/hyperactivity disorder (ADHD) group has a significantly thinner cortex vs healthy controls ($t>2$; unadjusted $P<.05$). B, Regions where carriers of the dopamine D_4 receptor (*DRD4*) 7-repeat allele have a thinner cortex vs noncarriers. C, Regions where the dopamine transporter (*DAT1*) 10-repeat allele homozygotes have a thinner cortex vs others. D, Regions where the dopamine D_1 receptor (*DRD1*) risk allele carriers have a thinner cortex vs others (B-D, combining all subjects regardless of diagnosis).

7-repeat allele, healthy 7-repeat allele carriers, and finally by healthy noncarriers. There was no significant interaction between genotype and diagnosis and thus no regions where possession of the 7-repeat allele had significantly different effects on cortical thickness in the ADHD compared with the healthy group. Longitudinal analyses demonstrated that the neuroanatomical correlates of genotype were most apparent early in development and resolved by late adolescence. Additionally, ADHD carriers of the 7-repeat allele had better clinical outcome and showed normalization of the right parietal cortex, a region we previously associated with better clinical outcome.[23] Two other polymorphisms of the dopamine transmitter system, in the *DRD1* and *DAT1* genes, lacked these neuroanatomical correlates. This highlights the specificity of our findings for the *DRD4* polymorphism and is consistent with the less robust evidence for association between single nucleotide polymorphisms in the *DRD1* gene and ADHD[36] and the paucity of cortical expression of the *DAT1* gene.[15,20]

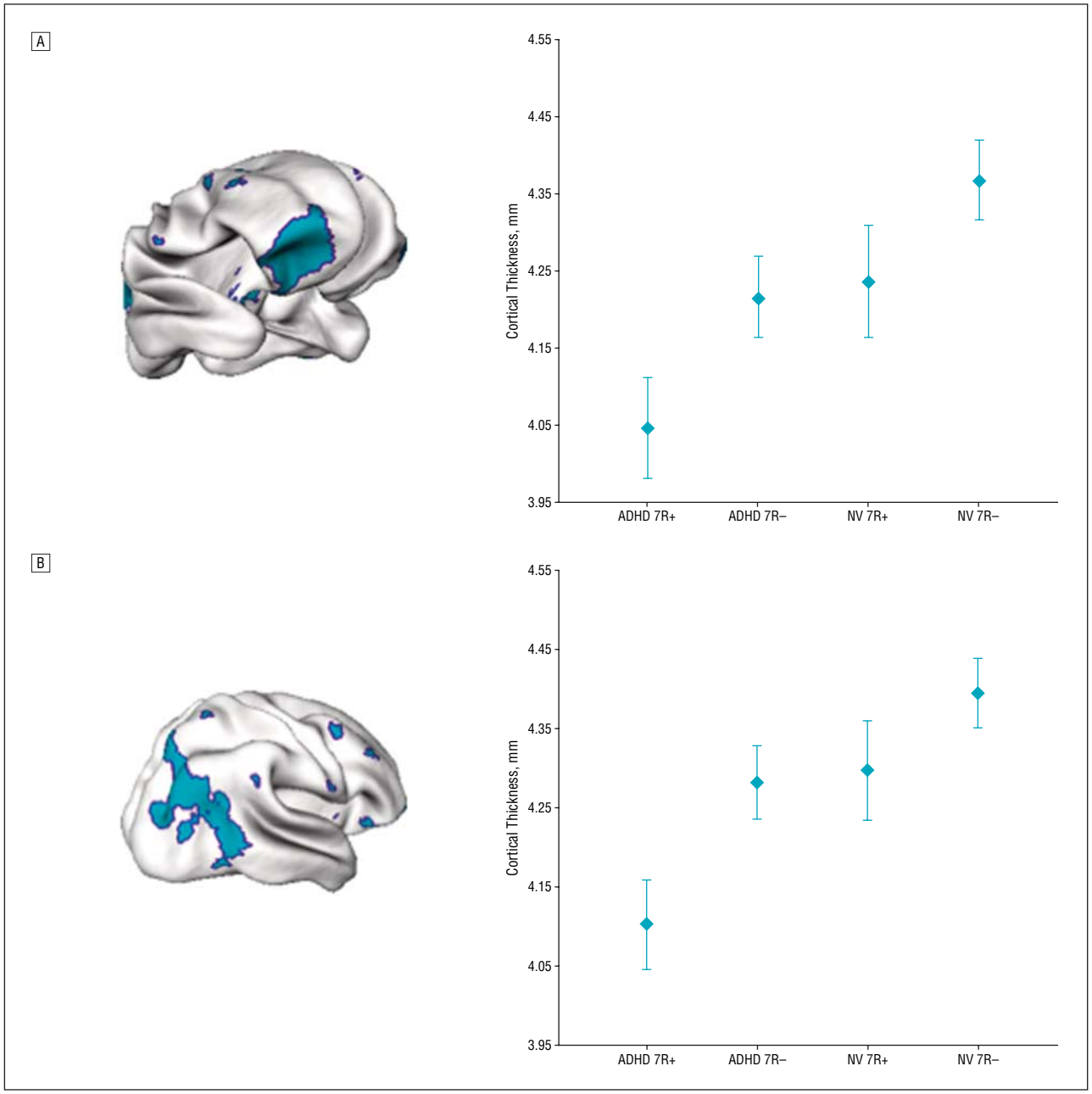

Figure 2. Brain maps showing the cortical regions where the main effects of diagnosis and dopamine D_4 receptor (*DRD4*) genotype overlapped. Accompanying graphs of mean (SEM) thickness in the right orbitofrontal/inferior frontal region (A) and right posterior parieto-occipital region (B). ADHD indicates attention-deficit/hyperactivity disorder; 7R+, carrier of the *DRD4* 7-repeat allele; 7R–, noncarrier of the *DRD4* 7-repeat allele; NV, healthy controls.

The overlapping main effects of diagnosis and *DRD4* genotype in regions important for the control of attention suggest similar neuroanatomical changes associated with the *DRD4* 7-repeat allele in those with ADHD and typically developing children. This is congruent with the concept of ADHD as representing the extremes of normally distributed traits with multiple genetic determinants.[37,38] According to this model, the effects of the relatively common *DRD4* 7-repeat allele summate or interact with multiple other genetic and nongenetic factors in the pathogenesis of ADHD.

It has been proposed that the *DRD4* 7-repeat allele defines a subtype of ADHD characterized by relatively intact cognition and perhaps even advantageous traits in keeping with the status of the 7-repeat allele as a relatively new genetic variant under positive selection.[39] In this context, our finding of a higher IQ in subjects with ADHD with the 7-repeat allele is of note, although there are contradictory findings.[6]

Cross-sectional studies have found regional increases in cortical thickness to correlate with cognitive function, including enhanced verbal declarative and extinction memory, and with "fluid" intelligence in older, healthy subjects.[40-42] In children, gains in verbal knowledge are mirrored by change in the cortical thickness of speech areas.[43] While our current study demonstrates changes in cortical thickness and symptoms occurring in tandem, a future goal is to refine further our appreciation of cortical thickness

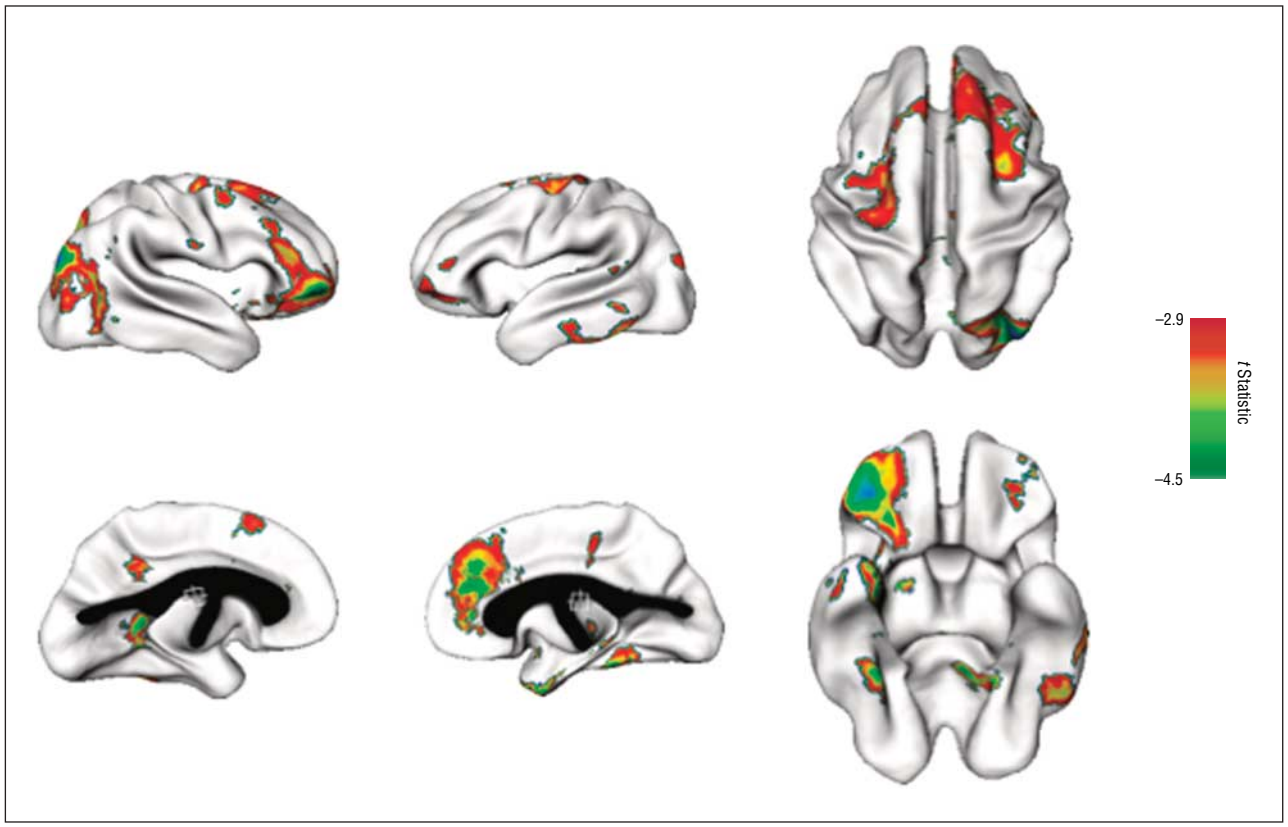

Figure 3. Cortical thinning in dopamine D_4 receptor (*DRD4*) 7-repeat allele carriers compared against healthy controls without the *DRD4* 7-repeat allele. Regions where the group differences were significant after adjustment for multiple comparisons are shown.

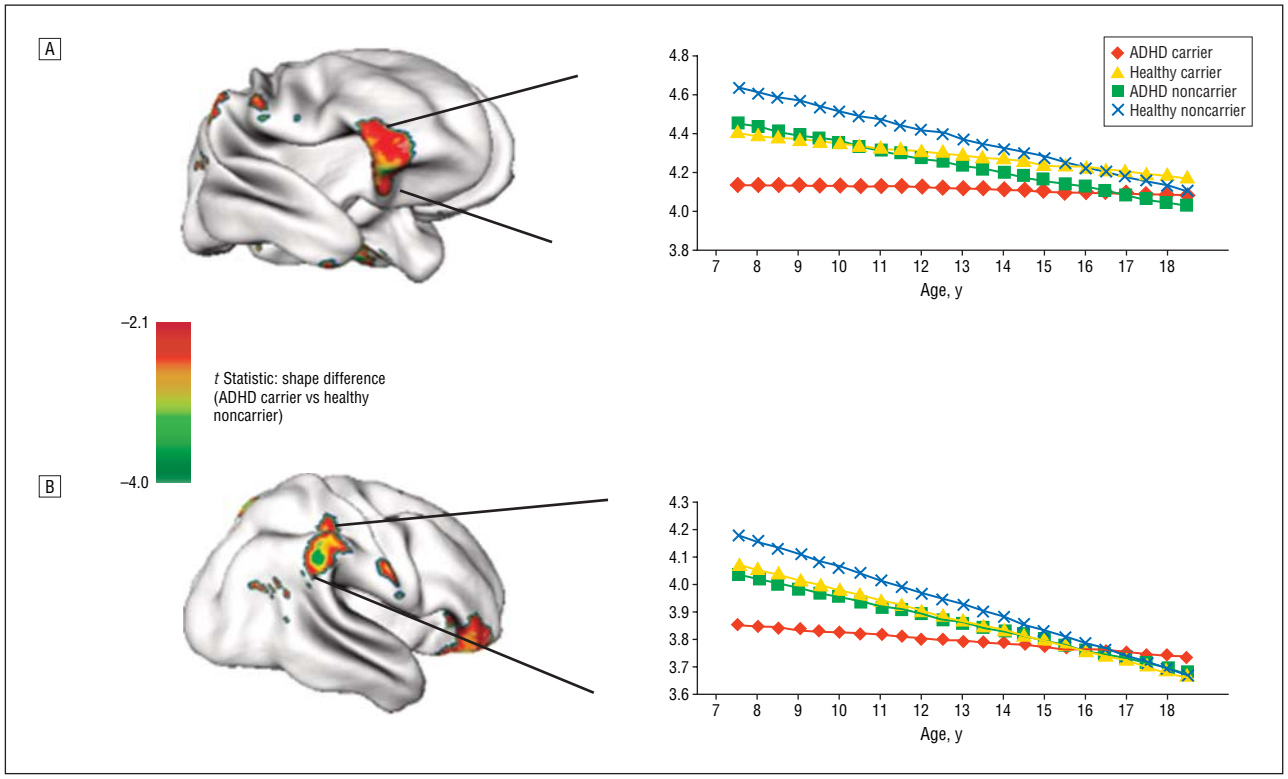

Figure 4. Brain maps of clusters where attention-deficit/hyperactivity disorder (ADHD) carriers of the 7-repeat allele differ in trajectory of cortical growth and graphs illustrating trajectories for these clusters. A, Right lateral orbitofrontal area, with a significant difference in shape between ADHD carriers and healthy noncarriers ($P = .01$). B, Right angular gyrus, with a significant difference between ADHD carriers and healthy noncarriers ($P = .007$).

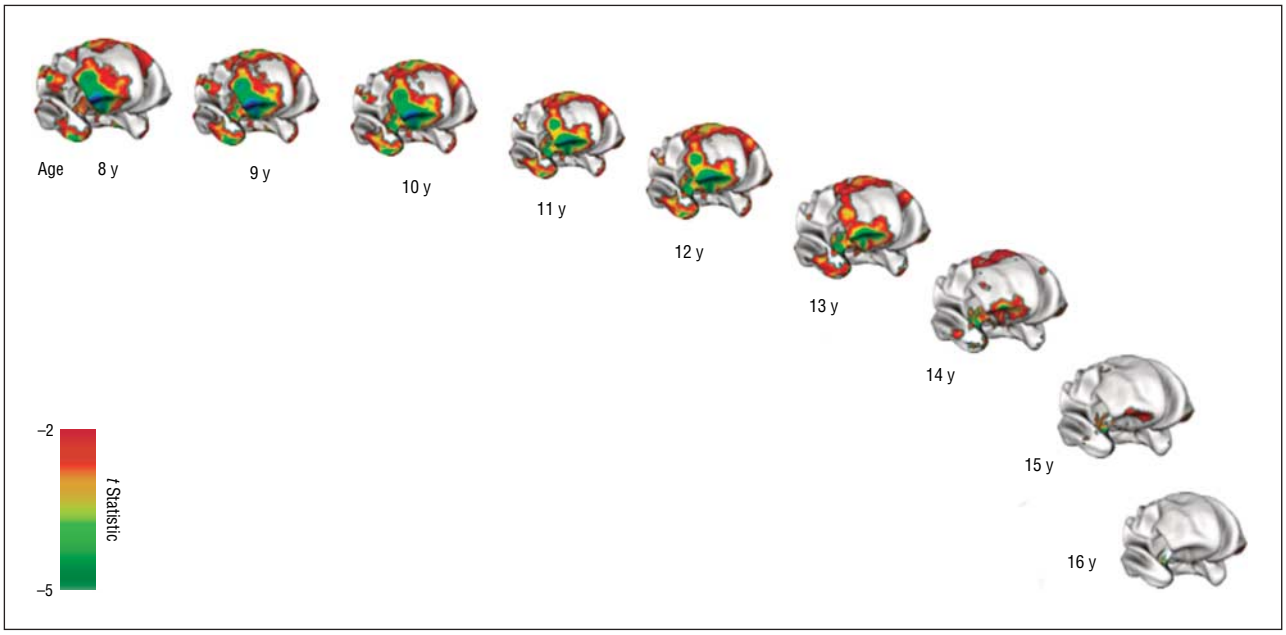

Figure 5. Cortical thinning at baseline in attention-deficit/hyperactivity disorder (ADHD) carriers of the 7-repeat allele corrects with age. Contrast between ADHD carriers and healthy controls without the 7-repeat allele from age 8 years through 16 years illustrating the resolution of regional cortical thinning with age (contrasts set at $t>2$).

by examining the links between this neuroanatomical variable and putative cognitive endophenotypes for ADHD, such as response inhibition and working memory.

LONGITUDINAL FINDINGS

Our longitudinal finding is unique in its links between genotype, clinical outcome, and cortical change. The localization of cortical normalization to right parietal cortical regions was predicted among the subjects with ADHD with the 7-repeat allele in view of their better clinical outcome. The region overlaps with the right inferior parietal cortical region, which showed functional plasticity in healthy controls following a working memory training intervention that ameliorates symptoms of ADHD in children.[44,45] The findings were also specific: neither the *DRD1* nor *DAT1* polymorphisms were individually linked with clinical outcome and neither polymorphism showed any pattern of cortical normalization. We cannot, however, infer causality between effects in our descriptive study; while genotype may determine better clinical outcome, which could in turn mold cortical development, it is equally plausible, for example, that genotype may directly influence cortical development and thus engender clinical improvement. While the *DRD4* genotype groups differed in baseline teacher ratings of hyperactivity, there was no difference in parental hyperactivity ratings at baseline, nor in any other clinical or treatment variable. Thus, initial clinical differences are unlikely to account for the different outcome related to genotype, especially because follow-up assessment mainly relied on information from the child and parent, but not the teacher. We were also unable to conduct analyses on the ADHD 7-repeat allele carriers further subdivided into those who improved and those who did not because there were only 6 subjects in the latter group, insufficient for our longitudinal analytic approach. This underscores the need for future multisite collaborations to allow an adequately powered test of the possibility that cortical normalization is particularly prominent within ADHD *DRD4* 7-repeat allele carriers who show clinical improvement. Theoretically, our longitudinal finding of cortical effects of the *DRD4* 7-repeat allele detected principally in childhood is compatible with differential gene expression during development, a finding congruent with twin studies, which suggests a combination of dynamic, as well as stable, genetic factors over time in ADHD.[46,47]

RELATIONSHIP WITH PREVIOUS STUDIES

We previously reported that (regardless of genotype) only the caudate, but not gray matter lobes, normalized in volume in ADHD[48] and did not find a link between lobar volumes and the *DRD4* 7-repeat allele.[16] We now expand on these earlier findings by demonstrating highly regional cortical change, undetectable at a volumetric lobar level, that related to *DRD4* genotype on an expanded genotyped sample of 103 subjects with ADHD (compared with 41 in our original genetic study). Our finding of a lack of effect of the *DAT1* risk genotype on cortical thickness is in keeping with a previous demonstration of no association with prefrontal gray matter volume among 30 subjects with ADHD and their unaffected siblings.[15] However, this study also found decreased prefrontal volume among carriers of the common *DRD4* 4-repeat allele (and thus presumably a relative increase in those with the 7-repeat allele), which is less congruent with our finding of a thinner cortex in the *DRD4* 7-repeat carriers.

Mill and colleagues[6] reported a worse outcome among *DRD4* 7-repeat allele carriers in a cohort of children with ADHD, an effect that was augmented when combined with

possession of the *DAT1* risk genotype. We found the reverse pattern, with significant additive effects favoring better clinical outcome, driven mainly by the association between better outcome and the *DRD4* 7-repeat allele. The discrepant results may reflect the relatively small sample sizes of both studies, with follow-up on 49 children with ADHD in the Mill and colleagues study and 65 children in our study. Also, in the Mill and colleagues epidemiological study, 62% of the ADHD population with outcome data had comorbid conduct disorder, compared with our selected clinical cohort, which had a high proportion of girls and low rates of comorbid conduct disorder. Given the possible relationship between the *DRD4* 7-repeat allele and combined ADHD and conduct disorder,[13,49] such differences are likely to be important. Additionally, to determine outcome, Mill and colleagues used an outcome scale measuring adult psychosocial adjustment rather than symptoms of ADHD, which we used. In the Mill and colleagues study, the worse outcome in the *DRD4* 7-repeat allele carriers did not remain after adjustment for differences in IQ, unlike our neuroanatomical findings, which became more pronounced after controlling for IQ. However, we did replicate the finding by Mill and colleagues of a lower IQ in association with homozygosity for the 10-repeat allele of the *DAT1* gene. Our phenotype of better outcome in ADHD carriers of the *DRD4* 7-repeat allele was not found by Barkley and colleagues.[10] However, the final assessment in their predominately male population was conducted at a later age (mean age, 20 years) than in our study and data on adolescent outcome are not given.

LIMITATIONS

Several features of our cohort limit the generalizability of our results. First, the selection criteria returned a phenotype of severe combined-type ADHD, which was relatively free of comorbidity. Also, girls with ADHD were deliberately overrepresented (given the relative neglect of studies in females with ADHD[50]) and the study drew from an affluent sociodemographic area, a feature that probably accounts for the high average IQ of the subjects with ADHD. Equally, these selection characteristics have the advantage of ensuring that statements can be made with some certainty about the neuroanatomical correlates of ADHD per se, relatively unconfounded by learning disability or serious comorbidity. The healthy controls were recruited from the same affluent sociodemographic area, which may contribute to their high average IQ but also has the advantage of enhancing comparability with the ADHD group.

To reduce the risk of artifactual findings, we restricted our analyses to candidate genes that either showed an association with ADHD within our sample (*DRD1* and *DRD4*) or are thought to have additive effects with the *DRD4* gene (*DAT1*).[6] Additionally, our longitudinal analyses were based on an a priori hypothesis. The findings are unlikely to be attributable to clinical differences because the groups were well matched on these variables. Follow-up data were not available on all subjects and it is impossible to exclude the possibility that attrition may have introduced bias. The rate of treatment with psychostimulants at baseline and throughout follow-up was high but critically did not differ by genotype or by clinical outcome.

CONCLUSIONS

The 48-bp VNTR in exon 3 in the *DRD4* gene appears to influence cortical structure in a similar manner in cortical regions important for the control of attention in those with ADHD and typically developing children. The effects of the polymorphism were detectable predominately in childhood, a finding of interest given our phenotype of better clinical outcome associated with the *DRD4* 7-repeat allele variant and the natural history of improvement in ADHD with age.

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REFERENCES

1. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry*. 2005;57(11):1313-1323.
2. Gornick MC, Addington AM, Shaw P, Bobb A, Sharp W, Greenstein D, Arepalli S, Castellanos FX, Rapoport JL. Association of the dopamine receptor D4 (DRD4) gene 7-repeat allele with children with attention-deficit/hyperactivity disorder (ADHD): an update. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144(3):379-382.
3. Swanson J, Oosterlaan J, Murias M, Schuck S, Flodman P, Spence MA, Wasdell M, Ding Y, Chi HC, Smith M, Mann M, Carlson C, Kennedy JL, Sergeant JA, Leung P, Zhang YP, Sadeh A, Chen C, Whalen CK, Babb KA, Moyzis R, Posner MI. Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention. *Proc Natl Acad Sci U S A*. 2000;97(9):4754-4759.
4. Langley K, Marshall L, van den Bree M, Thomas H, Owen M, O'Donovan M, Thapar A. Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. *Am J Psychiatry*. 2004;161(1):133-138.
5. Manor I, Tyano S, Eisenberg J, Bachner-Melman R, Kotler M, Ebstein RP. The short DRD4 repeats confer risk to attention deficit hyperactivity disorder in a family-based design and impair performance on a continuous performance test (TOVA). *Mol Psychiatry*. 2002;7(7):790-794.
6. Mill J, Caspi A, Williams BS, Craig I, Taylor A, Polo-Tomas M, Berridge CW, Poulton R, Moffitt TE. Prediction of heterogeneity in intelligence and adult prognosis by genetic polymorphisms in the dopamine system among children with attention-deficit/hyperactivity disorder: evidence from 2 birth cohorts. *Arch Gen Psychiatry*. 2006;63(4):462-469.
7. LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol Psychiatry*. 1996;1(2):121-124.
8. Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. *Mol Psychiatry*. 2000;5(4):396-404.

9. Mill J, Xu X, Ronald A, Curran S, Price T, Knight J, Craig I, Sham P, Plomin R, Asherson P. Quantitative trait locus analysis of candidate gene alleles associated with attention deficit hyperactivity disorder (ADHD) in five genes: DRD4, DAT1, DRD5, SNAP-25, and 5HT1B. *Am J Med Genet B Neuropsychiatr Genet*. 2005;133(1):68-73.
10. Barkley RA, Smith KM, Fischer M, Navia BA. An examination of the behavioral and neuropsychological correlates of three ADHD candidate gene polymorphisms (DRD4 7+, DBH TaqI A2, and DAT1 40 bp VNTR) in hyperactive and normal children followed to adulthood. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141(5):487-498.
11. Rowe DC, Stever C, Chase D, Sherman S, Abramowitz A, Waldman ID. Two dopamine genes related to reports of childhood retrospective inattention and conduct disorder symptoms. *Mol Psychiatry*. 2001;6(4):429-433.
12. Todd RD, Neuman RJ, Lobos EA, Jong YJ, Reich W, Heath AC. Lack of association of dopamine D4 receptor gene polymorphisms with ADHD subtypes in a population sample of twins. *Am J Med Genet*. 2001;105(5):432-438.
13. Holmes J, Payton A, Barrett J, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Gill M, Kirley A, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mill J, Gould A, Taylor E, Kent L, Craddock N, Thapar A. Association of DRD4 in children with ADHD and comorbid conduct problems. *Am J Med Genet*. 2002; 114(2):150-153.
14. Hamarman S, Fossella J, Ulger C, Brimacombe M, Dermody J. Dopamine receptor 4 (DRD4) 7-repeat allele predicts methylphenidate dose response in children with attention deficit hyperactivity disorder: a pharmacogenetic study. *J Child Adolesc Psychopharmacol*. 2004;14(4):564-574.
15. Durston S, Fossella JA, Casey BJ, Hulshoff Pol HE, Galvan A, Schnack HG, Steenhuis MP, Minderaa RB, Buitelaar JK, Kahn RS, van Engeland H. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. *Mol Psychiatry*. 2005;10(7):678-685.
16. Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, Sharp W, Marsh WL, Walter JM, Hamburger SD, Ginns EI, Rapoport JL, Sidransky E. Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses. *Mol Psychiatry*. 1998;3(5):431-434.
17. Bobb AJ, Addington AM, Sidransky E, Gornick MC, Lerch JP, Greenstein DK, Clasen LS, Sharp WS, Inoff-Germain G, Wavrant-De Vrieze F, Arcos-Burgos M, Straub RE, Hardy JA, Castellanos FX, Rapoport JL. Support for association between ADHD and two candidate genes: NET1 and DRD1. *Am J Med Genet B Neuropsychiatr Genet*. 2005;134(1):67-72.
18. Misener VL, Luca P, Azeke O, Crosbie J, Waldman I, Tannock R, Roberts W, Malone M, Schachar R, Ickowicz A, Kennedy JL, Barr CL. Linkage of the dopamine receptor D1 gene to attention-deficit/hyperactivity disorder. *Mol Psychiatry*. 2004; 9(5):500-509.
19. Kirley A, Hawi Z, Daly G, McCarron M, Mullins C, Millar N, Waldman I, Fitzgerald M, Gill M. Dopaminergic system genes in ADHD: toward a biological hypothesis. *Neuropsychopharmacology*. 2002;27(4):607-619.
20. Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ. Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet*. 1999;354(9196):2132-2133.
21. Kabani N, Le Goualher G, MacDonald D, Evans AC. Measurement of cortical thickness using an automated 3-D algorithm: a validation study. *Neuroimage*. 2001; 13(2):375-380.
22. Lerch JP, Evans AC. Cortical thickness analysis examined through power analysis and a population simulation. *Neuroimage*. 2005;24(1):163-173.
23. Shaw P, Lerch J, Greenstein D, Sharp W, Clasen L, Evans A, Giedd J, Castellanos FX, Rapoport J. Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention deficit/hyperactivity disorder. *Arch Gen Psychiatry*. 2006;63(5):540-549.
24. Reich W. Diagnostic interview for children and adolescents (DICA). *J Am Acad Child Adolesc Psychiatry*. 2000;39(1):59-66.
25. 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(3):217-229.
26. Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey BJ, Kozuch PL, Vaituzis AC, Vauss YC, Hamburger SD, Kaysen D, Rapoport JL. Quantitative magnetic resonance imaging of human brain development: ages 4-18. *Cereb Cortex*. 1996; 6(4):551-560.
27. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging*. 1998; 17(1):87-97.
28. Zijdenbos AP, Forghani R, Evans AC. Automatic "pipeline" analysis of 3-D MRI data for clinical trials: application to multiple sclerosis. *IEEE Trans Med Imaging*. 2002;21(10):1280-1291.
29. MacDonald D, Kabani N, Avis D, Evans AC. Automated 3-D extraction of inner and outer surfaces of cerebral cortex from MRI. *Neuroimage*. 2000;12(3): 340-356.
30. Pinheiro JC, Bates DM. *Mixed-effects Models in S and S-PLUS*. New York, NY: Springer; 2000.
31. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Statist Soc Ser B Methodological*. 1995;57:289-300.
32. Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage*. 2002;15(4): 870-878.
33. Kang AM, Palmatier MA, Kidd KK. Global variation of a 40-bp VNTR in the 3′-untranslated region of the dopamine transporter gene (SLC6A3). *Biol Psychiatry*. 1999;46(2):151-160.
34. Posner MI, Petersen SE. The attention system of the human brain. *Annu Rev Neurosci*. 1990;13:25-42.
35. Mesulam MM. Spatial attention and neglect: parietal, frontal and cingulate contributions to the mental representation and attentional targeting of salient extrapersonal events [published correction appears in *Philos Trans R Soc Lond B Biol Sci*. 1999;354(1352):2083]. *Philos Trans R Soc Lond B Biol Sci*. 1999; 354(1387):1325-1346.
36. Bobb AJ, Castellanos FX, Addington AM, Rapoport JL. Molecular genetic studies of ADHD: 1991 to 2004. *Am J Med Genet B Neuropsychiatr Genet*. 2005; 132(1):109-125.
37. Levy F, Hay DA, McStephen M, Wood C, Waldman I. Attention-deficit hyperactivity disorder: a category or a continuum? genetic analysis of a large-scale twin study. *J Am Acad Child Adolesc Psychiatry*. 1997;36(6):737-744.
38. Schmidt LA, Fox NA, Perez-Edgar K, Hu S, Hamer DH. Association of DRD4 with attention problems in normal childhood development. *Psychiatr Genet*. 2001; 11(1):25-29.
39. Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM, Moyzis RK. The genetic architecture of selection at the human dopamine receptor D4 (DRD4) gene locus. *Am J Hum Genet*. 2004;74 (5):931-944.
40. Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL. Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proc Natl Acad Sci U S A*. 2005;102(30):10706-10711.
41. Walhovd KB, Fjell AM, Dale AM, Fischl B, Quinn BT, Makris N, Salat D, Reinvang I. Regional cortical thickness matters in recall after months more than minutes. *Neuroimage*. 2006;31(3):1343-1351.
42. Fjell AM, Walhovd KB, Reinvang I, Lundervold A, Salat D, Quinn BT, Fischl B, Dale AM. Selective increase of cortical thickness in high-performing elderly—structural indices of optimal cognitive aging. *Neuroimage*. 2006;29(3): 984-994.
43. Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW. Longitudinal mapping of cortical thickness and brain growth in normal children. *J Neurosci*. 2004;24(38):8223-8231.
44. Klingberg T, Forssberg H, Westerberg H. Training of working memory in children with ADHD. *J Clin Exp Neuropsychol*. 2002;24(6):781-791.
45. Olesen PJ, Westerberg H, Klingberg T. Increased prefrontal and parietal activity after training of working memory. *Nat Neurosci*. 2004;7(1):75-79.
46. Kuntsi J, Rijsdijk F, Ronald A, Asherson P, Plomin R. Genetic influences on the stability of attention-deficit/hyperactivity disorder symptoms from early to middle childhood. *Biol Psychiatry*. 2005;57(6):647-654.
47. Rietveld MJ, Hudziak JJ, Bartels M, van Beijsterveldt CE, Boomsma DI. Heritability of attention problems in children: longitudinal results from a study of twins, age 3 to 12. *J Child Psychol Psychiatry*. 2004;45(3):577-588.
48. Castellanos FX, Lee PP, Sharp W, Jeffries NO, Greenstein DK, Clasen LS, Blumenthal JD, James RS, Ebens CL, Walter JM, Zijdenbos A, Evans AC, Giedd JN, Rapoport JL. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *JAMA*. 2002; 288(14):1740-1748.
49. Kirley A, Lowe N, Mullins C, McCarron M, Daly G, Waldman I, Fitzgerald M, Gill M, Hawi Z. Phenotype studies of the DRD4 gene polymorphisms in ADHD: association with oppositional defiant disorder and positive family history. *Am J Med Genet B Neuropsychiatr Genet*. 2004;131(1):38-42.
50. Castellanos FX, Giedd JN, Berquin PC, Walter JM, Sharp W, Tran T, Vaituzis AC, Blumenthal JD, Nelson J, Bastain TM, Zijdenbos A, Evans AC, Rapoport JL. Quantitative brain magnetic resonance imaging in girls with attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry*. 2001;58(3):289-295.