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Familial risk and ADHD-specific neural activity revealed by case-control, discordant twin pair design

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ABSTRACT

Individuals with ADHD, as well as their family members who do not meet clinical criteria, have shown deficits in executive function. However, it remains unclear whether underlying neural alterations are familial or ADHD-specific. To investigate this issue, neural activation underlying executive function was assessed using functional magnetic resonance imaging during performance of a Stroop task in three groups of individuals: 20 young adults who were diagnosed with ADHD in childhood, their 20 dizygotic co-twins without ADHD in childhood, and 20 unrelated controls selected from dizygotic twin pairs in which neither twin had ADHD in childhood (total $n=60$). Implicating the frontoparietal network as a location of effects specific to ADHD, activation in the superior frontal (Brodmann's Area — BA 6) and parietal regions (BA 40) was significantly reduced in twins with childhood ADHD compared to both their control co-twins and unrelated control twins. Consistent with familial influences, activity in the anterior cingulate and insula was significantly reduced in both the twins with ADHD and their co-twins compared to the unrelated controls. These results show that both ADHD-specific and familial influences related to an ADHD diagnosis impact neural systems underlying executive function.

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) is defined as a persistent pattern of inattentive and/or hyperactive or impulsive behavior that is beyond what is typically observed in individuals at a similar stage in development. ADHD symptoms are associated with a wide range of functional impairments, including difficulties in academic, social, and later occupational functioning (Polderman et al., 2011; Eiraldi et al., 2012; Willcutt et al., 2012). Consistent with familial influences on ADHD symptoms, siblings who do not have ADHD themselves (i.e., are discordant) exhibit phenotypes in between their siblings with the ADHD diagnosis and unrelated controls on a range of cognitive and behavioral indices (Schachar et al., 2005; Bidwell et al., 2007; Steinhausen et al., 2012). Despite much progress in our understanding of ADHD, it is unclear whether neurobiological alterations are specific to the disorder or better characterized as an underlying familial risk. Utilizing a three-group, discordant, control twin design, as in the current study, allows one to disentangle regional differences in neural activity related to a clinical diagnosis (ADHD-specific) from those that are shared between discordant twin pairs (familial). Hence, the use of twin pairs, as a natural experiment, is a potent way to further our understanding of these underlying neurobiological alterations (Plomp et al., 2009; Wood and Neale, 2010).

One of the core deficits observed in both children and adults with ADHD is poor executive function (EF) (Barkley, 1997; Willcutt et al., 2005). A classic way to measure EF — the ability to exert flexible goal-directed behavior — is the Stroop task (MacLeod, 2005). Meta-analyses indicate that both children and adults with ADHD perform worse than controls without ADHD on the Stroop task, with effect sizes ranging from small-to-medium (Hervey et al., 2004; van Mourik et al., 2005; Willcutt et al., 2005; Schwartz and Verhaeghen, 2008). In the typical version of the Stroop task (Stroop, 1935), individuals are asked to identify the ink color in which a word is presented. This task requires executive control at a number of different levels, including engaging in ink color identification (the task goal) rather than the more automatic process of word reading, as well as resolving conflict between potential responses (conflict). Studies of individual differences have shown that performance on the Stroop task loads highly on a factor

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common across EF tasks, which is thought to represent the ability to actively maintain a task goal in the face of distracting information (Miyake and Friedman, 2012).

Neuroimaging research has identified alterations in several networks underlying attentional deficits in individuals with ADHD, including the frontoparietal, cingulo-opercular, dorsal and ventral-attention and cerebellar networks (For reviews see Makris et al., 2009; Bush, 2011; Durston et al., 2011; Cortese et al., 2012; Posner, 2012). Recently our laboratory was interested in the degree to which these alterations are specific to ADHD in young adults and not to other co-morbid disorders (Banich et al., 2009). To do so, we examined brain activation during performance on the Stroop task in a highly selected sample of individuals with ADHD who had neither a comorbid psychiatric diagnosis nor any other form of learning disability, as compared with a group of age, gender, and IQ-matched healthy controls. The young adults with ADHD exhibited reduced activity in the mid-dorsolateral prefrontal cortex (dlPFC), anterior cingulate cortex (ACC), and parietal regions compared to the control group (Banich et al., 2009), suggesting alterations in these brain regions are likely due to effects related to ADHD and not other typically comorbid disorders.

In the present study we expand upon these findings to disentangle those neural alterations in young adults that are specific to an ADHD diagnosis in childhood from those related to familial influences. One approach to determining the degree of familial effects is to examine brain activation in control siblings of individuals diagnosed with ADHD as compared to unrelated controls. There are relatively few neuroimaging studies that have taken such an approach (e.g., Durston et al., 2006; Mulder et al., 2008, see also von Rhein et al., 2014 for a study in progress that takes this approach), and all have been with children and adolescents. Those studies show familial effects such that siblings who did not meet diagnostic criteria for ADHD showed reduced activity compared to unrelated controls in the ACC and ventrolateral prefrontal cortex, as well as the parietal cortex and cerebellum (Durston et al., 2006; Mulder et al., 2008). These studies used performance of a go/no-go task, which is often used to measure inhibitory aspects of executive control.

We are aware of only one functional neuroimaging study that examined neural activation during performance of an EF task in twins with attention problems (van t Ent et al., 2009). In that study, adolescent monozygotic twin pairs who both showed high levels of attentional problems, as assessed by the Child Behavior Checklist, exhibited decreased activity in the dlPFC during performance of the Stroop task and in the right parietal regions during performance of the Flanker task compared to twin pairs who both had low levels of attention problems. Unique to the discordant monozygotic twin design, this study also examined nonshared environmental influences by examining activation in twins with high levels of attention problems compared to their discordant co-twins with low levels of attention problems. Those twins high in attention problems showed reduced activity in the superior temporal gyrus, right fusiform gyrus, and right premotor regions compared to their discordant co-twins. These results suggest nonshared environmental influences since comparisons were between genetically identical twin pairs who shared a familial environment and only differed on nonshared environmental experiences.

In the current study, we aimed to determine the degree to which disorder-specific influences and familial influences affect the pattern of brain activation during performance of the Stroop task in young adulthood. To do so, we recruited a sample of twins from an on-going study of individuals who had been assessed for ADHD in childhood. We selected dizygotic (DZ) twin pairs who were discordant for receiving an ADHD diagnosis in childhood as well as a sample of unrelated controls selected from age- and gender-matched DZ twin pairs in which neither twin had a childhood diagnosis of ADHD. Dizygotic twins share on average 50% of their segregating genes and 100% of what is referred to as shared or familial environment. Unfortunately, monozygotic twins were not recruited for this study due to limitations in selecting a large enough sample of monozygotic twins discordant for ADHD.

Our design, nonetheless, allows us to examine a number of important issues. First, we can isolate alterations in brain activation specific to a childhood diagnosis of ADHD by contrasting brain activation of individuals who reached a clinical threshold of ADHD symptoms in childhood to that of both his/her co-twins and the unrelated controls, both of which had low levels of ADHD symptomatology in childhood. We predict that those individuals who reached diagnostic criteria for ADHD during childhood will show reduced brain activation in regions of the frontoparietal control network compared to their control co-twins and unrelated controls.

Second, familial effects associated with ADHD can be examined by comparing the sets of discordant twins to the unrelated controls. We predict, based on the existing literature suggesting familial effects in ADHD (Schachar et al., 2005; Bidwell et al., 2007; Steinhausen et al., 2012), that this contrast will also yield significant effects, although there is too little prior research to make more specific predictions. Of primary interest is whether these familial effects will occur within the same regions that are likely to show an effect of childhood diagnosis of ADHD, such as the frontoparietal control network, or whether other regions will be implicated.

2. Methods

2.1. Participants

A total of 20 DZ twins who met ADHD diagnostic criteria during childhood (TA), their 20 control co-twins without ADHD (CC), and 20 unrelated controls (UC) participated in the study for a total of 60 individuals. The mean age at the time of the childhood DSM interview was 12 with a range of 8 to 17. The average age at fMRI testing was 19 with a range of 17 to 23. We opted to select individuals based on childhood rather than current ADHD status. This procedure ensured that the twins with ADHD had exhibited symptoms during childhood and that criteria were not based on retrospective reports. While not all the individuals who met criteria during childhood did so during adulthood, this mainly reflects a slight reduction in symptomatology so as to no longer pass threshold for a current diagnosis. Moreover, recent evidence suggests that childhood ratings of ADHD by multiple informants, as we had for this sample (parent, teacher), have less rater-related measurement error than single self-report measures in early adulthood (Chang et al., 2013).

Twins were recruited from the Colorado Learning Disabilities Research Center (CLDRC) twin study, an ongoing study of the etiology of ADHD and learning difficulties (For details, see DeFries et al., 1997; Willcutt et al., 2010). Although the CLDRC study also recruited twins with reading difficulties, twin pairs with such difficulties were not included in the current study. All individuals signed informed consent, and protocols were approved by the Institutional Review Board.

Parent and teacher ratings of ADHD symptoms during childhood (Barkley and Murphy, 1998) were used to identify probands from DZ twin pairs who met criteria for ADHD during childhood. Items endorsed as occurring “often” or “very often” were coded as positive symptoms, and items that occurred “never” or “sometimes” were coded as negative symptoms. Parent and teacher ratings were then combined using the OR rule procedure in which a symptom is coded as positive if endorsed by the parent or the teacher (Lahey et al., 1994). Twins were identified as probands if they exhibited 6 or more symptoms of inattention or hyperactivity–impulsivity, exhibited significant functional impairment based on parent and teacher ratings, and had an age of onset of ADHD symptoms by the time they were seven years of age.

From a total sample of over 300 DZ probands with ADHD, 20 discordant twin pairs were selected in which the control co-twin did not meet criteria for ADHD during childhood. To control as much as possible for gender differences in ADHD diagnosis and due to limitations in recruitment from the sample, twin pairs were selected such that all participants in the ADHD group were males, while males and females were represented equally amongst their discordant twins. To complete these triads, twin pairs were then carefully matched for age, gender of the control co-twin, and geographical location of childhood home with an unrelated control

from a twin pair in which neither twin met DSM-IV criteria for ADHD during childhood. All participants did not meet criteria for a reading disability and control co-twins did not meet criteria for ADHD based on parent and teacher ratings during childhood. Participants were not selected or excluded based on other comorbid disorders in childhood. Comorbidity rates of diagnosis in the twins with ADHD as assessed during childhood, were similar to those in the literature for children with ADHD (Elia et al., 2008), with 50% oppositional defiant disorder (ODD), 30% conduct disorder (CD), 10% generalized anxiety disorder (GAD), and 5% major depression (MDD). Rates of some diagnoses in control co-twins without ADHD, were not as high as the twins with ADHD but still significantly higher than the unrelated controls (CT=20% ODD, 5% CD, 15% GAD, 0% MDD; UT=5% ODD, 0% CD, 5% GAD, and 0% MDD).

To assess current symptoms of ADHD and diagnostic history, participants completed the self-report version of the DSM-IV Current Symptoms Scale and a brief interview (Barkley and Murphy, 1998). At the time of the current assessment, 6 of the 20 participants who met DSM-IV criteria during childhood reported that they had received a formal diagnosis (3 combined type, and 3 predominantly inattentive type). Five of these individuals were prescribed medication, which included Adderall, Concerta, Ritalin, and Acidophilis. One participant who did not report receiving a formal diagnosis reported not remembering the medication he took (See Supplementary Results, Fig. S2, which presents data excluding individuals who reported taking medication). Although control twins, including both the co-twin of individuals with ADHD and the unrelated controls, did not meet clinical criteria for any subtype of ADHD during their childhood assessment, one control co-twin reported a diagnosis of mild ADD and was prescribed Adderall. Individuals who were currently taking stimulant medication were asked to not take the medication for 24 h prior to the scanning session.

2.2. Procedures and measures

Included in this report are behavioral data both on the standard clinical version of the Stroop task (e.g., Golden, 1978), which was performed during childhood, and a computerized Stroop task performed during fMRI scanning. In the standard clinical version of the Stroop task, the primary dependent measure for each of the three sets of trials is the number of items completed correctly in 45 s. The first set requires the participants to read the names of colors printed in black text (words correct). In the second set, participants name the colors of nonlinguistic letter strings (i.e., XXXXX) printed in red, green, or blue ink (colors correct). For the third set, the interference trials, participants name the color of the ink in which an incongruent word is printed (i.e., responding "blue" for the word "red" printed in blue ink).

The neuroimaging task utilized a hybrid-blocked/event-related fMRI design similar to that employed in Banich et al. (2009). Participants were instructed to identify, via a button press, the ink color of a word presented every 2 s. There were three trial types: neutral trials, in which the word did not contain any color-related information (e.g., the word "LOT" printed in *blue ink*), congruent trials in which the word and ink color are identical (e.g., the word "BLUE" printed in *blue ink*), and incongruent trials in which the word and ink color conflicted (e.g., the word "RED" printed in *blue ink*). Interference was calculated as (Incongruent–Neutral)/Neutral and facilitation as (Congruent–Neutral)/Neutral. These measures take into account baseline differences across individuals in reaction time and accuracy.

Each block consisted of 12 trials, half of which were specific to that block (i.e., congruent, incongruent, or neutral) and half of which were common neutral trials that were presented across all three blocks (see Banich et al., 2009 for more details). These three types of blocks were counterbalanced throughout the task for a total of six runs. Fixation blocks, which consisted of 12 trials in which a single fixation cross was shown, were interspersed between trial blocks.

The Stroop task is thought to measure various levels of attentional control. Common to all three trial types — incongruent, congruent, and neutral — as compared to a fixation baseline, is the need to engage in the less automatic process of color identification in the face of the more automatic process of word reading (I+C+N > Fixation). The contrast of incongruent-versus-congruent blocks (I > C) allows for a more stringent examination of control and, specifically, the ability required when the two sources of color information are conflicting as compared to when they are the same. Event-related effects comparing the block-specific trial (e.g., incongruent) to the common neutral trials within that block (e.g., common neutral trials within the incongruent block) did not yield significant group differences and therefore are not discussed further.

2.3. MRI data acquisition and pre-processing

Data were acquired using a 3T GE-Signa MR scanner located at the Anschutz Medical Campus of the University of Colorado Denver. Structural images were acquired using high-resolution, T1-weighted 3D-SPGR, anatomical images (repetition time=10.07 ms, echo time=0.03 ms, flip angle=10°, 256 × 256 matrix, 0.86 mm × 0.86 mm in-plane resolution, 122 slices, 1.3 mm slice thickness). Functional images were acquired using a T2*-weighted gradient echo (repetition time=2000 ms, echotime=32 ms, flip angle=70°, 29 slices parallel to the AC–PC line, thickness=4 mm, gap=0 mm, 64 × 64 acquisition matrix, 3.44 mm × 3.44 mm resolution, in-plane field-of-view=22 cm).

Image preprocessing was performed within FSL (Jenkinson et al., 2012; FMRIB Software library, Oxford, UK, www.fmrib.ox.ac.uk). Images were run with the brain extraction tool (BET) to remove skull and other non-brain features. Motion correction was performed using the rigid body translation and rotation algorithm (MCFLIRT). A high pass filtering cutoff of 100 seconds was used for temporal filtering and 8 mm full-width half maximum Gaussian Kernel was used for spatial smoothing. FMRIB's Improved Linear Model (FILM) was used for prewhitening before statistical analyses.

2.4. Statistical analyses

Statistical analyses were performed using General Linear Modeling as implemented in FSL's Expert Analysis Tool (FEAT). Montreal Neurological Institute standard stereotaxic space (MNI152) was used to register individual activity for comparison across individuals. Mixed effects analyses utilized FLAME 1+2 to account for mixed–effects variance, which modeled both fixed effects (within subject variability) and random effects (between-subjects variability). A voxel-wise threshold of $Z=2.58$ ($p<0.01$) was used with AlphaSim's cluster correction (3dClustSim) performed on lower-level data to further limit significant results to those reaching a cluster-wise threshold of $p<0.05$ (clusters size thresholds were 169–171 voxels).

Two main sets of contrasts were examined: i. Maintenance of task set (Incongruent+Congruent+Neutral > Fixation) and ii. Conflict (Incongruent > Congruent). To examine activity specific to ADHD during childhood, we utilized a conjunction analysis (Nichols et al., 2005). For ADHD-specific effects, we examined the overlap between results for the analyses of twins with ADHD compared to their discordant control co-twins (CC > TA) and the analyses of twins with ADHD compared to the unrelated controls (UC > TA). To identify familial effects, we performed a conjunction analysis between significant effects for the analysis comparing the twins with ADHD to the unrelated controls (UC > TA) and the analysis of the control co-twins compared to the unrelated controls (UC > CC). Activity that reached significance for incongruent, congruent, and neutral blocks versus fixation for each pair-wise group comparison individually can be found in Supplemental Table S2.

3. Results

3.1. Descriptive statistics

Descriptive statistics in Table 1 include the mean, standard deviation, and range for ADHD symptomatology assessed during childhood (mean age=12, SD=2.9) and young adulthood (mean age 19, SD=1.6). Consistent with our selection criteria, the twins with ADHD (TA) exhibited higher severity scores for inattention, hyperactivity, and total severity as compared to both their control co-twins and the unrelated controls during childhood ($p < 0.001$). Total severity scores and inattention were also significantly higher for the twins with ADHD compared to both their control co-twins and unrelated controls during the adulthood assessment ($p < 0.01$ and $p < 0.05$, respectively). Importantly, indicating a robust discordant design, the control co-twins and unrelated controls did not significantly differ in ADHD symptomatology either during childhood or young adulthood. WISC IQ scores tested in childhood (Table 1) did not significantly differ between control co-twins and twins with ADHD or unrelated controls. Twins with ADHD showed significantly lower scores compared to the unrelated control and an overall significant difference for the three groups ($F(2,56)=3.18$, $p=0.05$).

Performance on the clinical Stroop task as assessed during childhood and the Stroop task performed during the fMRI scan in adulthood is shown in Table 2. No significant differences were observed between groups in either reaction time in milliseconds or accuracy (percent correct) for measures obtained during childhood or adulthood. Table 2 also shows that there were no significant differences in interference and facilitation scores for the fMRI Stroop task, which incorporate baseline reading and response scores by dividing by neutral scores (*Interference=(incongruent-neutral)/neutral; Facilitation=(congruent-neutral)/neutral).*

To test for group differences in movement during scanning, motion was tested by comparing the range for *X*, *Y*, and *Z* points of translation and rotation. Analysis of variance across the three groups (ADHD, control co-twins and unrelated controls), as well as Tukey's pair-wise group comparisons were used and revealed no significant group differences (Supplemental Table S1).

3.2. Neuroimaging results

3.2.1. Maintenance of task set

(incongruent+congruent+neutral > fixation)

Fig. 1 shows activity for each group separately for maintenance of task set (I+C+N > fixation) with voxel-wise significance of $p < 0.01$ and a cluster-wise threshold of $p < 0.05$. As can be seen in Fig. 1, overall, the pattern appears to yield a gradient across groups, with less activation in the frontoparietal control network in twins with ADHD (TA) as compared to control co-twins (CC) who, in turn, have less activation compared to unrelated controls (UC). Formal comparisons of familial and ADHD-specific effects are shown in Fig. 2 and Table 3.

In terms of familial effects (see cyan regions in Fig. 2), the unrelated controls showed significantly greater activity compared to the discordant twin pairs in the bilateral anterior insula and anterior cingulate cortex (i.e., the conjunction of UC > TA in purple and UC > CC in navy). Specifically, the unrelated controls showed increased activation in these regions compared to fixation baseline, while the twins with ADHD and their discordant co-twins showed deactivation in these regions compared to baseline. Significantly greater activity was also found for unrelated controls compared to twin pairs in the amygdala, right orbital frontal gyrus (OFC) and left pre- and post-central gyrus. No regions yielded significant activity for the related twins greater than the unrelated controls (i.e., TA > UC and CC > UC).

In terms of ADHD-specific effects (see yellow regions in Fig. 2), both the control co-twins and unrelated controls showed significantly greater activity compared to the twins with ADHD in portions of the superior and middle frontal gyrus (i.e., conjunction of UC > TA in purple and CC > TA in red). They also exhibited greater activity in the right pre- and post-central gyrus, left supramarginal gyrus (BA40), and right lingual gyrus and fusiform. There were no regions yielding greater activity for the twins with ADHD compared to the controls.

3.2.2. Conflict (Incongruent minus Congruent)

No significant familial or ADHD-specific effects were observed in fMRI activity for the incongruent minus congruent contrast. However, activity in the right lingual gyrus extending into the fusiform was greater in the control co-twins compared to the unrelated controls (Table 3). This was the only contrast that showed less activity for unrelated controls compared to the related twins. Control co-twins were also found to have greater activity in the precuneus extending into postcentral gyrus compared to their co-twins with ADHD.

Table 1
Descriptive statistics for severity scores and between-subjects effects.

	Twins with ADHD (TA)			Control co-twins (CC)			Unrelated controls (UC)			Mean difference			Group
	M	SD	Range	M	SD	Range	M	SD	Range	TA > CC	TA > UC	CC > UC	F stat
Childhood severity													
Total severity	20.06	11.84	6.2–47	1.73	2.55	0–9.3	0.31	0.54	0–2.11	18.33***	19.75***	1.4	47.07***
Inattention	17.17	5.15	8.3–27	1.38	2.18	0–6.2	0.29	0.51	0–1.3	15.79***	16.87***	1.09	162.16***
Hyperactivity	5.84	7.21	0–25	0.84	1.62	0–4.9	0.13	0.34	0–1.1	5.91***	5.71***	0.7	10.11***
Adulthood severity													
Total severity	3.22	4.14	0–12.9	1.13	1.86	0–6	0.25	0.51	0–1.8	2.09*	2.97**	0.88	6.72**
Inattention	2.21	3.60	0–12	0.55	1.62	0–7.2	0.00	0.00	0–0	1.66*	2.2**	0.55	5.07**
Hyperactivity	1.38	2.07	0–8.3	0.97	2.33	0–10	0.39	0.86	0–3	0.41	0.98	0.58	1.4
Childhood WISC													
General IQ	107.4	9.66	87–122	112.1	14.5	78–139	116.8	10.8	102–142	−4.71	−9.4**	−4.69	3.18*

Note. TA=Twins with ADHD, CC=Control Co–Twins, UC=Unrelated Controls. Childhood severity was derived by the OR rule for teacher and parent ratings. Adulthood severity was self-report.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 2
Performance for stroop task and between-subjects effects.

Stroop performance	Twins with ADHD (TA)			Control co-twins (CC)			Unrelated controls (UC)			Mean difference			Group
	M	SD	Range	M	SD	Range	M	SD	Range	TA > CC	TA > CC	CC > UC	F stat
Childhood													
Number correct in 45 s													
Words correct	76.21	19.36	42–114	84	16.85	54–112	85.78	15.88	65–109	−7.79	−9.57	−1.78	1.58
Colors correct	55.42	15.93	27–81	57.33	13.94	36–81	61.56	12.8	44–82	−1.91	−6.13	−4.22	0.88
Interference correct	31.58	12.93	13–53	34.72	10.66	21–55	36.78	11.18	20–54	−3.14	−5.2	−2.06	0.94
Adulthood													
% Accurate													
Neutral	0.95	0.03	0.89–1	0.93	0.08	0.71–1	0.95	0.03	0.89–1	0.01	−0.02	−0.01	0.34
Congruent	0.95	0.04	0.88–1	0.95	0.04	0.87–1	0.96	0.04	0.89–1	0.00	0.00	−0.01	0.14
Incongruent	0.88	0.12	0.48–1	0.93	0.04	0.83–1	0.90	0.07	0.75–1	−0.05	−0.02	0.03	1.45
Interference	−0.07	0.13	−0.47–0.06	0.00	0.11	−0.14–0.37	−0.05	0.08	−0.18–0.09	−0.07	−0.02	0.05	2.04
Facilitation	0.01	0.04	−0.05–0.1	0.02	0.08	−0.06–0.23	0.01	0.01	−0.05–0.1	−0.02	0.00	0.01	0.42
Reaction time (ms)													
Neutral	718.1	124.5	552–1003.9	686.7	86.1	570.1–869.5	668.3	74.2	511.7–774.4	31.44	49.79	18.34	1.34
Congruent	698.2	123.7	511.1–994.6	707.2	111.5	545.1–902.5	658.2	79.2	495.4–815.6	−9.01	39.94	48.95	1.20
Incongruent	810.6	148.8	623.9–1136.6	835.3	151.3	648.8–1096.7	800.1	124.7	597.2–1060.5	−24.75	10.51	35.26	0.33
Interference	0.13	0.11	−0.03–0.4	0.22	0.17	0.02–0.69	0.20	0.11	0.01–0.38	−0.08	−0.06	0.02	2.07
Facilitation	−0.03	0.09	−0.17–0.15	0.03	0.11	−0.17–0.25	−0.01	−0.01	−0.1–0.16	−0.06	−0.01	0.04	1.99

Note. TA = Twins with ADHD, CC = Control Co-Twin, UC = Unrelated Control, Interference = (incongruent-neutral)/neutral; Facilitation = (congruent-neutral)/neutral, ms = milliseconds.

4. Discussion

The main findings of this study indicate that there are both disorder-specific and familial effects related to a childhood diagnosis of ADHD that manifest in patterns of brain activity during a demanding EF task in young adulthood. In a recent review, Bush (2011) summarized the neuroimaging findings on ADHD as hypofunction in the cingulate-frontal-parietal cognitive attention network. Our results are consistent with this conclusion as decreased activation in these regions was observed in individuals selected for having ADHD in childhood compared to unrelated controls. However, by utilizing a dizygotic, discordant-twin-pair design in the current study, we were able to show that these alterations are likely to reflect the combination of both diagnosis specific and familial effects. In particular, diagnosis-specific effects were more likely to involve portions of the frontoparietal control network, while familial effects were more likely to involve portions of the ACC, insula, and OFC.

More specifically, individuals with ADHD in childhood showed less activation compared to both the unrelated and related controls in the posterior portions of the superior frontal cortex (BA6), the right pre- and post-central gyrus (BA4), left supramarginal gyrus (BA40), and right lingual gyrus and fusiform (BA18) for the Stroop task as compared to the fixation baseline. The effects observed for BA 6 and BA40 are consistent with our prior findings in a carefully selected sample of individuals who met diagnostic

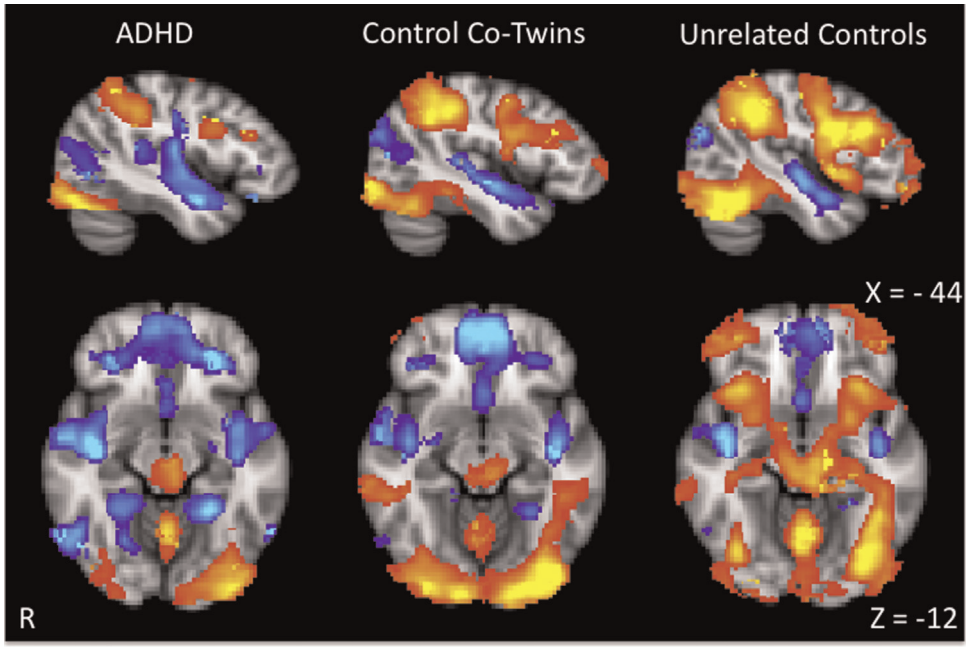

Fig. 1. Group average for maintenance of task set (I+C+N > fixation). Activation (red) and deactivation (blue) is shown for task blocks, which require maintenance of task set compared to fixation. Activation is shown as the group average activity for twins with ADHD, their discordant control co-twins, and unrelated controls, respectively.

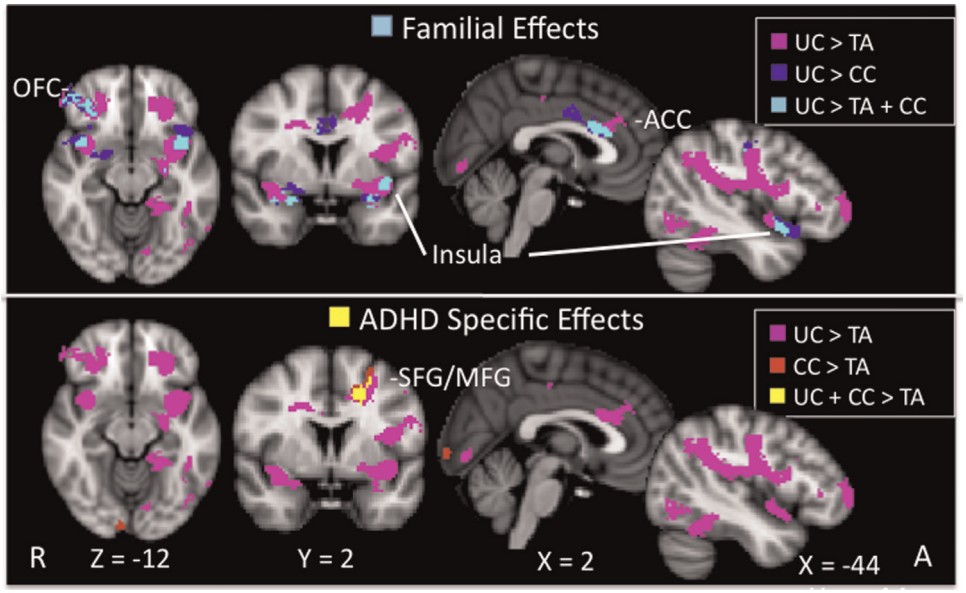

Fig. 2. Familial and ADHD specific conjunctions for maintenance of task set (I+C+N > fixation). Activity in cyan is representative of familial differences that were greater for unrelated controls (UC) compared to both the twins with ADHD (TA) and their discordant control co-twins (CC) (i.e., the conjunction between UC > TA and UC > CC). Activity in yellow is representative of ADHDspecific differences (i.e., the conjunction between UC > TA and CC > TA). These conjunctions are overlaid on UC > TA shown in purple, UC > CC in navy, and CC > TA in red. Abbreviations: Superior frontal gyrus (SFG); Middle frontal gyrus (MFG); Orbital frontal cortex (OFC); Anterior cingulate cortex (ACC).

Table 3
Activation differences for maintenance of task set and interference.

Brain region	BA	Max Z-stat	Voxels	*MNI coordinates*		
				x	*y*	*z*
Maintenance of task set						
Unrelated control > Twins with ADHD						
Supramarginal into Pre-central Gyrus (L)	BA40	5.09	9446	−64	−32	30
Insula (R)	BA13	4.83	3519	36	6	−14
Orbital Frontal Gyrus (R)	BA47	4.73	766	30	34	−10
Orbital Frontal Gyrus (L)	BA11	4.08	646	−28	40	−12
Lateral Occipital (R)	BA7	4.04	377	32	−70	52
Lingual Gyrus (L)	BA18	3.59	344	−8	−78	−6
Superior Parietal Lobule (R)	BA40	3.99	260	28	−40	60
Cingulate Gyrus (L)	BA31	4.02	246	−10	−30	40
Cuneus (R)	BA18	3.38	245	14	−88	26
Angular Gyrus (R)	BA39	3.42	205	38	−52	28
Frontal Pole (L)	BA10	3.57	185	−44	50	12
Parahippocampal Gyrus (L)	BA36	3.55	170	−22	−46	−10
Unrelated control > Control co-twins						
Insula (R)	BA13	3.84	732	40	10	−14
Orbital Frontal Gyrus (L)	BA47	3.68	645	−28	20	−18
Cingulate Gyrus (L)	BA24	3.56	541	−2	−4	34
Parahippocampus into amygdala (R)	BA28	3.68	239	16	−2	−14
Postcentral Gyrus (L)	BA3	4.1	224	−44	−16	48
Control co-twins > Twins with ADHD						
Supramarginal Gyrus(L)	BA40	3.9	396	−68	−30	40
Superior Frontal Gyrus(L)	BA6	3.7	231	−20	10	52
Lingual Gyrus(R)	BA18	3.12	207	6	−94	−8
Precentral Gyrus(R)	BA4	3.5	177	48	−8	42
Incongruent > Congruent						
Control co-twin > Twins with ADHD						
Precuneus into Postcentral Gyrus(R)	BA7	3.4	329	14	−42	56
Control co-twin > Unrelated Control						
Fusiform Gyrus(L)	BA19	3.47	169	−28	−74	−18

Note. No significant clusters for ADHD > Control Co-twin, ADHD > Unrelated controls, and Control Co-twin > Unrelated Controls. All results pass voxel wise threshold $p < .01$ and whole brain Alphasims Cluster correction $p < .05$.

criteria for ADHD in young adulthood in the absence of other co-morbid disorders (Banich et al., 2009). Less activity in these regions suggests that differences in cognitive and motor control may determine whether attention problems reach diagnostic criteria (Corbetta and Shulman, 2002; Bush, 2011).

In contrast, the pattern of activity in the medial and orbital frontal regions was more representative of a familial risk factor. While the unrelated controls showed moderate activation compared to fixation in the medial prefrontal cortex including the ACC and insula, both the twins with ADHD and their discordant co-twins showed deactivation in these regions. The ACC has long been known to play an important role in cognitive control, with various theories suggesting that it may be involved in late-stage control, response evaluation, and/or conflict monitoring (Milham and Banich, 2005; Mohanty et al., 2007; Banich, 2009). More recent work has shown that the insula may be co-activated with the ACC during these processes (see Petersen and Posner, 2012 for review). Familial effects were also observed for the amygdalae and OFC, suggesting that alterations in processing of emotional or salient material (see Etkin et al., 2011) may be contributing to this familial risk factor. Overall, these findings of alterations in patterns of brain activation in the discordant co-twins are consistent with the those observed in non-twin discordant siblings of children and youth with ADHD who show reduced activity in the ACC and ventrolateral prefrontal cortex compared to unrelated controls (Durston et al., 2006; Mulder et al., 2008). Such alterations may underlie the behavioral findings (Steinhausen et al., 2012) indicating that the non-ADHD siblings of youth with ADHD are still at risk for somatic complaints, anxious/shy behavior, and problems with peers.

We can only speculate on what these differences in neural activation between twins with ADHD, their control co-twins, and unrelated controls might reflect. The frontoparietal and cingulo-opercular networks are known to be involved in providing top-down control over task goals and are two networks commonly referred to as EF networks (Banich, 2009; Petersen and Posner, 2012). One possible explanation is that the frontoparietal network maintains the overall task goal while the ACC and related regions such as the insula, are more involved in reactive aspects of

cognitive control that cannot be planned for and/or exert control when a top-down bias is not strong (see for example, Silton et al., 2010; see Petersen and Posner 2012 for an alternative interpretation). If the neural machinery for top-down biasing is unaffected in control co-twins, they may be able to set and maintain task goals, despite the fact that both they and their twin with ADHD may have alterations in the more reactive aspects of cognitive control as well as potential alterations in motivational processes, reflected by alterations in activation of the OFC.

Despite the strength of the study's twin design, the study is not without limitations. Due to the highly specific nature of recruitment needs for a discordant twin sample, it was not possible to select females with ADHD. Hence, it is not known whether the results we report will generalize to females with ADHD. Control co-twins and their gender matched unrelated controls were half male and female. Therefore, results suggest that, on average, familial effects can be observed across both genders. As discussed in the supplementary materials, including gender as a covariate showed minimal differences between males and females in our control groups.

Another limitation was our inability to select groups that were homogenous in adulthood levels of ADHD, as well as, examine developmental changes in symptomatology from childhood to young adulthood. Whereas all twins with ADHD met criteria for ADHD during childhood, increases and decreases in ADHD symptomatology did occur. Exploratory results (see Supplementary Fig. 1) indicated that for the incongruent minus congruent contrast, individuals who continued to show ADHD symptomatology into adulthood showed greater activity in the superior occipital and parietal regions compared to those who did not. However, our small sample size limits our ability to clearly assess patterns of brain activation associated with developmental changes. Future studies assessing individual differences in symptomatology throughout development and their relationship to patterns of brain activation would provide important information on brain structures critical for either the maintenance or amelioration of attention problems in young adulthood.

In addition, because we utilized a sample of DZ twins, we can speak only to familial rather than genetic effects. DZ twins share on average 50% of their segregating genes but also share a similar home environment, which is why we refer to our findings as familial effects. Moreover, determining whether our findings would be observed with other tasks that involve executive control, such as reward-based tasks or measures of inhibitory control such as the go/no-go task, would be important to determine the generalizability of the observed effects.

Finally, while utilizing a discordant twin control sample has notable strengths, it does limit our ability to select a larger or more homogeneous sample without potential confounds such as medication or gender. Nonetheless, the analyses presented in the supplementary materials suggest that these potential confounds are not driving the pattern of results we observe. Future research will be important to confirm and clarify the findings reported in this paper.

5. Conclusion

By using a case control, discordant twin design, the present study provides evidence for distinct alterations in neural activation in individuals with ADHD that reflect both diagnosis-specific as well as familial effects. ADHD-specific effects were observed in portions of the frontoparietal executive control network. Specifically, individuals with ADHD showed less activation of the superior frontal gyrus, pre- and post-central gyrus, supramaginal gyrus, and lingual gyrus and fusiform compared to both their control co-twins and unrelated controls. In contrast, patterns of activity in the ACC, insula, amygdala, and OFC were consistently shared between twin pairs as compared to unrelated controls, providing strong evidence for an underlying familial risk factor that does not lead to a diagnosis of ADHD.

Overall, this study demonstrates that discordant twin-pair designs can help to disentangle the neurobiological differences specific to individuals with ADHD while facilitating our understanding of familial influences. We speculate that similarity in brain activation found between the twins with ADHD and their co-twins may reflect an underlying familial risk for decreased reactive control and alterations in emotional processes, while processes more specific to executive control and goal maintenance may determine whether these risk factors reach clinical significance.

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pscychresns.2015.07.019.

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