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## Neuroimaging of response interference in twins concordant or discordant for inattention and hyperactivity symptoms

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### Abstract

Attention deficit hyperactivity disorder (ADHD) is to a large extent influenced by genetic factors, but environmental influences are considered important as well. To distinguish between functional brain changes underlying primarily genetically and environmentally mediated ADHD, we used functional MRI to compare response interference in monozygotic twins highly concordant or discordant for attention problems (AP). AP scores were assessed longitudinally with the Child Behavior Check List attention problem scale (CBCL-AP). Response interference was measured during two executive function paradigms; a color-word Stroop and a flanker task. The neuroimaging results indicated that, across the entire sample, children with high CBCL-AP scores, relative to children with low CBCL-AP scores, showed decreased activation to response interference in dorsolateral prefrontal, parietal and temporal brain regions. Increased activation was noted in the premotor cortex and regions associated with visual selective attention processing, possibly reflecting compensatory mechanisms to maintain task performance. Specific comparisons of high and low scoring concordant twin pairs suggest that AP of genetic origin was characterized by decreased activation of the left dorsolateral prefrontal cortex during the Stroop task and right parietal lobe during the flanker task. In contrast, comparison of twins from discordant monozygotic pairs, suggest that AP of environmental origin was characterized by decreased activation in left and right temporal lobe areas, but only during Stroop interference.

The finding of distinct brain activation changes to response interference in inattention/hyperactivity of 'genetic' versus 'environmental' origin, indicate that genetic and environmental risk factors for attention/hyperactivity problems affect the brain in different ways.

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## Keywords

Functional MRI; Stroop task; flanker task; ADHD; genetic risk; environmental risk

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## INTRODUCTION

ADHD is a childhood onset psychiatric disorder characterized by symptoms of inattention, impulsivity and hyperactivity that affects about 3-5% of the child population (Goldman et al., 1998; Hudziak et al., 2000; Buitelaar and Kooij, 2000). In agreement with the general hypothesis that impaired executive function/inhibition is a core feature of ADHD (Barkley, 1997; Sergeant et al., 2002; Castellanos et al., 2006) brain imaging studies have pointed to impaired frontostriatal brain circuitry underlying ADHD symptoms (Bush et al., 2005; Seidman et al., 2005). For example, structural MR imaging in ADHD children has provided evidence for volume loss in orbitofrontal/inferior prefrontal cortex (Giedd et al., 2001; Carmona et al., 2005; Shaw et al., 2007b) and dorsolateral prefrontal lobes (Semrud-Clikeman et al., 2000; Overmeyer et al., 2001; Kates et al., 2002; Hill et al., 2003; Durston et al., 2004; Carmona et al., 2005; Shaw et al., 2007a), as well as regions of the basal ganglia (Filipek et al., 1997; Overmeyer et al., 2001; Qiu et al., 2009).

In addition to frontostriatal abnormalities, structural changes related to ADHD have also been observed in other brain regions, including the occipital, parietal and temporal lobes (Castellanos et al., 2002; Sowell et al., 2003; Durston et al., 2004; Shaw et al., 2007b), as well as the posterior corpus callosum (Hill et al., 2003) and cerebellum (Durston, 2003; Carmona et al., 2005; Krain and Castellanos, 2006). A recent meta-analysis of 22 region-of-interest studies found reductions of total and right cerebral volume, and volume loss for cerebellar regions, the posterior corpus callosum splenium, right caudate and several frontal regions to be most consistent (Valera et al., 2007). Another meta-analysis of 7 voxel-based studies identified significant gray matter reduction in the right putamen /globus pallidus region (Ellison-Wright et al., 2008). Brain volume increments associated with ADHD have also been reported for regions of both left and right parietal, frontal and temporal lobes (Sowell et al., 2003; van 't Ent et al., 2007; Brieber et al., 2007) as well as the right occipital lobe (Wang et al., 2007). Increased volumes may indicate a lack of synaptic pruning during brain development (Huttenlocher, 1979), or plastic adjustment to compensate for reduced processing of adjacent brain regions.

Functional brain imaging studies have generally provided support for a core role of frontostriatal deficits in ADHD but, again, other areas have been implied as well (for a meta-analysis see: (Dickstein et al., 2006)) and the exact pattern of ADHD related brain activation differences depends strongly on the type of task used. For example, fMRI during response inhibition tasks indicated that the decreased brain activation in ADHD children was restricted to the frontostriatal network (Durston et al., 2003; Booth et al., 2005). However, decreased brain activation was also found in right occipital-parietal brain regions in a spatial working memory task (Vance et al., 2007) and in frontal and parietal regions and putamen in a cued target detection task (Cao et al., 2008). During the interference condition of an fMRI compatible variant of the Stroop task (Stroop, 1935) decreased activation has been found for the anterior cingulate cortex (ACC), basal ganglia, insula and cerebellum (Zang et al., 2005). Reduced ACC activation has also been reported in adult ADHD patients with the counting Stroop (Bush et al., 1999). Functional MRI recordings during modified versions of the Eriksen flanker task (Eriksen and Eriksen, 1974) indicated decreased activation of frontostriatal (Konrad et al., 2006) and frontal-striatal-temporal-parietal networks (Vaidya et al., 2005) during response interference after incongruent flankers. Functional imaging studies also report increased brain activation in ADHD subjects compared to controls. Functional hyperactivation has been

observed most frequently in regions of the occipital and parietal lobes and basal ganglia, but also for frontal brain regions (for an overview see: Fassbender and Schweitzer, 2006).

Numerous twin and family studies indicate that attention problems have a major genetic component explaining up to 80% of the total variance (e.g., Thapar et al., 1999; Neuman et al., 1999; Rietveld et al., 2004; Hudziak et al., 2005; Derks et al., 2008). In agreement with the hypothesis of disturbed dopamine function in frontostriatal brain networks (Dickstein et al., 2006), genetic association studies have provided evidence for the involvement of dopamine transporter and receptor genes (Auerbach et al., 2001; Durston et al., 2005; Durston et al., 2008). However, involvement of other genes has also been reported (Brookes et al., 2006a). For example, in addition to confirmatory evidence for associations at previous candidate genes, a recent genome-wide association (GWA) study identified novel genetic associations for intronic regions of genes CDH13, a negative regulator of neural cell growth, and GFOD1, encoding glucose-fructose oxidoreductase (Lasky-Su et al., 2008).

Despite the high heritability, environmental risks, and possibly gene-environment interactions and correlations (Kahn et al., 2003; Brookes et al., 2006b), are also considered key elements underlying the development of ADHD. Consistent with the fact that ADHD is a childhood onset disorder, environmental risk factors primarily include problems that impair normal brain development, such as maternal alcohol abuse or smoking during pregnancy (Linnet et al., 2003; Huizink and Mulder, 2006), premature birth combined with low birth weight (Bhutta and Anand, 2001) and hypoxia at birth (Lou et al., 2004). Inattention and hyperactivity symptoms may also arise at later ages due to neurological complications, such as childhood stroke (Max et al., 2002; Max et al., 2005a), traumatic brain injury (Herskovits et al., 1999; Gerring et al., 2000; Max et al., 2005b), epilepsy (Powell et al., 1997; Schubert, 2005) and meningitis or autoimmune disorders (Peterson et al., 2000; Wait et al., 2002).

Except for cases with clearly identifiable brain lesions, the neurobiological pathways underlying environmentally driven ADHD are still unclear. Furthermore, it is largely unknown whether genetic and environmental etiologies follow similar or distinct pathways. A distinction between genetically and environmentally mediated neurobiological changes that underlie the development of behavioral traits such as ADHD, can be accomplished using a specific study design in twins, the concordant/discordant monozygotic (MZ) twin design. To assess the influence of environmental risk factors, neuroimaging results can be compared in MZ twin pairs strongly discordant for attention problems or ADHD. In these pairs, one twin scores very high, whereas the co-twin scores very low on ADHD symptoms. Because monozygotic twins are (nearly) always 100% identical at the DNA sequence level (Boomsma et al., 2002a), the discordance at the phenotypic level must arise from differential exposure to environmental influences. Differences in brain structure/function between the high-risk twin and the low-risk co-twin, therefore, also reflect environmental effects on the brain, including epigenetic effects, rather than effects of variation in DNA sequence. To maximize detection of the effects of genetic risk factors, neuroimaging results can be compared in twins that both score very high on symptoms of ADHD with twins that both score very low on symptoms of ADHD. These concordant high and low scoring twins are highly likely to come from families with either high or low vulnerability for ADHD. Because an influence of shared family environment on ADHD symptoms has hardly ever been found, whereas heritability is often over 80% (Rietveld et al., 2003), this familial vulnerability translates almost entirely to genetic vulnerability. Therefore, differences between the groups of low and high concordant twins are expected to highlight brain changes related to the genetically mediated risk for ADHD sequence.

In a previous study by our group we applied the concordant/discordant monozygotic twin design to study structural deviations in the brains of subjects with a genetic etiology of ADHD and in subjects with an environmental etiology of ADHD (van 't Ent et al., 2007). The results

from this study indicated that attention problems are associated with anatomical abnormalities of an action-attentional network including cerebellar, occipital/parietal and temporal brain regions interacting with the prefrontal cortex. This network appeared to be compromised at different brain areas for genetically and environmentally driven attentional problems, especially within the prefrontal lobes. In concordant high risk twins, there was volume loss in orbitofrontal brain areas, and a compromised posterior corpus callosum. In the high-risk twins from discordant twin-pairs, there was reduced volume in the right inferior dorsolateral prefrontal cortex.

In the present study we used the MZ concordant/discordant twin design (Lehn et al., 2007) to detect deviant *functional* brain activation that is specifically related to either genetic or environmental risk factors for ADHD. We recorded the fMRI BOLD (blood oxygen level dependent) signal during two different tasks that both probe selective attention and inhibitory control; the Stroop color-word task (Stroop, 1935) and a modified version of the Eriksen flanker task (Eriksen and Eriksen, 1974). We compared fMRI recordings of MZ pairs selected from the Netherlands Twin Register to be concordant high or low or discordant high-low for longitudinal parental ratings on the Child Behavior Checklist Attention/hyperactivity Problem scale (CBCL4/18: (Achenbach et al., 1991; Verhulst et al., 1996)). The CBCL is a widely used tool for the assessment of childhood behavioral abnormalities and correlates well with the clinical DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-4<sup>th</sup> edition: (American Psychiatric Association, 1994)) assessment of Attention Deficit Hyperactivity Disorder (ADHD) (Derks et al., 2006). MZ pairs selected to be highly concordant for parental ratings on the CBCL, either at the high or at the low end of the CBCL Attention Problems (CBCL-AP) scale are considered to represent a *genetic* contrast. MZ twin pairs in which one twin scores high and the other low on the CBCL-AP scale are considered to represent an *environmental* contrast.

## EXPERIMENTAL PROCEDURES

### Participants

The subjects were Dutch twins from the Netherlands Twin Register whose parents take part in a longitudinal study designed to examine genetic and environmental influences on the development of behavioral and emotional problems in twins from birth onwards (NTR: (Boomsma et al., 2002b)). Surveys are sent to the parents when the twins are 1, 2, 3, 5, 7, 10, and 12 years of age. The twins included in the present study were born between 1986 and 1993 and selected on basis of the maternal Child Behavior Checklist (Achenbach et al., 1991) ratings collected at the ages 7, 10, and 12 years. We considered only twin pairs whose mothers had completed the CBCL at ages 7, 10, and/or 12 years at least at two time-points ( $n = 4877$ ) and who were classified as monozygotic (MZ) ( $n = 1803$  pairs).

Each child was classified as either at *high risk* or *low risk* for ADHD based on his/her standardized CBCL-AP *T*-scores (mean = 50, SD = 10) at ages 7, 10, and 12. *T*-scores were calculated separately for boys and girls. High risk children had a *T*-score above 60 at all available time-points and a *T*-score above 65 at least once. Low risk children had a *T*-score below 55 at all available time-points. Prior research by our group and others have shown this to be a sensitive and specific technique to identify children at risk for clinical ADHD (Chen et al., 1994; Hudziak et al., 2004; Derks et al., 2006; Lehn et al., 2007). Using the CBCL-AP data we identified twin pairs who were concordant high (both twins high CBCL-AP scores), concordant low (both twins low CBCL-AP scores) and discordant (one twin high and co-twin low CBCL-AP score).

Twenty-seven monozygotic (MZ) twin pairs participated in the study. The selection of twin pairs was done at two time points. The first selection of twin pairs consisted of participants for

a structural MRI study ( $n = 25$ ; for a detailed description see (van 't Ent et al., 2007)). In this sample of twins, born between 1986 and 1991, functional MRI data during a Stroop and flanker task were also collected for 3 concordant AP high, 10 concordant AP low and 5 AP discordant MZ pairs. At the second selection, the sample was extended with concordant AP high and discordant AP twin pairs. There were 23 pairs from the birth cohorts 1992-1993 who met the inclusion criteria on basis of the Maternal CBCL-AP. Twins were excluded with chronic medical or neurological disorders ( $n = 1$ ) and mental disabilities ( $n = 4$ ), leaving 18 twin pairs to be invited. The families of 9 twin pairs (5 concordant high, 4 discordant) took part in the MRI experiments. Non-participants included families who could not be contacted ( $n = 3$ ), declined ( $n = 3$ ), or whose twins had orthodontic braces ( $n = 3$ ).

The total sample included 8 concordant high, 10 concordant low and 9 discordant twin pairs. The MZ status of all twin pairs was confirmed based on DNA zygosity testing of 15 genetic markers. Four individuals of the concordant high sample (twin and co-twin of one pair, and 2 twins from different pairs), and two twins of the concordant low sample (twin and co-twin of one pair) reported mild dyslexia. However these twins had no problems with reading single color words and showed normal Stroop task, as well as flanker task, performance. To assess DSM-IV diagnosis of ADHD, all mothers of the twins completed a telephone interview based on the Dutch version of the DISC-IV Parent Version (DISC-IV-P: (Shaffer et al., 1993)). In our final population, 4 twins from the concordant high-risk group (twin and co-twin of one pair and 2 twins from different pairs) and 2 twins from the discordant group met clinical criteria for ADHD diagnosis. For the twin and co-twin of the concordant high-risk sample that both met the ADHD criteria, the mother reported that the children had been diagnosed previously with PDD-NOS as well as Charcot-Marie-Tooth Disorder. Post hoc tests indicated that selective exclusion the twins with mild dyslexia or previously diagnosed of PDD-NOS did not significantly affect the results of the present study. All twins and their parents provided written informed consent. The study was approved by the university ethical review board.

### Task paradigms

In the Stroop task subjects had to report the ink color of written color words. The stimuli consisted of Dutch translations of the words 'red', 'yellow', 'blue' and 'green' and could be written in either of these four colors. Word meaning could be either congruent (e.g. the word 'red' written in red) or incongruent (e.g. the word 'blue' written in red) with ink color. A left index finger button press was required after ink colors red or yellow, and a right index finger button press after ink colors blue or green. The task was administered in an event-related design and split into six consecutive sessions separated by small breaks during which the subject was asked to lie still inside the scanner. In each session 12 congruent and 12 incongruent trials were presented in random order. Stimuli were shown for 2000 ms and the interstimulus interval consisted of a period of black screen after each stimulus (randomized between 1050 – 3050 ms) and a subsequent fixation cross for 950 ms before the next stimulus. Stimulus order was randomized for each run.

In the flanker task subjects had to indicate, as quickly as possible, the direction of a central target arrow (i.e., '<' left hand press; '>' right hand press) which was surrounded by four task irrelevant flankers of the same size and shape. The direction of the flanker arrows could be either congruent ('>>>>' or '<<<<') or incongruent ('<<><<' or '>><>>') to the direction of the central target arrow. Flankers and targets were displayed simultaneously. The complete experimental setup including stimulus presentation times, interstimulus interval and ratio of incongruent to congruent trials was the same as for the Stroop paradigm. Before the scan sessions, subjects practised both the Stroop and flanker task with a maximum of six trials. On average subjects were accustomed to each task after two or three stimuli. Inside the scanner, stimuli were back-projected onto a screen that subjects viewed through a mirror mounted on

the MRI head coil. Single characters of the stimuli in both tasks subtended  $1.1^\circ$  in height and  $0.9^\circ$  in width. Character spacing was  $0.16^\circ$ .

### Image acquisition

The MRI protocol consisted of a structural and functional scanning part and took about 60 minutes in total for each individual (30 minutes sMRI and 30 minutes fMRI). First, 3 separate whole head anatomical scans were consecutively acquired for each twin, with randomized scan order of the twins. Subsequently functional scanning during both tasks was performed, starting with the twin that had first entered the scanner for structural MRI measurement. Task order (Stroop followed by flanker or flanker followed by Stroop) was the same within twin pairs, but counterbalanced between twin pairs. During each individual scan session the twins remained inside the scanner and were asked to minimize head movement.

Magnetic resonance imaging of the brain was performed on a 1.5 T Sonata MR system (Siemens, Erlangen, Germany) with a standard circularly polarized head coil. Anatomical scans included 160 sagittal slices at 1 mm intervals acquired with a 3D gradient-echo T1-weighted sequence (flip angle  $15^\circ$ ; Repetition Time, TR = 1900 ms; Echo Time, TE = 3.93 ms; Inversion Time, TI = 1100 ms). In plane resolution was  $256 \times 224$  pixels (pixel size  $1 \text{ mm}^2$ ), gray level resolution was 16 bit. For fMRI, Echo Planar Images (EPI) sensitive to blood oxygen level dependent (BOLD) effects were obtained (flip angle =  $90^\circ$ , Repetition Time, TR = 2000 ms; Echo Time, TE = 60 ms; Field of View:  $200 \times 200$  mm, Bandwidth 888 hz/pixel, Echo Spacing 1.22 ms). For both reaction tasks, each of the 6 individual sessions consisted of 70 volumes of 20 axial slices (Slice Thickness = 5 mm; Interslice Gap 1 mm; In-plane Resolution:  $3.125 \times 3.125$  mm). The first 2 EPI volumes for every session were discarded to allow for magnetic saturation.

### Analysis of behavioral data

Analysis of task performance was performed in SPSS (SPSS Inc, Chicago, Illinois) using repeated measures ANOVA to correct for the dependency of twin data. For the concordant twin samples, the possibility of within-twin pair correlations of reaction performance was taken into account by defining a variable 'group' (group1 versus group2) as a within-twin pair factor, with one twin of each concordant pair randomly assigned to group1 and the co-twin to group2. Differences related to AP were modeled by using the variable 'twin AP status' (twins high on the CBCL-AP scale versus twins low on the CBCL-AP scale) as a between-twin pair factor. For the discordant pairs, 'twin AP status' (high CBCL-AP twin versus low CBCL-AP co-twin) was used as a within-twin pair factor. Variables 'selection time point' (twin pairs scanned at the first selection time-point versus twin pairs scanned at the second selection time-point) and 'sex' of the twins were included as additional between-pair covariates. A significance level of  $p < 0.05$  was applied throughout.

### fMRI analysis

fMRI data were analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK). EPI scans were slice time corrected, realigned and normalized to the standard MNI (Montreal Neurological Institute) brain in SPM. Subsequently, data were resliced to  $3 \times 3 \times 3$ -mm voxels and spatially smoothed using an 8-mm isotropic Gaussian kernel. After high-pass filtering (cut-off 128 seconds), functional scans were analyzed in the context of the general linear model using delta functions convolved with a canonical hemodynamic response function. Head-movement parameters were modeled as regressors of no interest. Post-hoc analysis of subject motion during the scans, based on the functional scan realignment parameters, indicated that the twins with high risk for ADHD did not exhibit significantly larger head movement compared to the low risk twins. For each subject and task, contrasts images were computed for simple main effects of congruent and incongruent trials, as well as the effect

of response interference (incongruent minus congruent trials). For all contrasts, only trials with correct reactions were included. Contrast images for interference were fed into second-level (random effects) analyses for assessment of AP-related differences using repeated measures ANOVA. Similar to the analysis of behavioral data, to account for within-twin pair correlations of fMRI, a within-twin pair variable 'group' was defined. For AP concordant pairs, twins were randomly assigned to group1 and group2 and variable 'twin AP status' was used as a between-twin pair factor. For the discordant samples, high-risk twins were assigned to group1 and the low-risk co-twins to group2 such that 'twin AP status' represented a within-twin pair factor. Variables 'selection time point' and 'sex' were included as between-pair covariates. For main effects across twins of selected contrasts we set an individual voxel threshold of  $p < 0.05$ , corrected for multiple comparisons (False Discovery Rate: FDR), with a minimal cluster extent of 25 voxels. Group differences, masked with the appropriate main effect (mask thresholded at  $p < 0.05$ , uncorrected), are reported at an uncorrected individual voxel threshold of  $p < 0.001$ .

## RESULTS

Demographics and mean CBCL-AP T-scores of the sample are summarized in Table 1. In line with our selection criterion, CBCL-AP T-scores were significantly higher for concordant CBCL-AP high compared to concordant CBCL-AP low twin samples ( $F(1,16) = 227.4$ ,  $p < 0.001$ ), as well as for the discordant CBCL-AP high twins relative to their CBCL-AP low scoring co-twins ( $F(1,8) = 67.1$ ,  $p < 0.001$ ). Table 1 also shows mean scores of DSM-Oriented scales for comorbid Anxiety problems, Oppositional Defiant problems and Conduct problems as well as mean birth weight. DSM-Oriented scores refer to composite scores on selected items from the CBCL4/18 inventory that are consistent with DSM-IV categories (Achenbach et al., 2003). The concordant CBCL-AP high sample exhibited higher scores, relative to the concordant low twins, on Anxiety problems ( $F(1,16) = 24.1$ ,  $p < 0.001$ ), Oppositional Defiant problems ( $F(1,16) = 39.1$ ,  $p < 0.001$ ) and Conduct problems ( $F(1,16) = 35.9$ ,  $p < 0.001$ ). CBCL-AP high twins from discordant pairs scored higher than their CBCL-AP low co-twins on Anxiety ( $F(1,8) = 10.5$ ,  $p = 0.012$ ), but not on Oppositional Defiant problems ( $F(1,8) = 1.2$ ,  $p = 0.306$ ) or Conduct problems ( $F(1,8) = 0.1$ ,  $p = 0.809$ ). Birth weight was significantly lower for CBCL-AP high twins from discordant pairs compared to their CBCL-AP low co-twins ( $F(1,8) = 9.2$ ,  $p = 0.016$ ). No difference in birth weight was seen for concordant CBCL-AP high compared to concordant CBCL-AP low twins ( $F(1,16) = 1.7$ ,  $p = 0.205$ ).

### Task Performance

Reaction times, across all individuals, for both the Stroop and flanker task were significantly delayed after incongruent compared to congruent stimuli [Stroop: incongruent  $659.1 \pm 203.4$  ms vs. congruent  $597.4 \pm 153.9$  ms,  $F(53,1) = 47.5$ ,  $p < 0.001$ ; flanker: incongruent  $611.5 \pm 131.4$  ms vs. congruent  $509.2 \pm 107.3$  ms,  $F(53,1) = 327.4$ ,  $p < 0.001$ ]. In addition, for both tasks, percentages of trials with correct reactions were significantly reduced after incongruent stimuli (Stroop: incongruent  $95.6 \pm 4.9$  vs. congruent  $96.6 \pm 4.3$ ,  $F(35,1) = 5.1$ ,  $p = 0.028$ ; Flanker: incongruent  $95.8 \pm 3.9$  vs. congruent  $99.2 \pm 2.6$ ,  $F(35,1) = 65.6$ ,  $p < 0.001$ ).

Effects of Stroop and flanker interference on reaction performance were quantified by computing differences in reaction times and reaction accuracy between incongruent and congruent stimulus trials. Consistent with previous findings in a larger sample (Stins et al., 2005), the interference effects on reaction time for the Stroop and flanker task were not significantly correlated (Pearson Correlation = 0.21,  $p = 0.13$ ). Response interference effects are displayed separately for the different cells of our twin design in table 2. Results from statistical tests on AP-related differences for the concordant and discordant twin samples ( $F$  and  $p$ -values) are also listed. The effect of response interference on reaction times was not significantly different between twins with high and low AP scores. For percentage of correct

responses, a larger effect of response interference was found in concordant high risk relative to concordant low risk twins during the flanker task.

### fMRI results

**Main effects**—Figure 1 shows brain areas with significant fMRI BOLD activations, across all participants, during performance of the Stroop task (left panels) and flanker task (right panels). Significant clusters for the different fMRI contrasts are also summarized in tables 3 and 4. The first two rows of figure 1 and first two columns of tables 3 and 4 illustrate brain areas with significant fMRI BOLD signal on congruent and incongruent stimulus trials. Brain areas activated during the Stroop and flanker task were highly similar. Task related activation was found in subcortical brain areas (thalamus and putamen), cortical regions related to sensory and motor processing (occipital/temporal junction, cerebellum, motor cortex and supplementary motor area) and brain regions involved in higher cognitive processing, including the parietal cortex, insula/claustum region and anterior cingulate. For the Stroop task there was also evidence for activation of left and right frontopolar areas. For the flanker task frontopolar activation was found only in the left hemisphere after incongruent stimuli.

Brain areas that showed increased fMRI BOLD signal on incongruent trials compared to congruent trials, reflecting enhanced activation to response interference, can be appreciated from the ‘*interference: (incongruent-congruent)*’ contrast. Results for this contrast are illustrated in the third row of figure 1 and third column of tables 3 and 4. Response interference was associated with enhanced activation of the cerebellum, left and right occipitotemporal, insula/claustum and parietal cortices as well as several frontal lobe regions including the left and right supplementary motor area (SMA), anterior cingulate cortex (ACC), inferior frontal gyrus (IFG) and dorsolateral prefrontal cortex (dlPFC). For the Stroop task, increased activation was also noted in the left and right cuneus. For the flanker task additional clusters were observed in left and right thalamus and a left frontopolar region.

### Genetic risk: High versus low risk twins from concordant pairs

Table 5 and left panels of fig. 2, show clusters of AP-related differences for brain activation to response interference between the concordant high and concordant low twin pairs. Compared to low risk twins from concordant pairs, decreased activation to response interference was found in high risk twins from concordant pairs, but located in different brain regions for the Stroop and flanker task. For the Stroop task, a single cluster of decreased activation was found in the left dorsolateral prefrontal cortex. For the flanker task hypoactivation was restricted to the right parietal cortex.

High-risk twins from concordant pairs showed *increased* activation to Stroop interference in a region overlying the caudal part of the left thalamus, including mainly the left pulvinar and extending rostrally to include the left posterior caudate body. For flanker interference, a single cluster of increased activation in high risk twins was located in the left premotor cortex.

### Environmental risk: high- versus low-risk twins from discordant pairs

Paired comparisons between the high-risk twin and the low-risk co-twin from discordant pairs revealed significant clusters of decreased activation to response interference only for the Stroop task (table 6 and fig. 2: right panels). High-risk twins from discordant pairs showed relatively reduced activity in regions of the left and right superior temporal gyrus, right fusiform gyrus and right precuneus.

High risk discordant twins also had areas of *increased* activation during response interference, but exclusively in the flanker task. Similar to findings in concordant high risk twins, one cluster



was located in the left premotor cortex. In addition there was evidence for increased activation in the right premotor cortex, right middle frontal gyrus and anterior cingulate.

## DISCUSSION

To examine functional brain changes related to genetic and environmentally mediated risk for ADHD, we measured functional MRI during performance of the Stroop and flanker tasks in genetically identical twins concordant or discordant for CBCL-AP attention and hyperactivity problem scores. High-risk concordant twin pairs are considered at higher genetic risk for ADHD than low-risk concordant twin pairs, while high-risk twins from discordant pairs are considered at higher environmental risk for ADHD than their low-risk co-twins (Castellanos et al., 2003; van 't Ent et al., 2007).

Analysis of task-related behavior indicated classical effects of response interference on reaction times and response accuracy for both the Stroop and flanker task. There was only marginal evidence for enhanced interference in children who were rated high on the CBCL-AP scale compared to children who were rated low on the CBCL-AP scale. That is, for the flanker task the negative effect of information conflict on reaction accuracy was significantly larger for high-risk twins from concordant pairs. Performance deficits in executive functioning for individuals with ADHD have been observed in several previous studies (for overviews see e.g., Sergeant et al., 2002; Doyle, 2006) including different versions of the Stroop task (Homack and Riccio, 2004) and flanker task (Mullane et al., 2008). However, a recent meta-analysis by van Mourik et al. did not confirm the presence of impaired Stroop interference in ADHD patients (van Mourik et al., 2005). There may be several reasons for inconsistent findings with regard to task performance such as differences in task instructions, performance measures or the format of the experimental task. For example, with regard to the Stroop task, Lansbergen et al. (Lansbergen et al., 2007) concluded from a recent meta-analysis that Stroop performance is more reliably affected in ADHD subjects when time based measures such as reaction time per item are used (although this disagrees with our findings) but also indicated more consistent behavioral impairment when the task is administered using a computerized version, instead of a set of cards.

The fMRI main effects of our study, across a large number of subjects (54 twins in total), at the same time of day for every individual and on the same scanner with the same scanning parameters, indicated that highly similar brain processes were active during Stroop and flanker task performance. CBCL-AP status clearly affected this brain activation such that high-risk twins from concordant and discordant pairs generally showed decreased activation during response interference compared to low-risk twins in frontal, parietal and temporal brain regions. Involvement of these brain areas generally corresponds with earlier functional imaging studies of ADHD, both with regard to hypofrontality (Bush et al., 2005; Dickstein et al., 2006; Rubia et al., 2008; Smith et al., 2008), and reduced parietal and temporal brain activation (Vaidya et al., 2005; Vance et al., 2007; Cao et al., 2008).

### Genetic contrast

Relative to concordant low-risk twins, concordant high-risk twins showed hypoactivation to response interference in the left dorsolateral prefrontal cortex (dlPFC) for the Stroop task and right parietal cortex for the flanker task. Reduced prefrontal cortex activation during a Stroop-like paradigm has also been found in boys with ADHD (Zang et al., 2005), in combination with hypoactivation of the anterior cingulate cortex (ACC). In addition, Bush et al. (Bush et al., 1999) reported hypofrontality in adult ADHD patients during a counting Stroop, but their main focus was on the ACC. Reduced parietal cortex activation during the flanker task is in line with a study by Vaidya et al. (Vaidya et al., 2005) that found a reduction in frontal-striatal-temporal-parietal network engagement in ADHD children during flanker interference

suppression. Konrad et al. (Konrad et al., 2006) also reported reduced brain activation in ADHD children during a modified flanker task, but restricted to frontostriatal brain regions.

Our results are only in partial agreement with results of family based studies by Durston and Mulder and colleagues (Durston et al., 2006; Mulder et al., 2008) to chart the effects of genetic vulnerability to ADHD on functional brain activation. In these studies that applied fMRI during a go no-go paradigm it was found that familial risk for ADHD was associated with hypoactivation on no-go trials in ventral prefrontal and inferior parietal cortical regions (Durston et al., 2006) and anterior cingulate cortex (Mulder et al., 2008). Durston et al. (Durston et al., 2008) also recently showed that the DAT1 dopamine transporter genotype interacts with the familiar risk of ADHD to influence activation of the striatum during this task. The present findings do not provide similar evidence for genetic impairment of the striatum or more medial-ventral regions of the frontal brain such as the ACC and inferior prefrontal cortex. This fits however with the notion that impairment of frontostriatal networks in ADHD is evident more consistently during tasks with specific emphasis on motor inhibition, such as the go no-go task (Booth et al., 2005; Smith et al., 2006).

The discrepancy between activation differences observed for the present Stroop and flanker tasks is somewhat surprising given the high correspondence between brain activation in response to the two tasks (see figure 1). However, the Stroop task may be more sensitive to AP-related effects on prefrontal brain areas than the flanker task. Correct Stroop performance seems to require a higher level of prefrontal brain involvement, as is supported by the fact that we found more consistent evidence for involvement of frontopolar brain regions during the Stroop task. Conversely, the flanker task may be more sensitive to AP related effects on parietal activation. This agrees with recent studies that measured the electroencephalogram (EEG) during the flanker task and found that arrow stimuli not only induce lateralized response activation, as indicated by the lateralized readiness potential, but also lateralized activation over parietal areas (Wascher et al., 1999; Willemsen et al., 2004). Parietal lateralization in the flanker task was interpreted as possibly indicative of an earlier coding of response side or lateralized stimulus- or attention related factors. In the present study we also found more robust statistical significance values in parietal lobe regions for main effects of interference in the flanker relative to Stroop task (compare Z score values for the response interference contrast of left and right parietal cortex clusters in Tables 3 and 4).

Increased functional activation to response interference in concordant high compared to concordant low risk twins, which may indicate compensatory mechanisms (Fassbender and Schweitzer, 2006), was noted in the left thalamus pulvinar for the Stroop task, and a left premotor region for the flanker task. The pulvinar is regarded as an important structure for visual attention function by facilitating communication between subcortical as well cortical structures related to visual processing (Leh et al., 2008), and damage to this structure results in visual attention deficit (Arend et al., 2008). Involvement of the thalamus in the genetic etiology of ADHD is in line with a recent study of resting-state brain function (Zhu et al., 2008), while increased probability of left thalamus activation associated with ADHD was also reported in a recent meta-analysis on 16 ADHD neuroimaging studies (Dickstein et al., 2006). Furthermore, in specific agreement with our present results, thalamic hyperactivation together with frontal underactivation in ADHD adolescents was recently observed in the context of a sustained attention task (Rubia et al., 2009). Thalamic hyperactivation may be more readily evident in the Stroop compared to the flanker task, because the Stroop paradigm requires a higher level of visual analysis. Conversely, increased premotor activation observed in the flanker task, may be more obvious because in this task there is a more direct coupling between the visual stimulus and required motoric action.

An interesting question is whether the brain areas that showed AP related functional activation differences are in agreement with our previous findings on AP related neuroanatomical differences between high- and low-risk twins (van 't Ent et al., 2007). In interpreting this correspondence it should be recognized that the relation between functional and structural brain changes is complex. Changes of brain function may relate to morphological alterations of gray or white matter or both, and the direction of functional and structural brain changes is not clearly defined, i.e., functional hypoactivation (and hyperactivation) may be associated with local brain tissue decrement or increment. In addition, our sMRI study included only a subset of the twin samples from the present study. Nonetheless, a close correspondence was found for some areas. The present cluster of dlPFC hypoactivation, for instance, is located near a region of CBCL-AP related prefrontal gray matter increase (van 't Ent et al., 2007: cluster labeled G in fig. 2, euclidean distance = 1.4 cm) and white matter decrease (van 't Ent et al., 2007: cluster J in fig. 3, distance = 1.3 cm). In other areas the correspondence between structural and functional findings was less immediate. The regions of deviant parietal lobe activation were at a relatively large distance (> 4 cm) compared to parietal gray and white matter reductions observed in our sMRI study which were located closer to the midline (van 't Ent et al., 2007: clusters A in fig. 2 and fig. 3). The present loci of hyperactivation in the left thalamus and left premotor cortex were not replicated in our structural MRI study. Structural changes in brain areas related to sensorimotor processing were found, but only in the right hemisphere (van 't Ent et al., 2007: increased gray and white matter in sensorimotor regions; cluster F in fig. 2 and cluster L in fig. 3).

### Environmental contrast

Difference in brain activation related to CBCL-AP scores in the discordant twin pairs were distinct from the functional changes observed between high- and low-risk twins from concordant pairs. Hypoactivation to response interference for discordant high risk twins was noted exclusively for the Stroop task and primarily restricted to regions of the temporal lobes, with an additional cluster in the right parietal precuneus. In particular temporal lobe changes may be evident primarily for the Stroop task due to the word processing requirements of this paradigm (Constable et al., 2004). Agreement of task-related hypoactivation with anatomical brain changes in high- relative to low-risk twins from discordant pairs was highest for the cluster in the left superior temporal gyrus (STG: close to a region of relative mid temporal white matter decrease for high-risk twins in van 't Ent et al. 2007: cluster A in fig. 5; distance = 1.4 cm).

Similar to what was found for high-risk twins from concordant pairs, clusters of hyperactivation were found in the high-risk twins from discordant pairs in cortical premotor regions. This is consistent with the theory that, as a result of impaired higher order processing (e.g., of the prefrontal brain), neural compensation in ADHD may be accomplished through enhanced recruitment of processes more proximal to the (visuo-)motor demands of the experimental task (Fassbender and Schweitzer, 2006). This appears to apply equally to AP of either genetic and environmental origin. High risk discordant twins further showed increased activation in regions of the right middle frontal gyrus and anterior cingulate cortex (ACC). This finding stands in sharp contrast to the frontal hypoactivations generally reported in ADHD (Bush et al., 2005; Dickstein et al., 2006). In particular, hypoactivation of the ACC has been noted repeatedly in ADHD patients for different executive tasks and in children/adolescents (Rubia et al., 1999; Durston et al., 2003; Tamm et al., 2004) as well as adults (Zametkin et al., 1990; Bush et al., 1999). Considering the major genetic component in ADHD these samples may have largely consisted of individuals at high genetic risk. Indeed, decreased frontal activation was found in the present study for concordant twins at high genetic risk, although not in the ACC but restricted to the left dlPFC. The contrast between decreased frontal activation in ADHD patient populations (and the high-risk twins from concordant pairs in this study) and increased frontal

activation in high-risk twins from discordant pairs suggests a clear distinction in the underlying neurobiology of genetically and environmentally mediated attention/hyperactivity problems.

Correspondence between the present functional hyperactivations and our previous sMRI findings in high-risk twins from discordant pairs were highest for the cluster in the left premotor cortex (near a cluster of decreased white matter in the left sensorimotor area, van 't Ent et al. 2007: cluster B in fig. 5, distance = 1.6 cm).

To summarize, the present functional neuroimaging study demonstrates decreases as well as increases in the amount of BOLD activation during the inhibition of distracting information that are related to parental report of inattention and hyperactivity symptoms. Furthermore, these AP related differences depended on the type of executive task used (color-word Stroop versus flanker task). In general agreement with neuroanatomical differences in twins with high relative to low CBCL-AP scores (van 't Ent et al., 2007), functional hypoactivation to response interference associated with high inattention/hyperactivity scores was primarily located in frontal, parietal and temporal brain regions. Functional hyperactivation, presumably related to compensatory mechanisms, was primarily found for brain regions involved in premotor (and visual selective attention) processing.

Comparison of the brain activation in the twins at genetic (high versus low concordant MZ pairs) or environmental risk (high-low discordant MZ pairs) for ADHD showed that different areas of the brain were affected by genetic and environmental risk factors. Consistent with previous findings in ADHD patients, attention deficit/hyperactivity problems of genetic origin were characterized by decreased activation of frontal brain regions (in the Stroop task) as well as parietal lobe regions (in the flanker task). In contrast, for attention deficit/hyperactivity problems of environmental origin there was evidence for increased, instead of decreased, activation of frontal brain regions and decreased activation was primarily restricted to areas of the temporal lobes. We conclude, that genetic and environmental risk factors for attention/hyperactivity problems act on partly different brain structures, and can influence the same structures in opposite ways.

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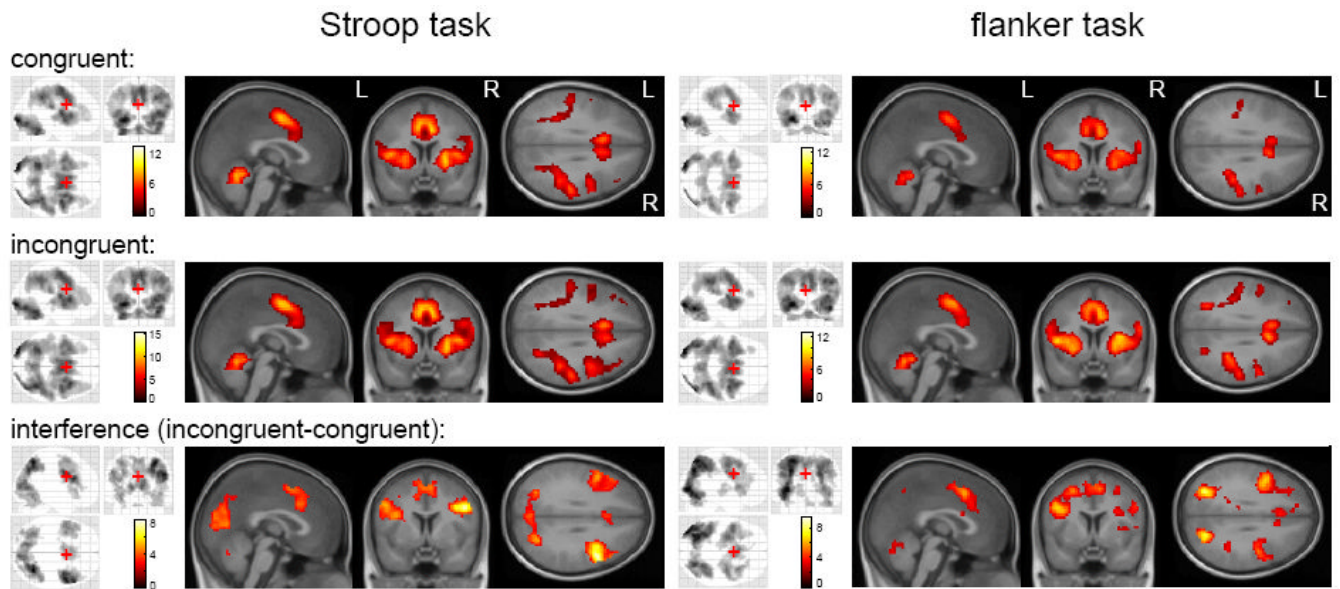
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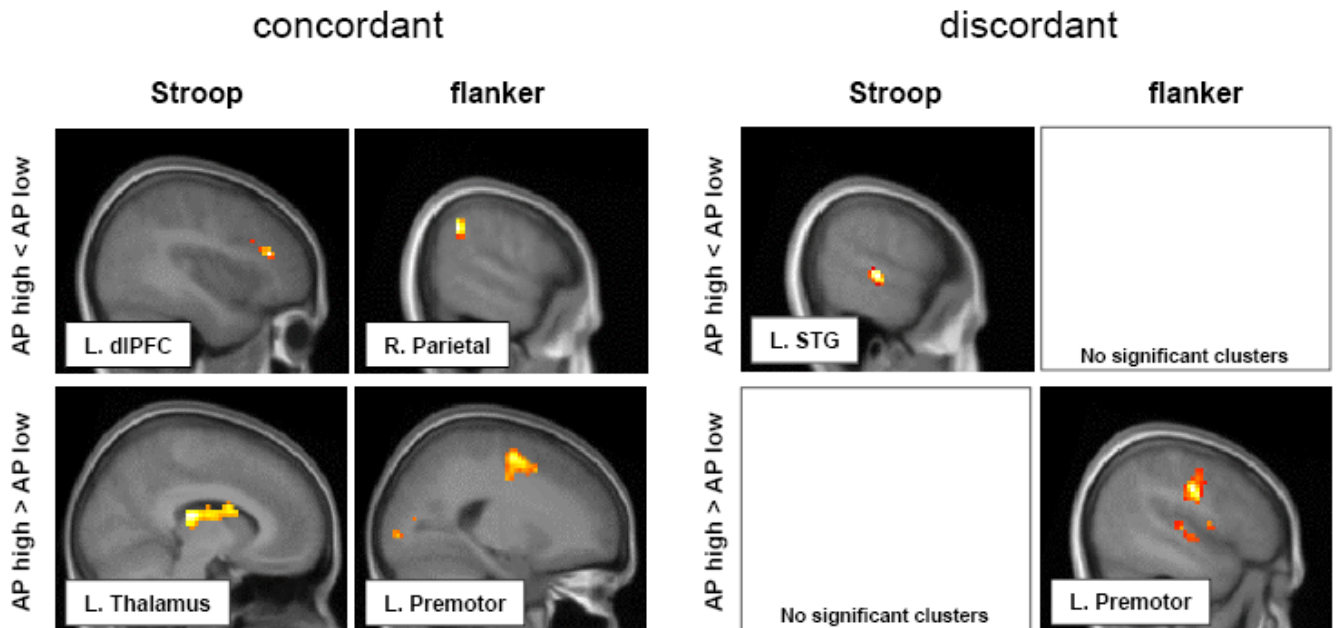
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**Figure 1.** Main effects of fMRI BOLD activation, across all participating twins, during Stroop task (left) and flanker task (right) performance. Significant clusters found after applying the *congruent*, *incongruent*, and *interference (incongruent-congruent)* contrasts are displayed from top to bottom. For each task and contrast, maximal intensity projections indicate voxels with statistical significance  $p < 0.05$  (FDR corrected and minimal cluster extent 25 voxels). Areas of significant activation are also overlaid on average structural MR sections, computed across all twins, with t-values mapped by the color bar. To facilitate comparisons, the location of the MRI slices is kept fixed and centered at MNI coordinates  $x = 0$ ,  $y = 6$ ,  $z = 30$  (also indicated by the red cross in the maximal intensity projections).

## CBCL-AP related differences



**Figure 2.**

Most significant clusters, overlaid on MR sections, from statistical evaluations of CBCL-AP related differences in brain activation to Stroop and flanker trials with response interference. Left panels: hypoactivations (top row) and hyperactivations (bottom) for concordant high compared to low risk twins (genetic contrast). Right panels: hypo- and hyperactivations activation in discordant high risk twins relative to their low risk co-twins (environmental contrast). For display only, a lower statistical threshold ( $p < .05$ ; uncorr.) is used. MNI coordinates and Z scores for voxel with maximum effect of each depicted cluster are listed in tables 5 and 6, respectively. dlPFC = dorsolateral prefrontal cortex; STG = superior temporal gyrus.

TABLE 1

**Twin sample demographics**

AP status: attention/hyperactivity status of twin pair; Twin pairs: number of female (F) and male (M) twin pairs; Age: age at time of MRI exam (in years); Mean AP T-score: mean CBCL-AP T-scores; Anxiety problems T-score: mean DSM-Oriented Anxiety problems T-scores; Oppositional Defiant problems T-score: mean DSM-Oriented Oppositional Defiant problems T-scores; Conduct problems T-score: mean DSM-Oriented Conduct problems T-scores. DSM-Oriented scales are based on CBCL4/18 items very consistent with the relevant DSM-IV category (Achenbach et al., 2003). All mean scores are across 2 or 3 time points (7, 10 and 12 years), and computed separately for boys and girls. Birth weight: birth weight of each twin in grams. Data separated by a forward slash refer to first and second born twins for concordant high and low risk twin pairs, and to the high versus low risk co-twin for the discordant pairs.

AP status	Twin pairs	Age	Mean CBCL-AP T-score	Anxiety problems T-score	Oppositional Defiant problems T-score	Conduct problems T-score	Birth weight
Concordant high	6F / 2M	mean	74.16 / 79.93	57.09 / 61.87	63.41 / 61.63	54.57 / 52.62	2547 / 2374
		st. dev	7.62 / 6.93	8.72 / 11.02	10.30 / 10.12	5.02 / 3.56	523 / 428
Concordant low	4F / 6M	mean	42.93 / 41.63	46.62 / 44.02	41.95 / 43.63	45.22 / 45.19	2642 / 2603
		st. dev	3.50 / 2.86	5.08 / 2.53	2.66 / 4.30	2.15 / 1.94	553 / 384
Discordant	7F / 2M	mean	72.53 / 46.64	60.70 / 48.98	56.54 / 51.60	55.51 / 54.34	1968 / 2489
		st. dev	7.77 / 4.45	11.41 / 7.48	10.39 / 12.21	8.26 / 14.01	604 / 466

TABLE 2

**Response interference effects on task performance**

Effects of information conflict on Stroop task (left) and flanker task (right) behavior, measured by computing differences in reaction times (top rows) and correct reaction rates (bottom rows) on incongruent relative to congruent stimulus trials. Columns 'F' and 'p' indicate results from statistical tests on AP-related differences between selected concordant or discordant twin samples. Degrees of freedom for the listed 'F' values are (1,14) and (1,6) for concordant and discordant twin comparisons, respectively. Asterisk indicates statistically significant difference.

measure	sample	Stroop incongruent-congruent			flanker incongruent-congruent			p
		CBCL-AP high	CBCL-AP low	F	CBCL-AP high	CBCL-AP low	F	
Reaction time	Concordant	66.27 ± 54.04	48.80 ± 58.91	0.54	128.98 ± 55.23	86.07 ± 23.34	0.09	0.769
	Discordant	59.98 ± 62.88	83.82 ± 99.68	1.02	94.24 ± 29.05	99.02 ± 27.50	0.00	0.966
Percentage correct	Concordant	-0.69 ± 3.13	-0.76 ± 3.59	0.17	-4.17 ± 4.09	-2.71 ± 2.32	6.28	0.025*
	Discordant	-3.09 ± 3.92	-0.15 ± 2.02	1.09	-3.40 ± 2.87	-3.24 ± 2.50	0.35	0.576

TABLE 3

**Brain regions active during performance of the Stroop task**

Anatomical locations of significant clusters for main effects of fMRI BOLD activations during the Stroop task, for simple *congruent*, *incongruent* contrasts and *interference* (*incongruent-congruent*). MNI coordinates and Z score are listed for the voxels with largest effect size. Dorsolateral PFC = dorsolateral prefrontal cortex.

Anatomical location	side	congruent						incongruent						interference (incongruent-congruent)					
		MNI coord's			Z score	MNI coord's			Z score	MNI coord's			Z score	MNI coord's			Z score		
		x	y	z		x	y	z		x	y	z		x	y	z			
Cerebellum	L	-30	-48	-21	7.47	-30	-48	-21	7.80	-27	-63	-30	2.98						
	M	0	-60	-18	6.55	0	-60	-18	6.87	-6	-69	-27	3.51						
Occipital/Temporal	R	30	-54	-27	5.94	21	-48	-24	6.60	33	-57	-24	3.51						
	L	-30	-90	-3	Inf.	-30	-90	-3	Inf.	-33	-54	-15	4.41						
Cuneus	R	42	-72	-9	7.35	42	-72	-9	Inf.	42	-60	-6	4.25						
	L									-15	-69	6	5.04						
Parietal	R									6	-84	9	5.18						
	L	-30	-30	48	5.48	-33	-63	42	6.08	-33	-54	55	4.56						
Motor Cortex	R	33	-42	42	7.26	42	-42	48	7.19	33	-57	54	6.32						
	L	-39	-18	54	4.88	-39	-18	54	5.22										
SMA	R	39	-15	60	5.34	39	-15	60	5.68										
	L	-12	-12	51	6.52	-12	-12	51	7.12	-6	3	57	4.14						
Anterior Cingulate	R	6	-3	57	7.47	6	-3	54	7.71	3	9	54	4.12						
	L	-9	12	36	6.94	-6	9	45	7.53	-6	18	36	4.20						
Thalamus	R	9	3	42	7.00	9	3	42	7.07	9	18	39	3.70						
	L	-9	-18	3	4.75	-9	-18	3	5.63										
Putamen	R	12	-18	3	5.28	12	-18	3	6.10										
	L	-27	0	6	6.36	-24	0	9	6.71										
Insula/Clastrum	R	27	3	0	7.06	27	6	0	7.30										
	L	-42	0	12	6.57	-33	12	9	6.92	-39	15	3	3.41						
Inferior Frontal Gyrus	R	36	-3	12	7.53	33	15	6	Inf.	39	15	0	2.80						
	L									-45	27	3	3.62						
dorsolateral PFC	R									45	15	6	3.28						
	L									-54	9	36	4.50						
	R									48	6	30	6.06						

Anatomical location	side	congruent			incongruent			interference (incongruent-congruent)			
		x	y	z	x	y	z	x	y	z	
Frontopolar	L	-33	39	21	3.49	-33	42	18	3.69		
	R	36	36	24	4.05	33	33	21	4.60		



**TABLE 4**  
**Brain regions active during performance of the flanker task**  
 Anatomical locations of significant clusters for main effects of fMRI BOLD activation during the flanker task.

Anatomical location	side	congruent						incongruent						interference (incongruent-congruent)					
		MINI coord's			Z score	MINI coord's			Z score	MINI coord's			Z score	MINI coord's			Z score		
		x	y	z		x	y	z		x	y	z		x	y	z			
Cerebellum	L	-30	-45	-24	4.03	-27	-48	-27	4.71	-39	-57	-27	3.42						
	M	-3	-51	-15	5.24	6	-45	-15	6.13	12	-72	-27	3.78						
Occipital/Temporal	R	36	-51	-30	5.58	33	-51	-30	6.21	30	-69	-27	3.62						
	L	-27	-90	0	Inf.	-27	-90	0	Inf.	-36	-84	3	7.05						
Cuneus	R	33	-81	-9	6.92	45	-69	-9	Inf.	33	-81	3	5.10						
	L																		
Parietal	R																		
	L	-39	-30	51	5.59	-42	-33	48	6.51	-30	-72	39	7.00						
Motor Cortex	R	45	-36	42	6.14	33	-42	45	7.18	24	-69	33	6.62						
	L	-36	-18	51	5.07	-36	-18	51	5.30										
SMA	R	39	-18	57	4.39	33	-15	60	5.09										
	L	-6	0	51	5.34	-6	0	51	6.37	-3	3	56	3.60						
Anterior Cingulate	R	6	-3	51	6.05	6	0	52	6.60	9	3	57	3.80						
	L	-9	9	39	5.49	-9	12	39	6.76	-1	15	51	5.03						
Thalamus	R	9	3	42	6.02	9	0	48	6.77	9	9	51	5.01						
	L	-15	-15	6	3.07	-15	-9	3	2.97	-12	-12	0	3.71						
Putamen	R	15	-15	6	3.98	15	-12	3	5.25	18	-9	12	2.93						
	L	-24	9	9	5.81	-30	3	0	6.47										
Insula/Clastrum	R	27	3	3	5.61	27	3	3	6.77										
	L	-39	0	9	6.23	-36	0	9	7.12	-36	21	3	4.01						
Inferior Frontal Gyrus	R	42	0	9	6.27	39	-3	15	7.19	33	15	9	4.80						
	L									-51	21	0	3.00						
dorsolateral PFC	R									48	18	-2	3.36						
	L									-45	6	30	5.64						
Frontopolar	R									36	-9	36	5.31						
	L									-33	39	24	3.49						

Anatomical location	side	congruent		incongruent		interference (incongruent-congruent)	
		MNI coord's	Z score	MNI coord's	Z score	MNI coord's	Z score
		x		x		x	
		y		y		y	
		z		z		z	

**Brain activation differences in concordant MZ twin pairs**

Differences in brain activation to trials with information conflict in concordant high compared to low risk twins. Test: test for significant hypo- or hyperactivation in twins high or low on the CBCL-AP scale; Location: anatomical location; MNI coord's: MNI coordinates for voxel with largest effect size; Z score: Z score of voxel with largest effect size. dIPFC = dorsolateral prefrontal cortex.

Test	Location	Stroop			Twins with high versus twins with low CBCL-AP scores			flanker			Z score
		x	y	z	Z score	Location	x	y	z		
high < low	L. dIPFC	-39	33	18	3.38	R. Parietal	60	-48	36	3.42	
high > low	L. Thalamus	-9	-24	15	3.16	R. Parietal	54	-48	39	3.15	
						L. Premotor	-21	-9	57	3.15	

TABLE 6

**Brain activation differences in discordant MZ twin pairs**

Differences in brain activation to information conflict in discordant high compared to low risk twins. STG = superior temporal gyrus; FFG = fusiform gyrus; ACC = anterior cingulate cortex; MFG = middle frontal gyrus.

Test	Location	Stroop			Twin with high versus co-twin with low CBCL-AP scores			flanker		
		x	y	z	Z score	Location	x	y	z	Z score
high < low	L. STG	-60	-24	0	4.02	No significant clusters				
	R. STG	63	-45	6	3.63					
	R. FFG	36	-42	-21	3.54					
	R. precuneus	6	-36	45	3.17					
high > low	No significant clusters					L. Premotor	-48	-12	33	3.95
						ACC	3	33	0	3.74
						R. Premotor	42	-9	33	3.48
						R. MFG	9	54	-3	3.14