Published in final edited form as:
Am J Med Genet B Neuropsychiatr Genet. 2010 March 5; 153B(2): 365–375. doi:10.1002/ajmg.b.31022.

Effect of Dopamine Transporter Gene (SLC6A3) Variation on Dorsal Anterior Cingulate Function in Attention-Deficit/ Hyperactivity Disorder

Ariel B. Brown, Ph.D.[1,2], **Joseph Biederman, M.D.**[1], **Eve M. Valera, Ph.D.**[1,3], **Alysa E. Doyle, Ph.D.**[1], **George Bush, M.D.**[3], **Thomas Spencer, M.D.**[1], **Michael C. Monuteaux, Sc.D.**[1], **Eric Mick, Sc.D**[1], **Susan Whitfield-Gabrieli, Ph.D.**[4], **Nikos Makris, M.D., Ph.D.**[5,6], **Peter S. LaViolette, M.S.**[1], **Marlene Oscar-Berman, Ph.D.**[2,7], **Stephen V. Faraone, M.D.**[8], and **Larry J. Seidman, Ph.D.**[1,9]

[1]Clinical and Research Programs in Pediatric Psychopharmacology and Adult ADHD, Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02114

Corresponding Author: Ariel Brown, Ph.D., Massachusetts General Hospital – East Campus, Department of Psychiatry, 149 13th St., Rm. 2603, Charlestown, MA 02129. Tel: 617-726-6043, Fax: 617-726-4078, ariel@nmr.mgh.harvard.edu.

Location of Work: Clinical and Research Programs in Pediatric Psychopharmacology and Adult ADHD, Massachusetts General Hospital, Boston, MA.

Financial Disclosures:

Dr. Ariel Brown reports no competing interests.

Dr. Joseph Biederman is currently receiving research support from the following sources: Bristol Myers Squibb, Eli Lilly and Co., Janssen Pharmaceuticals Inc., McNeil, Otsuka, Shire, NIMH, and NICHD. He is currently a consultant/advisory board member for the following pharmaceutical companies: Janssen, McNeil, Novartis, and Shire. He is currently a speaker for the following speaker's bureaus: Janssen, McNeil, Novartis, Shire, and UCB Pharma, Inc.

Dr. Valera has received travel support and/or honoria from Eli Lilly, Shire Pharmaceuticals, and divisions of Ortho-McNeil Janssen Pharmaceuticals as well as Remedica Medical Education and Publishing.

Dr. Alysa Doyle is currently a consultant to Pfizer Pharmaceuticals.

Dr. George Bush Dr. George Bush receives/d research support from, has received honoraria from, or has served as a consultant/ advisory board member for the Benson-Henry Institute for Mind-Body Medicine, the Centers for Disease Control, the David Judah Fund, Eli Lilly & Co, Intel, Janssen Pharmaceuticals, Johnson & Johnson, the McIngvale Fund, McNeil Pharmaceuticals, the Mental Illness and Neuroscience Discovery (MIND) Institute, the National Institute of Mental Health, the National Science Foundation, the National Alliance for Research in Schizophrenia and Depression (NARSAD), Novartis Pharmaceuticals, Pfizer, Inc, and Shire Pharmaceuticals.

Dr. Thomas Spencer receives research support from, is a speaker for or is on the Advisory Board of the following sources: Shire Laboratories, Inc, Eli Lilly & Company, Glaxo-Smith Kline, McNeil Pharmaceutical, Novartis Pharmaceuticals, Cephalon, Pfizer and the National Institute of Mental Health.

Dr. Michael Monuteaux reports no competing interests.

Dr. Eric Mick receives/d grant support is/has been a speaker for, or is/ has been on the advisory board for the following sources: McNeil Pediatrics and Janssen Pharmaceuticals, Pfizer, Shire and the National Institute of Mental Health (NIMH).

Dr. Stephen Faraone is currently receiving research support from the following sources: Pfizer, Shire, NIMH, and NICHD. He is currently a consultant for Shire.

Dr. Susan Whitfield-Gabrieli reports no competing interests.

Dr. Nikos Makris reports no competing interests.

Mr. Peter LaViolette reports no competing interests.

Dr. Marlene Oscar-Berman reports no competing interests.

Dr. Larry Seidman reports no competing interests.

[2]Ph.D. Program in Behavioral Neuroscience, Division of Graduate Medical Sciences, Boston University School of Medicine, Boston, MA, 02118

[3]Psychiatric Neuroimaging Research Program, Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston MA, 02129

[4]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

[5]Departments of Neurology and Radiology Services, Harvard Medical School, Boston, MA, 02118

[6]Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA 02129

[7]Departments of Psychiatry, Neurology, and Anatomy & Neurobiology, Boston University School of Medicine, and US Department of Veterans Affairs Healthcare System, Boston, MA

[8]Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, NY, 13210

[9]The Massachusetts Mental Health Center Public Psychiatry Division of the Beth Israel Deaconess Medical Center, Department of Psychiatry, Harvard Medical School Department of Psychiatry, Boston, MA, 02215

Abstract

Objective - Although Attention-Deficit/Hyperactivity Disorder (ADHD) is associated both with brain alterations in attention and executive function (EF) circuitry and with genetic variations within the dopamine system (including the dopamine transporter gene [SLC6A3]), few studies have directly investigated how genetic variations are linked to brain alterations. We sought to examine how a polymorphism in the 3' untranslated region (UTR) of SLC6A3, associated with ADHD in meta-analysis, might contribute to variation in dorsal anterior cingulate cortex (dACC) function in subjects with ADHD. Method - We collected fMRI scans of 42 individuals with ADHD, all of European descent and over the age of 17, while they performed the Multi-Source Interference Task (MSIT), a cognitive task shown to activate dACC. SLC6A3 3' UTR variable number tandem repeat (VNTR) polymorphisms were genotyped and brain activity was compared for groups based on allele status. Results - ADHD individuals homozygous for the 10R allele showed significant hypoactivation in the left dACC compared to 9R-carriers. Exploratory analysis also showed trends toward hypoactivation in the 10R homozygotes in left cerebellar vermis and right lateral prefrontal cortex. Further breakdown of genotype groups showed similar activation in individuals heterozygous and homozygous for the 9R allele. Conclusions - Alterations in activation of attention and EF networks found previously to be involved in ADHD are likely influenced by SLC6A3 genotype. This genotype may contribute to heterogeneity of brain alterations found within ADHD samples.

Keywords

dopamine transporter gene; functional magnetic resonance imaging; genetics; adhd; anterior cingulate

Introduction

Attention deficit/hyperactivity disorder (ADHD) affects up to 10% of children (Faraone et al., 2003), and 5% of adults worldwide (Faraone and Biederman, 2005). ADHD is increasingly recognized as a brain disorder, with alterations found across the lifespan in studies of both brain structure (Seidman et al., 2005) and function (Dickstein et al., 2006). The regions most frequently implicated in ADHD are anterior cingulate cortex (ACC),

lateral prefrontal cortex (PFC), striatum, and cerebellum (Dickstein et al., 2006; Valera et al., 2007), congruent with the executive and attentional processes that these networks support and that are often dysfunctional in the disorder.

ADHD has a strong genetic component, with results from twin studies estimating that the additive effect of multiple genes explains approximately 80% of its variance, making it one of the most heritable psychiatric disorders (Faraone et al., 2005). Although the genetic underpinnings of ADHD are complex, molecular genetic studies have identified several candidate genes associated with the disorder, most within catecholamine systems (Faraone et al., 2005). These associations are consistent with the longstanding "dopamine hypothesis" of ADHD, supported by multiple lines of evidence from neuroanatomic, pharmacologic and molecular imaging studies all implicating dopamine in the disorder (see Swanson et al., 2007 for review).

One of the candidate genes most extensively studied in ADHD is SLC6A3, which codes for the dopamine transporter (DAT), the primary protein responsible for clearing dopamine from the synaptic space in the striatum. The SLC6A3 gene is of particular interest to ADHD because DAT is the principal target of the two most effective pharmacological treatments for the disorder (both methylphenidate [MPH] and amphetamine [AMP]; Madras et al., 2005)), because altered levels of DAT availability have been found across studies of unmedicated patients with ADHD (Krause, 2008), and because the SLC6A3 knockout mouse is an animal model for ADHD with very high external validity (van der Kooij and Glennon, 2007). SLC6A3 contains a variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region (UTR). The 9-repeat (9R) and 10-repeat (10R) alleles are the most common forms of the gene, and it is the 10R allele that shows a weak but significant association with ADHD (Faraone et al., 2005; Yang et al., 2007). Although contrary reports have been published (van Dyck et al., 2005), the 9R allele has been found *in vivo* (Heinz et al., 2000), *ex vivo* (Mill et al., 2002), and *in vitro* (VanNess et al., 2005) to result in lower expression of DAT, presumably reducing synaptic DA clearance rates.

While genetic studies showing associations between genotype and diagnosis have made a significant impact on our understanding of the genes involved in ADHD, findings on any one particular gene, including SLC6A3, lack statistical strength, and studies are relatively equivocal (Faraone et al., 2005). Imaging genetics may be a more powerful technique for finding associations as it allows the observation of biologically-based endophenotypes, presumably under control of fewer genes than the complex sets of behaviors used to diagnose a disorder (Meyer-Lindenberg and Weinberger, 2006). Further, this technique can help us to understand the specific neurobiological consequences of genes previously identified in association studies. Specifically, imaging genetics can help us to understand how one variant of a gene might predispose towards one type of neural outcome, while another variant may have a different or additive effect. In a disorder with such substantial clinical and neurocognitive heterogeneity as ADHD (Sonuga-Barke, 2005), these types of studies can help elucidate the different causal paths leading from gene to brain to expression of the disorder. Identifying these heterogeneous pathophysiologic trends may lead eventually to refined diagnoses and more individualized treatments.

To date, only a handful of imaging genetics studies of ADHD have been published, most using structural imaging in children. These studies have focused on dopamine system genes previously associated with ADHD, several finding genetic effects on ADHD regions of interest (ROIs) such as frontal cortex, striatum and cerebellum (Durston et al., 2005; Durston et al., 2008; Monuteaux et al., 2008; Shaw et al., 2007). In the current study we investigated the role of the SLC6A3 VNTR in *adults* with ADHD, targeting its effect on the dorsal anterior cingulate cortex (dACC).

The ACC has been found to be affected in multi-modal neuroimaging studies of ADHD in both children and adults. It has shown reduced volume (Seidman et al., 2006), decreased cortical thickness (Makris et al., 2007; Shaw et al., 2006), and functional hypoactivation (Bush et al., 1999; Bush et al., 2005; Dickstein et al., 2006) in ADHD. Although the causative factors behind ACC pathology in ADHD are unknown, it does seem to be particularly affected by changes in brain dopamine levels, and specifically to changes in DAT function. For example, in studies of brain effects of MPH, which principally targets the DAT (Volkow et al., 1998), acute treatment increases fMRI BOLD signal (Bush et al., 2008) and rCBF (Udo de Haes et al., 2007) in ACC. An MR spectroscopic study found that chronic methylphenidate treatment affects NAA and choline levels in anterior cingulate but not in lateral frontal cortex (Kronenberg et al., 2008). In addition, at least two previous imaging genetics studies have found significant differences between SLC6A3 genotype groups on ACC functioning during cognitive tasks (Bertolino et al., 2006; Schott et al., 2006). Taken together, these findings suggest that changes in DAT function are linked to ACC function, and for this reason, we sought to investigate if variation in ACC activity within a group of subjects with ADHD might be partially explained by SLC6A3 genotype. We used the multi-source interference task (MSIT) to probe SLC6A3 effects on dACC as it was designed specifically for interrogating the dACC and related executive attention network (Bush and Shin, 2006), and appears to be sensitive to changes in DA system functioning as probed with MPH (Bush et al., 2008).

In this study we investigate if subjects with ADHD differ neurofunctionally based on SLC6A3 genotype. Based on results from previous fMRI studies showing regional hypoactivity in 10R homozygotes in both control and ADHD samples (Bertolino et al., 2006; Durston et al., 2008; Schott et al., 2006), the association of the 10R allele with ADHD, and the likely low activity nature of the 9R allele, we hypothesized that ADHD subjects homozygous for the SLC6A3 10R allele would show hypoactivity in the dACC.

Materials and Methods

This study is a secondary data analysis, integrating neuroimaging data collected in an NIH funded study (MH 062152), and genetic data previously collected in MH 062152 or 4 other NIH funded studies (MH 57934, HD 37694, MH 064019, HD 36317). All studies were conducted in accordance with the Declaration of Helsinki and the standards established by the Partners Healthcare Human Research IRB. Written informed consent was obtained from all adult subjects, and for subjects under 18 (17 year olds), consent was obtained from a parent and assent was obtained from the subject. Subjects described below include all individuals diagnosed with ADHD for whom both the fMRI task (MSIT) and DNA were collected, and who met the inclusion criteria for this analysis. There was insufficient overlap of control subjects between the neuroimaging and genetic studies to include a control sample in the study.

Subjects

Subjects were 42 participants (52% female), ages 17–59, who were diagnosed with ADHD, part of a ongoing study recruiting from Massachusetts General Hospital clinics and advertisements posted in the Boston area. Previous imaging reports have been published on subsets of this sample (Makris et al., 2007; Monuteaux et al., 2008; Seidman et al., 2006; Valera et al., 2005), but none using the MSIT, and the current report extends this work by examining the subset of ADHD subjects who had both genetic and MSIT functional imaging data available. Exclusion criteria for all subjects were as follows: an estimated Full Scale IQ < 80; lifetime history of psychosis; current alcohol or substance abuse; inadequate command of the English language; sensorimotor handicaps. In order to reduce the risk of stratification bias, for the current report we only included subjects who identified themselves as

Caucasian. Eight subjects had pharmacological intervention for ADHD in the past, and 16 were prescribed psychostimulants near the time of scan (the remaining 18 participants were naïve to pharmacological treatment for ADHD). All subjects prescribed stimulants at the time of scan underwent a 24 hour washout period before the MRI visit. Five subjects had taken medications for other psychiatric conditions within 24 hours of the MRI scan: 3 were on SSRIs, 2 on benzodiazepines.

Clinical and Behavioral Assessment

All subjects were assessed with the Structured Clinical Interview for DSM-IV (SCID-I; First et al., 1997). To assess childhood ADHD, an additional module was administered, derived from the Schedule of Affective Disorders and Schizophrenia for School Age Children (Kiddie SADS-E; Orvaschel and Puig-Antich, 1987). Previous work in our lab has shown that retrospective childhood diagnoses of ADHD can be made in a reliable and valid manner using this method (Faraone et al., 2000). We considered a subject positive for ADHD if DSM-IV diagnostic criteria were met in childhood. A committee of board-certified child and adult psychiatrists and psychologists blind to referral source resolved diagnostic uncertainties. To obtain a multi-dimensional assessment of mood near the time of brain imaging, we administered the Profile of Mood States (POMS; McNair et al., 1992). Block Design and Vocabulary subtests from the Wechsler Adult Intelligence Scale-III (WAIS-3; Wechsler, 1997) were used to estimate IQ. Academic achievement was assessed with the Reading and Arithmetic modules of the Wide-Range Achievement Test (WRAT-3; Jastak and Jastak, 1993).

fMRI Paradigm

The MSIT combines multiple dimensions of cognitive interference, including Stroop (Stroop, 1935), Eriksen (Eriksen and Eriksen, 1974), and Simon (Simon and Berbaum, 1990) effects with response competition and selection in order to maximally recruit dACC neurons. The task is described in detail elsewhere (Bush and Shin, 2006). To summarize, in each session we presented eight blocks of stimuli alternating between interference and control conditions beginning and ending with 30 seconds of fixation. In both conditions, subjects responded to the identity of the number that differed from two other numbers. In the control blocks, distractors were zeros, and target numbers were congruent with their positions on the button press. In the interference blocks, distractors were drawn from the set of potential target numbers (i.e., 1, 2, or 3), and targets were always placed incongruently with their button press positions (see Figure 1).

Each subject completed a 5-min practice session just prior to the fMRI scan and then completed two test runs during scanning, each 6 min, 42 sec. MSIT stimuli were generated using MacStim software (WhiteAnt Occasional Publishing, http://www.brainmapping.org/WhiteAnt) running on a Mac iBook G4, and projected onto a screen situated in the rear of the magnet bore. MSIT performance outcome measures were percent correct and reaction time during the control and interference conditions.

fMRI Data Acquisition and Analysis

Imaging was performed on a Siemens Sonata 1.5T full-body MR scanner equipped with high-speed imaging gradients and quadrature head coil. Head movement was minimized with padded stabilizers surrounding the head of each individual. A sagittal localizer scan was performed for correct placement of slices, followed by a coronal T2-weighted sequence to rule out unexpected neuropathology. A T1-weighted MPRAGE image was collected for anatomical coregistration of functional images. Functional imaging was performed using a gradient-echo EPI pulse sequence (22 sagittal slices, TR = 1500 ms, 5 mm thick, 1 mm interslice interval, TE = 21 ms, flip angle = 90°; 264 volumes + 4 dummy scans per session).

Imaging parameters (optimized for coverage of cingulate cortex) precluded full coverage of the anterior and posterior poles.

fMRI data were analyzed using Statistical Parametric Mapping (SPM2; Wellcome Department of Cognitive Neurology, London). Preprocessing included: 1) correction for bulk head motion, 2) coregistration of functional volumes to individual T1-weighted anatomy, 3) spatial normalization of T1 images to a template and application of normalization parameters to coregistered functional data for template space transformation, and 4) spatial smoothing with a Gaussian filter (8 mm full-width half maximum). Individual runs exhibiting a spike of more than three millimeters of scan to scan head motion and/or stimulus correlated motion of $r \geq 0.5$ were dropped. As a result, one run was dropped from a single subject.

Following preprocessing, statistical analyses were performed at the single-subject level. Each epoch of trials was modeled using a boxcar function convolved with a canonical hemodynamic response function. Low-frequency components of the BOLD signal were modeled as confounding covariates using a set of discrete cosine basis functions with a cutoff of 168 seconds in order to increase sensitivity. Six motion correction parameters were also included in the model as confounding covariates in order to increase sensitivity and reduce the possibility of motion artifacts. Voxel-wise *t*-tests were conducted at the individual subject level using our contrast of interest (Interference > Control), which isolates the signal associated with the MSIT interference effect (Bush and Shin, 2006). Second level two-sample *t*tests by genotype group were conducted for the whole volume, as well as restricted to the ROI that was hand drawn using MARINA (MAsks for Regions of INterest Analyses; Bender Institute of Neuroimaging, University of Giessen, Germany; Walter et al., 2003). The ROI was limited to dorsal anterior midcingulate cortex (anterior to $y = 0$, posterior to $y = 30$, and within 15 mm of midline), defined based on a meta-analysis of imaging studies reporting ACC activation during cognitively demanding tasks (Bush et al., 2000). The height threshold for genotype comparisons was set to $p < .005$-uncorrected, and only clusters with spatial extent of $k \geq 5$ were displayed. Cluster-level *p*-values for the dACC data were corrected for number of voxel-wise comparisons within the total bilateral ROI, while in other regions cluster level *p*-values were corrected for number of voxels in the whole volume. In our whole volume analysis, for exploratory purposes, we report any clusters emerging at significant or trend cluster-level uncorrected *p*-values ($p < 0.1$). Because of the wide age range in our sample, we also conducted an ANCOVA to assess for the potential confounding effect of age on genotype effects, testing effects both within the ROI and the entire volume, using the above thresholds.

In order to investigate whether resulting differences were due to an allele dosing effect, in SPSS© we ran Bonferroni corrected post-hoc tests on raw beta values averaged across the voxels of resulting clusters, with number of 9R alleles as the independent variable. In order to assess if an allele dosing effect could explain activity anywhere in the entire volume we additionally conducted a linear contrast within SPM2 with number of 9R alleles as the independent variable.

Genotyping Methods

Genotyping of SLC6A3 was conducted at the Massachusetts General Hospital Psychiatric and Neurodevelopmental Genetics Unit using the following protocol: Genomic DNA (5 ng) was amplified in a 7 μl reaction using HotStarTaq DNA Polymerase (0.2 U), the proprietary HotStarTaq Buffer (1X), dNTPs (200 μM), and the marker specific primers (0.2 μM). Primers were ordered from Applied BioSystems and are as follows: SLC6A3-F 6FAM-TGTGGTGTAGGGAACGGCCTGAG, SLC6A3-R CCTCCTGGAGGTCACGGCTCAAGG. The SLC6A3-R primer also contains the

proprietary tail. For amplification, samples were heated at 92° C for 9 min to activate the HotStarTaq Polymerase. This is followed by 12 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec beginning at 64.5°C and dropped 0.5° C every cycle, and primer extension at 72°C for 30 sec; 37 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec at 58° C, and primer extension at 72°C for 30 sec; 72°C for 1 hr. Amplified products were pooled and combined with size standard (LIZ-250) before being analyzed on an ABI-3730. GeneMapper v3.5 was used to analyze the raw results from the ABI3730, however, a genotype was not considered final until two laboratory personnel had independently checked (and corrected) the GeneMapper results and both individuals were in agreement.

Results

Genotype, Demographic, and MSIT Performance Data

Genotype frequencies of the SLC6A3 gene were as follows: 19 subjects were homozygous for the 10R allele and 23 were 9R carriers. Of the 9R carriers, six were homozygous for the 9R allele, 16 were 9R/10R, and one was 9R/11R. There was no evidence that these data were not in Hardy-Weinberg Equilibrium ($p = 0.89$).

As Table I shows, there were no significant differences between SLC6A3 genotype groups on any of the demographic, mood, or ADHD variables including age of onset, number of childhood symptoms, and number of symptoms at time of interview. No differences were found between groups on frequencies of lifetime alcohol or substance abuse, anxiety disorders, or major depression. MSIT performance data did not differ significantly between the two genotype groups, although there was a trend for higher accuracy on the interference task in the 10R/10R group ($p = 0.077$). We found no differences between our genotype groups in frequencies of subjects who were naïve to psychostimulant treatment, those who had been prescribed psychostimulants in the past, or those who were taking psychostimulants near the time of scan but that were washed out for at least 24 hours (Table II).

Main Effect of Task on BOLD Signal Change

Across the entire group of 42 subjects in our contrast of interest (interference > control), we found significant activation in BILATERAL inferior parietal lobules and cerebellar hemispheres, LEFT lateral PFC, precentral gyrus, caudate and insula, and RIGHT fusiform gyrus.

fMRI Results by Genotype

Table III and Figure 2 show these results. Within the dACC ROI, a significant cluster (left dACC) indicated hypoactivity in the 10R/10R group compared to the 9R carriers. Whole volume exploratory analyses additionally suggested hypoactivity in right lateral PFC and left cerebellar vermis (though neither of these clusters survived correction for multiple comparisons when considering the entire search volume). The ANCOVA treating age as a covariate of no interest yielded only minimal differences from the two sample t-test, most notably that the R PFC and L dACC findings dropped to trend level (p's = 0.052 and 0.068, respectively).

No areas, either within the dACC ROI or the whole volume showed greater activation in 10R/10R subjects than 9R carriers.

Regarding the effect of allele load, one-way ANOVAs revealed significant differences in activation in left dACC, left cerebellar vermis, and right lateral PFC (p's < 0.01) as

expected. *Post-hoc* Bonferroni corrected pairwise comparisons of mean beta values in dACC and right lateral PFC showed significantly more activation in the 9R-heterozygous group *vs.* 10R/10R (p's < 0.01), but not for 9R/9R group *vs.* 10R/10R (p's > 0.1). In the left cerebellar vermis, the 10R/10R group showed significant hypoactivity compared to both the 9R groups (p's < 0.01). In none of the regions did the 9R groups differ from each other. Although differences between the 9R/9R and 10R/10R groups did not reach statistical significance in dACC and lateral PFC, effect sizes were large (Cohen's d = 0.96 and 0.94 for dACC and lateral prefrontal, respectively, and power was restricted due to the small n of the 9R/9R group), and subjects with one 9R allele had very similar levels of activation to 9R homozygous subjects (see Figure 3). We were unable to find any areas in the entire volume which were significantly predicted by a linear dosing effect. This pattern of results is most consistent with the hypothesis that differences were due to homozygosity of the 10R allele and not to a dosing effect.

Discussion

In this study of adult subjects with childhood-onset ADHD, we found a significant effect of the SLC6A3 gene on activation in the dACC, as well as trend effects in the lateral PFC and cerebellar vermis. Specifically, homozygosity for the ADHD risk allele (10R), but not mere carriage of the allele, predicted hypoactivation in these areas on a cognitive interference task. These brain areas are all relevant to ADHD, implicated by structural (Seidman et al., 2006; Valera et al., 2007) and functional (Dickstein et al., 2006) neuroimaging studies, as well as neurobiological theories of the disorder (Krain and Castellanos, 2006).

The findings are of interest not only because they potentially elucidate the neurofunctional consequences of an ADHD candidate gene variant in the ADHD brain, but also because they help us to understand how SLC6A3 may produce endophenotypic heterogeneity *within* an ADHD sample even in the context of tight comparability of the two genotype groups on a series of demographic and phenotypic variables, such as psychiatric comorbidities, neurocognition and medication history (see Tables I and II). In addition, our findings extend to ADHD those of Bertolino et al (2006), who found ACC hypoactivity in 10R/10R control subjects compared to their 9R counterparts. Finally, our results suggest that the effect of the 10R allele on dACC function acts in a recessive manner, and that two copies of the 10R allele are necessary to produce an unexpected pattern of function (deactivation during an interference task).

While it did not reach significance, we did find a trend for better performance in our 10R/10R group during the inhibition task. This is consistent with several behavioral studies which found better performance in 10R/10R vs. 9R ADHD groups on measures of attention and executive function (Boonstra et al., 2008; Kim et al., 2006; Oh et al., 2003). These findings are also consistent with the theory that hypoactivity represents a more "focused" engagement resulting from increased SNR in the 10R group (see Bertolino et al., 2006). However, alternative explanations must also be considered as there is a literature suggesting no significant relationship between SLC6A3 and cognitive performance (see review in Rommelse et al 2008), and a robust imaging literature linking the ADHD diagnosis itself with hypoactivity in fronto-striatal regions (see Dickstein et al., 2006). It is possible that non-DAT1 effects across ADHD samples tip the scale towards less optimal functioning which compared to controls appears similar to the hypoactivity seen in 10R vs. 9R controls, but that indeed is associated with less efficient cognitive functioning. Future studies should investigate the significance of hypoactivity in ADHD vs. control groups (associated with less optimal functioning), and how its quality differs from the hypoactivity in 10R vs. 9R genotype groups (associated with more optimal functioning).

Our imaging findings are particularly interesting when viewed next to those of Bush et al (2008), who found that after a 6 week trial of MPH, dACC activation was increased during the MSIT in an adult ADHD sample independent of those reported in this paper. Given that one of the primary mechanisms of action of MPH is to block DAT (Volkow et al., 1998), the two studies can be seen as congruent, with our 9R-carriers analogous to the post-MPH group. In other words, increased dACC activation may be related to decreased DAT functioning, regardless of whether it is a function of genotype or of pharmaceutical intervention. Although we found no difference between our genotype groups in medication history, other groups have indeed found that 9R-carriers have an increased likelihood of response to methylphenidate (Kooij et al., 2008), findings which have been recently supported by meta-analysis (Purper-Ouakil et al., 2008). Future pharmacogenetic studies should be conducted that directly investigate the interaction between genotype, dACC activation, and response to psychostimulant medication.

We have found one other study investigating the impact of gene variants on fMRI measures in ADHD (Durston et al., 2008). Using a go no-go paradigm, Durston et al found that SLC6A3 predicted activation differences, as our study did, in cerebellar vermis (as well as striatum) in ADHD subjects. However, their vermis results were in the opposite direction to ours. Such varying results might be explained by differences in task demands and/or their use of a pediatric sample. Our findings are also novel in that to our knowledge it is the first imaging genetics study of ADHD in adults using fMRI, and that we probed and found differences in dACC, an area of much interest in ADHD.

The effect of the SLC6A3 genotype on cortical activation may be direct through synaptic effects on the relatively low amounts of DAT in these areas, indirect through striatal DAT effects on the cortex via thalamocortical pathways, through gene-gene interactions, or any combination of these factors. In contrast to Durston et al (2008), we did not find any effects of SLC6A3 on striatum. Although the reasons for the discrepant findings are not clear, it could be due to differences between the adult and pediatric samples studied, considering that caudate volumes tend to normalize in ADHD in adolescence (Castellanos et al., 2002), and/or the fact that our task is not specifically designed to probe the striatum.

Given our previous findings of anatomical abnormalities in the ADHD ACC as compared to a control group (Makris et al., 2007; Seidman et al., 2006), it is possible that our findings were influenced by differences in ACC morphology, such as cortical thickness or volume. Since, however, no studies that we know of have examined the effect of SLC6A3 on ACC structure, the potential impact of structure on our findings are unknown. Future genetic imaging studies involving dopamine genotypes should investigate the effect of brain structure on functional outcomes.

The current results not only link specific brain areas previously found to be altered in ADHD with a known ADHD risk genotype, but also support the complex heterogeneity of the disorder, providing insight into the likely multiple pathways to ADHD. Hypoactivations in frontocerebellar pathways as a result of SLC6A3 10R homozygosity may characterize one of several paths predisposing individuals towards ADHD. It may be that SLC6A3 is more closely linked to anterior cingulate network dysfunction (found in many psychiatric disorders) than to the ADHD phenotypic expression, and that previous association studies finding a link between SLC6A3 and ADHD may be more driven by associations with this network's dysfunction in a subset of subjects than to the clinical phenotype of ADHD itself. Further investigation of the effects of SLC6A3 on anterior cingulate function in controls and other psychiatric populations will be important to delineate whether this link is specific to ADHD or not.

Notably, in our analysis of main effects of task we did not find that the L dACC or R PFC were significantly activated in the interference vs. control condition. Even though the MSIT has been found to robustly activate dACC in *individual* control subjects (Bush et al., 2003), these findings were not very surprising given that dACC (as well as PFC and cerebellum) are frequently hypoactive during multiple tasks in subjects with ADHD (Booth et al., 2005; Bush et al., 1999; Konrad et al., 2006; Rubia et al., 1999; Valera et al., 2005). As can be seen in Figure 3, however, it is evident that activation is in fact in the opposite direction in the two genotype groups. These results are of great interest as they suggest that averaging across SLC6A3 genotypes groups in ADHD samples may wash away effects seen in these areas.

It is also important to note that in the dACC, cerebellar vermis, and lateral PFC, the 9R carriers had more activation in the interference condition as compared to the control condition, whereas 10R/10R subjects had less activation in the interference condition as compared to the control condition (see Figure 3). The lack of effect of SLC6A3 on activation in the control condition alone (results not shown) suggests that the variant likely impacts brain function associated with MSIT interference processes and/or baseline. Alternative methods of analysis should be employed to test the effect of SLC6A3 on "default-mode networks", which have indeed found to be altered in samples with ADHD (Castellanos et al., 2008;Fassbender et al., 2009).

Limitations of our study include modest power, which likely accounts for the cerebellar and frontal findings not surviving correction for multiple comparisons despite large Cohen's *d* effect size estimates (1.34 and 1.02, respectively). Given that classical association studies (including studies of this SLC6A3 polymorphism with ADHD) sometimes require hundreds of subjects for adequate power, these effect sizes are promising. Nonetheless, interpretations of statistically nonsignificant findings should be made with caution until demonstrated to be significant in larger samples. Future studies should test specific hypotheses in multiple ADHD ROIs including lateral PFC and cerebellum, as they are essential nodes of networks found to be altered in ADHD (Krain and Castellanos, 2006). Further, even though genotypes did not differ in terms of ADHD medication history, we would advise future replication using a medication-naïve sample given the effect of psychostimulants on both DAT and ACC, and the unknown effect of previous psychopharmacological treatment on brain function. Finally, as mentioned in the methods, since our primary hypothesis was regarding an effect in the ACC, we did not acquire data from the anterior or posterior poles. Therefore, we were unable to determine if any effect of SLC6A3 is present in these areas.

As mentioned above, our sample had a wide age range, potentially confounding our data with multiple stages of brain development. When we ran the ANCOVA removing variance associated with age, our significant findings did drop to trend level. Given that our results were just above trend level before removing the effects of age, we suggest the p-value reduction was likely due to a reduction of power by adding the additional covariate into the model, rather than to an actual effect of age. Future studies with better power should examine the effect of ageing on genotype effects.

Because this study is limited to only subjects with ADHD, we were unable to determine how the genotype effect we found is comparable to that seen previously in a healthy control sample. It is therefore possible that the DAT1 effect we found on interference processing is limited only to samples with ADHD, or that the magnitude of the effect would be greater or lesser than in a control sample. Because Schott et al (2006) found effects in the same direction (less signal change in 10R/10R) with a control group during an episodic memory task, as did Bertolino et al with a working memory task (2006), we expect that at least the directionality during the MSIT task would be similar in a control sample to what we found

in the ADHD sample. Future studies should include healthy controls and comparison psychiatric groups to test how the magnitude of this effect might differ between diagnoses.

Despite these considerations, our findings suggest that activation of dACC, lateral PFC, and cerebellar vermis, all component parts of attentional and EF networks affected in ADHD, are influenced by SLC6A3 genotype. These data help to elucidate the neurofunctional consequences of a risk gene in ADHD, and how this variation may produce endophenotypic heterogeneity within the disorder.

Acknowledgments

The authors would like to thank Sharmila Bandyopadhyay, Denise Boriel, Katie Crum, Kalika Kelkar, Alexandra Lomedico, Ksenija Marinkovic, Snezana Milanovic, Michael Schiller, Heidi Thermenos, Michael Vitulano, and our research study volunteers for their generous assistance.

Funding/Support: This research was supported by grants from: NIMH MH 62152 (LJS), MH 57934 (SF), MH 071535 (EV); Boston University School of Medicine, Division of Graduate Medical Sciences Graduate Student Research Fellowship (AB); US Department of Health and Human Services (MOB); NIAAA (R01-AA07112 and K05-AA00219); the National Alliance for Research on Schizophrenia and Depression Distinguished Investigator Award (JB); Janssen Pharmaceuticals (JB); Medical Research Service of the US Department of Veterans Affairs (MOB); the March of Dimes Foundation (LJS), the Mental Illness and Neuroscience Discovery (MIND) Institute (LJS); the Kimmerly-Neil Fund for the Study of Cognition and Psychiatric Disorders in Children; the Pediatric Psychopharmacology Council Fund; and the National Center for Research Resources (P41RR14075).

References

Anokhin AP, Heath AC, Myers E. Genetics, prefrontal cortex, and cognitive control: a twin study of event-related brain potentials in a response inhibition task. Neurosci Lett. 2004; 368(3):314–318. [PubMed: 15364418]

Bertolino A, Blasi G, Latorre V, Rubino V, Rampino A, Sinibaldi L, Caforio G, Petruzzella V, Pizzuti A, Scarabino T, et al. Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. J Neurosci. 2006; 26(15):3918–3922. [PubMed: 16611807]

Boonstra AM, Kooij JJ, Buitelaar JK, Oosterlaan J, Sergeant JA, Heister JG, Franke B. An exploratory study of the relationship between four candidate genes and neurocognitive performance in adult ADHD. Am J Med Genet B Neuropsychiatr Genet. 2008; 147(3):397–402. [PubMed: 17886261]

Booth JR, Burman DD, Meyer JR, Lei Z, Trommer BL, Davenport ND, Li W, Parrish TB, Gitelman DR, Mesulam MM. Larger deficits in brain networks for response inhibition than for visual selective attention in attention deficit hyperactivity disorder (ADHD). J Child Psychol Psychiatry. 2005; 46(1):94–111. [PubMed: 15660647]

Bush G, Frazier JA, Rauch SL, Seidman LJ, Whalen PJ, Jenike MA, Rosen BR, Biederman J. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. Biol Psychiatry. 1999; 45(12):1542–1552. [PubMed: 10376114]

Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci. 2000; 4(6):215–222. [PubMed: 10827444]

Bush G, Shin LM. The Multi-Source Interference Task: an fMRI task that reliably activates the cingulo-frontal-parietal cognitive/attention network. Nat Protoc. 2006; 1(1):308–313. [PubMed: 17406250]

Bush G, Shin LM, Holmes J, Rosen BR, Vogt BA. The Multi-Source Interference Task: validation study with fMRI in individual subjects. Mol Psychiatry. 2003; 8(1):60–70. [PubMed: 12556909]

Bush G, Spencer TJ, Holmes J, Shin LM, Valera EM, Seidman LJ, Makris N, Surman C, Aleardi M, Mick E, et al. Functional magnetic resonance imaging of methylphenidate and placebo in attention-deficit/hyperactivity disorder during the multi-source interference task. Arch Gen Psychiatry. 2008; 65(1):102–114. [PubMed: 18180434]

Bush G, Valera EM, Seidman LJ. Functional neuroimaging of attention-deficit/hyperactivity disorder: a review and suggested future directions. Biol Psychiatry. 2005; 57(11):1273–1284. [PubMed: 15949999]

Castellanos FX, Lee PP, Sharp W, Jeffries NO, Greenstein DK, Clasen LS, Blumenthal JD, James RS, Ebens CL, Walter JM, et al. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. JAMA. 2002; 288(14):1740–1748. [PubMed: 12365958]

Castellanos FX, Margulies DS, Kelly C, Uddin LQ, Ghaffari M, Kirsch A, Shaw D, Shehzad Z, Di Martino A, Biswal B, et al. Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. Biol Psychiatry. 2008; 63(3):332–337. [PubMed: 17888409]

Dickstein SG, Bannon K, Castellanos FX, Milham MP. The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. J Child Psychol Psychiatry. 2006; 47(10):1051–1062. [PubMed: 17073984]

Durston S, Fossella JA, Casey BJ, Hulshoff Pol HE, Galvan A, Schnack HG, Steenhuis MP, Minderaa RB, Buitelaar JK, Kahn RS, et al. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Mol Psychiatry. 2005; 10(7):678–685. [PubMed: 15724142]

Durston S, Fossella JA, Mulder MJ, Casey BJ, Ziermans TB, Vessaz MN, Van Engeland H. Dopamine transporter genotype conveys familial risk of attention-deficit/hyperactivity disorder through striatal activation. J Am Acad Child Adolesc Psychiatry. 2008; 47(1):61–67. [PubMed: 18174826]

Eriksen BA, Eriksen CW. Effects of noise letters upon the identification of a target letter in a nonsearch task. Perception Psychophysics. 1974; 16:143–149.

Faraone S, Biederman J. What is the prevalence of adult ADHD? Results of a population screen of 966 adults. Journal of Attention Disorders. 2005; 9(2):384–391. [PubMed: 16371661]

Faraone SV, Biederman J, Feighner JA, Monuteaux MC. Assessing symptoms of attention deficit hyperactivity disorder in children and adults: Which is more valid? J Consult Clin Psychol. 2000; 68:830–842. [PubMed: 11068969]

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; 57(11):1313–1323. [PubMed: 15950004]

Faraone SV, Sergeant J, Gillberg C, Biederman J. The worldwide prevalence of ADHD: is it an American condition? World Psychiatry. 2003; 2(2):104–113. [PubMed: 16946911]

Fassbender C, Zhang H, Buzy WM, Cortes CR, Mizuiri D, Beckett L, Schweitzer JB. A lack of default network suppression is linked to increased distractibility in ADHD. Brain Res. 2009; 1273:114–128. [PubMed: 19281801]

First, M.; Spitzer, R.; Gibbon, M.; Williams, J. Structured Clinical Interview for DSM-IV Axis I Disorders. Washington, D.C.: American Psychiatric Press; 1997. 84 p.

Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M, Weinberger DR. Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology. 2000; 22(2):133–139. [PubMed: 10649826]

Jastak, J.; Jastak, S. Wide Range Achievement Test-Third Edition. Wilmington, DE: Jastak Associates; 1993.

Kim JW, Kim BN, Cho SC. The dopamine transporter gene and the impulsivity phenotype in attention deficit hyperactivity disorder: a case-control association study in a Korean sample. J Psychiatr Res. 2006; 40(8):730–737. [PubMed: 16368111]

Konrad K, Neufang S, Hanisch C, Fink GR, Herpertz-Dahlmann B. Dysfunctional attentional networks in children with attention deficit/hyperactivity disorder: evidence from an event-related functional magnetic resonance imaging study. Biol Psychiatry. 2006; 59(7):643–651. [PubMed: 16197925]

Kooij JS, Boonstra AM, Vermeulen SH, Heister AG, Burger H, Buitelaar JK, Franke B. Response to methylphenidate in adults with ADHD is associated with a polymorphism in SLC6A3 (DAT1). Am J Med Genet B Neuropsychiatr Genet. 2008; 147B(2):201–208. [PubMed: 17955457]

Krain AL, Castellanos FX. Brain development and ADHD. Clin Psychol Rev. 2006; 26(4):433–444. [PubMed: 16480802]

Krause J. SPECT and PET of the dopamine transporter in attention-deficit/hyperactivity disorder. Expert Rev Neurother. 2008; 8(4):611–625. [PubMed: 18416663]

Kronenberg G, Ende G, Alm B, Deuschle M, Heuser I, Colla M. Increased NAA and reduced choline levels in the anterior cingulum following chronic methylphenidate. A spectroscopic test-retest study in adult ADHD. Eur Arch Psychiatry Clin Neurosci. 2008; 258(7):446–450. [PubMed: 18330668]

Madras BK, Miller GM, Fischman AJ. The dopamine transporter and attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; 57(11):1397–1409. [PubMed: 15950014]

Makris N, Biederman J, Valera EM, Bush G, Kaiser J, Kennedy DN, Caviness VS, Faraone SV, Seidman LJ. Cortical thinning of the attention and executive function networks in adults with attention-deficit/hyperactivity disorder. Cereb Cortex. 2007; 17(6):1364–1375. [PubMed: 16920883]

McNair, DM.; Lorr, M.; Droppelman, LF. EdITS Manual for the Profile of Mood States (POMS). San Diego, CA: EdITS/Education and Industrial Testing Service; 1992.

Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci. 2006; 7(10):818–827. [PubMed: 16988657]

Mill J, Asherson P, Browes C, D'Souza U, Craig I. Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet. 2002; 114(8):975–979. [PubMed: 12457396]

Monuteaux MC, Seidman LJ, Faraone SV, Makris N, Spencer T, Valera E, Brown A, Bush G, Doyle AE, Hughes S, et al. A preliminary study of dopamine D4 receptor genotype and structural brain alterations in adults with ADHD. Am J Med Genet B Neuropsychiatr Genet. 2008; 147B(8):1436–1441. [PubMed: 18951431]

Oh KS, Shin DW, Oh GT, Noh KS. Dopamine transporter genotype influences the attention deficit in Korean boys with ADHD. Yonsei Med J. 2003; 44(5):787–792. [PubMed: 14584093]

Orvaschel, H.; Puig-Antich, J. Schedule for Affective Disorders and Schizophrenia for School-Age Children: Epidemiologic Version. Fort Lauderdale, FL: Nova University; 1987.

Peper JS, Brouwer RM, Boomsma DI, Kahn RS, Hulshoff Pol HE. Genetic influences on human brain structure: a review of brain imaging studies in twins. Hum Brain Mapp. 2007; 28(6):464–473. [PubMed: 17415783]

Purper-Ouakil D, Wohl M, Orejarena S, Cortese S, Boni C, Asch M, Mouren MC, Gorwood P. Pharmacogenetics of methylphenidate response in attention deficit/hyperactivity disorder: Association with the dopamine transporter gene (SLC6A3). Am J Med Genet B Neuropsychiatr Genet. 2008; 147B(8):1425–1430. [PubMed: 18563707]

Rubia K, Overmeyer S, Taylor E, Brammer M, Williams SC, Simmons A, Bullmore ET. Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. Am J Psychiatry. 1999; 156(6):891–896. [PubMed: 10360128]

Schott BH, Seidenbecher CI, Fenker DB, Lauer CJ, Bunzeck N, Bernstein HG, Tischmeyer W, Gundelfinger ED, Heinze HJ, Duzel E. The dopaminergic midbrain participates in human episodic memory formation: evidence from genetic imaging. J Neurosci. 2006; 26(5):1407–1417. [PubMed: 16452664]

Seidman LJ, Valera EM, Makris N. Structural brain imaging of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; 57(11):1263–1272. [PubMed: 15949998]

Seidman LJ, Valera EM, Makris N, Monuteaux MC, Boriel DL, Kelkar K, Kennedy DN, Caviness VS, Bush G, Aleardi M, et al. Dorsolateral prefrontal and anterior cingulate cortex volumetric abnormalities in adults with attention-deficit/hyperactivity disorder identified by magnetic resonance imaging. Biol Psychiatry. 2006; 60(10):1071–1080. [PubMed: 16876137]

Shaw P, Gornick M, Lerch J, Addington A, Seal J, Greenstein D, Sharp W, Evans A, Giedd JN, Castellanos FX, et al. Polymorphisms of the dopamine D4 receptor, clinical outcome, and cortical structure in attention-deficit/hyperactivity disorder. Arch Gen Psychiatry. 2007; 64(8):921–931. [PubMed: 17679637]

Shaw P, Lerch J, Greenstein D, Sharp W, Clasen L, Evans A, Giedd J, Castellanos FX, Rapoport J. Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry. 2006; 63(5):540–549. [PubMed: 16651511]

Simon JR, Berbaum K. Effect of conflicting cues on information processing: the 'Stroop effect' vs. the 'Simon effect'. Acta Psychol (Amst). 1990; 73(2):159–170. [PubMed: 2343770]

Sonuga-Barke EJ. Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. Biol Psychiatry. 2005; 57(11):1231–1238. [PubMed: 15949993]

Stroop JR. Studies of interference in serial verbal reactions. J Exp Psychol. 1935; 18:643–662.

Swanson JM, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N, Taylor E, Casey BJ, Castellanos FX, Wadhwa PD. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol Rev. 2007; 17(1):39–59. [PubMed: 17318414]

Udo de Haes JI, Maguire RP, Jager PL, Paans AMJ, den Boer JA. Methylphenidate-induced activation of the anterior cingulate but not the striatum: A [15O] H20 PET study in healthy volunteers. Human Brain Mapping. 2007; 28:625–635. [PubMed: 17080442]

Valera EM, Faraone SV, Biederman J, Poldrack RA, Seidman LJ. Functional neuroanatomy of working memory in adults with attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; 57(5):439–447. [PubMed: 15737657]

Valera EM, Faraone SV, Murray KE, Seidman LJ. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biol Psychiatry. 2007; 61(12):1361–1369. [PubMed: 16950217]

van der Kooij MA, Glennon JC. Animal models concerning the role of dopamine in attention-deficit hyperactivity disorder. Neurosci Biobehav Rev. 2007; 31(4):597–618. [PubMed: 17316796]

van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M, Baldwin RM, Innis RB, Gelernter J. Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. J Nucl Med. 2005; 46(5):745–751. [PubMed: 15872345]

VanNess SH, Owens MJ, Kilts CD. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. BMC Genet. 2005; 6:55. [PubMed: 16309561]

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS, Hitzemann R, Pappas N. Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. Am J Psychiatry. 1998; 155(10):1325–1331. [PubMed: 9766762]

Walter, B.; Blecker, C.; Kirsch, P.; Sammer, G.; Schienle, A.; Stark, R.; Vaitl, D. MARINA: An easy to use tool for the creation of MAsks for Region of INterest Analyses [abstract]. 9th International Conference on Functional Mapping of the Human Brain; New York, NY. 2003.

Wechsler, D. Wechsler Adult Intelligence Scale III [manual]. San Antonio, TX: The Psychological Corporation; 1997.

Yang B, Chan RC, Jing J, Li T, Sham P, Chen RY. A meta-analysis of association studies between the 10-repeat allele of a VNTR polymorphism in the 3'-UTR of dopamine transporter gene and attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet. 2007; 144(4): 541–550. [PubMed: 17440978]

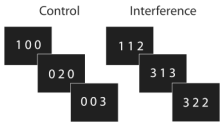

Figure 1.
The MSIT Paradigm. Example stimuli from the control and interference conditions of the multi-source interference task.

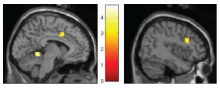

Figure 2.
Activation differences in whole volume analysis of 9R-carriers > 10R/10R (Interference > Control contrast). Colorbar represents *t* statistic. The height threshold is $p < .005$-uncorrected, and only clusters with spatial extent of $k \geq 5$ are displayed.

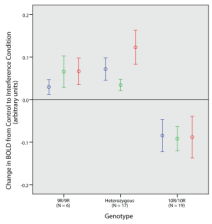

Figure 3.

Signal change in clusters that differed in main genotype comparison. Error bars represent ± 1 standard error. Zero baseline represents no difference in activity between interference and control conditions. One way ANOVAs for each of the 3 regions all revealed significant differences (all *p*'s < .01); *Post-hoc* Bonferroni corrected pairwise comparisons: [a] 9R/9R > 10R/10R (*p* = 0.221), Heterozygous > 10R/10R (p = 0.003), Heterozygous > 9R/9R (*p* = 1.00); [b] 9R/9R > 10R/10R (*p* = 0.004), Heterozygous > 10R/10R (p = 0.001), Heterozygous > 9R/9R (*p* = 1.000); [c] 9R/9R > 10R/10R (*p* = 0.174), Heterozygous > 10R/10R (p = 0.008), Heterozygous > 9R/9R (*p* = 1.000).

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2011 March 5.

Table I

Demographic and MSIT performance data for genotype groups

	10R/10R (N = 19)		9R carriers (N = 23)		Statistic	
	N	%	N	%	χ^2	*p*
Left-handers	3	16	6	26	.655	.418
Female	10	53	12	52	.001	.976
Psychiatric Comorbidities (Lifetime History)						
Multiple (≥2) Anxiety Disorders	5	26	5	22	.521	.470
Major Depressive Disorder	11	58	10	43	.865	.352
Alcohol Abuse	9	47	9	39	.288	.591
Substance Abuse	4	21	4	17	.090	.764
	Mean	SD	Mean	SD	*t*	*p*
Age	37.4	(15.0)	33.4	(12.2)	.949	.349
IQ	114.4	(9.2)	117.2	(14.2)	.731	.469
WRAT-Arithmetic	105.1	(7.8)	103.4	(14.0)	.464	.646
WRAT-Reading	107.4	(5.3)	107.6	(13.3)	.045	.965
POMS – Tension/Anxiety	34.8	(5.7)	36.7	(7.6)	.874	.387
POMS - Depression	38.5	(4.6)	39.8	(5.0)	.902	.373
Age ADHD Onset	5.3	(2.5)	5.3	(2.5)	.070	.944
Inattentive Symptoms, Childhood	7.1	(1.6)	7.7	(1.4)	1.347	.186
Hyperactive Symptoms, Childhood	5.6	(2.1)	5.8	(2.6)	.206	.838
Inattentive Symptoms, Recent	5.5	(2.0)	5.7	(2.5)	.234	.816
Hyperactive Symptoms, Recent	4.1	(2.1)	3.8	(2.8)	.412	.683
MSIT Performance Data						
Neutral %	98.8	(1.8)	98.4	(2.4)	.560	.578
Neutral RT	583.2	(57.0)	565.0	(74.4)	.867	.392
Interference %	96.7	(3.0)	93.6	(6.9)	1.818	.077
Interference RT	893.1	(109.3)	910.2	(114.2)	.485	.631
Interference RT – Neutral RT	309.8	(70.5)	345.3	(104.0)	1.25	.219

IQ = Estimated Full Scale IQ; WRAT = Wide Range Achievement Test; POMS = Profile of Mood States; RT = reaction time. All RTs in milliseconds. Due to computer error, MSIT performance data was lost for two 9R carriers.

Table II

Stimulant Medication History in Genotype Groups

	10R/10R (N = 19)		9R carriers (N = 23)	
	N	%	N	%
Currently Prescribed Stimulants[a]	8	42	8	35
Stimulants Prescribed only in Past	4	21	4	17
Stimulant Naïve	7	37	11	48

χ^2 (2) = 0.513, p = 0.77;

[a]Subjects prescribed stimulant medications near time of scan were washed out for at least 24 hours

Table III

Areas of greater activation in 9R-carriers than in subjects with 10R/10R (Interference > Control Contrast)

Cluster	x	y	z	K (cluster extent)	*T*	z	*p*-value
Region of Interest							
L dACC (BA 24/32)[a]	−3	0	30	26	3.54	3.28	.036
Whole Volume Analysis							
R Lateral PFC (BA 46)[b]	42	18	27	28	3.50	3.25	.049
L Cerebellar Vermis[b]	−6	−48	−6	25	4.73	4.19	.061

Coordinates are given in Montreal Neurological Institute (MNI) standard space;

[a] Cluster defined by ROI analysis, *p*-value corrected for number of voxels across the entire bilateral ROI;

[b] Clusters defined by whole-volume analysis, *p*-values are uncorrected.

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2011 March 5.