THE ROLE OF CATECHOL-O-METHYLTRANSFERASE AND DOPAMINE RECEPTOR D4 IN ADHD SYMPTOM VARIATION AMONG INDIVIDUALS WITH DOWN SYNDROME

By

GINA MARIE MASON

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Approved by:

Dr. Nadel
Department of Psychology

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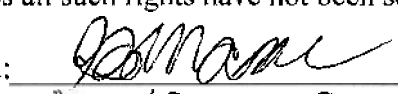
:The University of Arizona Library Release	I hereby grant to the University of Arizona Library the nonexclusive worldwide right to reproduce and distribute my dissertation or thesis and abstract (herein, the "licensed materials"), in whole or in part, in any and all media of distribution and in any format in existence now or developed in the future. I represent and warrant to the University of Arizona that the licensed materials are my original work, that I am the sole owner of all rights in and to the licensed materials, and that none of the licensed materials infringe or violate the rights of others. I further represent that I have obtained all necessary rights to permit the University of Arizona Library to reproduce and distribute any nonpublic third party software necessary to access, display, run or print my dissertation or thesis. I acknowledge that University of Arizona Library may elect not to distribute my dissertation or thesis in digital format if, in its reasonable judgment, it believes all such rights have not been secured. Signed:  Date: May 10, 2010

Abstract

Individuals with Down syndrome (DS) have shown difficulties in executive function, including the ability to implement new rules, inhibit responses, and maintain attention. Attention-deficit hyperactivity disorder (ADHD) is also related to executive function deficits, and it is not uncommon for those with DS to exhibit ADHD symptoms. Studies have indicated a link between variation in two genes, catechol-o-methyltransferase (*COMT*) and dopamine receptor D4 (*DRD4*), and differences in executive ability and ADHD symptoms among typically-developing individuals. We examined whether these genes also relate to differences in executive function and ADHD symptoms within DS. Participants included 77 individuals with DS (7-40 yrs) and 50 mental-age matched controls (3-6 yrs). Participants were tested using prefrontal tasks from established assessments (e.g. CANTAB Eclipse, the Dots task), and caretakers completed questionnaires measuring ADHD symptoms. Consistent with past research, those with DS showed executive difficulties and higher inattention levels compared to controls. Within a subset of those with DS (7-20 yrs.), *COMT val-val* carriers displayed more omission errors on an attention task, and more impairment on parent reports of working memory. While some trends were found for *DRD4*, greater sample size is needed. This research adds to our knowledge of gene function under different developmental conditions, and may also lead to better pharmacological treatments for those with DS.

Introduction

Since Gregor Mendel's (1865) early discoveries of genes and inheritance, it has become widely accepted that our genes play some role in determining our characteristics. In psychology, advances in gene-identification technologies have allowed us not only to discover genes related to specific psychological disorders and developmental disabilities, but also to pinpoint genes relating to differences in more general processes underlying learning and attention.

Down syndrome (DS) is one of the most common developmental disabilities with a recognized genetic basis, and was one of the first diseases causing early intellectual impairment to be widely recognized and classified (Down 1866). The genetic pathology of DS was first described by LeJeune, Gautier and Turpin (1959), when they discovered the presence of an extra copy of the genetic material of chromosome 21 within the fibroblast (skin) cells of 9 individuals with DS. This phenomenon came to be known as *trisomy 21*, indicating the presence of 3 copies of chromosome 21 in all of the patient's cells rather than the typical 2 found in those without DS.

Regarding the effects of *trisomy 21* on outcomes for those with DS, research is ongoing; however, some common traits existing between those carrying the extra chromosome have been indicated. Among various other characteristics, it has been observed that many individuals with DS display difficulties with *executive function* (EF), described broadly as the ability to acquire and apply new rules, inhibit inappropriate social responses, and harness attention towards achieving a specific plan or goal (Robbins, Weinberger, Taylor & Morris, 1996). Specific to those with DS, Rowe, Lavender and Turk (2006) found significant impairments on cognitive measures of set-shifting (the *Weigl Colour-Form Sort Test*), planning and problem solving of the kind typically associated with frontal lobe lesions (*Raven's Coloured Progressive Matrices*), and attention (*Attention Sustained–Leiter International Performance Scale –revised*). Interestingly,

similar executive difficulties have also been frequently perceived in individuals diagnosed with Attention-Deficit Hyperactivity Disorder (ADHD) (Willcutt, Doyle, Nigg, Faraone, & Pennington 2005), and in observer scales of behavior and psychopathology for those with DS, symptoms falling within attentional and self-control domains such as distractibility, impulsivity, impatience and disobedience have been some of the highest and most frequently indicated (Clark & Wilson 2003). This suggests that in addition to executive function difficulties similar to those seen in ADHD, it is not uncommon for individuals with DS to display other behavioral characteristics associated with ADHD as well.

While EF and ADHD symptoms are indeed common for those carrying *trisomy 21*, the severity of the difficulties expressed can vary significantly among individuals (Edgin et. al., in review), and the causes of this variability are not entirely known. In typically-developing individuals and other populations, variants of certain background genes have been associated with differences in executive abilities and ADHD symptom risk. Among these genes are *catechol-o-methyltransferase* (*COMT*), and *dopamine receptor D4* (*DRD4*). Initially described by Axelrod (1957), *COMT* functions to create enzymes that break down various types of hormones known as *catecholamines* via o-methylation, which is simply the addition of a methyl (CH_3) group to the hydroxide (OH) group of the chemical to be degraded (see Napolitano, Cesura & Da Prada 1995 for illustration). Among the catecholamines degraded by *COMT* is dopamine, and while *COMT* is found to work in various brain regions, studies have suggested that *COMT* is particularly important for the degradation of dopamine in the prefrontal cortex (PFC) due to a scarcity of dopamine transporter (DAT) in this structure relative to other brain areas and a greater subsequent dependence on other means to clear dopamine from synapses (Yavich, Forsberg, Karayiorgou, Gogos & Männistö 2007).

Regarding *COMT*'s role in executive function, variation at codon 108/158 of *COMT* (a single base-pair mutation resulting in the polymorphic substitution of Methionine (Met) for Valine (Val) at codon 108/158) has been associated with differences in certain executive abilities among different populations along with differences in dopamine regulation (various studies have indicated that Met allele substitution in *COMT* causes the enzyme produced to degrade dopamine more slowly than it does in those carrying the Val/Val genotype (Meyer-Lindenberg et. al. 2005; Yavich et. al. 2007)). For instance, Diamond, Briand, Fossella & Gehlbach (2004) demonstrated that typically-developing children homozygous for the Methionine *COMT* allele (Met/Met) performed significantly better than those homozygous for Valine (Val/Val) on the "dots-mixed" task, a task of working memory and inhibition related to prefrontal function. Similar patterns of executive difficulty have been found in patients with schizophrenia, as a study conducted by Egan et.al. (2001) found that those carrying the Val/Val genotype had decreased performance on the Wisconsin Card Sorting task (a set-shifting EF task). Additionally, in a study involving individuals with Parkinson's disease (PD), variation in *COMT* was found to correlate with different strategies on the CANTAB ID/ED set-shifting task—although in this case, those with the Val/Val genotype employed more profitable strategies than those carrying Met alleles (Williams-Gray, Hampshire, Barker, & Owen 2008). The effects of dopaminergic medication on performance also depended on genotype among these patients (doses of the dopamine precursor levadopa decreased performance among those carrying the Val/Val genotype, while no effect on base performance was found for those homozygous for Methionine), suggesting that *COMT*'s effects on EF tasks were related to its role in dopamine degradation for these individuals.

DRD4 is another gene whose function is related to the prefrontal cortex, and specific variants of the gene have been associated with differences in ADHD susceptibility as well as the

greater persistence of symptoms within the pathology of ADHD. Expressed mainly in prefrontal grey matter (Durston et.al. 2005), DRD4 works to inhibit adenylyl cyclase, which is responsible for making a hormone known as cyclic AMP (cAMP) that activates many different pathways in the brain (Asghari et.al. 1995). A 48-base pair variable nucleotide tandem repeat (VNTR) in exon III of the gene creates allelic variants that depend upon the number of 48-base pair repeats present. The 7-Repeat (7R) allele variant appears to have less of an affinity for dopamine (Asghari et.al. 1995), which could possibly cause a reduction in dopamine-activated adenylyl cyclase/cAMP level inhibition. In any case, a meta-analysis conducted by Faraone, Doyle, Mick & Biederman (2001) associated the 7R allele with ADHD risk both through case-control and family-based studies. Furthermore, in a longitudinal study assessing the persistence of ADHD difficulties in children diagnosed or suspected to have the disorder, Langley et.al. (2009) found via parent, teacher, and child interviews addressing clinically-relevant symptom criteria (the Child and Adolescent Psychiatric Assessment (CAPA); the Child ADHD teacher telephone interview (ChATTI)) that children carrying the DRD4 7R allele had significantly less of a decline in ADHD symptoms compared to the decline observed in those without the 7R variant. These studies suggest not only that risk for ADHD in general might be partly mediated by differences in DRD4, but also that DRD4 differences might have an effect on the perseverance of ADHD-symptoms in those with ADHD.

Although research has addressed the effects of *COMT* and *DRD4* gene variants in typical populations as well as in some others (e.g. PD patients), little research has examined the effects of these common variants on the EF and ADHD difficulties exhibited by those with Down syndrome. Such research is important for a number of reasons. First, it might help to explain the variability in symptoms observed among those with DS, which in turn may lead to better

pharmacological treatments and interventions for those with greater symptom severity. Secondly, researching the effects of background genetics in the context of *trisomy 21* might help to illuminate the possible pathways by which *trisomy 21* works to create the DS phenotype. For instance, it is possible that *trisomy 21* might interact with non-chromosome 21 genes such as *COMT* and *DRD4* to either dampen or enhance their effects. If *trisomy 21* completely dampens the effects of background genes, we should expect no significant differences in symptoms between individuals carrying different gene variants; however, if *trisomy 21* works to enhance their effects, we should observe significant differences between groups with different alleles. Another possibility is that background genes may contribute an additive effect on symptoms commonly associated with DS without interacting with *trisomy 21* as well. Considering these possibilities, *COMT* and *DRD4* are particularly relevant candidates for study within DS, not only because they have been related to particular cognitive and behavioral difficulties (EF and ADHD symptoms) commonly observed in DS, but also because they are important players within the prefrontal cortex, a brain region whose development is theorized to be affected by *trisomy 21* (Nadel 1999).

In the present study, we examined the effects of variation in *COMT* and *DRD4* on differences in executive function and ADHD risk in individuals with Down syndrome. Our first aim was to confirm that individuals with DS do indeed display higher levels of EF difficulties and ADHD symptoms when compared to individuals of relative intellectual level without DS. Next, we analyzed the effects of the *COMT Met/Val* and *DRD4 R7* polymorphisms on specific executive abilities and ADHD symptoms within those with DS, to determine whether these variants contribute to the symptom variability we observe in these individuals. The results of this study will help us to establish whether background genes should be taken into account when

considering pharmacological and cognitive/behavioral interventions for those with DS, and will also set a premise for further research on how trisomy 21 might or might not interact with background genes to create the DS phenotype.

Methods

Participants

Seventy-seven individuals with DS ages 7-40 years (39 male, 38 female) were recruited from 3 sites including the University of Arizona, Tucson; Johns Hopkins University, Baltimore; and, Emory University, Atlanta. These participants were found via advertisement, doctors' referral or local organizations supporting Down syndrome (e.g., Southern Arizona Network for Down Syndrome (SANDS)). Exclusion criteria included history of head injury; presence of mosaic Down syndrome or Robertsonian translocation rather than full trisomy 21 (individuals without any medically-verified diagnosis were also excluded); and, loss of consciousness for over 5 minutes.

To evaluate the general presence of executive difficulties in Down syndrome, 36 children ages 3-6 years were also recruited from University participant databases to serve as Mental-Age (MA)-matched controls. These children were matched on their combined average verbal and non-verbal IQ scores as assessed through the Kaufmann Brief Intelligence Test, Second Edition (K-BIT II, described in Measures below). Taken as a whole, their average score was approximately equivalent to the average of 58 of the total participants with DS (**TABLE 1**). Thus, following exclusions and mental-age matching, final EF and ADHD general comparisons were made between 58 participants with DS and 36 MA-matched controls.

For genetics analyses, only individuals ages 7-20 (n= 27) were analyzed to avoid possible gene-expression differences associated with aging. Of these, genotyping was inconclusive in 2 cases for *COMT* and in 4 cases for *DRD4*. Thus, final genetics comparisons were made among 24 individuals for *COMT*, and 22 for *DRD4*.

All participants received $40 in gift cards (Target or Wal-Mart) as compensation, along with stickers and toy prizes.

Measures

Arizona Cognitive Test Battery (ACTB): The ACTB (Edgin et. al., under review) is a comprehensive neuropsychological test battery used to assess general IQ as well as cognitive functions associated with the parts of the brain most commonly seen to be effected in DS, including the hippocampus, cerebellum and prefrontal cortex (Pennington et. al. 2003; Rowe et. al. 2006). Tasks within this battery especially relevant to executive function and ADHD symptoms are as follows:

- *CANTAB Simple Reaction Time* (SRT): The CANTAB Simple Reaction Time (*CANTAB* 2009) is a continuous performance measure of attention and motor ability. A white box is shown intermittently on a black computer screen, and participants are instructed to press a keypad button as soon as the box appears. Attention errors are encoded either through commission (pressing the button before the stimulus appears) or omission (not pressing the button after the stimulus has remained on the screen for an extended time). Differentiating between the type of error (commission vs. omission) and pressing pattern (e.g. repetitive quick presses in the absence of stimuli vs. absence of presses even in the presence of stimuli) seen among different participants might assist us in determining

whether the inattention observed is driven by hyperactivity, or by general difficulties in maintaining concentration toward the task.

- *CANTAB Intra-Extra Dimensional Set Shift Task* (IED): The IED (2009) assesses working memory, set-shifting and inhibitory abilities. Participants are first presented with two colored shapes on a computer screen, and must determine which shape is "correct" through trial-and-error. As trials progress, the rule for "correctness" shifts, making the previously incorrect shape correct and vice-versa; and in later trials, a second shape is transposed onto the colored shapes, adding another dimension that participants must monitor when determining which shape is now "correct". Participants must recognize when the rule changes during the game and adapt their responses accordingly. This task has been used to assess executive ability differences in diverse populations such as those with Parkinson's disease, and performance differences appear relatable to *COMT* genotype and to specific PFC differences in dopamine regulation as demonstrated through levadopa administration and fMRI/Region of Interest (ROI) analyses (Williams-Gray, Hampshire, Barker & Owen 2008).
- *"Frogs and Cats" Modified DOTS task*: the Frogs and Cats task is adapted from Davidson, Amso, Anderson and Diamond's (2006) "Dots" (dots-mixed) task measuring working memory and inhibition. In the first phase (congruent rule), a picture of a cat is displayed on the left or right side of a computer touch-screen along with two buttons corresponding to the left and right, and participants must press the button located on the same side as the cat. In the incongruent rule phase, a frog appears on the left or right side of the screen, and participants are instructed to choose the button on the opposite side of the frog. The final "mixed" phase employs random-order trials in which either a frog or

cat appears; participants must respond to each stimulus using the rules learned in the previous phases. The Dots task has been related to *COMT* variation in typical children and to the PFC via neuroimaging (Diamond et. al. 2004), making it especially relevant for this study.

General IQ: In addition to these tasks above, another assessment within the battery important for this study was the Kaufmann Brief Intelligence Test, 2nd Edition (Kaufman & Kaufman 2004). This assessment measures both verbal and non-verbal IQ through a series of picture questions in which participants are asked to "point to the picture corresponding to the word" stated by the experimenter (verbal); riddles(verbal); and, analogical matrices(non-verbal) (e.g. having participants identify a picture corresponding to a dog in the same way a carrot relates to a rabbit). Verbal and nonverbal scores are added and standardized (M= 100, SD= 15) to form a composite. This task provides a basis by which IQ can be compared and ruled-out as a possible cause of EF and ADHD differences between participant groups.

ADHD and Executive Function behavioral rating questionnaires: These measures included the Behavior Rating Inventory of Executive Function (BRIEF) Parent Form, and the Conners 3™ Parent Rating Scales, Revised for ADHD Symptoms. The BRIEF-A Parent Form consists of 86 questions designed to measure 8 different domains of executive function falling within one of two broader validity scales. The "Behavioral Regulation" Scale includes questions measuring Inhibition, Shifting and Emotional Control, while the "Meta-Cognition" scale measures aspects of executive function including Initiation, Working Memory, Planning/Organizing, Organization of Materials, and Monitoring. The parent response form has been found to have reasonably high

test-retest reliability (r= 0.82) and convergent validity when compared to similar measures of attention, learning and impulsivity (PAR 2009).

The Conners 3™ Parent Rating Scales, Revised for ADHD Symptoms includes 24 items rated on a 0-3 scale (0 being "not true at all", and 3 indicating "very much true") assessing risk for inattentive (e.g. "has trouble keeping his/her mind on work or play for long") and hyperactive (e.g. "fidgets or squirms in seat") -type ADHD. These scales also include a 10-item ADHD index screening for general (non-specific) ADHD risk. Preliminary studies of reliability for the Conners short form scales suggested a 2-4 week test-retest reliability of 0.93 for the Inattention scale and 0.94 for Hyperactive-Impulsive ADHD (Rzepa, Conners, Gallant, Pitkanen, Sitarenios, & Marocco 2007), and other statistical analyses have suggested that the scales have strong discriminative validity in terms of differentiating children with ADHD from typical children as well as children with other disorders such as disruptive behavior and learning disorders (Conners 2009).

Genetics collection: Samples were collected using Oragene OG-250 DNA kits in disk format, along with Oragene CS-1 "DNA for children" test kits (DNA Genotek). These kits were chosen for their non-invasive nature as they require saliva rather than blood, and for their ease of handling and shipping (each kit contains preservative that allows the samples to remain stored without refrigeration). An optional board game was also constructed specifically for saliva sample collection to make the procedure as appealing for the children as possible. This board game contained "sugar monsters" and the way to advance to the next step was to place saliva in the collection cup. Many participants appeared to respond well to this approach.

Procedure

All procedures were approved by the Human Subjects Committee at the University of Arizona. The Arizona Cognitive Test Battery was administered either in the UA's Down Syndrome Research Group laboratory or in participants' homes with few distractions, and the entire testing session was approximately 3 hours in length. The first task administered was the K-BIT II, following standard protocols outlined in the K-BIT II test manual (Kaufman & Kaufman 2004). Other tasks were presented in counter-balanced order to avoid fatigue effects among measures. Computer measures (all CANTAB tasks, the Modified DOTS task) were presented on a touch-screen computer, and other devices including a button-box for the CANTAB SRT were added when needed. For non-computerized measures (e.g. the K-BIT II), experimenters placed themselves across from participants in a way that allowed them to score participants' performance discreetly so as not to influence participants' future responses. During the testing, parents completed the BRIEF and Conners questionnaires. Participants were allowed a break approximately halfway through the testing; if they became especially anxious or tired, more breaks were allowed.

During the break period, DNA samples were collected using the Oragene technology described in Materials. Once large batches of DNA samples were collected, they were shipped to Emory University for genetics analysis. *COMT* genotype was identified via a standard Taqman® SNP assay (Applied Biosystems). This assay includes two PCR primers used to amplify the region surrounding the SNP, and two Taqman® probes (one corresponding to each allele type) labeled with fluorescent reporter dye at the 5' end of the probe and a 3' quencher that initially controls the degree of fluorescence emitted. As PCR cycles progress, the probe(s) corresponding to the allele(s) present in the sample are cleaved, freeing the fluorescent reporter from the

quencher and thus increasing the fluorescence intensity. Once PCR amplification is finished, the relative fluorescence of the alleles are read, and genotype is identified (De La Vega, Lazaruk, Rhodes, & Wenz 2005).

DRD4 VNTR copy number was identified through PCR amplification and fluorescent labeling using primers flanking the regions corresponding to the repeat length polymorphism. Once fluorescence was amplified and the VNTR region separated from other PCR products via the ABI 3100 genetic analyzer (Applied Biosystems), Gene Mapper bioinformatics software was used to determine the lengths of the VNTRs for each sample.

Statistical Analyses.

The various parts of this study each employed a between-subjects design. In the first task, the independent variable was Down syndrome diagnosis (DS vs. MA control), and dependent variables included the level of EF difficulties and ADHD symptoms as measured by the cognitive tasks (SRT, IED and Frogs and Cats task) and caregiver questionnaires (BRIEF and Conners) outlined in *Measures*. To evaluate symptom differences between those with Down syndrome and MA controls, we performed an independent-subjects t-test comparing scores on these measures, along with scores on the K-BIT verbal/nonverbal IQ assessment to verify that IQ was approximately equivalent between groups.

In the genetics analyses conducted only among those with DS, the independent variable was genotype. For *COMT*, individuals with DS ages 7-20 were divided into two groups: those homologous for Valine (Val/Val, n=11), and those carrying at least one Methionine (Met) substitution (n=13). An independent-subjects t-test was used to compare these groups on K-BIT general IQ assessment, cognitive EF and attention tasks, and behavioral ADHD and EF reports

(Conners' and BRIEF). For *DRD4* comparison, those with DS aged 7-20 were divided into those carrying at least one 7R VNTR (n=7) and those without the 7R allele (n=15). Again, an independent-subjects t-test was conducted analyzing differences among groups on the same measures analyzed between groups for *COMT* variation.

Results

Mental-Age matched comparison of general ADHD and Executive Function symptoms. Table 1 shows all means, t-values and probability scores for each of the measures evaluated. First, we conducted an independent-subjects t-test between those with DS and MA control participants to confirm that the two groups were matched on K-BIT verbal and nonverbal raw scores. No significant differences were found between groups, $p>0.05$.

Regarding cognitive executive function and attention measures, individuals with DS showed higher rates of errors on the CANTAB ID/ED set-shifting task ($p<0.05$) and scored lower in the combined rule phase of the Modified Dots task ($p=0.05$), suggesting that those with DS have greater difficulty with tasks requiring set-shifting (adapting to different rules) even when controlling for IQ. Interestingly, there were no significant differences between groups on the CANTAB SRT attention task ($p>0.05$), possibly due to the chronological age difference between the two groups (and the fact that children ages 3-6 from a developmental perspective are naturally inattentive—see discussion).

Examining behavioral reports of executive function and ADHD, we observed that those with DS were reported to have significantly greater working memory and monitoring difficulties on the BRIEF: Behavior Rating Inventory of Executive Function than MA controls ($p<0.01$). In addition, individuals with DS were reported to have significantly higher inattention levels

according to caregiver report on the Conners' 3™ Parent Rating Scales, Revised for ADHD Symptoms ($p<0.01$), although no significant differences in ratings of hyperactivity/impulsivity were found between groups ($p>0.05$). There was a trend toward significance regarding general ADHD risk ($t(92)=1.84$, $p=0.08$), with individuals with DS having a higher overall probability score for having some form of ADHD.

COMT variability effects within sample with Down syndrome (Table 2; Figs. 1 &2). Table 2 indicates all means, t-values and probability scores between groups. Comparing scores on the K-BIT verbal and non-verbal assessments, an independent-subjects t-test revealed no significant IQ differences among individuals carrying the *COMT* Val/Val genotype vs. those carrying at least one Met allele ($p>0.05$), suggesting not only that *COMT* variation does not affect global IQ for those with DS, but furthermore, that any EF and ADHD symptom differences found between these two groups likely are not explainable through IQ differences.

For cognitive EF and ADHD tasks, no significant differences in correct responses were found between groups on any phase of the Modified Dots task ($p>0.05$). On the SRT attention task, no significant differences in commission errors were found ($p>0.05$); however, those carrying the Val/Val genotype appeared to have significantly greater omission errors (Fig. 1), suggesting higher levels of inattention but not hyperactivity on this task. Interestingly, those carrying the Val/Val genotype also made significantly fewer set-shifting errors on the CANTAB ID/ED task (Fig. 1), possibly reflecting a greater overall ability to adapt to rule changes.

Scores on the BRIEF behavioral rating inventory indicated greater working memory difficulties for individuals with the Val/Val genotype ($p<0.01$), while non-significant trends were found between groups on scales of monitoring ($t(24)= -1.94$, $p=0.07$) and inhibition ($t(24)= -$

1.99, p=0.06). Regarding the Conners' ADHD scales, no significant differences were found for hyperactivity or total ADHD probability (p>0.05), though a non-significant trend emerged with regard to inattention levels (t(24)= -1.57, p= 0.13).

DRD4 variability effects within sample with Down syndrome (Table 3, Figs. 3 & 4). All means, t-values and probability values are indicated in Table 3. No significant differences were observed among groups for the verbal, non-verbal and IQ composite scores of the K-BIT (p>0.05), suggesting that any ADHD and executive function differences observed among groups likely are not related to differences in IQ. Additionally, no significant differences in performance were found for any of the cognitive EF/ADHD tasks, including the Modified Dots task, SRT attention task, and CANTAB ID/ED (p>0.05). For behavioral measures, no significant differences were found on the BRIEF Inhibition and Monitoring scales or on the Conners' scale of Hyperactivity (p>0.05); however, non-significant trends were observed on the BRIEF scale of Working Memory (t(22)= -1.60, p=0.13) and on the Conners' scales of inattention (t(22)= -1.46, p=0.19) and general ADHD risk (t(22)= -1.56, p=0.16). These trends suggest that individuals with the R7 risk allele might have higher levels of working memory difficulties and ADHD symptoms (particularly inattentive type); however, a more equal distribution of participants between groups is needed before conclusions can be drawn.

***Table 1*. Mental-Age Matched Comparison of Executive Function and ADHD Symptoms**

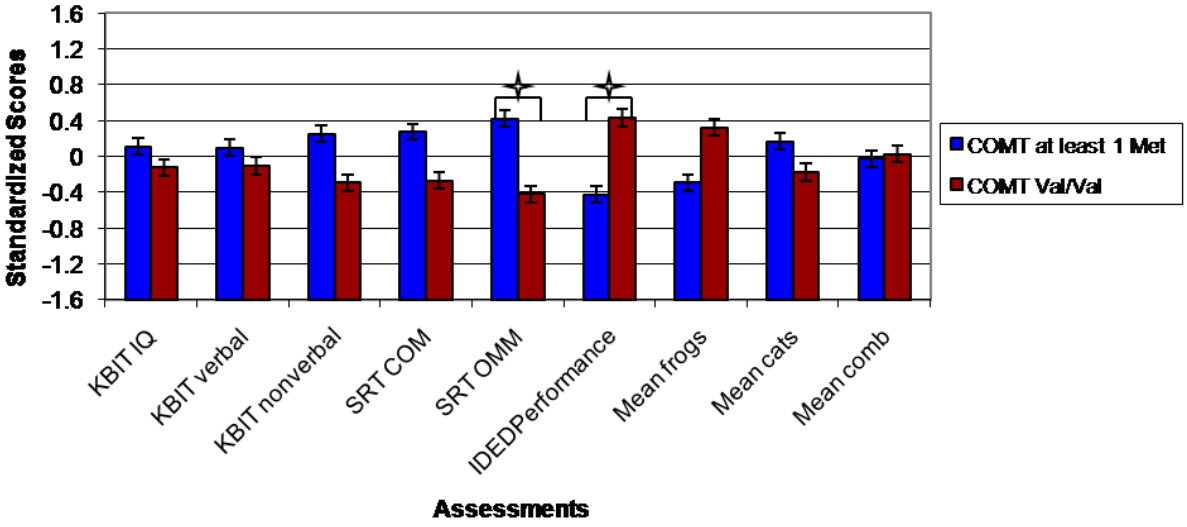
	Individuals with DS (N=58)	**Typically-Developing MA-Matched Controls (N=36)**	***t*** (92)	**p**
Background and Benchmark Measures				
KBIT-II IQ	45.66	110.03	-35.37	0.00*
KBIT-II verbal sum	26.22	27.39	-0.71	0.48
KBIT-II non-verbal sum	13.54	13.97	-0.50	0.62
Cognitive Executive Function Measures				
CANTAB ID/ED total errors	32.96	25.78	2.90	0.01*
CANTAB SRT total commission errors	8.49	10.32	-0.93	0.36
CANTAB SRT total omission errors	4.34	2.35	1.71	0.09
Modified Dots Task mean cats correct	0.87	0.90	0.051	0.61
Modified Dots Task mean frogs correct	0.67	0.76	-1.51	0.14
Modified Dots Task mean combined correct	0.57	0.67	-2.03	0.05*
Executive Function/ADHD Behavioral Measures				
Brief inhibit rating[a]	17.40	n/a	n/a	n/a
Brief working memory rating[a]	20.35	n/a	n/a	n/a
Brief monitor rating[a]	17.03	n/a	n/a	n/a
Conners' Rating Scale ADHD Inattentive	3.00	0.33	3.79	0.00*
Conners' Rating Scale ADHD Hyperactive-Impulsive	2.38	2.33	0.04	0.97
Conners' ADHD Index Probability Score[b]	5.13	1.80	1.84	0.08

a: Data for control individuals not available for this measure.
b: When analyzing only the ages 7-20 sample used for genetic analyses (n=27) and including individuals omitted due to MA-matching, the Conners' 3 overall ADHD Index Probability score was also found to be significant (p= 0.002). This result did not appear significantly related to differences in verbal and nonverbal IQ according to univariate analysis.

Table 2. **DS Executive Function and ADHD Scores as a Function of *COMT* Val158Met variation**

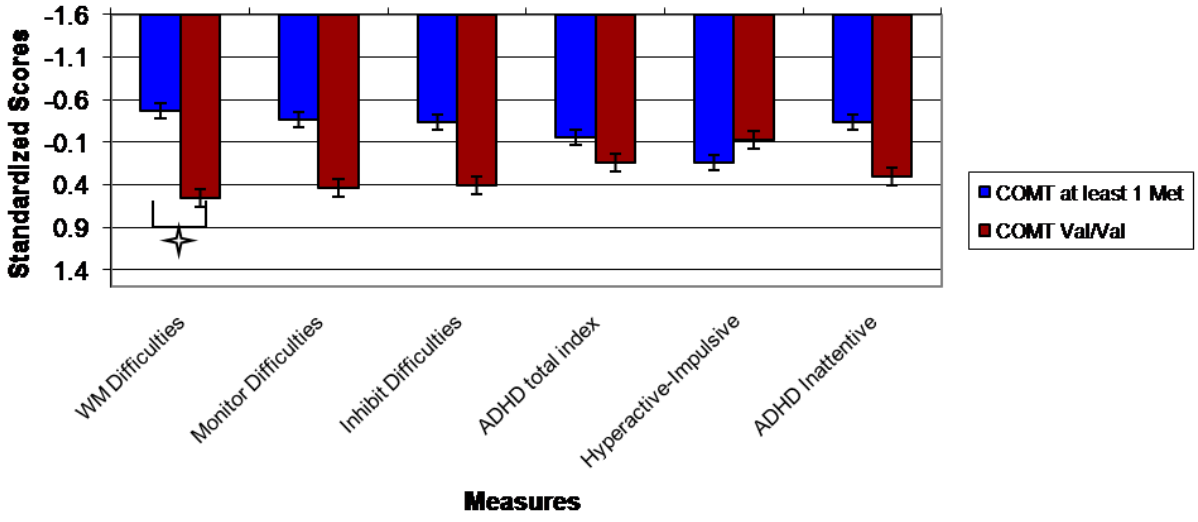
	DS *COMT* at least 1 Met allele mean (N=13)	**DS *COMT* Val/Val mean (N=11)**	***T***(24)	**p**
Background and Benchmark				
KBIT-II IQ	46.31	44.55	0.64	0.53
KBIT-II verbal sum	21.62	18.91	0.63	0.53
KBIT-II non-verbal sum	12.85	0.36	1.24	0.23
Cognitive Executive Function Measures				
CANTAB ID/ED total errors	33.00	26.55	2.09	0.05*
CANTAB SRT total commission errors	11.18	19.09	-1.33	0.19
CANTAB SRT total omission errors	1.55	11.09	-2.16	0.05*
Modified Dots Task mean cats correct	0.85	0.75	0.94	0.36
Modified Dots Task mean frogs correct	0.59	0.71	-0.98	0.34
Modified Dots Task mean combined correct	0.55	0.55	0.03	0.98
Behavioral Executive Function/ADHD Measures				
Brief inhibit rating	16.62	20.80	-1.99	0.06
Brief working memory rating	18.77	23.40	-2.78	0.01*
Brief monitor rating	15.77	18.50	-1.94	0.07
Conners' Rating Scale ADHD Inattentive	2.50	4.30	-1.57	0.13
Conners' Rating Scale ADHD Hyperactive-Impulsive	3.20	2.60	0.43	0.67
Conners' ADHD Index Probability Score	5.00	7.20	-0.77	0.45

Fig. 1. Cognitive Executive Function Scores as a Measure of COMT Variation



***p<0.05**

Fig. 2. ADHD Behavioral Symptoms as a Measure of COMT Variation



***p<0.05**

Table 3. **DS Executive Function and ADHD Scores as a Function of *DRD4* R7 allele variation.**

	DS *DRD4* no R7 risk allele (N=15)	**DS *DRD4* at least 1 R7 allele (N=7)**	***T***(22)	**p**
Background and Benchmark				
KBIT-II IQ	45.87	46.29	-0.13	0.90
KBIT-II verbal sum	21.60	19.29	0.47	0.65
KBIT-II non-verbal sum	12.33	11.00	0.56	0.58
Cognitive Executive Function Measures				
CANTAB ID/ED total errors	28.71	32.67	-0.99	0.34
CANTAB SRT total commission errors	16.36	14.67	0.23	0.82
CANTAB SRT total omission errors	7.86	4.83	0.52	0.61
Modified Dots Task mean cats correct	0.84	0.68	1.30	0.23
Modified Dots Task mean frogs correct	0.67	0.56	0.82	0.42
Modified Dots Task mean combined correct	0.58	0.48	1.09	0.29
Behavioral Executive Function/ADHD Measures				
Brief inhibit rating	18.36	18.57	-0.08	0.94
Brief working memory rating	19.64	23.00	-1.60	0.13
Brief monitor rating	16.64	17.57	-0.53	0.60
Conners' Rating Scale ADHD Inattentive	2.67	4.86	-1.46	0.19
Conners' Rating Scale ADHD Hyperactive-Impulsive	2.33	4.14	-1.71	0.31
Conners' ADHD Index Probability Score	4.42	9.71	-1.56	0.16

Fig. 3. Cognitive Executive Function Scores as a Measure of DRD4 Variation

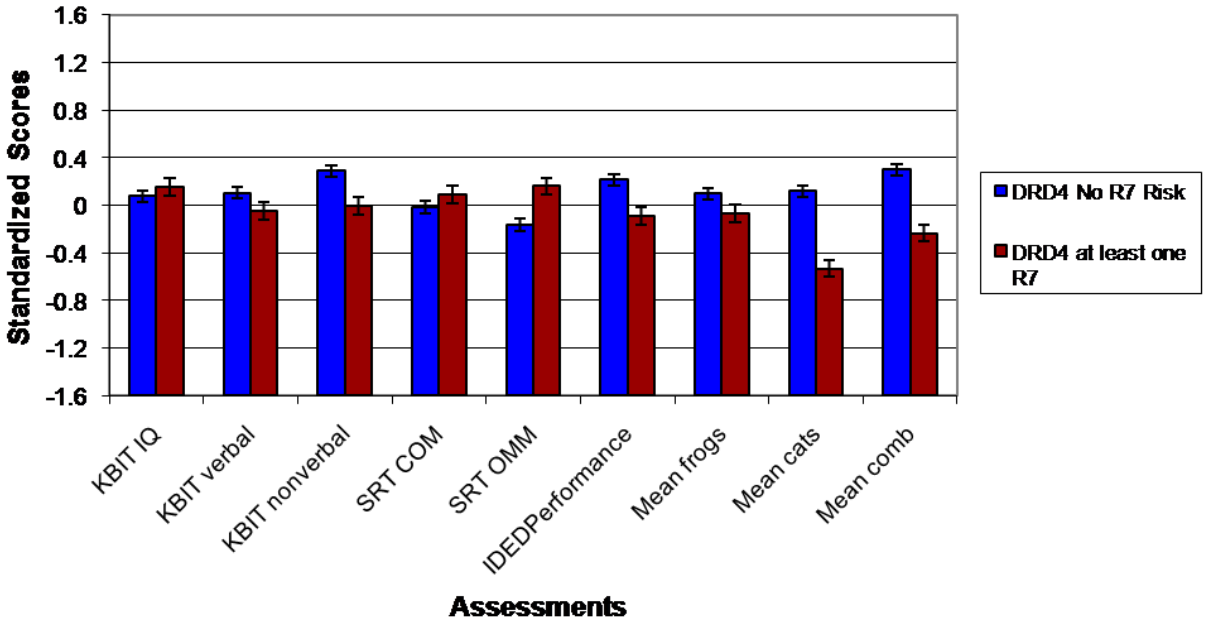
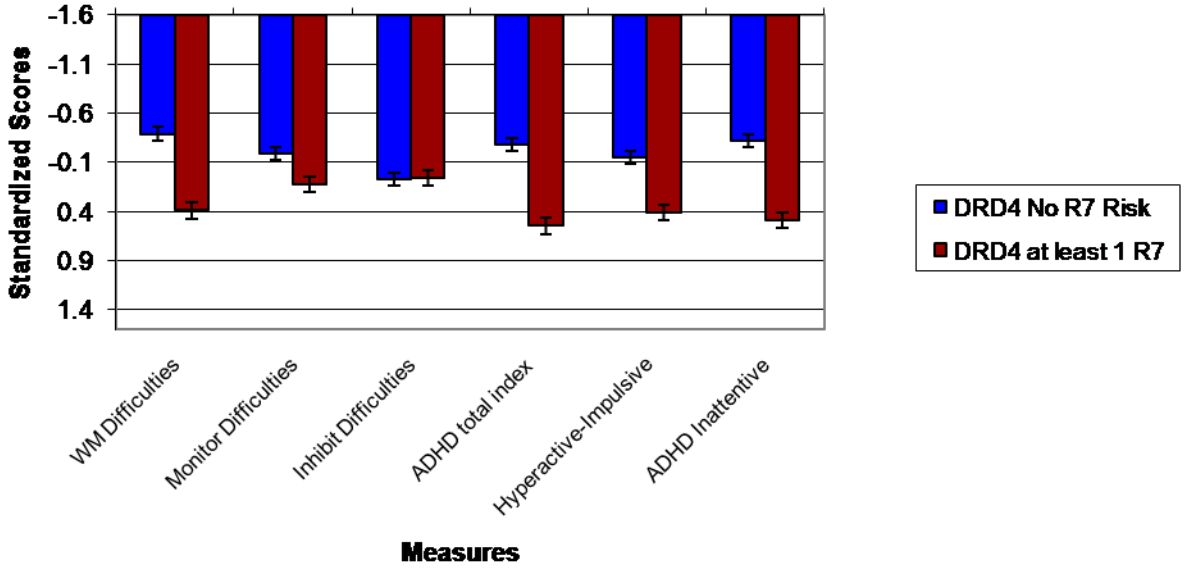


Fig. 4. ADHD Behavioral Symptoms as a Measure of DRD4 Variation



Discussion

This study explored the possible relationship between variation in two background genes, *COMT* and *DRD4*, and differences in executive function and ADHD difficulties in children with Down syndrome. Our first goal was to confirm previous findings that EF and ADHD symptoms are generally elevated in Down syndrome independent of IQ. Comparing individuals with DS to MA controls, we found that those with DS show greater levels of set-shifting difficulties along with parent-reported difficulties in attention, monitoring, working memory and general ADHD risk. Though attention scores appeared similar between groups on one cognitive task (the CANTAB SRT), we believe we can attribute this result to the fact that the controls used were 3-6 years of age while those with DS were 7-20, and that in typical development, significant attention span differences can be observed between the age of 3 and later years (Plude, Enns & Brodeur 1994). In contrast, the Conners 3 parent reports allow parents to take into consideration their children's ages and what is expected developmentally at that age when appraising their levels of inattention; thus, the fact that higher inattention levels were implicated in those with DS according to these reports suggests that we can more-or-less safely regard individuals with DS as having higher inattention levels even when controlling for IQ.

With the heightened presence of EF and ADHD difficulties among those with DS thus substantiated, our next aim was to investigate whether genetic variants of *COMT* and *DRD4* contribute to the variation in EF and ADHD symptoms observed between individuals with DS. Regarding *COMT*, individuals carrying the Val/Val genotype resulting in faster prefrontal dopamine clearance exhibited significantly greater working memory difficulties according to parent report (BRIEF), as well as higher levels of inattention on the CANTAB SRT. These results lend support to the idea that genes not located on chromosome 21 can in fact influence

executive function abilities and ADHD risk in Down syndrome. Interestingly, individuals with the same Val/Val genotype displayed fewer overall errors on the CANTAB IED set-shifting task when compared to those with at least one Met allele. Considering the effect of the Met/Val substitution on the rate of dopamine breakdown in the PFC, this result suggests that for those with Down syndrome, differences in dopamine regulation might produce differing effects specific to the type (and possibly also to the complexity) of executive function examined. For instance, individuals with DS having higher attention levels and better working memory associated with the *COMT* Met allele might also be more likely to perseverate on the shape originally presented as "correct" for each set of trials on the IED even after the rules change, while those with working memory and attention difficulties associated with Val/Val might not form enough of an initial mental representation of the original "correct" shape to perseverate. It is also possible that those with the Met allele are anticipating the rule change (before administering the task we inform the participants that the rule will change at some point, but that it doesn't happen often and occurs only when the computer thinks they have mastered the rule) and treating each trial of a set as independent, while those with Val/Val do not perseverate on this instruction. If this were the case, it would be similar to the strategy differences observed by Williams-Gray et.al. (2008) in Parkinson's disease patients carrying different *COMT* genotypes. In this study, those with Val/Val shifted attention when the rule had changed and applied the rules learned through experience to subsequent trials until the computer indicated that the rule had changed. For those with DS, future studies evaluating the strategies employed on the IED by individuals of differing *COMT* genotype will be necessary to determine how *COMT* interacts in DS to affect specific EF processes.

In analyzing the possible effects of *DRD4* on EF and ADHD symptoms, statistical analyses were somewhat inconclusive given the unequal distribution of participants carrying the R7 risk allele (n=7) vs. those not carrying the R7 allele (n=15). Although no significant differences in EF or ADHD symptoms were found for *DRD4*, the fact that non-significant trends were found in the areas of parent-reported working memory (BRIEF), inattention (Conners) and general ADHD risk (Conners) in spite of the unequal group size suggests that *DRD4* variation might significantly affect EF and ADHD, with the R7 allele corresponding to increased risk of EF and ADHD difficulties. Overall, while a more evenly distributed sample is needed before conclusions can be drawn, the differences we observed between our groups are promising with regard to the notion that non-trisomy 21 genes can influence EF and ADHD symptom outcome for individuals with DS.

The effects shown between variation in *COMT* and differences in certain EF and ADHD symptoms, along with the implications of the trends found for *DRD4* are not only important in terms of discovering these genes' specific effects within DS, but also for deciphering the broader questions regarding trisomy 21's effects on background genes in creating the DS phenotype. Regarding specific effects, it must be noted that while *COMT* has been established as highly important for dopamine degradation in the PFC, it is also responsible for the breakdown of other catecholamines such as norepinephrine(NE) (Axelrod 1957), and the limits of this study do not allow us to truly confirm whether the effects observed are due to variation in the degradation of dopamine or of norepinephrine. In future research, it may be helpful to include neuroimagining of the PFC along with dopamine/norepinephrine tracers to determine relative breakdown rates of each catecholamine in relation to working memory and performance on EF tasks. It might also be beneficial to administer pharmacological dopamine/NE precursors to determine if

performance is altered between genotype in the context of medication, similar to what was done in Williams-Gray et.al. (2008)'s study with PD patients.

Considering the overall question of how trisomy 21 might interact with background genes, three distinct hypotheses currently exist regarding how trisomy 21 works to create the symptoms observed in DS. The gene-dosage effect hypothesis posits that most phenotypic aspects of DS are specific to the genes located on chromosome 21, and that triplication of these particular genes directly causes the symptoms expressed (Antonarakis, Lyle, Dermitzakis, Reymond & Deutsch 2004; Pritchard & Kola 1999). In contrast, the amplified developmental instability hypothesis suggests that non-specific gene triplications cause an overall genetic imbalance affecting the expression of all genes, and that the symptoms observed in DS are the result of global gene expression abnormalities (Pritchard & Kola 1999). An alternative to these theories suggests that while some the genes located on *trisomy 21* are directly involved in creating certain phenotypic effects, the interactions of these genes with genes not located on chromosome 21 and the effects of triplication on the balance of genes in general also contribute to the symptoms expressed (Olson, Richtsmeier, Leszl & Reeves 2004). While the results of the present study imply that genes not located on *trisomy 21* can have some effect on the severity of symptoms found in DS, one limitation in the context of the above question is that the size of these effects are not known. Thus, the next step in teasing apart the competing hypotheses surrounding trisomy 21 is to determine the size of the effect associated with these background genes in DS compared to the gene effect size seen in typically-developing controls. If the effect size is enhanced in individuals with DS, it would suggest a possible interaction between trisomy 21 and other genes, lending support to either the alternative/synthesis or amplified developmental hypotheses (although for the amplified developmental hypothesis, it is important

to remember here that other trisomies observed in humans, e.g. full trisomy 16 have higher fatality rates (Antonarakis et. al. 2004) and thus it isn't likely that the specific genes copied in trisomy 21 are entirely irrelevant in determining phenotype). On the other hand, comparative effect sizes between individuals with DS and controls might lend support more to the dosage-imbalance hypothesis, as the typical amount of variability could produce an effect adding on to the already-present effects caused by genes on trisomy 21. Dampened effect size in DS might also lend support to a negative interaction between trisomy 21 and non-trisomy 21 genes, or could simply indicate that the effects of trisomy 21 are so strong standing alone that background genetic effects are simply "washed out". In any case, future studies analyzing the background genotype of both chronological and MA controls will be immensely helpful in further revealing the possible genetic pathway by which trisomy 21 creates DS phenotype.

Overall, while this study cannot fully resolve the exact mechanisms by which background genes—specifically, *COMT* and *DRD4*—relate to the variation in EF and ADHD difficulties that we observe in DS and to *trisomy 21*, the fact that significant differences in symptoms were found relating to genotype suggests that background genes should be taken into account when examining symptom variability within DS. This promising finding sets a basis for future research that may be geared toward planning possible pharmacological or cognition-based interventions for those with DS, and unraveling the genetic mysteries surrounding *trisomy 21*.

References

Antonarakis, S.E., Lyle, R., Dermitzakis, E.T., Reymond, A., & Deutsch, S. (2004). Chromosome 21 and Down syndrome: from genomics to pathophysiology. *Nature Reviews (Genetics), 5*, 725-738.

Asghari, V., Sanyal, S., Buchwaldt, S., Paterson, A., Jovanovic, V. et.al. (1995). Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *Journal of Neurochemistry, 65*(3), 1157-1165.

CANTAB Simple Reaction Time (2009). Cambridge Cognition. http://www.camcog.com/science/attention.asp

Conners, C.K. (2009). *Conners 3rd edition™: a thorough and focused assessment of ADHD and its most common comorbid problems and disorders in children and adolescents* (Brochure). Available electronically at: http://downloads.mhs.com/conners/Conners3_Brochure_09.pdf

Davidson MC, Amso D, Anderson LC, Diamond A. Development of cognitive control and executive functions from 4 to 13 years: Evidence from manipulations of memory, inhibition, and task switching. *Neuropsychologia* 2006; 44(11):2037- 2078.

De La Vega, F.M., Lazaruk, K.D., Rhodes, M.D., & Wenz, M.H. (2005). Assessment of two flexible and compatible SNP genotyping platforms: Taqman® SNP genotyping assays and the SNPlex™ genotyping system. *Mutation Research 573*, 111-135.

Diamond A, Briand L, Fossella J, Gehlbach L. (2004). Genetic and neurochemical modulation of prefrontal cognitive functions in children. *Am J Psychiatry*. 161(1):125-132.

Down, J. L. H. (1866). Observations on an ethnic classification of idiots. In: London Hospital Reports, 3: 259-262.

Durston, S., Fossella, J.A., Casey, B.J., Hulshoff Pol, H.E., Galvan, A., Schnack, H.G., et.al. (2005). 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. *Molecular Psychiatry, 10*, 678-685.

Edgin, J.O., Mason, G., Allman, M.J., Capone, G., DeLeon, I., Maslen, C.L. et.al. (under review). Development and validation of the Arizona Cognitive Test Battery for Down syndrome. *The Journal of Adolescent Psychology and Psychiatry; Association for Child and Adolescent Mental Health*.

Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., Goldman, D. and Weinberger, D.R. (2001). Effect of COMT val 108/158 met genotype on frontal lobe function and risk for schizophrenia. *Proceedings of the National Academy of Sciences*, 98(12), 6917-6922.

Faraone, S.V., Doyle, A.E.D., Mick, E., & Biederman, J. (2001). Meta-analysis of the association between the 7-repeat allele of the dopamine D4 receptor gene and attention deficit hyperactivity disorder. *American Journal of Psychiatry, 158*, 1052-1057.

Kaufman AS, Kaufman NL. Kaufmann Brief Intelligence Test- Second Edition. Pearson Assessments, 2004.

Langley, K., Fowler, T., Grady D., Moyzis R., Holmans P., van den Bree M., et. al. (2009). Molecular genetic contribution to the developmental course of attention-deficit hyperactivity disorder. *European Journal of Child and Adolescent Psychiatry*, 18, 26-32.

LeJeune, J., Gautier, M., & Turpin, R. (1959). Etudes des chromosomes somatiques de neuf enfants mongoliens. *Comptes Renus de l'Academie les Sciences, 48, 1721-1722*.

Mendel, G. (1865). Experiments in plant hybridization. *Presented at the meetings of February 8th, and March 8th, 1865*. Available online at: http://www.mendelweb.org/Mendel.html

Meyer-Lindenberg, A., Kohn, P.D., Kolachana, B., Kippenhan, S., McInerney-Leo, A., Nussbaum, R. et.al. (2005). Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. *Nature Neuroscience* 8, 594-596.

Nadel, L. (1999). Down syndrome: a genetic disorder in biobehavioral perspective. *Down syndrome: Genes to Brains to Behavior*, 1-35.

Napolitano, A., Cesura, A. M., & Da Prada, M. (1995). The role of monoamine oxidase and catechol-O-methyltransferase in dopaminergic neurotransmission. *Journal of Neural Transmission*, 45: 35-45.

Olson, L.E., Richtsmeier, J.T., Leszl, J., & Reeves, R.H. (2004). A chromosome 21 critical region does not cause specific Down syndrome phenotypes. *Science, 306*, 687-690.

PAR Inc. (2009). *Behavior Rating Inventory of Executive Function (BRIEF)*. http://www4.parinc.com/Products/Product.aspx?ProductID=BRIEF

Pennington, B.F., Moon, J., Edgin, J., Stedron, J., & Nadel, L. (2003). The neuropsychology of Down syndrome: Evidence of hippocampal dysfunction. *Child Development, 74*(1), 75-93.

Plude, D.J., Enns, J.T., & Brodeur, D (1994). The development of selective attention: a life-span overview. *Acta Psychologica 86*, 227-272.

Pritchard, M. & Kola, I (1999). The "gene dosage effect" hypothesis vs. the "amplified developmental instability" hypothesis in Down syndrome. *Journal of Neural Transmission, 57,* 293-303.

Robbins, T.W., Weinberger, D., Taylor, J.G. & Morris, R.G. (1996). Dissociating executive functions of the prefrontal cortex (and discussion). *Philosophical Transactions: Biological Sciences, 351*(1346), 1463-1471.

Rowe, J., Lavender A., & Turk V. (2006). Cognitive executive function in Down's syndrome. *British Journal of Clinical Psychology*, 45, 5-17.

Rzepa, S. R., Conners, C. K., Gallant, S., Pitkanen, J., Sitarenios, G., & Marocco, M. (2007). *Development and Psychometric Properties of the Conners 3 Short Forms.* Poster presented at the annual meeting of the American Psychological Association, San Francisco, CA.

Williams-Gray, C., Hampshire, A., Barker, R.A., & Owen, A.M. (2008). Attentional control in Parkinson's disease is dependent on COMTval158met genotype. *Brain*, 131, 397-408.

Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., & Pennington, B.F. (2005). Validity of the executive function theory of Attention-Deficit/Hyperactivity Disorder: a meta-analytic review. *Biological Psychiatry*, 57, 1336-1346.

Yavich, L, Forsberg, M.M., Karayiorgou, M., Gogos, J.A., & Männistö, P.T. (2007). Site specific role of catechol-o-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum. *The Journal of Neuroscience*, *27*(38), 10196-10202.