CATECHOL-O-METHYLTRANSFERASE (COMT) VAL$^{108/158}$ MET POLYMORPHISM AND ADHD: PHARMACO-BEHAVIOURAL GENETIC AND NEUROCOGNITIVE STUDY.

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Abstract

The *catechol-O-methyltransferase* (*COMT*) gene is the predominant means of dopamine deactivation within the prefrontal cortex (PFC), a brain locus implicated in Attention deficit/hyperactivity disorder (ADHD). Dopamine dysregulation is a significant contributor to the pathophysiology of ADHD and Methylphenidate (MPH), an effective treatment for ADHD, and acts at least in part, through modulation of dopamine levels in the PFC. Thus, we tested the hypothesis that the *Catechol-O-Methyltransferase (COMT) Val*$^{108/158}$ *Met* polymorphism modulates behavioral dimensions relevant for ADHD and/or response of these behavioral dimensions to MPH and/or neuropsychological functions considered relevant for ADHD. No genotype or genotype by treatment interaction effects were observed for behavioral response to MPH. No genotype effects were observed using the family-based approach. Marginal genotype effects were observed between the *Met/Met* genotype and some but not all aspects of executive functioning. Overall, these results do not support the implication of the *COMT Val*$^{108/158}$ *Met* polymorphism in ADHD, ADHD relevant behaviours or response to methylphenidate, but weakly implicate *COMT* gene in some aspects of executive functions in children with ADHD. Given that gene effects on behaviours are likely to be very small, a much large sample would be needed in order to establish these results, both negative and positive, with better confidence.

Résumé:

Le gene *catechol-O-methyltransferase (COMT)* est le moyen le plus important de la désactivation de la dopamine dans le cortex préfrontal (CPF), région du cerveau impliquée dans Trouble Déficit de l'Attention avec Hyperactivité (TDAH). Les anomalies de la régulation de la dopamine est le facteur le plus important contribuant à dans la pathophysiologie de la TDAH. Le Methylphenidate (MPH), un traitement efficace de TDAH, agit ou moins en partie, par l'intermédiaire de la régulation des niveaux de la dopamine dans le CPF. Par conséquent, nous avons émis l'hypothèse que le *COMT* $Val^{108/158}$ *Met* polymorphism module les dimensions comportementales définissant le TDAH et/ou la réponse de ces dimensions au MPH et/ou les performance neuropsychologiques qui ont été impliquées dans le TDAH. Aucun effet principal de génotype ou interaction entre génotype et traitement ont été observés. Une association marginale entre le genotype *Met/Met* et un nombre limité de tests évaluant les fonctions exécutives ont été observée. En conclusion, cette étude n'implique pas le *COMT* $Val^{108/158}$ *Met* polymorphism dans le TDAH, la réponse du TDAH au MPH mais suggère une possible implication de ce polymorphisme dans certains trait cognitifs. Étant donnée que les effets des gènes sur les comportement sont vraisemblablement très petits, une étude avec un échantillons beaucoup plus important serait nécessaire pour établir ces résultats de manière robuste.

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Zia Ulhaq Choudhry performed the data analysis, drafted the manuscript, aided in patient recruitment and data collection. Natalie Grizenko was involved in the conception of the study and provided clinical support. Sarojini M. Sengupta provided clinical support and aided in data collection. Valentin Mbekou aided in neuropsychological testing and data collection. Rosherie Deguzman performed the genotyping for the study and aided in data management. Marina Ter-Stepanian coordinated the clinical aspects of the study and was involved in data management. Anna Polotskaia and Johanne Bellingham performed provided help in patient recruitment, data collection, and neuropsychological testing. Ridha Joober was responsible for the conception of the study, drafting of the manuscript, and supervision of the research project.

Zia Ulhaq Choudhry , Natalie Grizenko, Steven Sanche, Sarojini M. Sengupta, Norbert Schmitz, Valentin Mbekou, Rosherie Deguzman, Anna Polotskaia, Marina Ter-Stepanian, Johanne Bellingham, and Ridha Joober. *Catechol-O-Methyltransferase Val* $^{108/158}$ *Met polymorphism and ADHD: no association with quantitative traits or response to Methylphenidate.*

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"To look at natural phenomena we must first consider both true logic and the approach of Hippocrates. First are the phenomena simple or complex in their origin and in their actions? And, if simple, study their basis and also on what they act, and by what means. And if complex, to enumerate the elements one by one, and to know what each does, and how it causes suffering".

Socrates' contribution to Plato's *Phaedrus.*

CHAPTER I

INTRODUCTION

1 RATIONALE

Attention-deficit/hyperactivity disorder (ADHD) is the most common mental-health disorder in children with an estimated 5 % world-wide prevalence (Polanczyk et al., 2007). Its core symptoms include hyperactivity, impulsivity, and/or inattention, which commonly coexisting with conduct (CD) and/or oppositional disorders (ODD) (Dulcan & Benson, 1997).

ADHD is a familial disorder, with heritability estimates of around 60%-88% (Dulcan et al., 1997; Thapar, 2002). Environmental factors are also believed to play a role in the pathogenesis of ADHD (Bhutta et al., 2002; Langley et al., 2005). The interplay between genetic and environmental factors during development is believed to result in deregulation of neural transmission, particularly in the dopamine (DA) brain systems, which in turn result in behavioural deviances that are collectively grouped under the clinical syndrome of ADHD.

Several studies suggest that dysregulation in the fronto-subcortical DA system is important for the emergence of ADHD symptoms (Aman et al., 1998; Durston, 2003; Giedd et al., 2001). Indeed, executive function, regulated by the prefrontal cortex (PFC), and goal directed motor activity, regulated by interconnections between the PFC and the striatum, are abnormal in children with ADHD. Clinically, the symptoms associated with ADHD are effectively treated by psycho-stimulant drugs such as Methylphenidate (MPH), which acts primarily by blocking the dopamine transporter and thus increases

synaptic DA levels (Biederman & Faraone, 2005). Although it is believed that MPH acts mainly at the striatal level, more recent studies in animals indicate that it may also increase DA in the prefrontal cortex (Berridge et al., 2006).

Numerous associations between ADHD and candidate genes coding for proteins involved in the DA neurotransmission pathway have been reported (Eisenberg et al., 1999; Faraone & Khan, 2006). The *Catechol-O-methyltransferase* (*COMT*) gene codes for the *COMT* enzyme, which is involved in the metabolism of catecholamines, especially dopamine (Weinshilboum et al., 1999). *COMT* is expressed in the frontal lobes where it has an important role in regulating the level of extra-synaptic dopamine levels compared to the dopamine transporter (*DAT*) (Moron et al., 2002; Karoum et al., 1994).

A functional polymorphism of the *COMT* gene exist in humans, involving a substitution of Valine (*Val*) for Methionine (*Met*) at codon 108/158 ($Val^{108/158}$ *Met*) of the soluble and the membrane bound forms of the *COMT* protein respectively (Lachman et al., 1996); it has been the focus of several studies in ADHD and other psychiatric disorders. This substitution results in a 3- to 4-fold increase in *COMT* activity, an effect particularly important for PFC functioning because of its poor concentration in dopamine transporter (Chen et al., 2004; Lachman et al., 1996). Thus, variations in the *COMT* enzyme activity, as dictated by the $Val^{108/158}$ *Met* polymorphism, might modulate synaptic DA levels and in consequence performance in cognitive and behavioral domains mediated by this brain region (executive functioning, attention, and goal oriented behaviour), and/or behavioral response to DA agonists such as MPH in healthy individuals, as well as individuals with reduced/ increased PFC basal dopamine levels. Several studies have implicated this polymorphism in modulating executive functions in normal controls and in patients with

various mental disorders. A recent meta-analysis concluded that there is small but significant relationship between *Val*$^{108/158}$ *Met* genotype and executive function in healthy individuals, the *Met/Met* genotype performing better than the *Val/Val* genotype (d=0.29; 95% confidence interval (CI) 0.02–0.55, P=0.03)]; however this relationship was not observed in patients with schizophrenia (Barnett et al., 2007).

Results from studies exploring association between the *Val*$^{108/158}$ *Met* polymorphism and ADHD have been inconsistent. Some studies have reported an association with the *Val* variant (Eisenberg et al., 1999), while others with the Meth variant (Qian et al., 2003), and further more many have reported no association (Barr et al., 1999; Hawi et al., 2000; Payton et al., 2001; Zhang et al., 2003). The difficulties in identifying a role for the *Val*$^{108/158}$ *Met* polymorphism of the *COMT* gene in ADHD may be explained, at least in part, by the heterogeneity of this disorder. Indeed, it has been postulated that ADHD may be due to relatively independent deregulations of either executive functions or regulation of the emotional valence associated with task performance. The DA meso-cortical and meso-limbic pathways play an important role in executive and emotional function respectively and, due to its differential role in these two brain dopamine pathways, the *COMT* may be more relevant for the former rather than the latter function. To date, all studies investigating the *COMT* in ADHD have focused exclusively on its implication in ADHD as a syndrome, and did not investigate its role in modulating different behavioral dimensions relevant for ADHD or the response of these behavioral dimensions to psychostimulant drugs.

Given the importance of the *COMT* enzyme on modulating synaptic DA levels within the DA meso-cortical pathway and the role of these pathways in mediating

performance in cognitive and behavioral domains, it can be speculated that the *Val*$^{108/158}$ *Met* polymorphism of the *COMT* gene may also modulate behavioral response to DA agonists such as MPH in ADHD individuals.

2 OBJECTIVES

The aims of our research protocol were to determine:

1. Whether or not the *COMT Val*$^{108/158}$*Met* polymorphism of the COMT gene is associated with ADHD as a syndrome, behaviors relevant to ADHD and their response to MPH.

2. Whether or not the *COMT Val*$^{108/158}$*Met* polymorphism of the COMT gene is associated with more refined endophenotypes e.g. executive functioning in ADHD children.

3 HYPOTHESES

1. Given (a) the involvement of the *COMT* enzyme, in the metabolism of DA (Weinshilboum et al., 1999)within the frontal lobes and its importance in regulating the level of extra-synaptic dopamine levels compared to the dopamine transporter (*DAT*) within this region (Karoum et al., 1994; Moron et al., 2002), and (b) the importance of dysregulated fronto-subcortical DA system for the emergence of ADHD symptoms (Aman et al., 1998; Durston, 2003; Giedd et al., 2001), we hypothesized **(A)** that the *COMT Val*$^{108/158}$*Met* polymorphism will be associated with ADHD as a clinical syndrome, and/or with behaviors relevant to ADHD.
2. Given that ADHD symptoms are effectively treated by the use of psycho-stimulant drug MPH, which acts primarily by blocking the dopamine transporter and thus increases synaptic DA levels (Biederman et al., 2005)within the striatum and the

prefrontal cortex (Berridge et al., 2006), we further hypothesized **(B)** that the *COMT* *Val*$^{108/158}$*Met* polymorphism is associated with ADHD behavioral (therapeutic) response to MPH (0.5 mg/kg/day)

3. Given the accepted role of *COMT* in DA metabolism within the PFC, and the paucity of *DAT* within this region (Chen et al., 2004; Lachman et al., 1996), we hypothesized **(C)** that the *COMT Val*$^{108/158}$*Met* polymorphism is associated with variations in performance in cognitive domains mediated by this region i.e. it may be associated with modulation of performance in tasks of executive functioning(Weinberger, 1993). And, as ADHD children exhibit dysfunctional DA neurotransmission (Elia et al., 1994) and deficits in tasks of executive functioning(Sergeant et al., 2002), we hypothesized that the association between the *COMT Val*$^{108/158}$*Met* polymorphism and performance on tasks of executive functioning would be more credible for children with ADHD. Additionally, as previous research exploring relationship between *COMT* activity and PFC mediated cognition suggests that the *Met* homozygotes show superior performance, relative to *Val* homozygotes (Bilder et al., 2004; Egan et al., 2001; Goldberg et al., 2003), we further hypothesized that specifically ADHD children expressing the high enzymatic activity *Val* allele (H), having reduced synaptic DA within PFC(Lachman et al., 1996), will exhibit more deficits in neuropsychological task performance than their low enzymatic activity *Met* allele (L) counterparts.

4 EXPERIMENTAL DESIGN

To test these hypotheses, we constructed an experimental design consisting of two parts:

1. In part A of our study, we wanted to investigate, concomitantly, the association of the *Val*$^{108/158}$ *Met* polymorphism in the *COMT* gene with ADHD as a syndrome, the effect of this polymorphism on behaviors relevant to ADHD and their response to MPH (0.5 mg/kg/day) using a double-blind placebo-controlled crossover trial. ADHD relevant behaviors were assessed by parents and teachers using the Conners' Global Index for parents (CGI-Parents) (Conners CK, 1997b), and the Conners' Global Index for teachers (CGI-Teachers)(Conners CK, 1997a). This rating scale has been recommended for titrating and monitoring treatment with psychostimulant drugs, and consists of two factors: 'Emotional lability' and 'Restless-impulsive behavior' (Conners CK, 1997c; Conners CK, 1999). The assessments were completed during the week preceding the clinical trial (ie, at baseline) while the children were not taking any medication, and similarly, at the end of each week of the clinical trial with MPH.

2. In part B of our study, we wanted to determine whether *COMT Val*$^{108/158}$ *Met* polymorphism mediates neuropsychological task performance in ADHD children. Thus we used three measures of executive function selected according to their ability to tap into various performance domains of executive function. Executive functioning

(EF) was measured with the following tasks: Wisconsin Card Sorting Test (WCST)(Grant & Berg, 1948), a measure of set-shifting, abstraction and concept formation ability, capable of differentiating between ADHD children and normal controls (Kado et al., 2005; Oner & Munir, 2005) and also associated with *COMT* $Val^{108/158}$ *Met* polymorphism in normal adults (Barnett et al., 2007); the Tower of London test (Shallice, 1982; Krikorian et al., 1994), a measure used to assess planning and problem solving aspects of executive functioning which has been shown to differentiate ADHD children from controls (Sergeant et al., 2002; Taerk et al., 2004); and the Self Ordered Pointing Task (SOPT) (Petrides & Milner, 1982), a measure of visual working memory previously shown to detect significant differences in performance between ADHD children and normal controls (Shue & Douglas, 1992). These assessments were completed while the children were not taking any medication, and followed a previous one-week medication 'wash-out' period.

CHAPTER II

LITERATURE REVIEW

1 HISTORICAL EVOLUTION OF ADHD

In the 1854, a German physician *Heinrich Hoffman* wrote about the traits of two boys he called "*Johnny Look-in-the Air*" and "*Fidgety Philip*"; these traits were the features of the clinical syndrome which is now called Attention-Deficit/Hyperactivity Disorder (ADHD) (Greydanus et al., 2007; Lin-Dyken & Wolraich, 1992; Thome & Jacobs, 2004). ADHD was initially described as a childhood disorder, mainly noted in boys exhibiting hyperactivity and unruly behaviour. Additionally, between 1916 and 1927, deficits in attention and conduct disorder-like behavior were noted in children diagnosed mostly, but not always, with *encephalitis lethargica* (Von Economo's Disease). Patho-physiologically these deficits were thought to be a result of *minimum brain damage* or *dysfunction* (Greydanus et al., 2007; Adler & Chua, 2002; Clements, 1996). In the 20th century, ADHD has also been referred to as *hyperkinetic syndrome* and *hyperactive reaction of childhood* (Ounsted, 1955; Dodson, 2005). This disorder was classified by the American Psychiatric Association (APA) in their 1980, 1987, and 1994 *Diagnostic and Statistical Manual of Mental Disorders (DSM)* as ADD and ADHD. However, clinicians in European countries using the criteria based on the *International Classification of Diseases (ICD)*, use the term attention-deficit/hyperkinetic disorder (Greydanus et al., 2007; American Psychiatric Association, 2000; Faraone et al., 2003).

2 DEFINITION OF ADHD

ADHD is a complex, multifaceted neurodevelopmental condition defined as "a persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequent and severe than is typically observed in individuals at a comparable level of development" (American Psychiatric Association, 1994; Stefanatos & Baron, 2007). ADHD is distinguished by the increased and situationally inappropriate motor activity (Halperin et al., 1992), by the decreased inhibitory control over responses (Nigg, 2001), and by the display of an impaired ability to focus, sustain, and switch attention during task (Cepeda et al., 2000)by ADHD subjects(Stefanatos & Baron, 2007).

3 EPIDEMIOLOGY OF ADHD

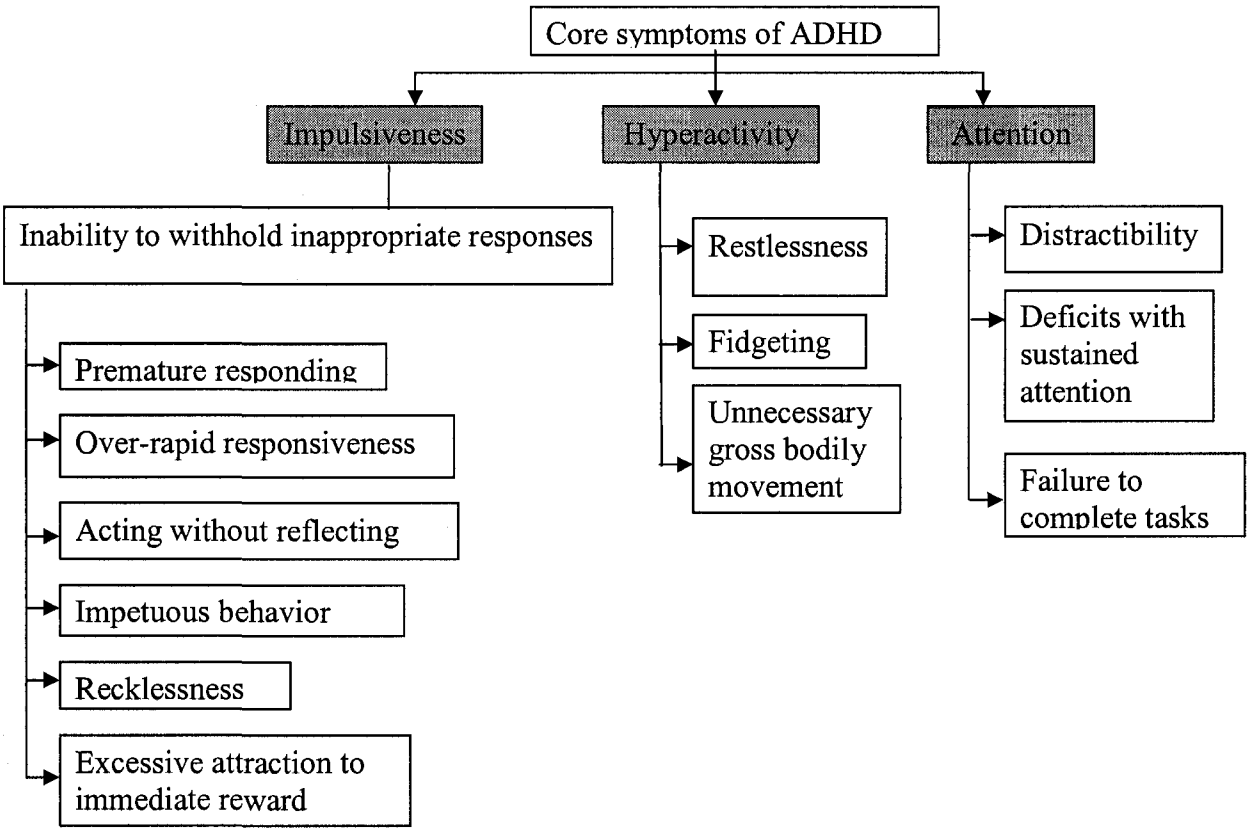
Attention-deficit/hyperactivity disorder (ADHD) is the most common mental-health disorder of children; prevalence rates range from 2% to 9% in North America (Biederman & Faraone, 2005). It is three times more common in males than females; if the diagnosis is based on the APA's *DSM* (Faraone et al., 2003; American Psychiatric Association, 2000; Gingerich et al., 1998). In contrast, European clinicians have reported a much lower prevalence for ADHD, as they evaluate their subjects according to the criteria described in the *ICD-10*. However, studies conducted in different countries using similar diagnostic criteria confirm the widespread prevalence of ADHD (Biederman & Faraone, 2005). ADHD symptoms though begin at an early age, and may continue throughout the lifespan in 50% of the cases, with a prevalence of approximately 3 to 5% is found in adults over age 20 (Kessler et al., 2006; Asherson, 2005; Greydanus et al., 2007). In 2006, 5 million individuals in the US were prescribed psychostimulant medication; out of these 3.5 million were aged between 3 and 19 years, where as the remaining 1.5 million were

between ages 20 and 64 years (Greydanus et al., 2007). ADHD results in academic failure, strained peer and family relations, poor self-image, antisocial behaviour, delinquency, and low occupational performance. (Fergusson et al., 1997; Fischer et al., 1993; Leslie & Wolraich, 2007).

4 CLINICAL FEATURES OF ADHD

ADHD is heterogeneous in its clinical expression, with the core symptoms being poor sustained attention, impulsiveness, and hyperactivity (Sagvolden & Sergeant, 1998) (Figure 1). Clinically, ADHD is six times more common in boys than girls (6:1) (Gingerich et al., 1998). The clinical difference may be due to differences in expressed phenotypes by boys and girls with ADHD; boys exhibit higher levels of overactive/disruptive behaviour, where as girls show higher levels of inattentive symptoms. This might result in referral bias (Stefanatos & Baron, 2007).

Figure 1 Description of the three core symptoms of ADHD (Sagvolden & Sergeant, 1998)



5 DIAGNOSIS OF ADHD

5.1 Pre DSM-IV ADHD diagnoses and assessment

ADHD was initially considered as a unitary disorder, with two core symptoms, attention deficit and hyperactivity. This unitary definition of ADHD was changed by the third version of the Diagnostic and Statistical Manual (American Psychiatric Association, 1980), which elaborated ADHD as a bi-dimensional condition, occurring with or without hyperactive symptoms (Morgan et al., 1996). As per DSM III, there were two groups of patients: ADD without hyperactivity, and ADD with Hyperactivity. This bi-dimensional concept was abandoned in the revised version of the DSM-III, which again suggested a uni-dimensional classification of the disorder (American Psychiatric Association, 1987).

5.2 DSM-IV criteria for ADHD diagnoses and assessment

Clinically, "attention-deficit/hyperactivity disorder" subjects as per DSM-IV (American Psychiatric Association, 1994) are differentiated into three distinct defined types: the *predominantly inattentive type (PIA);* the *predominantly hyperactive/impulsive type (PHI);* and *combined type (C).* The clinical diagnosis for ADHD as per DSM IV diagnostic criteria is made, when the child in question demonstrates six or more symptoms from either of the two nine-item lists set forth in the DSM-IV-Text Revision manual (American Psychiatric Association, 2000) and adapted here in table 1. These symptom lists were compiled by taking into consideration previous experimental data, field trials and the consensus of leading experts in the field (Lahey et al., 1994) and comprise of items chosen to index problems related to "inattention," and "hyperactivity-impulsivity."

To be diagnosed as a particular subtype of ADHD (*PIA, PHI, or C*), at least six symptoms on either the inattention *(PIA)* and/or the hyperactivity-impulsivity *(PHI)* list or on both *(C)* the lists should be exhibited by the patient. Additionally, to reach a formal clinical diagnosis of ADHD it is also required that: the onset of symptoms should be prior to the age of seven years; these symptoms should exist for a minimum of 6 months; they should be pervasive, that is, observed in more than one setting i.e. academic (school) and home; and they are out of keeping with developmental level, maladaptive, and interfere with academic, social, or occupational functioning. A child can not be diagnosed as ADHD if the symptom occur exclusively in the course of other psychiatric disorders such as: pervasive developmental disorder (PDD); schizophrenia (SC); or a psychotic disorder (PD); or these symptoms could be better accounted for another specific mental disorder, such mood disorder (MD), anxiety disorder (AD), dissociative disorder (DD), or personality disorder (PD).

Table 1 DSM-IV-TR criteria for ADHD (American Psychiatric Association,2000; Stefanatos & Baron, 2007)

A. Either (1) or (2)

(1) Six (or more) of the following symptoms of inattention have persisted for at least six months to a degree that is maladaptive and inconsistent with developmental level:

Inattention

—Often fails to give close attention to details or makes careless mistakes in school work, work, or other activities.

—Often has difficulty sustaining attention in tasks or play activities.

—Often does not seem to listen when spoken to directly.

—Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not to do to oppositional behavior or failure to understand instructions).

—Often has difficulty organizing tasks and activities.

—Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework).

—Often loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books, or tools).

—Is often easily distracted by extraneous stimuli.

—Is often forgetful in daily activities.

—Symptom total

(2) Six (or more) of the following symptoms of hyperactivity-impulsivity have persisted for at least six months to a degree that is maladaptive and inconsistent with developmental level:

Hyperactivity

—Often fidgets with hands or feet or squirms in seat.

—Often leaves seat in classroom or in other situations in which remaining seated is expected.

—Often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, they are limited to subjective feelings of restlessness).

—Often has difficulty playing or engaging in leisure activities quietly.

—Is often "on the go" or often acts as if "driven by a motor."

—Often talks excessively.

Impulsivity

—Often blurts out answers before questions have been completed.

—Often has difficulty awaiting turn.

—Often interrupts or intrudes on others (e.g., butts into conversations or games).

— Symptom total

Some hyperactive-impulsive or inattentive symptoms that caused impairment were present before age seven years.

Some impairment from both symptoms is present in two or more settings (e.g., at school or work, and at home).

Clear evidence of clinically significant impairment in social, academic, or occupational functioning.

Note. Adapted from (American Psychiatric Association,2000; Stefanatos & Baron, 2007)

The DSM-IV classification of ADHD compared to the DSM-IIIR classification has shown to be superior, in terms of diagnostic sensitivity; DSM-IV classification has helped in identifying more ADHD cases (McBurnett et al., 1999). Studies using DSM-IV classification have shown: (i) ADHD-Inattentive type to be more common in the community sample compared to the clinical population; these include children which are either females, or belong to an older age group (Baeyens et al., 2006; Woo & Rey, 2005); (ii) ADHD-Hyperactive subtype is associated with younger age and is found to be relatively rare (Woo & Rey, 2005); and (iii) ADHD-Combined subtype is shown to be more prevalent (55%) in the clinical population compared to the other two subtypes (Baeyens et al., 2006). Some authors have debated the reliability and validity of the ADHD subtypes diagnosed as per DSM-IV (Woo & Rey, 2005; Hinshaw, 2001). This literature suggests that though DSM-IV ADHD subtype diagnosis is reliable, it might be affected by informant bias (Woo & Rey, 2005)i.e. considering a single informant most individuals appeared to have hyperactive only *(PIH)* or inattentive only *(PIA)* diagnosis, but when integrated reports from two informants were considered most children often met the combined subtype criterion. It is further suggested that the differences amongst informants may be due to cross-situational differences, and thus diagnosis based on considering only one informant may not be so reliable. Furthermore, studies focusing on ADHD subtype consistency across time have shown varied results i.e. children initially diagnosed as ADHD hyperactive subtype shifted to combined subtype over time (Lahey et al., 2005). This observation could help in understanding the low rates of ADHD-Hyperactive subtype in the clinical population observed as per DSM-IV. There has also been questioning as to the distinction of ADHD combined type and ADHD predominantly inattentive type as subtypes of ADHD (Milich et al., 2001). Milich et al.,

(2001) following an exhaustive review of related publications concluded that ADHD-Combined types suffer mostly from distractibility, whereas ADHD-Inattentive type exhibit slow cognition. Thus these two subtypes may in fact be distinct disorders. This observation of distinct set of attention problems associated with the ADHD-Combined and Inattentive types has been proposed much earlier (Barkley, 1997) and has been supported more recently(Diamond, 2005).

5.3 Other Instruments for ADHD diagnoses and assessment

In some clinical settings the diagnosis of ADHD requires additional use of a number of other well-validated instruments (Table 2) in conjunction with DSM-IV criteria for ADHD (Greydanus et al., 2007). These Rating scales can be assessed to measure individual assessments of attention, disruptive and oppositional behaviors.

Table 2 Instruments for ADHD diagnoses and assessment (Greydanus et al., 2007)

Child Behavior Check List
Conners Parent and Teacher Rating Scales
ADHD Rating Scale-IV (SNAP-IV)
Wender-Utah Rating Scale (WURS)
Conners Adult ADHD Rating Scales (CAARS)
Brown Attention Deficit Disorder Scale
Conners/Wells Adolescent Self-Report of Symptoms (CASS)
Others

Note. Adapted here from (Greydanus et al., 2007)

5.4 ADHD and differential and/or comorbid diagnoses

Accumulating evidence suggests that a number of conditions may be differentiated from or comorbid with ADHD. These include (Table 3 a, b, c, d): (i) other mental health (MH) disorders; (ii) Cognitive dysfunction and learning disabilities (LD) (Grizenko et al., 2006); (iii) other medical disorders; and (iv) Chaotic environment related issues (Leslie & Wolraich, 2007; Biederman et al., 1991). These coexisting conditions complicate assessment, diagnosis, and the determination of the most needed intervention (Greydanus et al., 2007).

Table 3a Mental health conditions that may be differentiated from or comorbid with ADHD (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006)

Mental Health Disorders
Anxiety disorders (generalized anxiety disorder, separation anxiety)
Affective (mood) disorders
Substance abuse disorders (stimulants, cocaine, phencyclidine, others)
Conduct disorder
Oppositional defiant disorder
Impulse-control disorders
Mental retardation
Autism spectrum disorder (including Asperger's Disorder)
Tic disorders
Schizophrenia and other psychotic disorders
Personality disorders (as antisocial personality disorder)
Developmental coordination disorder
Adjustment disorders

Note. Adapted here from (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006).

Table 3b Cognitive dysfunction and learning disabilities that may be differentiated from or comorbid with ADHD (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006)

Cognitive dysfunction and learning disabilities
Disorders of mathematics
Disorders of Reading
Disorders of written expression

Note. Adapted here from (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006).

Table 3c Medical disorders that may be differentiated from or comorbid with ADHD (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006)

Medical Disorders
Hyperthyroidism
Early stages of progressive neurodegenerative disorders
Subclinical epilepsy
Frontal lobe tumor or abscess
Fetal alcohol syndrome
Klinefelter syndrome
Angelman syndrome
Williams syndrome
Velocardiofacial syndrome
Sotos syndrome

Note. Adapted here from (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006).

Table 3d Conditions that may be differentiated from or comorbid with ADHD (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006)

Environment Related Issues (symptoms predominantly related to chaotic environment)
Child and adolescent abuse and neglect
Severely dysfunctional family dynamics
Highly gifted student placed in unchallenging regular curriculum
Cognitively challenged student placed in a regular curriculum/classroom

Note. Adapted here from (Greydanus et al., 2007; Culpepper, 2006; Spencer, 2006; Grizenko et al., 2006)

6 NEURO-PSYCHOLOGICAL THEORIES OF ADHD

ADHD children exhibit certain distinct deficiencies in: (i) attention to detail; (ii) consecutive maintenance of attention over a length of time; and (iii) high variability in performance during assigned task. Most of the theories regarding ADHD have tried to explore and identify the mechanism pertinent to the understanding of these impairments. Douglas et al. (1970), introduced the problems associated with sustained attention and impulse control observed in subjects with ADHD (Douglas, 1972). Following suit, many theories focusing on cognitive and behavioural aspects of ADHD have emerged, these include: (i) executive dysfunction (Pennington & Ozonoff, 1996); (ii) behavioural inhibition deficit (Barkley, 1997); (iii) deregulated arousal/activation (Sergeant, 2000); and (iv) delay aversion (Sonuga-Barke, 2002).

6.1 Executive Dysfunction Model

Pennington and Ozonoff (1996) proposed the executive dysfunction deficit theory of ADHD(Pennington & Ozonoff, 1996). Executive functions (EFs) are neurocognitive processes that maintain an appropriate problem solving set to attain a future goal (Welsh & Pennington, 1988). More specifically, this model concludes that ADHD children may exhibit: (i) EF deficit; (ii) motor inhibition; and (iii) other general cognitive deficits. Though this core EF deficit model has been widely accepted (Barkley, 1997), it has not been able to explain comorbidity between ADHD and developmental disorders, e.g. dyslexia. Recently to better explain (i) the complexity of ADHD, (ii) its risk and protective factors, and (iii) comorbidities associated with ADHD, the multiple deficit model has been proposed instead of the previous single core model (Pennington, 2006).

6.2 Behavioural Inhibition Model

Barkley (1997) suggested a unifying theory of ADHD, in which behavioural inhibition deficit, i.e. dysfunctional suppression of behaviour (Molnar, 2007; Nigg, 2006), is the central deficiency in ADHD. According to this theory deficiencies in behavioural inhibition are likely to affect abilities to: (i) suppress irrelevant responses; (ii) resist external interference; and (ii) perform complex sequences of responses i.e. executive dysfunction. Furthermore, behavioural inhibition deficiencies are believed to affect abilities such as: (i) working memory; (ii) self-regulation of affect, motivation, and arousal; (iii) internalization of speech, reconstitution and (iv) motor control, fluency, and syntax. And behavioural inhibition dysfunction is believed to directly influence the motor

system leading to executive dysfunction, which in turn again affects the motor control (Barkley, 1997). The behavioural inhibition deficit model holds true for the ADHD combined subtype (Oosterlaan et al., 2005).

6.3 Cognitive-energetic Model

Sergeant (2000) suggested this model; it focuses on the energy state of the children, and links motor behaviour (energy state) to EF (Livesey et al., 2006). In this model it is specifically argued that the deficiency observed in inhibition is dependent on the energy state and the effectiveness of information processing across three levels: (i) process: a computational mechanism that consists of encoding, searching, decision making and motor organization; (ii) state: made up of the energetic pools such as effort, arousal and activation; and (iii) management/evaluation: linked with planning, monitoring detection and correction of error.

6.4 Dual-pathway Model

This model is proposed by Sonuga-Barke (2002) (Sonuga-Barke, 2002)and debates that the unifying model of ADHD proposed by Barkley fails to explain the heterogeneous nature of ADHD. In this model, Sonuga-Barke assumes that ADHD children express a different motivational style i.e. they are motivated to avert delay (Antrop et al., 2006). In this model, two distinct pathways involved in the executive and reward circuits are implicated in ADHD (Sonuga-Barke, 2002). Dysregulation of thought and action pathway (executive circuits) results in inhibitory dysfunction, which in turn deregulates cognition and behaviour affecting task regulation. Cognitive dysregulation

directly mediates task engagement; whereas behavioural dysregulation mediates its effects through the manifestation of ADHD symptoms. Dysregulation of the motivational style pathway (reward circuits) exhibits as "delay aversion", which mediates the behavioural representation of ADHD symptoms and thus affects the task engagement.

7 AETIOLOGY OF ADHD

ADHD is a complex neurobehavioral disorder; its aetiology centers around the nature (genetics)/ nurture (environment) debate (Williams et al., 1999). Accumulating evidence suggests that multiple risk factors interact to increase liability to ADHD (Doyle et al., 2005b); both environmental and genetic factors are implicated in the disorder (Taylor, 1998; Faraone et al., 1995; Faraone & Doyle, 2001; Smalley et al., 2001; Sonuga-Barke, 1998; Wolraich et al., 1996). Genetic studies have shown ADHD to be a familial disorder with heritability estimates of around 76% (Biederman & Faraone, 2005). Twin (Nadder et al., 1998; Eaves et al., 1997; Sherman et al., 1997a; Sherman et al., 1997b; Levy et al., 1997; Stevenson, 1992; Goodman & Stevenson, 1989), family (Hechtman, 1996; Samuel et al., 1999; Faraone et al., 1994), and adoption studies (Cadoret & Stewart, 1991)suggest a significant role of genetic factors in predisposing and perpetuating the development of ADHD. Genetic association studies have reported some evidence for association between ADHD and genes coding for proteins involved in the DA neurotransmission pathway (*SLC6A3, DRD4, DRD5, and COMT*) (LaHoste et al., 1996; Swanson et al., 1998; Cook, Jr. et al., 1995; Daly et al., 1999; Eisenberg et al., 1999). Yet it has been difficult to implicate a specific gene beyond reasonable doubt from linkage(Ogdie et al., 2003; Smalley et al., 2002; Bakker et al., 2003)and association studies (Faraone et al., 2005), and it has been suggested that ADHD is caused by multiple genes each having a small

effect. Additionally, many studies have suggested that environmental risk factors such as pregnancy, labor/delivery, and neonatal complications, marital distress, family dysfunction, and drug abuse during pregnancy have all been implicated in the pathogenesis of ADHD (Wolraich et al., 1996; Ben Amor et al., 2005; Knopik et al., 2006; Astbury et al., 1987; Milberger et al., 1997). However it has been observed that not all infants who are exposed to these environmental risk factors develop ADHD. Furthermore, it is increasingly being recognized that gene-environment (GXE) interactions, where the genotype of the individual modulates the sensitivity or response to the environmental risk factor, may play a pivotal role in the disorder (Swanson et al., 2007).

8 PATHOPHYSIOLOGY OF ADHD

8.1 Neurochemical hypothesis

The most compelling *Neurochemical hypothesis (Biological factors)* of ADHD postulates that ADHD is a dysfunction of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) pathways (Faraone et al., 1995; Aman et al., 1998; Durston, 2003; Sagvolden & Sergeant, 1998). DA and NE neurons originate in the mesencephalon; the DA (substantia nigra, and anterior tegmentum) and NE (locus ceruleus) nuclei project diffusely to the entire brain. Projections that go to the PFC and the anterior cortex (AC) are highly involved in ADHD. Neuroimaging and neuropsychological studies have shown that the PFC and its connections to the striatum and cerebellum are implicated in ADHD (Arnsten, 2006a). Initial theory involving the DA and NE pathways in ADHD was based on the observed effects of pharmacological agents in ameliorating symptoms of ADHD(Arnold et al., 1997), and by animal models of ADHD (animals showing lesions in

DA pathways) in rats (Shaywitz et al., 1978; Sagvolden, 2000) and monkeys (Schneider et al., 1994). DA is believed to be important in the neuro-modulation of various key functions such as locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation. Similarly, NE has been shown to be essential for modulation of cognitive processes, including attention, alertness and vigilance, as well as autonomic function (Jasmin et al., 2002; Hahn et al., 2003; Svensson, 1987). MPH is a psychostimulant widely used for the treatment of ADHD. It is a potent DA and NE agonist, which acts by blocking the DA and NE transporters (DAT & NET) (Krause et al., 2000), and thus in turn results in increasing the synaptic DA and NE concentration (Volkow et al., 2001; Madras et al., 2005). Similarly, animal studies show that MPH and atomoxetine both increase the rate of DA and NE release in the PFC (Bymaster et al., 2002).

8.2 Neuropsychological hypothesis and neurobiology of ADHD

Since ADHD individuals commonly, but not always exhibit deficits of executive functioning, executive dysfunction is a major driving force behind the *Neuropsychological theory* of ADHD (Doyle et al., 2005a; Sonuga-Barke et al., 2002). Executive Functions are cognitive processes that help in selecting an optimal action by integrating stored information from working memory with current information about the present context (Willcutt et al., 2005) (figure 2). The frontal-subcortical circuits, which are implicated in ADHD (Castellanos, 1997), are believed to control EFs' such as: (i) *Inhibition,* a function enabling suppression of response to stimuli (Arnsten, 2006a)mediated by right prefrontal cortex and basal ganglia (Casey et al., 1997); (ii)

Working Memory, a function holding and manoeuvring information enabling task completion(Leffard et al., 2006)controlled by dorso-lateral prefrontal cortex (Dowker, 2006); (iii) *Set-Shifting,* a function monitoring and adjusting action as per need (Crone et al., 2004) modulated by the dorsolateral prefrontal cortex (Ortuno et al., 2006); (iv) *Interference Control;* (v) *Planning,* a function guiding responses to achieve goal (Asato et al., 2006)facilitated by prefrontal cortex and the fronto-parietal-occipital network (Boghi et al., 2006)); and (vi) *Sustained Attention* (Biederman & Faraone, 2005). To date, all ADHD neuropsychological studies have debated about a core neuropsychological deficit (see Neuro-psychological theories of ADHD section for description), which might be the basis of ADHD symptoms and associated neuropsychological impairments. As no individual neuropsychological theory can fully explain all the features associated with ADHD, the neuropsychological impairments observed in ADHD individuals are heterogeneous (Sonuga-Barke, 2005; Nigg et al., 2004).

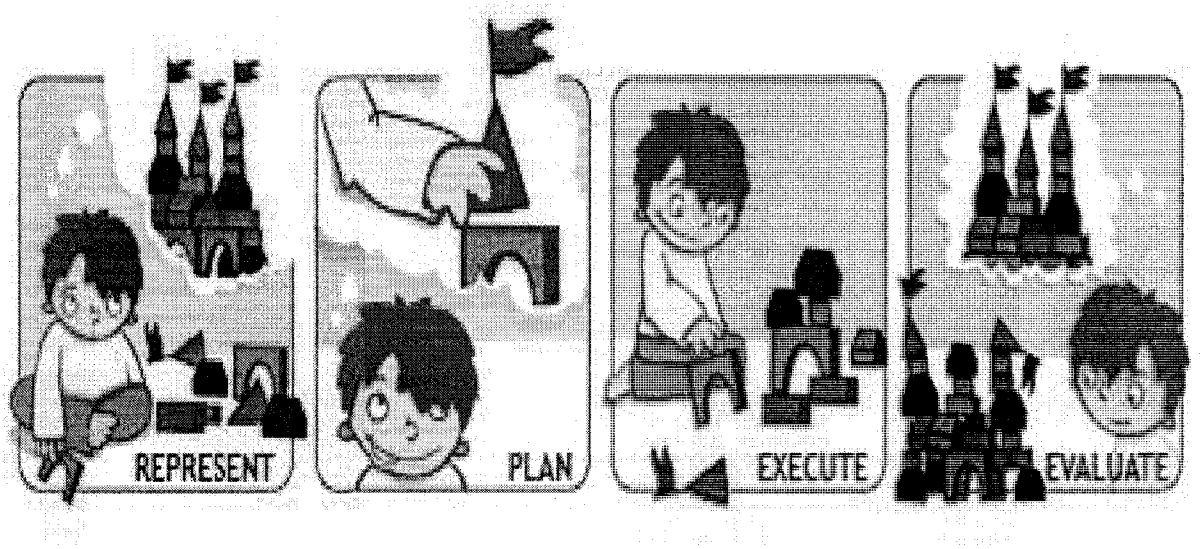
A body of literature suggests that neuropsychological deficit within ADHD subjects may be due to deregulated frontal-subcortical circuits (Castellanos, 1997). Neuro-imaging studies support this theory (Rubia et al., 1999; Castellanos, 1997; Faraone, 2004; Casey et al., 1997; Dowker, 2006; Ortuno et al., 2006) (Boghi et al., 2006), but additionally also implicate other brain structures with ADHD executive dysfunction. Structural brain imaging studies have shown *anatomical abnormalities* (Hynd et al., 1991; Hynd et al., 1993), and an overall reduction in size for different brain regions in ADHD individuals (Castellanos et al., 2002a; Shaw et al., 2006). Swanson, et al. (2007) analyzing structural imaging data *(ADHD vs. controls)* noted that: (i) ADHD group compared to control group had smaller caudate nucleus and globus pallidus (two regions with high density of DA

receptors); (ii) ADHD groups had smaller anterior brain regions but larger posterior regions; and (iii) sub-regions such as the rostrum and splenium of the corpus callosum and the cerebellum vermis lobules VIII-X are smaller in ADHD groups (Swanson et al., 2007). Similarly, Valera et al. 2007 in a meta-analysis quantitatively estimated the reductions in size ($d = 0.408$) and implicated regional reductions in caudate (right), cerebellum (vermis), and corpus callosum (splenium) (Valera et al., 2007)in ADHD individuals. These findings emphasize the biological nature of ADHD.

Functional neuroimaging studies assessing the degree of brain activation and neural systems associated with neuropsychological tasks in ADHD individuals initially showed decreased activation of the DA pathway (the cortical-striatal-thalamic brain circuit) in ADHD (Rubia et al., 1999; Durston et al., 2003). However recent data shows that the patterns of hypo-activation of ventral prefrontal and inferior parietal regions related to attentional networks appear in both ADHD subjects and their unaffected siblings(Durston et al., 2006). Studies exploring acute effects of stimulant medication in the treatment of ADHD cases showed that MPH, a DA-agonist exerting its effects by binding to dopamine transporters, mostly within the striatum partially corrected the pattern of frontal hypo-activation (Volkow et al., 1998; Volkow & Swanson, 2003; Lee et al., 2005). Studies in medication naïve ADHD children with neuropsychological tasks showed hypo-activation of frontal lobe (motor inhibition) and prefrontal, temporal, and parietal regions (task switching)(Smith et al., 2006; Pliszka et al., 2006; Tamm et al., 2006; Casey & Durston, 2006). Additionally, earlier Single Photon Emission Computed Tomography (SPECT) studies showed reduction in blood flow to the frontal lobes and basal ganglia, but increased blood flow to occipital lobes (Lou et al., 1990) in children with ADHD

compared to controls. Meanwhile, Positron Emission Tomography (PET) showed lower glucose metabolism in frontal lobes of the adult ADHD group when performing an auditory attention task compared to control group (Zametkin et al., 1990).

Figure 2 What is Executive Function (EF)?



EFs are top-down cognitive processes that facilitate the performance of a task. As depicted in the cartoon below (*http://www.aboutkidshealth.ca/ofhc/news/SREF/4144.asp*), these cognitive processes:

- Maintain and update information about possible choices in working memory;
- Integrate this knowledge into the current context so as to plan and organize the optimal action (strategic planning and organization);
- Facilitate self-initiation; regulate impulse and interference control, and vigilance in order to execute the task.

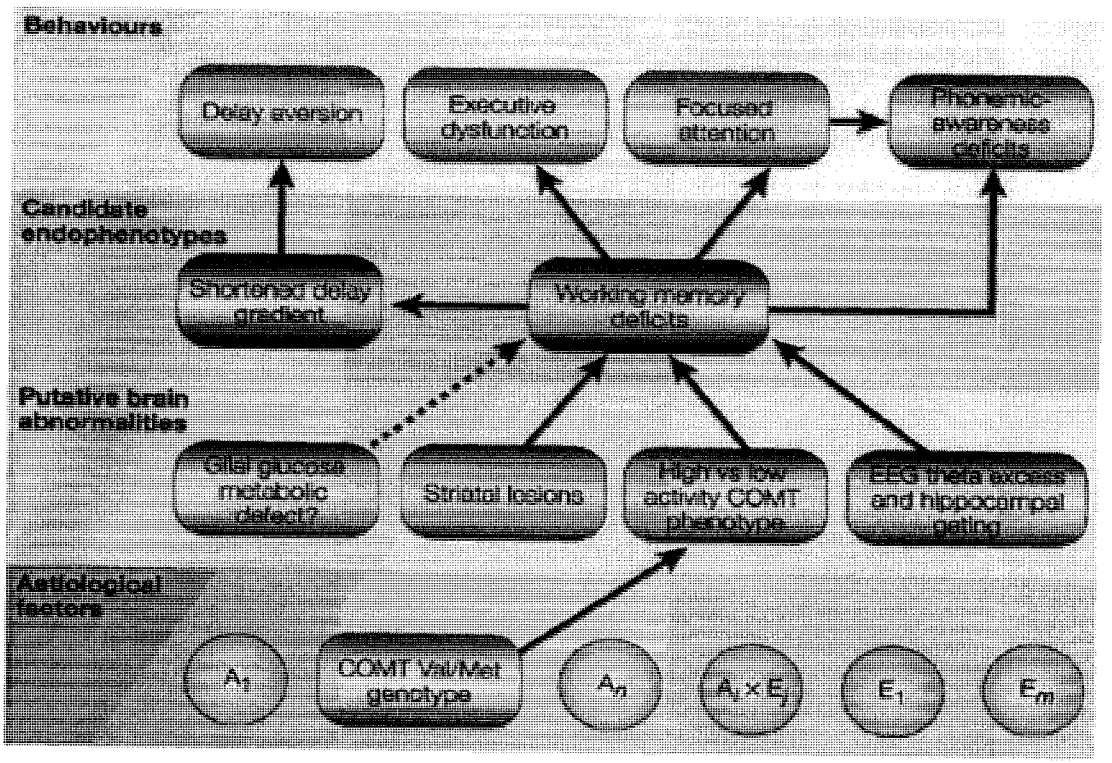
9 QUANTITATIVE PHENOTYPES RELEVANT TO ADHD

ADHD is a heterogeneous psychiatric disorder which fails to follow Mendelian patterns of inheritance. Thus ADHD, as diagnosed by the DSM-IV criteria, may limit success of studies investigating the genetic determinants of the disorder (Szatmari et al., 2007). Genetic epidemiological studies strongly support the relevance of decomposing ADHD into several behavioural dimensions (pertinent phenotypes) since each is likely to have its own genetic determinants (Hudziak et al., 1998; Martin et al., 2002; Sherman et al., 1997). This approach might have potential in unravelling the genetics of complex neuro-psychiatric conditions (Castellanos & Tannock, 2002b; Gottesman & Gould, 2003; Carlson et al., 2004; Joober et al., 2002). As eloquently formulated by Gottesman and Gould (2003), "It stands to reason that more optimally reduced measures of neuro-psychiatric functioning should be more useful than behavioural "macros" in studies pursuing the biological and genetic components of psychiatric disorders"(Gottesman & Gould, 2003).

Endophenotypes in psychiatry retain the notion of an internal process, but one that can be objectively measured, ideally in a robust and reliable fashion. This is a characteristic often lacking in the diseases with which they are associated (Flint & Munafo, 2007), elegantly defined by Gottesman (2003): "An endophenotype should be heritable, co-segregate with a psychiatric illness, yet be present even when the disease is not (i.e. state independent), and be found in non-affected family members at a higher rate than in the population (Gottesman & Gould, 2003). Castellanos and Tannock (2002), in a selective review, concluded that such endophenotypes should be solidly grounded in the

neurosciences, and further proposed three endophenotypes: (i) a specific abnormality in reward-related circuitry that leads to shortened delay gradients; (ii) deficits in temporal processing that result in high intrasubject intertrial variability; (iii) and deficits in working memory, as most compelling quantitative traits worth considering during investigations aiming to uncover the causes of ADHD (Castellanos & Tannock, 2002b)(figure 3) .

Figure 3 Working memory deficits as a candidate endophenotype (Castellanos & Tannock, 2002b)



Adapted from, Castellanos and Tannock (2002) (Castellanos & Tannock, 2002b); "Such deficits might arise as a result of brain abnormalities, including striatal lesions and alterations in catechol-O-methyl transferase (COMT) activity. Attention-deficit/hyperactivity disorder (ADHD)-associated behaviours that are influenced by working memory might include attentional processes and learning disorders. Broken arrows indicate untested proposed causal links; A_1, dopamine transporter (DAT1) polymorphism; A_{2-n}, additive genetic factors; A_3, catechol-O-methyl transferase (COMT) Val/Met polymorphism; $A_i \times E_j$, gene–environment interactions; EEG, electroencephalogram; E_{1-m}, environmental factors."

10 TREATMENTS OF ADHD AND METHYLPHENIDATE

The research literature exploring treatment outcomes of ADHD suggests that the primary symptoms of ADHD are most successfully treated by medication, and the secondary symptoms by behavioural approaches(Pelham, 1993; Williams et al., 1999). Gittelman and colleagues (1976) showed that hyperactive school children receiving Methylphenidate (MPH) had significantly better behaviour modification compared to children receiving placebo (Gittelman-Klein et al., 1976). Data from the MTA study (N=579, multi-center) concluded that use of MPH was by itself more efficacious than the other approaches for hyperactivity, impulsivity, inattention, and even aggression(National Institute of Mental Health, 2001; Jensen et al., 2001), whereas the combined approach was important for children with co-morbid anxiety disorders and disruptive disorders(Wells et al., 2000a; Wells et al., 2000b). Faraone (2003), reviewing the effect size of the most common medication treatments of ADHD, reported that the stimulants had an effect size greater than 0.9 (Faraone & Wilens, 2007).

MPH is considered the first line treatment of ADHD (Culpepper, 2006). It is a potent DA and NE agonist, which is believed to enhance neurotransmission of DA and NE (Spencer et al., 2004). MPH selectively binds to the presynaptic DAT in central nervous system areas (predominantly prefrontal and striatal), leading to DA increase in the synapse and enhanced DA neurotransmission; it also blocks the NET (Krause et al., 2000; Greydanus et al., 2007), resulting in increased synaptic NE concentration(Volkow et al., 2001; Madras et al., 2005). Approximately 75% of ADHD individuals note some benefit using psycho-stimulant medication (MPH, n = 133 trials) compared to placebo (Pliszka, 2007;

Greydanus et al., 2007). Data exploring the effect of short-term use of MPH and other Phenylethylamine stimulant medications in children shows a marked decrease in errors of commission and omission, improvement in vigilance tasks, improvement in search tasks, improvements in arithmetic and spelling tasks, and a marked increase in the ratio of children handing in correctly done work. But similar results have not been observed in long term treatment (Weiss et al., 1975; Barbaresi et al., 2006; Gittleman-Klein & Klein, 1976; Rie et al., 1976a; DuPaul & Rapport, 1993; Pelham et al., 1990; Cunningham & Barkley, 1978; Rie et al., 1976b; Williams et al., 1999). Additionally, MPH use in children also results in reduction in disruptive behaviours(Pelham et al., 1990), diminished aggression(Murphy et al., 1992), and improved family and peer relations(Barkley & Cunningham, 1979; Barkley, 1988; Whalen et al., 1989).

DA is pathophysiologically important for ADHD (see Pathophysiology of ADHD section for description). Solanto (2002) suggested that cognitive impairments associated to ADHD may result from hypodopaminergic state of PFC, while hyperactivity results from hyperdopaminergic state in striatum, possibly secondary to hypodopaminergic PFC (Solanto, 2002), thus the normal functioning of the PFC is dependent on DA (Goldman-Rakic et al., 2000). And as MPH increases prefrontal DA levels, it improves dopaminergic neurotransmission, resulting in improvement in both cognitive and behavioral symptoms of ADHD (Durston, 2003). Studies have reported that ADHD children receiving psychostimulant medication had improved executive control compared to ADHD children not receiving medication. (Barnett et al., 2001; Konrad et al., 2004). Also MPH in pediatric ADHD has shown improvement in response inhibition (Tannock etr al., 1989). MPH administration has been shown to enhance cerebral blood flow to the

dorsolateral prefrontal cortex (DLPFC), a region considered important in executive control (Mehta et al., 2000). More recently, Grizenko et al (2006) showed that ADHD children without learning disabilities responded adequately to MPH (75%) (Grizenko et al., 2006). Oral therapeutic doses of MPH block more than 50% of the DAT in the striatum (Volkow et al., 1998). This finding suggests that blockage of DAT in the striatum is needed for the efficacy of MPH (Durston, 2003). This idea is also supported by the fact that DAT sites are reduced after treatment with MPH (Krause et al., 2000). Additionally, extracellular DA levels in the striatum are significantly increased following an acute oral dose of MPH (Volkow et al., 2001). Hence, it can be concluded that increased DA in the striatum following MPH dose may be an amplification of spontaneously released DA, secondary to environmental stimulation (Durston, 2003). Thus DA increase may increase the signal-to-noise ratio of striatal cells, and decrease background dopaminergic firing, enhancing task-related neuronal cell firing (Volkow et al., 2001; Durston, 2003).

Studies exploring role of DA in modulation of PFC function have shown that DA modulates PFC functions through its action at the D1 (D1, D5) and D2 (D2, D3, D4) families of receptors (R) (Arnsten, 2006b). The D2 R family is still ambigous, but a great deal is know about D1 R family. Drugs stimulating D1 R family produces an inverted 'U'-shaped dose–response on the working memory and attention regulation processes of the PFC (Zahrt et al., 1997; Granon et al., 2000). According to this hypothesis, optimum PFC performance is observed for medium DA synaptic levels. Lower and higher levels of DA will result in a deterioration of PFC functions (working memory and attention regulation) compared to this optimal concentration. The D1/D5 R actions are mediated via

cAMP/protein kinase intracellular signaling mechanisms (Arnsten et al., 2005). Moderate D1/D5 R stimulation enhances spatial tuning by the suppression of delay-related firing for nonpreferred spatial directions (Williams & Goldman-Rakic, 1995; Arnsten et al., 2005), whereas high D1/D5 R stimulation erodes spatial tuning, as it suppresses delay-related firing for all directions (Arnsten et al., 2005). Studies using nonselective agonists confirm the inverted 'U'-shaped dose–response hypotheses in both humans and animals(Kimberg et al., 1997); these also suggest that D1 agonists compared to D2 agonists may be more helpful in improving working memory (Muller et al., 1998). Taken together, these data suggest that genes (e.g. DAT, COMT) and/or drugs (MPH) influencing synaptic DA levels may modulate PFC mediated regulation of behavior.

11 CANDIDATE GENES IN THE DOPAMINE PATHWAY POTENTIAL FOR ADHD

ADHD is often considered an inherited disorder; genes coding for molecules in the DA pathway have been associated with ADHD (Faraone et al., 2005). Family (Hechtman, 1996), twin (Nadder et al., 1998), and adoption (Cadoret & Stewart, 1991) studies strongly suggest a genetic component of ADHD. The multifactorial polygenic model of transmission fits the genetic epidemiological data and predicts a high heritability of ADHD (75 - 90%). Association studies have been numerous and report relatively consistent evidence for association between ADHD and candidate genes coding for proteins involved in the DA neurotransmission pathway, the *SLC6A3* gene (Cook, Jr. et al., 1995), the *DRD4* gene (LaHoste et al., 1996), the *DRD5* gene (Tahir et al., 2000), and

catechol-O-methyltransferase (*COMT*) gene (Eisenberg et al., 1999). Although these genetic results are promising, there are difficulties in replicating them which may originate, at least in part, in the heterogeneity of this disorder.

The *Catechol-O-methyltransferase* gene codes for the *COMT* enzyme, which is involved in the metabolic degradation of catecholamine neurotransmitters such as dopamine and norepinephrine by an extraneuronal transfer of a methyl group to catechol compounds (Weinshilboum et al., 1999; Tenhunen et al., 1994). *COMT* is expressed in the frontal lobes and plays an important role in regulating the level of extra-synaptic dopamine levels compared to the dopamine transporter (*DAT*) (Karoum et al., 1994; Moron et al., 2002). In humans, the *COMT* gene has been localized to the chromosomal region 22q11.1-q11.2. A functional polymorphism of the human *COMT* gene exists; it involves a substitution (valine → methionine) at position 108 or 158 of the coding sequence of the soluble and the membrane bound forms of the *COMT* protein respectively. Given the paucity of DAT within the PFC (Moron et al., 2002; Lewis et al., 2001; Sesacket al., 1998), it is reasonable to consider that variations in the *COMT* enzyme activity, as dictated by the $Val^{108/158}$ *Met* polymorphism, might modulate synaptic DA levels and in consequence performance in cognitive domains mediated by this region i.e. modulation of performance in tasks of executive functioning in healthy individuals, as well as individuals with reduced/ increased PFC basal dopamine levels. Supporting this assumption, the *Valine* allele (less synaptic DA) of the *COMT* gene compared to the *methionine* allele (high synaptic DA), seems to be associated with low scores in executive functioning tasks in

healthy and schizophrenic adults (Bilder et al., 2002; Egan et al., 2001; Joober et al., 2002; Mattay et al., 2003).

Studies in ADHD children are relatively scant; few studies investigated the effect of *COMT* $Val^{108/158}Met$ polymorphism on cognitive tasks known to tap the PFC(Mills et al., 2004; Taerk et al., 2004). Mills et al (2004) (N=124) reported no effect of this polymorphism on cognitive impulsiveness, sustained attention, and response inhibition. Similarly, Taerk et al (2004) (N=118) did not identify any association of this polymorphism with working memory, planning and set shifting. However, Bellgrove et al. (2005), analyzing sustained attention capacity in relation to *COMT* genotype, identified a specific and unexpected impairment in sustained attention that was most pronounced in children possessing at least one copy of the methionine variant (Bellgrove et al., 2005). Given the fact that prefrontal cognition can be impaired by both hypo- and hyper-dopaminergic states (Arnsten, 2006a), it was suggested that the methionine variant of the *COMT* associated with slower clearance of dopamine may be disadvantageous to cognition in ADHD (Bellgrove et al., 2005).

To date, all studies investigating the *COMT* in ADHD have focused exclusively on its implication in ADHD as a syndrome, its effects on PFC mediated cognitive tasks, and did not investigate its role in modulating different behavioral dimensions relevant for ADHD or the response of these behavioral dimensions to psychostimulant drugs.

CHAPTER III

METHODS

1 Subjects

In part A of our study, two hundred and forty four ADHD children (203 boys and 41 girls), were recruited from the Disruptive Behavior Disorders Program and the child psychiatry outpatient clinic at the Douglas Hospital in Montreal. **In part B** of our study, two hundred and thirty two of the previous sample (193 boys and 39 girls, a subset of subjects in part A of our study), were analysed for cognitive traits relevant for ADHD.

All of the subjects were referred to these specialized care facilities by school principals, community social workers, and paediatricians. Participant in the present study were between 6 to 12 years, and met DSM-IV diagnostic criteria for ADHD (Lahey et al., 1994). The diagnosis was based on clinical examination of the child and an interview of at least one parent by a child psychiatrist. Additonally, the DISC-IV (parental report), a structured clinical interview (National Institute of Mental Health, 1998), was administered to parents, and the school reports were collected. In the majority of the cases, the mothers were the primary informants. Furthermore, parents completed the Child Behavioral Checklist(Achenbach, 1991), a scale that assesses several behavioral domains. All of the above mentioned assessments were completed during the week preceding the clinical trial when the children were not taking any medication (drug wash out period).

Children with an IQ less than 70, history of Tourette's syndrome, pervasive developmental disorder, psychosis, or any other medical condition/impairment were excluded from the study.

The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Parents were explained the study and they provided written consent. Children were explained the study and they gave their assent to participate.

2 Study Design, Procedure and Assesments

2.1 Part A

After the completion of the baseline evaluations, the children proceeded into a 2-week double-blind, placebo-controlled, within-subject (crossover) experimental design that was used to assess the behavioral response to MPH as compared to placebo (PBO) (hereafter referred to as MPH response). The children were given a single fixed and moderate dose (0.5 mg/kg/day) of MPH. This dose, although sometimes not optimal to achieve therapeutic response, has been shown to have a significant effect on behavior, often reaching clinical significance, (Schachter et al., 2001), and conforms with the recommendations of initiating treatment with MPH at low to moderate doses followed by titration to higher doses if no adequate response is seen (American Academy of Pediatrics, 2001). Thus, at this moderate dose, it is expected that variability of behavioral response to methylphenidate will be greater than at higher or lower dose, allowing better detection of a genetic effect on response to methylphenidate.

After the compeltion of 1 week of baseline assessments (this period also served as a wash-out period for previously MPH medicated children), the subjects received 1 week of

treatment with placebo and one week of treatment with 0.5 mg/kg/day of MPH in a divided dose (0.25 mg/kg morning and noon). The order of treatments (PBO and MPH) administration was determined by random assignment.

Both PBO and MPH were prepared individually in opaque gelatin capsules in weekly blister packs by a pharmacist not otherwise involved in the study to maintain the blind allocation of treatments. At the end of each week of treatment, all the blister packs were collected and adherence to the medication was checked.

ADHD Behaviour Assessment

Conners' Global Index for parents and teachers

ADHD behaviour was assessed by parents using the Conners' Global Index for parents (CGI-Parents; CGI-P)(Conners CK, 1999), and by teachers using the Conners' Global Index for teachers (CGI-Teachers; CGI-T)(Conners CK, 1999). Both the CGI-Parents and CGI-Teachers are subsets of the original Conners' Rating Scales, which is widely used for assessing behavioural and emotional problems in children. These scales have been widely used for the assessment of ADHD symptoms and other psychopathology in children between 3 and 17 years of age; normative data for these rating scales have been well established (Conners CK, 1997c).

Each CGI scale consists of a total of 10 items, representing the Hyperactivity Index of the original Conners' scale and is comprised of two main factors: 'Emotional lability' (EL) and 'Restless-impulsive behavior' (RIB). The raw total and factor scores are transformed into normalized T-scores; a total score of 65 or higher is considered to be clinically

significant. This rating scale has been recommended for titrating and monitoring treatment with psychostimulant drugs (Conners CK, 1997c).

In our present study, the CGI-P and CGI-T assessments were completed during the week preceding the clinical trial (ie, at baseline) while the children were not taking any medication. Similarly, at the end of each week of the clinical trial with MPH, the child's teacher and parents were contacted by the research assistant and asked to provide information as per the CGI-P and CGI-T, respectively. The teachers were asked to take into consideration the behavior of the child during the entire week at school (five days) under treatment, whereas the parents were asked to consider the behavior of the child during the entire week, including weekends at home (seven days) under treatment.

2.2 Part B

Executive functions (EFs) are defined as a set of mental control cognitive processes that permit the use of goal directed behaviour and help in sustaining suitable problem solving (Pasini et al., 2007; Taerk et al., 2004). EFs' include several domains including response inhibition, planning, cognitive flexibility/set shifting and working memory (Pasini et al., 2007; Sergeant et al., 2002). A comprehensive neuropsychological test battery assessing different aspects of the central executive functions was administered to all children by trained research personnel. Tests were selected according to their ability to tap into various performance domains of executive function. We restricted the number of tests in each domain in order to balance comprehensiveness with the co-operation of patients. Executive functioning (EF) was measured with the following tasks: Wisconsin Card Sorting Test (Sergeant et al., 2002); Tower of London (Krikorian et al., 1994; Shallice,

1982); Self Ordered Pointing Task (SOPT) (Petrides & Milner, 1982). All study subjects were assessed subsequent to a one-week medication "wash-out" period. They were permitted to take breaks upon request and, in some cases, testing was carried out over two sessions. An average testing procedure lasted 1.5 hours.

Neurocognitive assessment

(a) Wisconsin Card Sorting Task (WCST)

Abstraction and concept formation of ADHD children was evaluated by means of a computerized version of the WCST (Grant & Berg, 1948; Sergeant et al., 2002). In this task, children are required to sort cards according to three different criteria (colour, number, or shape of signs presented on cards). Feedback on whether the child achieved a correct or incorrect match is given after each trial. The matching criterion changes after ten consecutive correct matches (referred to as completing a category) and the child has to identify the new matching criterion using the feedback (correct/incorrect) provided to them. In this study, we have included the following WCST scores: (1) total number of correct responses; (2) total number of errors; (3) number of perseverative responses; (4) number of perseverative errors; (5) number of non-perseverative errors (standard/raw score); (6) number of categories completed; (7) number of trials to complete the first category; and (8) number of failures to maintain set. Although these different scores may reflect specific aspects of executive functions, they are not independent.

(b) Tower of London (TOL)

Planning capacity of ADHD children was evaluated using the TOL (Shallice, 1982). This test is used to assess planning and problem solving aspects of executive functioning. The validity and reliability of the TOL has been reported in numerous studies (Sergeant et al., 2002; Taerk et al., 2004). Standardized administration and scoring procedures as well as normative data have been developed for paediatric populations (Shallice, 1982; Taerk et al., 2004). The TOL also involves twelve levels of task difficulty, thus in this study, we have included the following TOL scores: (1) Standard Score; (2) total correct in 1 trial; (3) Solution time for each level of task difficulty; (4) Item Score achieved at each level of the task.

(c) Self ordering pointing Task (SOPT)

We used the abstract version of the SOPT to evaluate visual working memory (Petrides & Milner, 1982; Taerk et al., 2004). In this task, series of matrices of 6, 8, 10, and 12 images are presented to the child. The child is asked to select, by pointing, one different image on each page. Errors occur when the child points to images previously selected on the preceding pages. Each set is presented to the child three times. Successful performance on this task involves working memory as well as planning and monitoring skills. Shue & Douglas (1992) have reported significant differences in performance between ADHD children and normal controls on the SOPT(Shue & Douglas, 1992; Taerk et al., 2004). As the SOPT, involves four levels of difficulty, in this study, we have included the following SOPT scores: (1) total score; (2) scores at the four different levels i.e., series of matrices of 6, 8, 10, and 12 images respectively.

3 Molecular Genetics

Blood or saliva samples were collected from each child participating in this study. Blood samples were also collected from parents whenever possible. The *Val*$^{108/158}$ *Met* polymorphism of the *COMT* gene was genotyped using a PCR based method as previously described (Lachman et al., 1996; Taerk et al., 2004). The PCR was performed in a 25 µl total reaction volume containing 1X PCR buffer, 200 uM dNTPs, 200 ng of primers (5'-GCGATGGTGGCACTCCAAGC; 5'-TTGGAGAGGCTGAGGCTGAC), 1 unit of Taq DNA polymerase, and 100 ng of genomic DNA. PCR products were electrophoresed on agarose-TAE gel along with 1 kb ad 100 bp DNA ladders, visualized under UV-light and coded according to the length of the PCR product. Genotypes were called by two independent and experienced technicians who were blind to all clinical data. No discordance in any of the readings was noted. Children were stratified according to genotype only after all neuropsychological task data was collected as per protocol.

4 Statistical Analyses

The *Val*$^{108/158}$ *Met* polymorphism consists of both the low-activity *Met* and high-activity *Val* alleles. Study subjects were stratified into three groups: two homozygous genotype groups (*Met/ Met*, *Val/Val*) and one heterozygous genotype group (*Met/Val*).

4.1 Part A

ANOVA (analysis of variance) and Chi-square statistic tests were used to explore group differences in demographic variables, clinical characteristics and IQ. To test the effects of genotype (*Met/ Met*, *Met/Val, and Val/Val*), treatment (placebo and MPH) and genotype-

by-treatment interaction on the main outcome variables (CGI-Parents or CGI-Teachers ratings) during medication weeks, we used mixed model analyses of variance using SPSS. Treatment, order of treatment, genotype, cross-over, and treatment-by-genotype interaction were fixed effects; individuals were random effects. Main effects and any interactions were regarded as statistically significant when $p<0.05$. Baseline value of the outcome scores (CGI-P or CGI-T ratings) were included as covariate (Senn S, 2007).

To test for linkage/association between the *COMT* $Val^{108/158}Met$ polymorphism and ADHD considered as a syndrome, we calculated a transmission disequilibrium statistic using fBAT statistical software program. The effect of this polymorphism on the quantitative phenotypes was assessed utilizing the quantitative module of fBAT, statistical software package. The numbers of transmitted alleles from parents to affected children were counted using UNPHASED 2.4.

4.2 Part B

Group (*Met/Met*, *Val/Met* and *Val/Val*) differences in demographic and clinical characteristics were assessed with ANOVA (analysis of variance) or Chi-square statistics as appropriate. ANCOVA (analysis of covariance with age as a co-variate) was used to test for associations between WCST indices across the three genotype groups. Mixed design ANCOVA, where genotype and level of task difficulty were the between and within subjects independent variables, respectively, neuropsychological task performances were the dependent variables, and age was the covariate was used to test for the effect of *COMT* on SOPT and TOL indices.

To test for linkage/association between the *COMT* $Val^{108/158}Met$ polymorphism and ADHD considered as a syndrome, we calculated a transmission disequilibrium statistic using UNPHASED 2.4 statistical software program. The effect of this polymorphism on the quantitative phenotypes was assessed utilizing the FBAT, statistical software package.

CHAPTER IV

RESULTS

1 Part A

Clinical and Demographic Characteristics

Tables 4 and 5 show demographic and clinical information for the children stratified according to their genotypes. The three groups were similar with regard to sex, age, average household income, severity of behavioural problems as assessed by the CBCL mean number of inattention and hyperactivity items according to the DISC-IV and the distribution of ADHD subtypes. No significant differences existed between the groups in IQ as measured by the WISC-III. The frequency of co-morbid disorders was equally distributed between the genotype groups, although the *Val/Met* group had a trend to lower comorbidity with general anxiety disorder compared to the other homozygote groups (χ^2=5.92, df=2, p=0.051). The proportion of subjects who had never received medication for ADHD within each genotype group was also remarkably similar. Children with the *Met/Met* genotype had marginally lower CGI-T scores at baseline (p=0.03) (Table 6). No differences were observed for CGI-P. Thus, we included baseline scores as covariates in subsequent analsyses (Senn S, 2007).

Table 4: Demographic characteristics of children with ADHD separated according to their genotypes in the *COMT Val*$^{108/158}$*Met* polymorphism.

	Met/Met (n=49)	*Val/Met* (n=128)	*Val/Val* (n=67)	Statistic and p-value
Sex, M/F	42/7	103/25	58/9	χ^2=1.44, df=2, p=0.48
Age, yrs	9.1 (1.9)	9.1 (1.8)	8.9 (1.7)	$F_{2,241}$=0.23, p=0.78
Household income (% < $20,000 per yr)	40.90%	39.83%	37.31%	χ^2=0.17, df=2, p=0.91
Ethnic origin (Caucasian/other)	43/6	112/16	54/13	χ^2=1.92, df=2, p=0.38

M= male, F= female. Values are Mean (SD).

Table 5: Clinical characteristics of children with ADHD separated according to their genotypes in the *COMT Val*$^{108/158}$*Met* polymorphism.

	Met/Met (n=49)	*Val/Met* (n=128)	*Val/Val* (n=67)	Statistic and p-value
WISQ-III, full scale IQ	97.3 (14.3)	98.8 (14.6)	96.8 (12.7)	$F_{2,222}$=0.45, p=0.63
CBCL (total score)	68.5 (8.7)	70.1 (9.2)	69.5 (8.1)	$F_{2,234}$=0.56, p=0.56
CBCL (Attention problems)	70.1 (9.7)	70.2 (10.7)	71.4 (9.5)	$F_{2,234}$=0.37, p=0.68
CBCL (externalisation)	67.8 (9.9)	70.7 (10.4)	69.7 (8.6)	$F_{2,234}$=1.57, p=0.20
CBCL (internalisation score)	63.0 (11.8)	64.1 (10.5)	64.2 (10.2)	$F_{2,234}$=0.20, p=0.81
DISC-IV, Inattention Items	7.1 (1.7)	6.9 (2.2)	7.2 (2.1)	$F_{2,238}$=0.32, p=0.72
DISC-IV, Hyperactivity Items	5.2 (2.5)	5.9 (2.4)	6.2 (2.5)	$F_{2,238}$=2.13, p=0.12
DSM-IV, ADHD Subtype (I/H/C)	19/4/14	22/10/47	12/3/25	χ^2=7.66, df=4, p=0.10
Comorbidity (%) with:				
Conduct Disorder	20.0%	33.6%	26.1%	χ^2=4.12, df=6, p=0.66
Opposition Defiant Disorder	36.7%	42.5%	41.5%	χ^2=0.49, df=2, p=0.78
Generalized Anxiety Disorder	8.6%	1.8%	10.1%	χ^2=5.92, df=2, p=0.051
Major Mood Disorder	8.6%	9.4%	1.6%	χ^2=3.7, df=2, p=0.15
Previous medication status (%)	44.1%	46.0%	53.7%	χ2=1.30, df=2, p=0.52

WISC=Wechsler Intelligence Scale for Children, 3rd edition; CBCL = Child Behavioral Checklist. DISC-IV = Diagnostic Interview Schedule for Children fourth edition. ADHD Subtypes: I = Inattentive, H = Hyperactive, C = Combined. Values are Mean (SD).

Table 6: ADHD behaviours assessed by parents and teachers at baseline in children with ADHD separated according to their genotypes in the *COMT Val*$^{108/158}$*Met* polymorphism.

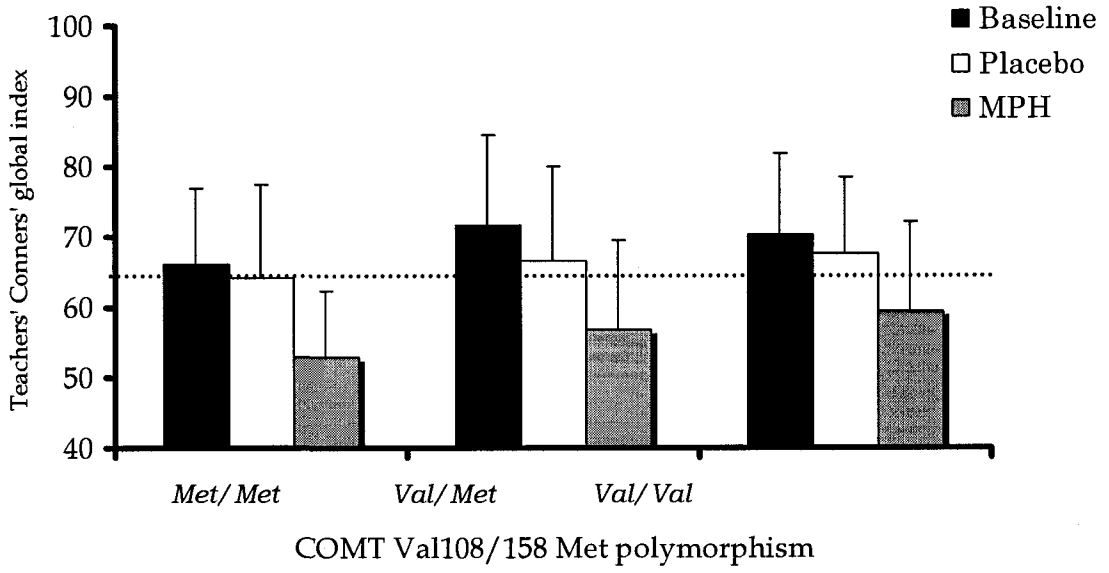
	Met/Met (n=49)	*Val/Met* (n=128)	*Val/Val* (n=67)	Statistic and p-value
CGI-Parents	74.0 (10.2)	75.1 (10.6)	74.9 (9.6)	$F_{2,235}=0.20$, $p=0.81$
CGI-P-RI score	74.7 (9.9)	75.3 (9.9)	75.9 (8.9)	$F_{2,235}=0.21$, $p=0.80$
CGI-P-EL score	67.2 (13.5)	68.7 (12.6)	66.8 (12.8)	$F_{2,235}=0.54$, $p=0.57$
CGI-Teachers	66.0 (10.8)	71.5 (13.0)	70.3 (11.5)	$F_{2,212}=3.28$, $p=0.03$
CGI-T-RI score	65.4 (9.5)	69.4 (11.7)	68.2 (10.5)	$F_{2,212}=2.07$, $p=0.12$
CGI-T-EL score	61.8 (15.2)	69.1 (16.7)	68.5 (15.3)	$F_{2,212}=3.38$, $p=0.03$

Values are Mean (SD). CGI-Parents = Conners' Global Index for parents, CGI-P-RI score = Conners' Global Index for parents, Restless-impulsive behavior, CGI-P-EL Conners' Global Index for parents, Emotional lability. CGI-Teachers = Global Index for teachers, CGI-T-RI score = Conners' Global Index for teachers, Restless-impulsive behavior, CGI-T-EL = Conners' Global Index for teachers, Emotional lability

COMT Val$^{108/158}$ *Met* polymorphism and the response of ADHD relevant behaviours to MPH

For CGI-T, the mixed model analysis of variance revealed a highly significant main effect of treatment ($F_{1,200}$=33.20, p=0.00), and no significant main effects of the order of treatment ($F_{1,386}$=2.34, p=0.12), genotype ($F_{2,204}$=1.27, p=0.28), cross-over ($F_{1,205}$=0.86, p=0.35) and genotype by treatment interaction ($F_{3,203}$=0.37, p=0.77). The same analysis for the "emotional lability" and "restless impulsive" dimensions scores yielded parallel results (data not shown).

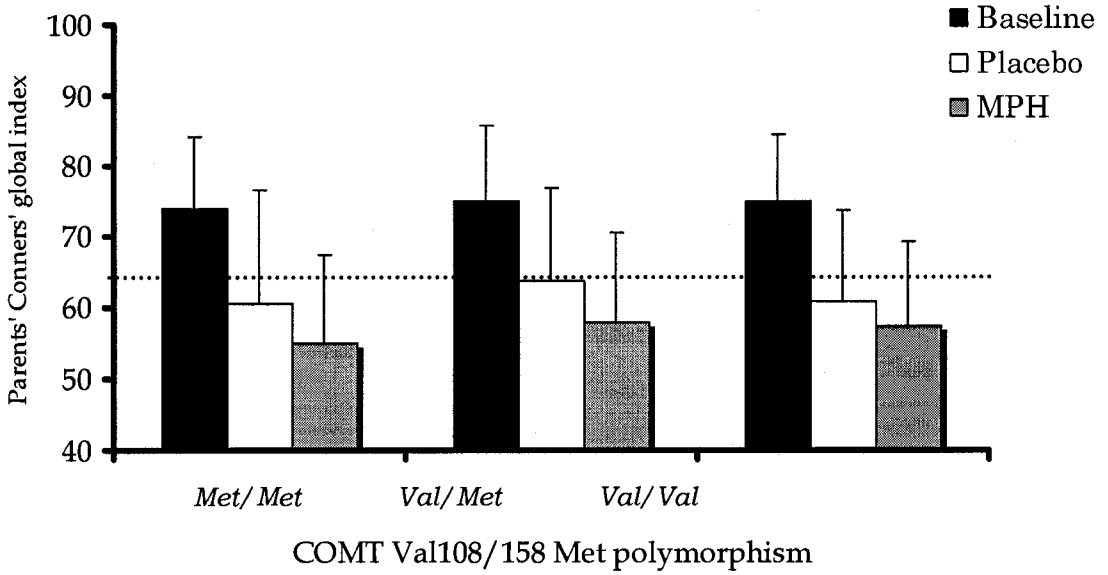
Figure 4 Conners' Global Index scores (±SD) for teachers for COMT genotype in children with ADHD.



Conners' Global Index scores (±SD) for teachers in children with ADHD separated according to their genotype in the COMT gene during baseline evaluation, treatment with placebo and treatment with methylphenidate (MPH). Dashed line represents the threshold for clinical significance on the Conners' scales (≥ 65).

For CGI-P, a significant main effect of treatment ($F_{1,223}$=6.78, p=0.01) was observed but no significant main effects of the order of treatment ($F_{1,418}$=1.27, p=0.26), genotype ($F_{2,226}$=1.11, p=0.34), cross-over ($F_{1,227}$=2.77, p=0.09) and no genotype by treatment interaction ($F_{3,224}$=0.73, p=0.53) were observed. Similarly "emotional lability" and "restless impulsive" dimensions scores yielded parallel results.

Figure 5 Conners' Global Index scores (±SD) for parents for COMT genotype in children with ADHD.



Conners' Global Index scores (±SD) for parents in children with ADHD separated according to their genotype in the COMT gene during baseline evaluation, treatment with placebo and treatment with methylphenidate (MPH). Dashed line represents the threshold for clinical significance on the Conners' scales (≥ 65).

Family-based association analyses

One hundred and seven and 101 children had one or two parents respectively who gave blood for the purpose of genetic analyses. Among these parents, 146 were heterozygous (Mothers = 89 and Fathers = 57). Using UNPHASED 2.4 program revealed 28 transmissions and 36 non-transmissions of the *Met* allele from heterozygous parents to affected children. This difference was not statistically significant as tested by fBAT [$\chi 2$ = 1.00; df = 1, p = 0.31]. The TDT test using the fBAT program under additive, dominant, recessive and genotype models also revealed no preferential transmission of either one of the two alleles from parents to affected offspring (Table 7).

Table 7 – Results of the family-based analysis using FBAT

Model	Allele/genotype (%)	Families[b]	*Z*- score	*P*- value
Additive	*Met* (46)	69	- 0.61	0.54
	Val (54)	69	0.61	0.54
Dominant	*Met* (46)	51	0.28	0.77
	Val (54)	39	1.34	0.17
Recessive	*Met* (46)	39	− 1.34	0.17
	Val (54)	51	− 0.28	0.77
Genotype	*Met/Met* (24)	39	− 1.34	0.17
	Val/Met (46)	69	1.16	0.24
	Val/Val (30)	51	− 0.28	0.77

[b] *Number of informative families. (Analyses performed if ≥ 10 informative families).*

Since we observed a significant difference in CGI-T during the baseline evaluation between the three genotype groups, we conducted a family-based quantitative analysis on CGI-P and CGI-T as measured at baseline, during the week of treatment with placebo, during the week of treatment with methylphenidate, and change scores (methylphenidate - placebo) for both CGI-P and CGI-T. None of these traits were significantly associated with the *COMT Val/Met* polymorphism according to this quantitative trait analysis (data not shown).

2 Part B

Clinical and Demographic Characteristics

Table 8 and 9, shows demographic and clinical information for the children separated according to genotype [n = 46 for *Met/Met* (19.82%), n = 123 for *Val/Met* (53.01%) and n = 63 for *Val/Val* (27.15%)]. Genotype distribution did not depart from Hardy Weinberg equilibrium [χ^2 = 1.01; df = 2, p = 0.40]. The three groups were similar with regard to gender, age, average household income, severity of behavioural problems as assessed by the CBCL, mean number of inattention items according to the DISC-IV, and distribution of ADHD subtypes. The three groups showed a trend towards significant difference with regard to mean number of hyperactivity items [$F_{2,226}$=2.59, p=0.07], and externalization behavioural problems as assessed by the CBCL [$F_{2,222}$=2.37, p=0.09]. No significant differences existed between the groups in IQ as measured by the WISC-III. The frequencies of conduct and oppositional defiant disorders were equally distributed between the genotype groups. The *Val/Met* group had a trend towards lower comorbidity with general anxiety disorder compared to both homozygote groups [χ^2=5.73, df=2, p=0.05], while the *Met/Met* group had a trend towards lower comorbidity with conduct

disorder compared to both *Val/Met* and *Val/Val* groups [χ^2=4.81, df=2, p=0.09]. The proportions of subjects who had never received medication for ADHD within each genotype group were similar.

Table 8: Demographic characteristics of children with ADHD separated according to their genotypes in the *COMT Val*$^{108/158}$*Met* polymorphism.

	Met/Met (n=46)	*Val/Met* (n=123)	*Val/Val* (n=63)	Statistic and p-value
Sex, M/F	40/6	99/24	54/9	χ^2=1.39, df=2, p=0.49
Age, yrs	9.1 (1.9)	9.1 (1.8)	8.9 (1.6)	$F_{2,229}$=0.28, p=0.74
Household income (% < $20,000 per yr)	39.0%	40.70%	38.09%	χ^2=5.19, df=10, p=0.87
Ethnic origin (Caucasian/other)	41/5	110/13	51/12	χ^2=2.8, df=2, p=0.23

M= male, F= female. Values are Mean (SD).

Table 9: Clinical characteristics of children with ADHD separated according to their genotypes in the *COMT Val*$^{108/158}$*Met* polymorphism.

	Met/Met (n=46)	*Val/Met* (n=123)	*Val/Val* (n=63)	Statistic and p-value
WISQ-III, full scale IQ	97.4 (14.5)	99.0 (14.6)	97.2 (12.7)	$F_{2,212}$=0.39, p=0.67
CBCL (total score)	68.3 (8.9)	70.2 (9.3)	69.2 (8.2)	$F_{2,222}$=0.85, p=0.42
CBCL (Attention problems score)	70.3 (9.9)	69.8 (10.7)	71.5 (9.8)	$F_{2,222}$=0.53, p=0.58
CBCL (externalisation score)	67.4 (10.1)	71.0 (10.2)	69.3 (8.6)	$F_{2,222}$=2.37, p=0.09
CBCL (internalisation score)	62.7 (11.8)	64.2 (10.4)	64.0 (10.4)	$F_{2,202}$=0.38, p=0.68
DISC-IV, Inattention Items	7.1 (1.7)	6.8 (2.2)	7.1 (2.2)	$F_{2,226}$=0.35, p=0.70
DISC-IV, Hyperactivity Items	5.1 (2.5)	6.0 (2.3)	6.1 (2.5)	$F_{2,226}$=2.59, p=0.07
DISC-IV, ADHD Subtype (I/H/C)	19/4/14	22/10/47	12/3/25	χ^2=7.66, df=4, p=0.10
Comorbidity (%) with:				
Conduct Disorder	17.3 %	34.1%	26.2%	χ^2=4.81, df=2, p=0.09
Opposition Defiant Disorder	39.1%	42.6%	40.9%	χ^2=0.17, df=2, p=0.91
Generalized Anxiety Disorder	6.9%	1.9%	10.9%	χ^2=5.73, df=2, p=0.05
Major Mood Disorder	13.9 %	12.8 %	7.1 %	χ^2=1.4, df=2, p=0.47
Previously medicated (%)	45.0%	46.3%	57.1%	χ2=2.22, df=2, p=0.32

WISC=Wechsler Intelligence Scale for Children, 3rd edition; CBCL = Child Behavioral Checklist. DISC-IV = Diagnostic Interview Schedule for Children fourth edition. ADHD Subtypes: I = Inattentive, H = Hyperactive, C = Combined. Values are Mean (SD).

Quantitative trait analyses:

(a)WCST

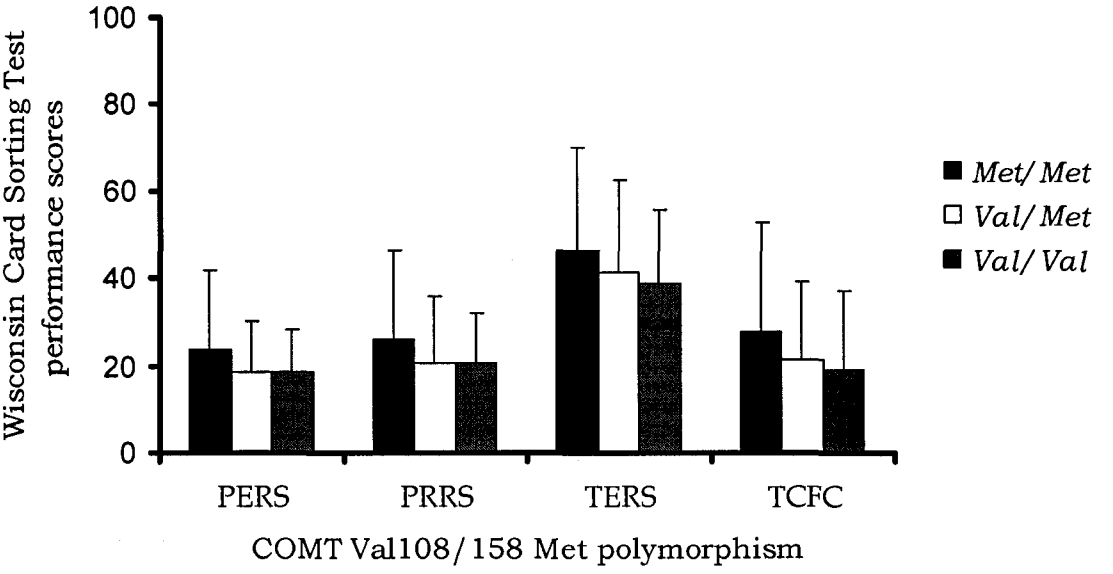
A one-way ANCOVA (covariate for age) revealed a significant effect of genotype on WCST perseverative errors [$F_{2, 198} = 3.10$, p=0.04] and trends suggesting the same on total error [$F_{2,198} = 2.42$, p=0.09;], perseverative responses [$F_{2,198} = 2.41$, p=0.09] , and number of trials to complete first category [$F_{2,198} = 2.71$, p=0.06] (Table 10).

Table 10: Comparison of Wisconsin Card Sorting Test performance scores for each genotype and results of ANCOVA in children with ADHD

	Met/Met (n=46)	*Val/Met* (n=123)	*Val/Val* (n=63)	Statistic and p-value
All subjects;				
Perseverative error raw score	23.6 (18.2)	18.5 (11.8)	18.52 (9.7)	$F_{2, 198} = 3.10$, p=0.04
Perseverative responses raw score	26.1 (20.4)	20.7 (15.2)	20.5 (11.7)	$F_{2,198} = 2.42$, p=0.09
Total error raw score	46.6 (23.4)	41.5 (20.8)	38.7 (16.9)	$F_{2,198} = 2.41$, p=0.09
Trials to complete first category	28.0 (24.8)	21.7 (17.5)	19.0 (18.1)	$F_{2,198} = 2.71$, p=0.06

Values are mean (±SD).

Figure 6 Mean Wisconsin Card Sorting Test performance scores (± SD) for COMT genotype in children with ADHD.



PERS = Perseverative error raw score, PRRS = Perseverative responses raw score, TERS = Total error raw score, TCFC = Trials to complete first category.

Post-hoc pairwise comparisons (using the Least Significant Difference method) for WCST perseverative errors revealed significant differences between the *Met/Met* (average ± SD: 26.16 ± 20.43) genotype and both the *Val/Met* (20.76 ± 15.29; $p<0.02$) and the *Val/Val* (20.58 ± 11.71; $p<0.04$) genotypes, suggesting that the *Met* allele may be acting according to a recessive model. Indeed, when the *Val/Met* and *Val/Val* genotypes were grouped and contrasted with the *Met/Met* genotype, significant differences were observed for WCST perseverative responses [$F_{1,199} = 4.75$, $p=0.03$], perseverative errors [$F_{1,199} = 6.15$, $p=0.01$] and WCST trials to complete first category [$F_{1,199} = 4.57$, $p=0.03$]. Compared to carriers of the *Val* allele, patients with the *Met/Met* genotype showed

significantly more perseverative responses (26.16 ± 20.43, *vs.* 20.70 ± 14.21; $p<0.03$), more perseverative errors (23.64 ± 18.24 *vs.* 18.56 ± 11.15; $p<0.01$), and required more trials to complete first category (28.02 ± 24.84 *vs.* 20.89 ± 17.75; $p<0.03$) (Table 11).

Table 11: Comparison of Wisconsin Card Sorting Test performance scores for each genotype and results of recessive model analysis in children with ADHD

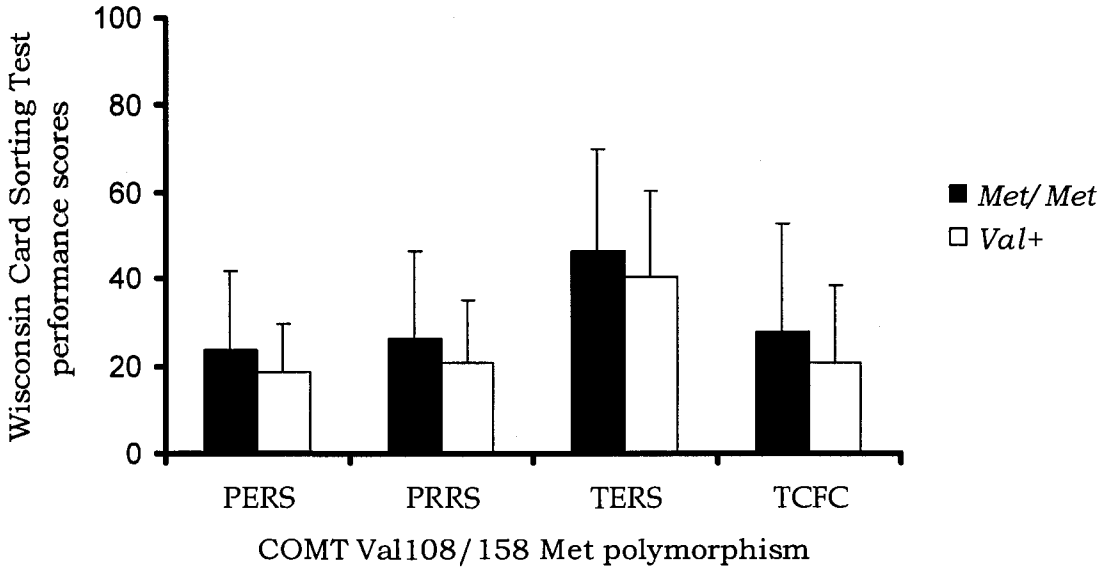
	Met/Met (n=46)	*Val+* (n=186)	Statistic and p-value	Cohen's *d*
All subjects;				
Perseverative error raw score	23.6 (18.2)	18.5 (11.1)	$F_{1,199} = 6.15$, p=0.01	0.33
Perseverative responses raw score	26.1 (20.4)	20.7 (14.2)	$F_{1,199} = 4.75$, p=0.03	0.30
Total error raw score	46.6 (23.4)	40.6 (19.6)	$F_{1,199} = 3.48$, p=0.06	0.27
Trials to complete first category	28.0 (24.8)	20.8 (17.7)	$F_{1,199} = 4.57$, p=0.03	0.33
Caucasian subjects;				
perseverative errors raw score	23.7 (18.4)	18.6 (11.1)	$F_{1,196} = 6.13$, p=0.01	0.33
perseverative responses raw score	26.3 (20.6)	20.8 (14.2)	$F_{1,196} = 4.81$, p=0.02	0.31
Total error raw score	46.4 (23.6)	40.7 (19.6)	$F_{1,196} = 3.11$, p=0.07	0.26
Trials to complete first category	27.1 (24.4)	20.9 (17.8)	$F_{1,196} = 3.37$, p=0.06	0.29

Values are mean (±SD). Cohen's d effect size of COMT Val$^{108/158}$Met polymorphism [Met/Met vs. Val/Val+ Val/Met genotypes (Val+)].

The same results were observed when we restricted the analysis to Caucasians (n = 202).

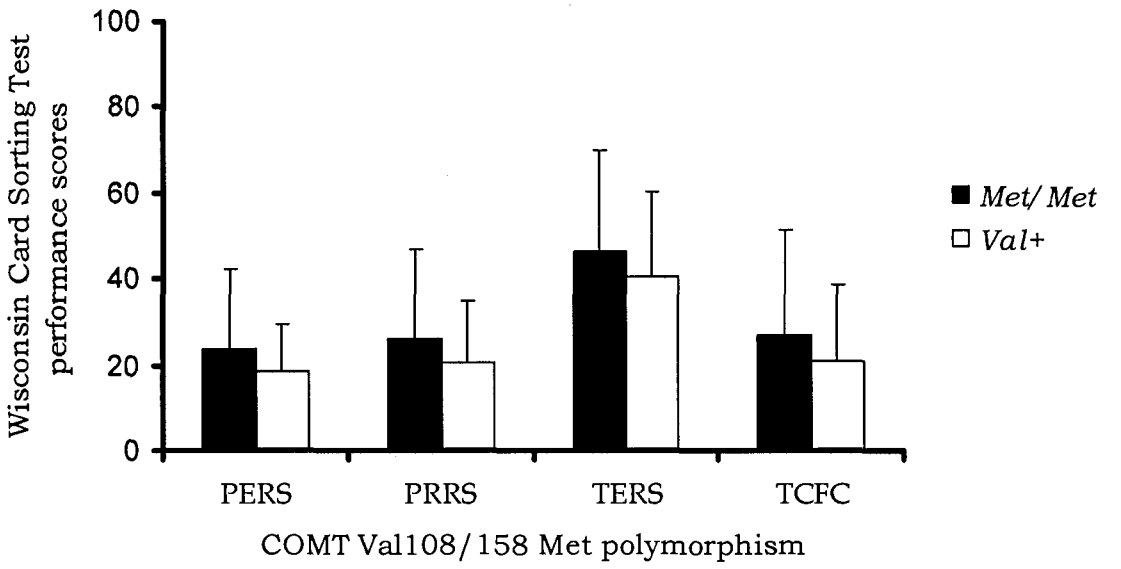
Figure 7 Mean Wisconsin Card Sorting Test performance scores (± SD) for COMT genotype recessive model in children with ADHD.

(a) All subjects



PERS = Perseverative error raw score, PRRS = Perseverative responses raw score, TERS = Total error raw score, TCFC = Trials to complete first category.

(b) Caucasian subjects



PERS = Perseverative error raw score, PRRS = Perseverative responses raw score, TERS = Total error raw score, TCFC = Trials to complete first category.

(b) TOL

A one-way ANCOVA (covariate for age) revealed no effect of genotype on TOL Total standardized score [$F_{2,197}$ = 0.58, p=0.55], and TOL Total correct in 1st trial score [$F_{2,226}$=0.42, p=0.65] (Table 12).

Table 12: Comparison of Self-ordered pointing test and Tower of London test performance scores for each genotype in children with ADHD

	Met/Met (n=46)	*Val/Met* (n=123)	*Val/Val* (n=63)	Statistic and p-value
All subjects;				
SOPT Total raw scores	14.2 (7.4)	14.1 (7.6)	15.0 (7.3)	$F_{2,221} = 0.13$, p=0.87
TOL Total standardized score	104.6 (15.1)	104.8 (14.9)	107.3 (13.9)	$F_{2, 197} = 0.58$, p=0.55
TOL Total correct in 1st trial score	10.6 (1.38)	10.5 (1.50)	10.6 (1.46)	$F_{2,226}$=0.42, p=0.65

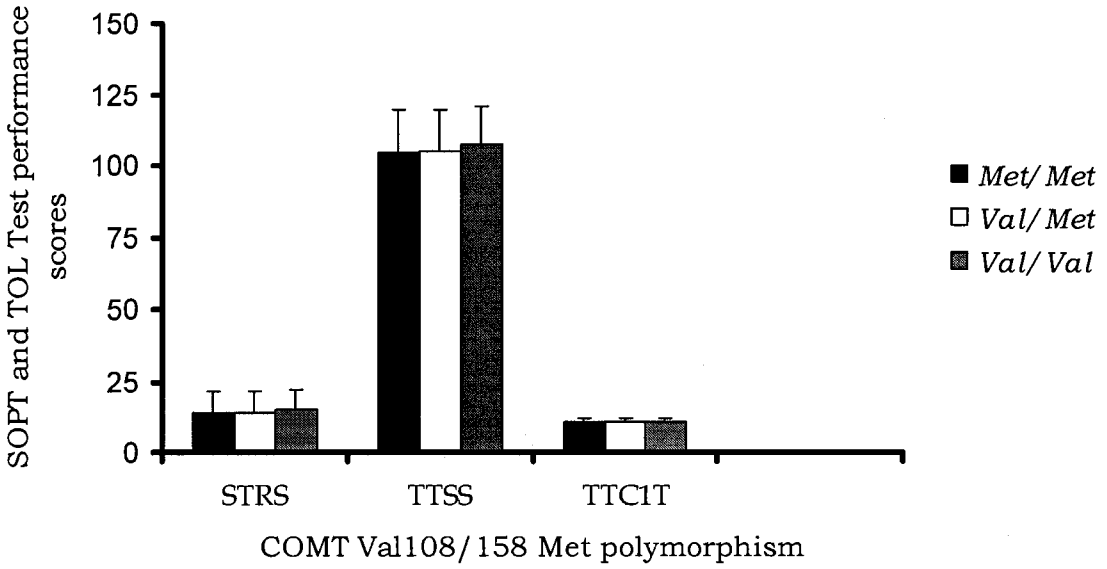
Values are Means (±SD). SOPT = Self-ordered pointing test, TOL= Tower of London test.

A repeated-measure, mixed design ANCOVA (covariate for age) performed on TOL Solution time scores showed a significant effect of task difficulty [$F_{11,2442}$ = 110.41, p<0.001], no effect of *COMT* genotype [$F_{2,221}$ = 0.34, p=0.70], and no genotype by task difficulty interaction [$F_{22,2442}$ = 1.14, p=0.28] (data not shown). Similarly the same analysis on TOL raw item score showed a significant effect of task difficulty [$F_{11,2431}$ = 117.26, p < 0.001], no effect of *COMT* genotype [$F_{2,220}$ = 0.43, p=0.64], and no genotype by task difficulty interaction [$F_{22,2431}$ = 1.19, p=0.24] (data not shown).

(c) SOPT

A repeated-measure, mixed design ANCOVA (covariate for age) revealed a significant effect of task difficulty [$F_{3,666} = 109.42$, $p<0.001$], no effect of genotype on SOPT raw error scores [$F_{2,221} = 0.13$, $p=0.87$], and no genotype by task difficulty interaction [$F_{6,666} = 0.28$, $p=0.94$] (Table 12).

Figure 8 Mean Self-ordered pointing test and Tower of London test performance scores (± SD) for COMT genotype in children with ADHD.



SOPT = Self-ordered pointing test, TOL = Tower of London, STRS = SOPT Total raw scores, TTSS = TOL Total standardized score, TTC1T = TOL Total correct in 1st trial score.

Family-based association study

Two hundred and ninety five parents participated in the study and gave blood samples. Among these parents, 145 were heterozygous (M = 53 and F = 92). Using UNPHASED 2.4 program, we found that the *Met* allele was transmitted to the affected children in 45 occurrences, whereas this same allele was not transmitted in 51 occurrences [$\chi 2 = 0.37$; df = 1, p = 0.54]. Additionally, we also used the FBAT program to test for association/linkage between EFs parameters considered as quantitative traits and the *COMT Val/Met* polymorphism. None of the traits that were associated with the *COMT Val/Met* polymorphism according to the quantitative trait analysis were found to be significant according to the family based analysis (data not shown).

CHAPTER V

DISCUSSION

1 Part A

The purpose of the part A of this research was to examine, concomitantly, the effects of the *Val*$^{108/158}$ *Met* polymorphism in the *Catechol-O-Methyltransferase (COMT)* gene on ADHD as a syndrome, behaviors relevant to ADHD and their response to MPH (0.5 mg/kg/day) using a double-blind placebo-controlled crossover trial. We used both quantitative trait and family-based analyses to assess the relation of this polymorphism to ADHD, some behavioral traits relevant for ADHD and the response of these behaviours to methylphenidate.

Previous investigations of the *COMT Val*$^{108/158}$*Met* polymorphism on ADHD, considered as a clinical syndrome lead to various results, some suggesting positive(Eisenberg et al., 1999; Qian et al., 2003)while others concluding no association (Barr et al., 1999; Hawi et al., 2000; Payton et al., 2001; Zhang et al., 2003). Our family based association analyses did not reveal a significant over-transmission of either one of the investigated alleles from parents to affected children. This result is consistent with most of the literature published on this topic until now.

As many researchers have suggested, it is likely that genetic effects may be more readily detectable when considering more refined quantitative phenotypes (simpler behavioural dimensions) rather than the ADHD clinical syndrome(Sherman et al., 1997; Hudziak et al., 1998; Joober et al., 2007; Martin et al., 2002), since ADHD as a syndrome may be

heterogeneous. In this sample of 244 ADHD children, we observed a genotype effect on CGI-T (p=0.03) but not on CGI-P during the baseline evaluation. However, this genotype effect was not observed during the weeks of treatment with placebo or the week of treatment with methylphenidate suggesting that this difference is likely due to chance. This conclusion is supported by the observation that the family-based analysis did not confirm this association and was negative for all the CGI scores (parents and teachers) on all measurement occasions.

In addition to investigating the role of the *COMT* $Val^{108/158}$ *Met* polymorphism in ADHD and relevant quantitative behavioral dimensions for this disorder, we also investigated the role of this gene in modulating response of these behaviors to methylphenidate, a question that has not been addressed in the previous literature. This question is relevant because it has been recently suggested that, at therapeutic doses, methylphenidate may be exerting its effect though an enhancement of dopamine transmission in the prefrontal cortex where the *COMT* enzyme is critical for dopamine clearance. Our data did not identify a genotype-by-treatment interaction, indicating that there is no association between the *COMT* $Val^{108/158}$*Met* polymorphism and the profile of behavioral response to MPH as compared to placebo as evaluated by both CGI-Parents and CGI-teachers.

Some limitations should be considered when interpreting this study. First, the dose used in this study is considered low to medium compared to the average dose used in clinical practice. However, it has been reported that at this dose, over 50% of the dopamine transporter is blocked (Volkow et al., 2005) resulting in a dopamine increase, which is likely to supersede the effect of COMT genotype. Thus, it may be concluded, that at the

doses used in clinical practice, which are often higher than those used in this trial, the effect of COMT *Val*$^{108/158}$ *Met* polymorphism on dopamine (and possibly other catecholamines) and its consequences on behaviors as assessed by CGI-P and CGI-T, is negligible if at all existent compared to much higher effect of methylphenidate. Second, our sample comprised relatively few females (41 girls, 16.8% of total sample). It is noteworthy that there is some evidence for sexual dimorphism with regards to the COMT gene (Pooley EC, 2007; Gogos et al., 1998; Chen et al., 2004; Kates et al., 2006). However, when we restricted our analysis to males (the largest group) our results remained the same. Analyses in a larger sample of females may be needed in the future. Third, although the majority of our patients are of Caucasian genetic background, it is known that mixing patients from different ethnic backgrounds in the same analysis may result in false positive and negative results. However, when we restricted our analyses to patients with Caucasian background (n = 207), the results remained the same. Finally, it is possible that the outcome measures used in this study (CGI-Parents and CGI-Teachers) may not capture the essence of the behavioral phenotype relevant for this polymorphism. Indeed, several studies have reported an association between this polymorphism and various aspects of behavioral outcomes. Future studies investigating the effect of the COMT gene on other behavioral manifestations of ADHD and their response to MPH may be informative.

2 Part B

Previous investigations of the *COMT Val*$^{108/158}$*Met* polymorphism on various quantitative traits that tap into the prefrontal cortex functions in adults lead to various results. Two recent meta-analyses have concluded that the *Val* allele may be associated with a small degree of alteration in EFs (explaining less than 1% of the variance) (Barnett et al., 2007; Flint & Munafo, 2007). Studies in children remain relatively scant. In a preliminary study, we have reported on a sample of 118 ADHD patients and did not identify any association between the *COMT Val*$^{108/158}$*Met* polymorphism and various indices of EF (Taerk et al., 2004). Similarly, Mills et al have reported no effect of this polymorphism on measures of cognitive impulsiveness, sustained attention, and response inhibition (Mills et al., 2004). However, Bellgrove et al (2005) reported that ADHD carriers of the *Met* allele tended to perform worse than those with the *Val/Val* genotypes on two sub-tests of the "Test of Everyday Attention for Children" (Bellgrove et al., 2005). Similarly, Baker et al (2005) compared *COMT* hemizygous for the *Met* and the *Val* alleles in patients (mean age 16 years) with the Velo-Cardio-Facial Syndrome (a disorder caused by a 22q11 chromosomal deletion and associated with high risk for ADHD), they reported poorer neuropsychologic performance and frontal auditory mismatch negativity in *Met* compared with *Val* hemizygous (Baker et al., 2005). In contrast, Diamond, et al. have reported an association between the *Val/Val* genotype and poor scores on measures of working memory and inhibition when compared with a *Met/Met* genotype group in a sample of 39 normally developing children (Diamond et al., 2004).

In this extended sample of 232 children with ADHD, a marginal association between the *Val*$^{108/158}$*Met* polymorphism and cognitive flexibility/set shifting, measured by the WCST

is observed. Patients with *Met/Met* genotype had higher perseverative responses, higher WCST perseverative errors, higher WCST total errors, and greater trials to complete first category of the WCST compared to carriers of the *Val* allele.

The finding that the *Met/Met* genotype of the COMT gene polymorphism impairs cognition in ADHD runs counter to earlier results in healthy adults where it is believed that the Val allele has a recessive or co-dominant negative effect on EF. However, several lines of evidence suggest that the effect of the *Val* and *Met* alleles on EFs may depend on the overall level of dopamine availability in the synapse. For example, in patients with Parkinsonism (a disorder characterised by a deficit of DA in the nigro-stratial pathway) the *Met* allele was associated with relatively impaired performance on working memory tasks (Foltynie et al., 2004). Also, Mattay et al (2003) reported that following amphetamine administration the *Met* homozygotes showed a decrease in prefrontal efficiency at high working memory load, compared to the *Val* homozygotes (Mattay et al., 2003). These observations have been interpreted according to the inverted "U" shaped curve relating dopamine availability to behavioural performance proposed by the late Goldman-Rakic (Goldman-Rakic et al., 2000). According to this hypothesis, optimum behavioural performance is observed for medium dopamine synaptic levels. Lower and higher levels of dopamine will result in a deterioration of behavioural performances compared to this optimal concentration. Consequently, the *Met* and *Val* allele will exert a positive or negative effect on PFC sensitive performances depending on the background dopamine levels. The observation that patients homozygous for the *Met* allele have poorer performances on some WCST indices that depend on PFC in the present study replicate a similar observation made by Bellgrove et al (2005) and may be explained using the

proposed inverted "U" curve relating dopamine levels to cognitive performances. Developmental changes in enzymatic activity (Galva et al., 1995; Stramentinoli et al., 1978; Venero et al., 1991), various receptors(Boyson & Adams, 1997; Meng et al., 1999), and transporter densities (Meng et al., 1999; Moll et al., 2000) have been reported and are compatible with the idea that the optimal level of dopamine concentration in the PFC may vary with age. More specifically, observations in both rats(Demarest et al., 1980; Estes & Simpkins, 1980; Lee et al., 2001) and humans(Carlsson & Winblad, 1976; Riederer & Wuketich, 1976) suggest that monoamine content and metabolism decrease with age. Accordingly, it may be expected that the *Met* allele, associated with a relatively lower enzymatic activity, will result in an optimal behavioural output in adults whereas this same allele will shift the "U" inverted curve to suboptimal behavioural output in children.

Alternatively, the significant associations observed here with some WCST indices may reflect a chance finding. This possibility is supported by the fact that we did not observe a significant effect of this polymorphism on any of the tasks when we used a family based association analysis. Also, the absence of significant effect on the two other outcome variables (TOL and SOPT) is compatible with a chance finding. However, it is well known that family-based association studies are statistically less powerful than quantitative trait analsyses. In addition, the sample used to perform the family based analysis was restricted because of non availability of DNA from a substantial proportion of parents or non-informative parental genotypes. The fact that we observed a positive association in only some indices of executive functioning may also be due to differential sensitivity of different tasks to PFC input.

Some limitations need to be considered when interpreting the results of this study. First, our sample comprised a significant proportion of children who were previously receiving stimulant medication. It has been suggested that the *Met* homozygotes have poorer and less efficient prefrontal function at high working memory loads when treated with dextroamphetamine (stimulant) (Mattay et al., 2003). Although we have tested the children during a washout period, it may be possible that the effect of previous treatment may affect differentially the performances of children with different genotypes. However, this potential confounder is not likely to affect our results since the proportion of children who were previously treated are equivalent in the three genotype groups. Second, the presence of patients from different ethnic backgrounds may result both in false positive and false negative results. However, the proportions of non-Caucasians were equivalent in the three genotype groups. Furthermore, the effects of the *COMT* $Val^{108/158}$*Met* polymorphism on WCST indices were observed when we restricted the analyses to Caucasians only.

CONCLUSION

The discussion continues as to whether *COMT* $Val^{108/158}$*Met* polymorphism is associated with ADHD. The results of the present study give some clues and provide us a piece of this puzzle in the allusive wider picture. Unquestionably, the complex relation existing between genes coding for proteins in the dopamine pathway and ADHD, its behavioral/neurocognitive symptoms and their response to MPH needs to be clarified. In the present study we attempted to explore these questions: the role of *COMTVal*$^{108/158}$*Met* polymorphism in modulating behaviors relevant to ADHD, the behavioral response to

MPH, and executive functioning in ADHD children. We hypothesised that the *COMT Val$^{108/158}$Met* polymorphism in will be associated with ADHD as a clinical syndrome, and/or with behaviors relevant to ADHD. The lack of significant association observed in our family based study quantitative trait analyses does not support an association between *COMT Val$^{108/158}$Met* polymorphism and ADHD as a syndrome, and/or with behaviors relevant to ADHD. Our findings are consistent with many previous reports (Barr et al., 1999; Hawi et al., 2000; Payton et al., 2001; Zhang et al., 2003). With respect to the role *COMT Val$^{108/158}$Met* polymorphism in the modulation of therapeutic response to MPH (0.5 mg/kg/day), our findings again do not support our hypothesis. The main finding of our study suggests that the *Val$^{108/158}$ Met* polymorphism of the *COMT* gene may modulate some aspects executive functions in children with ADHD. Contrary to studies in adults and in line with a previous study in children (Bellgrove et al., 2005), our results show that the Met allele is associated with poorer performances, although the effect size might be very modest. Our study has many strengths: it has the biggest sample size (N=232) of ADHD children; it has a robust double- blind, placebo-controlled, crossover design; it uses both a family based and a quantitative approach to identify for potential genetic associations; the Behavioral and neurocognitive assessments used are widely accepted and used for the assessment of ADHD symptoms and other psychopathology in children; and normative data for these rating scales and neurocognitive tests have been well established. Our observations suggest developmental effects need to be considered while conducting association studies in children. More studies, with much larger samples, are required to elucidate the role *COMT Val$^{108/158}$Met* polymorphism in ADHD. Large samples are needed, as it is more and more likely that genes have very modest effect on behaviour (Flint & Munafo, 2007) due to the complex genetic architecture for

phenotypes. Future work will need to determine whether the effect of the *Met* variant are reflected in brain activation (using brain imaging) and behaviours (using more ecologically relevant behavioural measures).

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APPENDIX:

I.

INFORMATION

Clinical and Pharmaco-genetic study of Attention Deficit with Hyperactivity Disorder (ADHD)

(Douglas Hospital Research Centre)

Drs Ridha Joober and Natalie Grizenko

Who is conducting this study?

This study is being conducted by a team of researchers including child psychiatrists (Drs Natalie Grizenko, Philippe Lageix), psychiatric geneticist (Dr Ridha Joober) and psychologist (Dr Valentin Mbekou) working in the field of attention deficit hyperactivity disorder (ADHD). The team is highly committed to exploring the causes and developing better treatments for ADHD. About 4-5% of youngsters in Canada and worldwide are affected with ADHD. The high frequency and the severe consequences of this disorder motivate our research.

Is ADHD inherited?

It has been known for a long time that ADHD tends to run in families. Also, ADHD may appear in families without a past history of this disorder. On the other hand, a child of two affected parents (that is, both parents having ADHD themselves) will not necessarily be affected. Recent scientific studies have confirmed these observations and show that genes

are very important in predisposing an individual to ADHD. However, we still do not know which specific genes are involved in this disorder

What is the goal of this study?

There is strong evidence that one or several genes can lead to the behavioural changes observed in ADHD. Genes may also be involved in the way the disorder evolves (how severe it is and whether it responds to treatment medications or not). The purpose of this project is to identify genes that are involved in ADHD or that are involved in the way this disorder responds to methylphenidate (Ritalin), the medication usually prescribed for treatment.

What are genes?

Genes are the basis of heredity between relatives. Each person inherits one set of genes from his/her father and a set from his/her mother. In our laboratory, we can identify and locate genes. The principal goal of our study is to find the genes inherited by children who have developed ADHD ("affected children"). For this purpose, we need the participation of affected children as well as their siblings (brothers and sisters) and parents.

Why are we looking for a different gene?

We believe that searching for a gene is one of the best strategies we have to find a cause for ADHD. Afterward, we can discover exactly what the gene does and what is different about it in people who have ADHD. We are hopeful this knowledge will help researchers to improve the treatment of this disorder by focusing their treatment on the specific problem.

What will be your role in this study?

Child with ADHD: Your child will be asked to have an interview with a doctor. He/she also will be asked to participate in neuropsychological testing that involves, performing computer tests where he/she will be asked to push a button when he/she sees specific signals, sorting cards, classifying figures, memorizing sequences of movements, drawing simple lines, choosing between different rewards and performing school type work. The child will also be asked to wear on his wrist a small watch-like device (actiwatch) that measures motor activity on two occasions to measure changes in activity levels during treatment.

In order to assess the quality of response to medication, each child who gives his/her assent (agrees) to participate will be given 0.5 mg/Kg of Ritalin per day divided in a twice a day dosing for one week and a sham medication (called "placebo" a capsule that looks exactly like the real medication but not containing Ritalin) for another week. Ritalin is the most used medication to treat ADHD and the dose of 0.5 mg/Kg is the usual dose prescribed by physicians. The reason we give placebo and Ritalin for one week each is that the behaviour of the child may fluctuate from day to day. We therefore need a longer period to evaluate the changes under medication. The child, his/her parents and the treating physician will not know which week the child is receiving Ritalin or placebo. The child will be asked to put a small watch-like machine on his wrist (actiwatch). This watch is sensitive to movement and will measure the quality of his sleep. This watch will be put on the child's wrist when she/he goes to bed and take it off when she/he wakes up. During the two weeks, the child will be evaluated by his/her treating team, teacher(s) and parents.

Furthermore, in order to better determine the optimal dose for each child, three additional days will be included following the 2 week evaluation to observe the quality of response at 0.3, 0.5, and 1.0 mg/Kg doses of Ritalin under additional testing conditions.

In order to study genes, we will need to take, on one occasion, a 40 cc (about 1 ounce or 40 ml) sample of blood. This small amount of blood is about twice the amount taken in a routine clinical blood test. Blood samples will be used to determine the frequency of some genes in children with ADHD in order to compare them to those of non-affected subjects (parents and siblings).

Parents: Parents will provide information about family history, complications which may have arisen during pregnancy and delivery, as well as the donation of a blood sample (40 ml) in order to carry out a comparison between their genes and those of their affected and unaffected children. Parents will be asked to complete a behavioural evaluation of their child on three occasions. Before the start of the medication trial, the parent will fill out a questionnaire regarding the presence and severity of sleep problems in his child. The parent will be asked to put and take off the motion sensitive watch on the wrist of his child during the two weeks of treatment. The parent will also complete a sleep log where he will note the time of turning off the light in the evening and turning the light on the next morning and document very briefly the quality of the sleep of their child. Parents will also be asked to complete a brief behavioural questionnaire and a diagnostic interview for each of their children.

Siblings: We will ask one non-affected sibling (the one who is closest in age and with the same gender whenever possible) to provide a blood sample. This blood sample will allow

us to compare his/her genes with those of his/her affected sibling and his/her parents. Only siblings who assent to participate in this study will be included.

Withdrawal from the study

Parents can withdraw their children from this study at any time. The refusal to participate in the study will not affect the quality of care provided to the child.

Will participants benefit in any way from the study?

The advantages for your child in participating in this clinical and genetic study are that he/she will receive a comprehensive clinical and neuropsychological evaluation, free of charge. Results of this evaluation will be available to parents and the treating team of the child, if the parents agree. The results of the detailed clinical and neuropsychological evaluations may help his/her treating team and the school personnel in determining appropriate treatment for ADHD. The fact that the child will be treated one week with placebo and one other week with Ritalin will allow us and his/her treating team to see how the child responds to Ritalin under carefully monitored conditions and with a variety of specialized behavioural rating instruments and tests. The subsequent administration of several different doses of Ritalin will allow the treating team to fine-tune the dose of medication most beneficial to the child.

Your participation will help to increase knowledge about ADHD and may eventually lead to a better understanding of what causes ADHD, and may one day be useful in the treatment and prevention of ADHD.

Although it is not the primary purpose of this study, the genetic results may possibly lead to commercial applications. In this case, patients or their parents will not benefit from the commercial application resulting from having given blood.

Are there any risks to participating?

Our study includes the administration of a clinically relevant dosage of Ritalin, a medication that has been the treatment of choice for ADHD, in use for years. Sometimes the medication may induce side effects such as loss of appetite or nausea, difficulties with sleep, upset stomach, tension, anxiety, dizziness, blurred vision or headaches. If these or other side effects occur, the treating physician could adjust the dose or, if necessary, stop the research study.

If your child is currently receiving Ritalin and is enrolled in the study, the fact that your child will not receive medication for a one week period is not expected to be associated with clinically significant discomfort. Indeed, it is rather good clinical practice to give medication holidays for children treated with Ritalin to reassess the need for medication.

The actiwatch, which measures motor activity during sleep, runs on battery and is insulated. It poses no risk. For parents and siblings, there are no inconveniences except for having blood drawn, which can sometimes lead to bruising that disappears in a few days.

CONSENT FORM

CLINICAL AND PHARMACO-GENETIC STUDY OF ADHD

(FOR CHILDREN IN THE DAY HOSPITAL PROGRAM)

(McGill University)

Dr. R Joober & Dr. N Grizenko

I, _____, consent to have my children participate

(please print)

and participate myself in the clinical and genetic study on ADHD to be carried out by Drs Ridha Joober, Natalie Grizenko and associates of the Douglas Hospital Research Centre.

By signing this form:

1. I understand that the purpose of the study is to improve our knowledge about children with ADHD and to look for genes which may play a role in the cause of this disorder or in the way children with ADHD respond to medication. This investigation involves the study of children with ADHD, their parents and their siblings.
2. I confirm that my child and one of his/her non-affected siblings have agreed to participate in this study.
3. I agree to be interviewed by a health professional. During the interview I will be asked questions about my general health and whether I or members of my family have a history of mental illness, and, if so, the nature of the mental illness. The interview will

last approximately 60-90 minutes. I will be interviewed about children's behaviour. The interview will last about 90-180 minutes. I will also be interviewed on events related to the pregnancy and birth of my child who is involved in this study and his/her siblings. This interview will also last 60 minutes. Finally, I will be asked to observe and assess the behaviour of my child on three occasions.

4. I agree that my child will undergo clinical and neuropsychological evaluations. All the evaluations are part of usual and good clinical practice for children with ADHD.

5. I agree that my child affected with ADHD will be treated for a period of one week with Ritalin at appropriate doses and one week with a sham medication (placebo) that looks exactly like Ritalin but does not contain medication. My child, the treating physician, and myself will not know which week Ritalin or placebo are prescribed. I also agree that at the end of the two weeks of treatment, my child will receive 0.3, 0.5 and 1.0 mg/Kg of Ritalin on three different days. On each of these days, my child will receive neuropsychological and clinical evaluations that will allow the treating team to determine the optimal dose of Ritalin he/she may need.

6. I agree to document the quality of sleep of my child, and to complete a sleep schedule every day during the two weeks of the medication trial. This will be done with the help of a small watch-like machine which is sensitive to movement and which will measure the quality of sleep of my child. I will put this "watch" on my child's wrist when she/he goes to bed and take it off when she/he wakes up.

7. Before the start of the medication trial, I will fill out a questionnaire regarding the presence and severity of sleep problems in my child. My child will also be asked to rate his/her sleepiness.

8. I agree that my child affected with ADHD, one of his/her siblings not affected with ADHD and myself give a single 40 cc sample of blood (about 1 ounce, same as 40 ml) that will be drawn from an arm vein. This amount of blood is about twice the amount normally drawn for routine blood tests. Aside from minor discomfort and possibly some bruising, the procedure is quite safe. The blood sample will serve as a source of genetic material so that the investigators may look for genes that may be responsible for ADHD.

9. I have been informed and I agree that the results of clinical investigations and neuropsychol-ogical tests relevant for the clinical management of my child's condition will be available to the treating team. These tests and interviews are the following: Continuous Performance Test (CPT), Wisconsin Card Sorting Test (WCST), Self Ordered Pointing Test (SOPT), Tower of London, Wide Range Assessment of Memory and Learning (WRAML), Wide Range Achievement Test (WRAT-R), Delay Aversion Task, Wechsler Intelligence Scale for Children (WISC-III), interactive self report interview for children (Dominic), Child Behavioural Checklist, Conner's Global Index and the Diagnostic Interview Schedule for Children (DISC-IV) and Line-Drawing.

10. I have been informed that all information obtained during the study, except the tests listed in point 9, will be available only to researchers involved in the study and will remain confidential. To ensure confidentiality, information will be coded (i.e. names removed) and maintained in locked files. Genetic material will be identified by codes and stored in a secure facility accessible only to researchers involved in this study. This genetic material will be destroyed after 15 years of storage. If any scientific publication arises from the study my identity will not be revealed.

11. I have been informed that my child may benefit from a thorough clinical and neuropsychological evaluation. There is no charge for this evaluation. The results of the study may also help the treating team to adapt the treatment (including medication) that my child is receiving. Although it is not the primary purpose of this study, results from genetic testing may lead to commercial applications. In this case, I will not benefit from the commercial applications that would result, in part, from the blood and information I gave to the researchers.

12. I have the option, for myself and for my children, to withdraw from the study at any time without prejudice to any future care my relatives or I may require. In the case I want to withdraw, my genetic material will be destroyed.

13. I have been informed that if I have any questions about this research, I may contact by telephone either Dr Natalie Grizenko at (514) 761-6131 ext. 2113 or Dr. Ridha Joober at (514) 761-6131 ext. 2404 who will answer my questions. If I have any question about my rights as a patient, or as a research subject, I can phone the Douglas Hospital Ombudsman at (514) 762-3010 ext. 2255.

14. I have been given a copy of this information and consent form. The content of the information form has been explained to me to my satisfaction and my child has been explained the content of the information form in terms that are comprehensible to him/her.

_____	_____
Signature of Parent/Guardian	Signature of Witness
Date:_____	Date:_____
_____	_____
Signature of Parent/Guardian	Signature of investigator:
Date:_____	Date:_____

CONSENT FORM FOR FUTURE CONTACTS

GENETIC AND CLINICAL STUDY OF ADHD

(MCGILL UNIVERSITY)

DR. R JOOBER & DR. N GRIZENKO

I, _____, give researchers conducting the clinical and genetic study on ADHD or their collaborators permission to contact me in the future:

Check the box corresponding to your choice. You can check one or both choices

- ☐ To inform me of any relevant medical condition concerning my children or myself that they may discover while they are conducting their research.
- ☐ To inform me about the general findings of their research.

II.

Consent Document

Genetic Research and DNA Banking

Clinical and Pharmaco-genetic study of Attention Deficit with Hyperactivity Disorder (ADHD)

Principal researcher responsible for the project:

Dr. Ridha Joober – psychiatric geneticist and Dr. Nathalie Grizenko – child psychiatrist

Collaborators:
Dr. Philippe Lageix – child psychiatrist and Dr. Valentin Mbekou – psychologist

Institution:
Douglas Hospital Research Centre

Project sponsored by: **Canadian Institutes of Health Research (CIHR)**

1. RESEARCH PROJECT DESCRIPTION

1.1 Justification:

- **Purpose of the research**

This study is being conducted by a team of researchers including child psychiatrists, psychiatric geneticist and psychologist, working in the field of attention deficit hyperactivity disorder (ADHD). The team is highly committed to exploring the causes and developing better treatments for ADHD. About 4-5% of youngsters in Canada and worldwide are affected with ADHD. The high frequency and the severe consequences of this disorder motivate our research.

It has been known for a long time that ADHD tends to run in families. Also, ADHD may appear in families without a past history of this disorder. On the other hand, a child of two

affected parents (that is, both parents having ADHD themselves) will not necessarily be affected. Recent scientific studies have confirmed these observations and show that genes are very important in predisposing an individual to ADHD. However, we still do not know which specific genes are involved in this disorder

The principal goal of our study is to find the genes inherited by children who have developed ADHD ("affected children"). For this purpose, we need the participation of affected children as well as their siblings (brothers and sisters) and parents.

- Simplification of relevant terms

DNA: Molecule containing all the transmissible genetic information, which controls the activities of the body cells. DNA provides the instructions for determining the hereditary characteristics of a person such as eye colour or blood type.

Genes: Genes are the basis of heredity between relatives. Each person inherits one set of genes from his/her father and a set from his/her mother. In our laboratory, we can identify and locate genes.

1.2 Description of the research project:

There is strong evidence that one or several genes can lead to the behavioural changes observed in ADHD. Genes may also be involved in the way the disorder evolves (how severe it is and whether it responds to treatment medications or not). The purpose of this project is to identify genes that are involved in ADHD or that are involved in the way this disorder responds to methylphenidate (Ritalin), the medication usually prescribed for treatment.

1.3 Request for participation

We are asking for your participation in this research project.

2. PROGRESSION OF THE RESEARCH PROJECT

2.1 Procedures

Child with ADHD: Your child will be asked to have an interview with a doctor. He/she also will be asked to participate in neuropsychological testing that involves, performing computer tests where he/she will be asked to push a button when he/she sees specific signals, sorting cards, classifying figures, memorizing sequences of movements, drawing simple lines, choosing between different rewards and performing school type work.

In order to assess the quality of response to medication, each child who gives his/her assent (agrees) to participate will be given 0.5 mg/Kg of Ritalin per day divided in a twice a day dosing for one week and a sham medication (called "placebo" a capsule that looks

exactly like the real medication but not containing Ritalin) for another week. Ritalin is the most used medication to treat ADHD and the dose of 0.5 mg/Kg is the usual dose prescribed by physicians. The reason we give placebo and Ritalin for one week each is that the behaviour of the child may fluctuate from day to day. We therefore need a longer period to evaluate the changes under medication. The child, his/her parents and the treating physician will not know which week the child is receiving Ritalin or placebo. During the two weeks, the child will be evaluated by his/her treating team, teacher(s) and parents.

Furthermore, in order to better determine the optimal dose for each child, three additional days will be included following the 2 week evaluation to observe the quality of response at 0.3, 0.5, and 1.0 mg/Kg doses of Ritalin under additional testing conditions.

In order to study genes, we will need to take, on one occasion, a 40 cc (about 1 ounce, same as 40 ml) sample of blood. This small amount of blood is about twice the amount taken in a routine clinical blood test. Blood samples will be used to determine the frequency of some genes in children with ADHD in order to compare them to those of non-affected subjects (parents and siblings).

Parents: Parents will provide information about family history, complications which may have arisen during pregnancy and delivery, as well as the donation of a blood sample (40 cc) in order to carry out a comparison between their genes and those of their affected and unaffected children. Parents will be asked to complete a behavioural evaluation of their child on three occasions. Parents will also be asked to complete a brief behavioural questionnaire and a diagnostic interview for each of their children.

Siblings: We will ask one non-affected sibling (the one who is closest in age and with the same gender whenever possible) to provide a blood sample. This blood sample will allow us to compare his/her genes with those of his/her affected sibling and his/her parents. Only siblings who assent to participate in this study will be included.

2.2 Duration of the research project

The project will be continuing until March 2004, and will be asking for five year renewal.

2.3 Scope of the research project

This research will take place at Douglas Hospital (Montreal, Quebec).

2.4 Access to your medical record

The research team will consult your medical record to obtain information, which is pertinent to the research project.

2.5 Combination with other information

Not applicable.

3. STORAGE AND SAFEKEEPING OF DNA SAMPLES

3.1 Identification of the sample

We will protect the confidentiality of the samples by assigning them a specific code. Your DNA sample will not be specifically identified but a code will link you to the sample. Decoding can only be performed by the principal researcher or an individual authorized by the latter.

3.2 Length of storage

Samples of your DNA will be kept at Douglas Hospital under the responsibility of Dr. Ridha Joober for 15 years after the end of the research project. After this time, all the samples will be destroyed.

Samples of your DNA will be kept in the form of immortalized cell lines, hence for a 15 year period at Montreal General Hospital

3.3 Other research

A new consent will be necessary for the use of your coded DNA sample in other research. May we contact you in the future for other research?

Yes No

4. BENEFITS

You will receive no personal benefit from your participation in this research project. We hope, however, that the results obtained will permit us to further our knowledge in the area of ADHD by increasing the knowledge about ADHD and may eventually lead to a better understanding of what causes ADHD, may one day be useful in the treatment and prevention of ADHD and eventually, benefit society as a whole.

5. RISKS

5.1 Physical risks

Although the taking of the blood sample causes no serious problems for most people, it can cause some bleeding, bruising, malaise, dizziness, infection and/or discomfort at the injection site.

5.2 Socio-economic Risks

One of the risks associated with this research project relates to the disclosure of the results or the disclosure of your participation to third parties. Mere participation in genetic research projects could compromise or diminish your chances and the chances of your family of obtaining insurance (life insurance, disability, mortgage, or health) or certain types of employment.

6. CONFIDENTIALITY

6.1 Safety/security of the data

All of the information obtained about you and the results of the research will be treated confidentially. This information will be coded, and kept under lock and key. The study file will be kept at Douglas Hospital under the responsibility of Dr. Ridha Joober and also in the Electronic files of Douglas Hospital. Your participation and the results of the genetic part of the research will not appear in your medical record. The results of this study may be published or communicated in other ways, but it will be impossible to identify you.

6.2 Third-party Access to Results

Unless you have provided specific authorization or where the law permits or a court order has been obtained, your personal results of the genetic part of the study will not be made available to third parties such as employers, governmental organizations, insurance companies or educational institutions. This also applies to your spouse, other members of your family and your physician. However, for the purposes of ensuring the proper management of the research, it is possible that a member of an ethics committee, a Health Canada representative may consult your research data as well as your medical record.

7. COMMUNICATION OF RESULTS

You can communicate with the research team to obtain information on the general progress or the results of the research project. However, we will not communicate any individual results to you. You can communicate with the research team to obtain information on the status of the work or the general results of the research project. In the case where there are scientifically validated results with possible impact for your health and preventive measures or treatment are available, would you like to be informed through a physician?

Yes No

The communication of this type of information carries risks for you and your family, such as anxiety, discrimination (employment, insurance), and has implications for reproductive decisions. The results of the analysis may uncover non-paternity but this will not be communicated to you.

8. GENETIC COUNSELLING SERVICES

At any time, you can be referred to a genetic counsellor.

9. COMMERCIALIZATION/RENUNCIATION

The analysis of your DNA sample may contribute to the creation of commercial products from which you will receive no financial benefit.

10. CONFLICT OF INTEREST

The principal researcher and/or the institution is/are paid by the Fonds de la Recherche en Santé du Québec (FRSQ) and Canadian Institutes of Health Research (CIHR) company, which is sponsoring this project.

11. RECRUITMENT OF OTHER FAMILY MEMBERS

Over the course of the study, it is possible that we may ask you or a person you will designate, to contact other members of your family to offer them the opportunity to participate in the study. The researchers and their medical team cannot personally contact your family members for recruitment purposes.

12. FREEDOM OF PARTICIPATION AND PERIOD OF REFLECTION

Your participation is completely free and voluntary. The quality of medical services available to you will not be affected by your decision. You may take the time necessary to reflect on your decision and discuss your participation in the project with persons close to you before giving us your answer. You are free to withdraw from the study at any time. If you withdraw, your DNA sample will be retraced and destroyed.

13. COMPENSATION FOR EXPENSES INCURRED AND FOR INCONVENIENCE

Expenses incurred by reason of your participation will not be reimbursed.

14. CIVIL LIABILITY

If you suffer any injury as a result of your participation in this project, you retain all legal recourses against the research collaborators.

15. RESOURCE PERSONS

Members of the research team

If you would like additional information regarding the progression of the research project or would like to communicate any change of address to us, you can contact Dr. Natalie Grizenko at (514) 761-6131 ext 2113 or Dr. Ridha Joober at (514)761-6131 ext. 2404 during the regular office hours.

Ombudsman, ethics committee or authorized person

If you would like to discuss your participation with an individual not directly involved in the project, we invite you to contact Douglas Hospital Ombudsman at (514) 762-3010 from outside the hospital or ext. 3287 from Douglas Hospital internal telephone.

16. FINAL WORD: UNDERSTANDING, FREEDOM, QUESTIONS

_____ (name) explained the nature and the progression of the research project. I have familiarized myself with the consent form and have received a copy. I have had the opportunity to ask questions that have been answered. Upon reflection, I agree to participate in this research project.

17. SIGNATURE, NAME, DATE

Name: _____ Surname: _____

Address: _____

Tel. (home): _____ Tel. (work): _____

I will inform the principal researcher of any change of address.

Signature of participant: _____ Date: _____

18. VERBAL TRANSLATION

I was present during the meeting between the research team member and the participant. I translated, for the participant, the consent form and all information conveyed/presented regarding the research project.

Name: _____ Signature: _____ Date: _____

19. AGREEMENT OF THE RESEARCHER

The research project, as well as the conditions of participation, was described to the participant. A member of the research team answered any questions and explained that participation was free and voluntary.

Name: _____ Signature of the researcher: _____ Date: _____

20. ETHICS BOARD APPROVAL

The research project was approved by the research ethics committee of Douglas Hospital, in October 1999.