Use of multiple strategies to understand the complex genetic architecture of ADHD

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"What the mind of man can conceive and believe, it can achieve"
Napoleon Hill
Author, 1883-1970

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ABSTRACT

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent, clinically heterogeneous neurodevelopmental disorder with a complex etiology implicating both genetic and environmental factors. Although it is well accepted that multiple genes are involved in the pathophysiology of ADHD, no genetic risk variants have been identified beyond doubt. In addition, environmental factors, including, maternal smoking and maternal exposure to stress during pregnancy have been consistently associated with this disorder.

This thesis will describe multiple genetic strategies that may help reduce the "clinical heterogeneity" and "etiological complexity" of ADHD phenotype facilitating the identification of genetic variants, which may help, in dissecting pathways to the disorder.

1. By using the "endophenotypes" approach and selecting *COMT* gene, which is firmly implicated in the modulation of brain catecholamines, we found a tentative association between Catechol-O-Methyltransferase alleles/haplotypes and the modulation of Executive Functions in ADHD children.

2. We used "gene/environment interplay" i.e. stratifying ADHD children based on exposure to maternal smoking during pregnancy and maternal stress during pregnancy and investigated the implication of latrophilin3 gene *LPHN3*, a candidate gene consistently shown to be involved in ADHD (based on linkage studies, and candidate association studies) in increasing the risk for ADHD. This approach allowed the

uncovering of differential associations between single nucleotide polymorphisms (SNPs) within the *LPHN3* and a number of endophenotypes in patients according to their exposure to maternal stress during pregnancy.

3. "Comorbidity" with obesity was employed as a tool to index a more homogenous subgroup of ADHD children and facilitate the identification of genetic variants implicate in ADHD. Using this scheme, we comprehensively (behaviorally and clinically) characterized children with ADHD in relation to their BMI/weight categories. We showed that, self-regulation deficits, usually hypothesized to mediate obesity in children with ADHD, are not more present in children with ADHD and obesity compared to the non-obese ADHD children. Furthermore, in a group of children not exposed to maternal smoking during pregnancy, we observed a novel association between ADHD pertinent phenotypes and a Fat Mass and Obesity (*FTO*) gene polymorphism that has been strongly associated to obesity by genome-wide association studies (GWAS).

In summary, this research work demonstrates the usefulness of multiple strategies to reduce the clinical heterogeneity and etiological complexity of ADHD which may facilitate identification of genetic risk variants and the interaction of these with environmental factors. This in turn may help in elucidating the pathophysiology of ADHD.

RÉSUMÉ

Le trouble déficit de l'attention avec hyperactivité (TDAH) est un trouble neuro-développemental très répandu, ayant une présentation clinique hétérogène et une étiologie complexe impliquant des facteurs génétiques et environnementaux. Bien qu'il soit généralement admis que plusieurs gènes sont impliqués dans la physiopathologie du TDAH, aucune variante génétique augmentant le risque n'a été identifiée avec certitude. En outre, les facteurs environnementaux, y compris, le tabagisme maternel et l'exposition maternelle au stress pendant la grossesse ont été systématiquement associés à ce trouble.

Cette thèse décrira comment l'utilisation de stratégies multiples mettant à profit les données épidémiologiques peut aider à réduire "l'hétérogénéité clinique" et la "complexité étiologique" du TDAH. Ces stratégies facilitent l'identification des variantes génétiques, qui à leur tour peuvent aider à disséquer les différentes trajectoires physiopathologiques conduisant au TDAH.

1. En utilisant l'approche des "**endophénotypes**" cognitifs, et en sélectionnant le gène *COMT (*Catéchol-O-Méthyltransferase) qui est impliqué dans le métabolisme des neuroamines, nous avons identifié une association entre les allèles/haplotypes de ce gène et la modulation des certaines fonctions exécutive (EF) chez les enfants ayant le TDAH.

2. Nous avons utilisé "**la stratification**" des enfants ayant le TDAH en fonction de l'exposition au tabagisme et au stress maternel pendant la grossesse pour investiguer l'implication du gène *LPHN3*, un gène candidat impliqués dans le TDAH (sur la base d'études de liaison et d'association). Cette stratégie a permis la découverte d'associations

différentielles entre des polymorphismes (SNP) du gène *LPHN3* et un certain nombre d'endophénotypes chez les patients en fonction de leur exposition au stress maternel pendant la grossesse.

3. Enfin, nous avons utilisé la "**comorbidité**" fréquemment rapporté entre obésité et TDAH comme un outil pour indexer un sous-groupe plus homogène d'enfants TDAH et faciliter l'identification des variantes génétiques communes au TDAH et à l'obésité. Nous avons comparé des enfants atteints de TDAH catégorisés selon leur Indice de masse corporelle (IMC)/catégories de poids par rapport à leurs caractéristiques comportementales et cliniques. Nous avons montré que les déficits d'autorégulation, une hypothèse souvent avancée pour expliquer la grand prévalence de l'obésité chez les enfants TDAH, ne sont pas associés avec l'obésité observées chez les enfants TDAH. Dans un deuxième temps, nous avons exploré l'association entre des phénotypes pertinents au TDAH et un gène hautement impliqué dans la régulation de la masse adipeuse appelé *FTO*. Nous avons identifié une association hautement significative entre ce gène et un grands nombre de traits pertinent pour le TDAH, particulièrement chez les enfants qui n'out pas été exposés au tabagisme au cours de la grossesse.

En conclusion, ce travail suggère que l'utilisation de plusieurs stratégies visant à réduire "l'hétérogénéité clinique" et "La complexité étiologique" du TDAH peuvent faciliter l'identification des variantes de risques génétiques et l'interaction de celles-ci avec les facteurs environnementaux, ce qui à son tour peut aider à élucider les mécanismes neurobiologiques qui mènent au TDAH.

CONTRIBUTIONS OF AUTHORS

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CHAPTER 8 – Summary, Discussion, and Conclusion

Writing of text = Z. Choudhry

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• Chapter three aimed to study *"endophenotypes"* as a tool to understand ADHD. More specifically, it investigates the relation between the catechol-o-methyltransferase gene and executive function in children with ADHD. The manuscript has been published as: Choudhry Z, Sengupta S, Thakur G, Page V, Schmitz N, Grizenko N, Joober R.(2012) **"Catechol-O-Methyltransferase Gene and Executive Function in Children With ADHD"** in *J Atten Disord*.

• Chapter four aimed to study *"gene-environment interplay"* as an approach to understand ADHD. It deals with investigating the relation between the Latrophilin 3, maternal stress during pregnancy, and ADHD. The manuscript has been accepted as: Choudhry Z, Sengupta SM, Grizenko N, Fortier ME, Thakur GA, Bellingham J, Joober R. (2012) **"LPHN3 and attention-deficit/hyperactivity disorder: interaction with maternal stress during pregnancy"** in *J Child Psychol Psychiatry*.

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• Chapter seven aimed to study *"comorbidity"* as a tool to understand ADHD. The manuscript has been published as: Choudhry Z, Sengupta SM, Grizenko N, Thakur GA, Fortier ME, Schmitz N, Joober R. (2013) **"Association between obesity-related gene FTO and ADHD"** in *Obesity (Silver Spring)*.

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Choudhry Z, Sengupta S, Thakur G, Page V, Schmitz N, Grizenko N, Joober R. (2012) Catechol-O-Methyltransferase Gene and Executive Function in Children With ADHD. *J Atten Disord*.

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LIST OF ABBREVIATIONS

ADHD: Attention-deficit/hyperactivity disorder

ANOVA: Analysis of variance

APA: American Psychiatric Association

ATX: Atomoxetine

CBCL: Child Behavioral Checklist

CBT: Cognitive Behavioral Therapy

CDT: Choice Delay Task

CE: Commission Errors

CGAS: Candidate gene association studies

CGI: Clinical Global Impression

CI: Confidence Interval

CNV: Copy number variant

Conners'-P: Conners' Global Index-Parents

Conners'-T: Conners' Global Index-Teachers

COMT: Catechol-o-methyltransferase

CPT: Continuous Performance Test

DA: Dopamine

D-AMP: Dextroamphetamine

DAT: Dopamine transporter

DISC-IV: Diagnostic Interview Schedule for Children-version IV

DMHUI: Douglas Mental Health University Institute

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition

DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revisions

DSM-V: Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

DR: Dopamine Receptor

EF: Executive Function

EFD: Executive Function Deficit

ERFs: Environmental risk factors

ES: Effect Size

FBAT: Family-Based Association Tests

FW: Finger Windows Test

FTO: Fat Mass and Obesity

G-E: Gene-environment interplay

GXE: Gene-environment interaction

rGE: Gene-environment correlation

GWAS: Genome-Wide Association Studies

HTR: Serotonin receptor

5-HT: Serotonin

IQ: Intelligence Quotient

ICD: International Classification of Diseases

IPT: Interpersonal psychotherapy

IRR: Incidence Rate Ratio

ISI: Inter-Stimulus Interval

LD: Linkage disequilibrium

LPHN: Latrophilin

MAO: Monoamine oxidase

MPH: Methylphenidate

MRI: Magnetic Resonance Imaging

MSDP: Maternal smoking during pregnancy

MESDP: Maternal exposure to stress during pregnancy

nAChR: n-acetylcholine receptor

NE: Norepinephrine

NET: Norepinephrine transporter

NTs: Neurotransmitter systems

NCP: Neurochemical pathways

OCs: Obstetrical complications

OE: Omission Errors

OR: Odds Ratio

PBO: Placebo

PDD: Pervasive developmental disorder

PD: Psychotic disorder

PERFs: Prenatal ERFs

PFC: Prefrontal cortex

RASS: Restricted Academic Situation Scale

SCZ: Schizophrenia

SD: Standard Deviation

SE: Standard Error

SES: Socioeconomic Status

SNAP: Synaptosomal-associated protein

SNP: Single nucleotide polymorphism

SPD: Self-perceived distress

SOPT: Self-Ordered Pointing Task

TOL: Tower of London

VNTR: Variable number of tandem repeats

WCST: Wisconsin Card Sorting Test

WISC: Wechsler Intelligence Scale for Children

WRAML: Wide Range Assessment of Memory and Learning

CHAPTER 1

Introduction

1. BACKGROUND

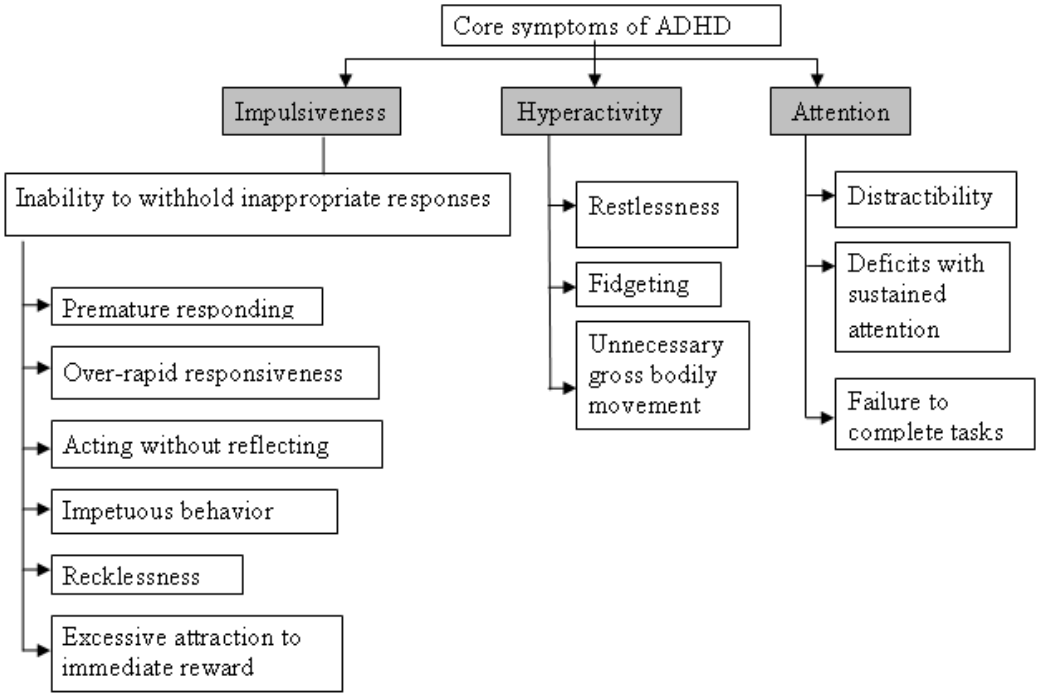
Attention Deficit/ Hyperactivity Disorder (ADHD) is a complex and multifaceted neurodevelopmental condition with a polygenetic and multifactorial etiology (Biederman, 2005). It is the most common childhood psychiatric disorder (Biederman & Faraone, 2005) and affects nearly 8-12% of the childhood population (Faraone, Sergeant, Gillberg, & Biederman, 2003). Children with ADHD usually present with a persistent pattern of inattention, impulsiveness, and hyperactivity (Figure 1.1) compared to other children with the same level of development (Gmitrowicz & Kucharska, 1994; Stefanatos & Baron, 2007). According to the definition in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (*APA, DSM IV*, 1994), there are three behavioural subtypes of the disorder: primarily inattentive, primarily hyperactive/impulsive and the combined subtype.

The core symptoms of ADHD are often aggravated by comorbid conditions including oppositional defiant, conduct, anxiety, mood disorders and learning disabilities (Biederman, 2005). Recent data suggests that subjects with childhood ADHD may continue to suffer ADHD symptoms in their adulthood (Wilens, Faraone, & Biederman, 2004). It is estimated that between 1 to 7 % of the adult population experience symptoms of ADHD (Fayyad et al., 2007) throughout their lifetime. Furthermore, ADHD in adulthood is associated with significant psychiatric morbidity (antisocial personality disorder, smoking and other addictive behaviours, mood and anxiety disorders) (Biederman, Monuteaux, et al., 2006; Biederman et al., 2010).

By virtue of these side effects and the high prevalence of this disorder, ADHD adds stress to the families of patients and places a significant financial burden on the whole society (Newlove-Delgado & Stein, 2012; Verster & Cox, 2008). Consequently, ADHD is considered a serious public health concern (Newlove-Delgado & Stein, 2012).

Stimulant medications are the main pharmacological treatment modality used to ameliorate ADHD symptoms in children and adults (Biederman & Faraone, 2005). It is estimated that, approximately 6% of the school-aged population in Canada use these medications for ADHD (Romano et al., 2005). Although 70% of the children respond adequately to a well conducted trial of stimulants, there is significant variability in clinical response associated with these medications (Spencer, Biederman, Wilens, & Faraone, 2002). Alternative to psychostimulants other medications (atomoxetine, clonidine, antidepressants such as bupropion) (Biederman, Arnsten, et al., 2006; Huang & Tsai, 2011; Rains, Scahill, & Hamrin, 2006) have also been used to treat ADHD, but, these have been associated with greater side effects (Spencer, et al., 2002). In addition to pharmacological interventions, psychological interventions including cognitive behavioral therapy (CBT) (Safren, 2006), academic remediation (Loe & Feldman, 2007) and social skill training (Biederman & Faraone, 2005) have also been shown to be efficacious in ADHD (Emilsson et al., 2011).

Figure 1.1: Description of the three core symptoms of ADHD (adapted here from (Sagvolden & Sergeant, 1998))



2. HISTORICAL EVOLUTION OF THE CONCEPT OF ADHD

The current concept of ADHD as defined by the DSM-IV-Text Revisions (DSM-IV-TR) (Lange, Reichl, Lange, Tucha, & Tucha, 2010) is comparatively new. A review of historical literature shows that symptoms of inattention, hyperactivity, and impulsivity in childhood distinctive of this disorder have been previously noted and described by quite a few authors, philosophers, healers and physicians. These include *Plato (428 – 347 BC),*

Eucharius Roesslin (C. 1470 – 1526), and *Heinrich Hoffman (1854)* (reviewed by (Greydanus, Pratt, & Patel, 2007; Lin-Dyken, Wolraich, Hawtrey, & Doja, 1992; Thome & Jacobs, 2004). Furthermore, in the last century, deficits in attention and conduct disorder-like behavior were noted in children diagnosed mostly, but not always, with *encephalitis (Von Economo's Disease)* (reviewed by (Greydanus, et al., 2007; Lin-Dyken, et al., 1992; Thome & Jacobs, 2004). These behavioral deficits were thought to result from *"minimum brain damage or dysfunction"* (Adler & Chua, 2002; Greydanus, et al., 2007; Strother, 1973). More recently, ADHD was referred to as *"hyperkinetic syndrome"* (Baldursson, Guethmundsson, & Magnusson, 2000) and *"hyperactive reaction of childhood"* (Dodson, 2005; Rubia et al., 2001).

Additionally, this disorder was classified by the *American Psychiatric Association (APA)* in their *1968, 1980, 1987, 1994, and 2000* DSMs as *"ADD and ADHD"* (Maurer & Stewart, 1980; Morgan, Hynd, Riccio, & Hall, 1996; Trujillo-Orrego, Pineda, & Uribe, 2012). Alternatively, *European clinicians* have described this syndrome as *"attention-deficit/hyperkinetic disorder"* (Bruchmuller, Margraf, & Schneider, 2012; Dopfner, Breuer, Wille, Erhart, & Ravens-Sieberer, 2008) inline with the International Classification of Diseases (ICD).

3. ADHD - EPIDEMIOLOGY

ADHD is one of the most common childhood mental-health disorder and its prevalence rate as per DSM IV diagnostic criterion range from 2% to 10% (Biederman & Faraone, 2005; Froehlich et al., 2007; Merikangas et al., 2010; Wolraich et al., 2011). According to the DSM-IV, ADHD has three behavioral subtypes: (i) primarily

inattentive, (ii) primarily hyperactive-impulsive, and (iii) the combined subtypes (Froehlich, et al., 2007). These subtypes account for 43%, 24%, and 33% of the total ADHD subjects respectively. Although the inattentive subtype is most commonly observed, the combined subtype is more likely to receive clinical attention and services (Willcutt, 2012). Further, ADHD is three times more common in males than females (Biederman & Faraone, 2004).

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).

Although ADHD symptoms begin at an early age, these may continue throughout the lifespan in 50% of the cases. The prevalence of adult ADHD was estimated to be between 3 to 5% in adults over 20 years of age (Copeland et al., 2013; Dalsgaard, Mortensen, Frydenberg, & Thomsen, 2013; Fletcher, 2013; Greydanus, et al., 2007; Kessler et al., 2006; McCarthy et al., 2013). 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, whereas the remaining 1.5 million were between ages 20 and 64 years (Greydanus, et al., 2007).

4 ADHD – A CLINICALLY HETEROGENEOUS DISORDER

4.1 CLINICAL FEATURES OF ADHD

Clinically, ADHD diagnosis is achieved if the behavioral symptoms are present before the age of 7 and they impair functioning of children in at least two different settings, such as home and school (Biederman & Faraone, 2005). Interestingly, ADHD is more commonly diagnosed in boys (12%) than in girls (5%) (Bloom, Cohen, & Freeman, 2010; Gingerich, Turnock, Litfin, & Rosen, 1998; Rucklidge, 2010), and this difference may be due to the differences in expressed phenotypes by boys and girls presenting with ADHD (Burke & Stepp, 2012). More specifically, ADHD boys exhibit higher levels of overactive/disruptive behaviour (Sibley et al., 2011; Trepat & Ezpeleta, 2011), whereas ADHD girls show higher levels of inattentive symptoms (Newcorn et al., 2001). This might result in referral bias (Stefanatos & Baron, 2007).

It is noteworthy that the presently accepted clinical definition ADHD is currently being amended (Al-Yagon et al., 2013; DuPaul, Gormley, & Laracy, 2013; Montague & Cavendish, 2013). Indeed, the DSM 5th (DSM V) committee for ADHD has suggested some changes that will affect the future diagnosis of ADHD. Amongst these changes, it is proposed that the age of onset criterion should be changed from age 7 to age 12 in children. The idea behind this suggestion is that since ADHD is a neurodevelopmental disorder its symptoms will present in early childhood, without necessarily causing impairment (Polanczyk et al., 2010). Furthermore, it has been suggested that the term

"subtype" should be changed to "presentation" and a fourth category termed "restrictive inattentive" (Fernandez-Perrone, Martin Fernandez-Mayoralas, & Fernandez-Jaen, 2013; Nikolas & Nigg, 2013) should be added. Restrictive inattentive subjects may present with six or more symptoms of inattention but no more than two symptoms of hyperactivity-impulsivity for the past six months.

The clinical complexity, phenotype heterogeneity, and constant evolution of the definition of ADHD as a behavioral disorder illustrates that its definition remains yet to be established. Thus, identifying potential pathways mediated by particular genetic and environmental factors leading to the emergence of this clinical syndrome may help researchers better define and characterize ADHD.

4.2 DIAGNOSES AND ASSESSMENT OF ADHD

Given the clinical heterogeneity of ADHD syndrome, its diagnosis has been a challenge for psychologists, physicians and healthcare professionals. Till to-date the criteria, guidelines, and terminologies used to describe the characteristic symptoms of ADHD are in constant evolution. This has been done to facilitate diagnosis and to reduce complexity of the disorder.

4.2.1 The Diagnostic and Statistical Manual of Mental Disorders (DSM) Guidelines

4.2.1.1 DSM-IV guidelines

Clinically, as per DSM-IV, the diagnosis for ADHD is made, when a subject demonstrates six or more symptoms from either or both of the two nine-item lists set forth in the DSM-IV-TR manual (APA, 2000). Additionally, a formal clinical diagnosis of ADHD can only be reached if the onset of symptoms happens prior to the age of seven years. The symptoms exist for a minimum of 6 months, are pervasive, and are observed in more than one setting i.e. academic (school) and home. Moreover, these symptoms are age and developmental level inappropriate, maladaptive, and interfere with academic, social, or occupational functioning. Furthermore, a child cannot be diagnosed as ADHD if the symptoms occur exclusively in the course of other psychiatric disorders such as: pervasive developmental disorder (PDD), schizophrenia (SCZ), or a psychotic disorder (PD). Also, if these symptoms could be better accounted for by another specific mental disorder, such mood disorder, anxiety disorder, dissociative disorder, or personality disorder, then the diagnosis of ADHD is unwarranted (APA, 2000).

Studies to-date using DSM-IV classification have shown that ADHD-Inattentive subtype appears to be less common in the clinical population compared to the community sample; also this subtype is more prevalent in female children and individuals belonging to an older age group (Baeyens, Roeyers, & Walle, 2006; Woo & Rey, 2005). Further these studies also show suggest that subjects with ADHD-Hyperactive subtype are of younger age and are relatively rare (Woo & Rey, 2005), and that ADHD-Combined subtype is most prevalent in the clinical population compared to the other two subtypes (Baeyens, et al., 2006).

4.2.1.2 DSM V guidelines; work in progress

Given the chronicity and social impacts associated with ADHD, a considerable body of data has been collected in the past decade in order to understand the nature of ADHD as a disorder and also to treat subject suffering from symptoms of ADHD (Bell, 2011). These empirical findings have helped in the development of the DSM V guidelines, which are to be published latter this year. The DSM V will include multiple changes in the ADHD diagnostic definition focusing on criterion items, cross-situational requirement, age of onset, reorganization of subtypes, and comorbidity with autism (Coghill & Seth, 2011). The DSM V promises to incorporate the current state of knowledge and research in ADHD. This will help in harmonizing APA guidelines with ICD-10; which will help clinicians in providing better care for patients with ADHD.

4.2.2 International Statistical Classification of Diseases (ICD) – 10 Guidelines

The tenth edition of the *International Statistical Classification of Diseases and Related Health Problems* (ICD-10) is a standardized diagnostic classification used for epidemiology, health management and clinical purpose. ICD-10 is developed by the World Health Organization (WHO) and classifies symptoms of ADHD under the label of *"hyperkinetic disorders"*. However, if subject exhibits comorbid conduct disorder with ADHD symptoms, the condition is referred to as *"hyperkinetic conduct disorder"*. Additionally, ADHD symptoms in this classification system have also been referred to as *"disturbance of activity and attention"*, *"other hyperkinetic disorders"* and/or

"hyperkinetic disorders, unspecified" (Adam, Dopfner, & Lehmkuhl, 1999; Hara, 1994; Koster et al., 2004; Rasmussen, 2002)

4.2.3 Other Instruments

In addition to the DSM-IV and ICD-10 criteria, some other diagnostic and evaluation instruments have been extensively used in certain clinical settings to augment and validate the diagnosis of ADHD. These are described in Table 1.1 (Greydanus, et al., 2007; Weisler & Goodman, 2008; Wolraich, et al., 2011). Further, these diagnostic instruments may be used to measure supplementary individualized assessments of behaviours associated with attention, disruption and opposition.

Table 1.1 Instruments for ADHD diagnoses and assessment

Instruments for ADHD diagnoses and assessment

- Child Behavior Check List (CBCL)
- Conners Parent and Teacher Rating Scales
- Conners Adult ADHD Diagnostic Interview
- ADHD Rating Scale-IV (SNAP-IV)
- Barkley's Current Symptoms Scale with supplemental Barkley Scales
- Kiddie Schedule for Affective Disorders and Schizophrenia Diagnostic Interview–ADHD module
- Wender-Utah Rating Scale (WURS)
- Conners Adult ADHD Rating Scales (CAARS)
- Brown Attention Deficit Disorder Scale
- Conners/Wells Adolescent Self-Report of Symptoms (CASS)
- Barkley's Current Symptoms Scale
- Adult ADHD Self-Report Scale
- Adult ADHD Investigator Symptom Report Scale
- Adult ADHD Clinical Symptom Rating Scale

Note. Adapted here from (Greydanus, et al., 2007; Weisler & Goodman, 2008)

4.3 ADHD AND DIFFERENTIAL AND/OR COMORBID DIAGNOSES

Converging lines of evidence suggest that ADHD may be associated with a number of somatic and psychiatric conditions. It is important to distinguish ADHD symptoms from these differential and/or comorbid conditions (Table 1.2) for the proper assessment, diagnosis, and clinical treatment of subjects with ADHD.

4.3.1 ADHD and Comorbid Psychiatric Diagnoses

From a neuropsychiatric point of view, approximately 50-80% of subjects with ADHD display symptoms of *externalizing disorders* (Tureck, Matson, May, & Turygin, 2013) such as oppositional defiant disorder and conduct disorder. Alternatively, ADHD subjects may exhibit behaviours associated with *internalizing disorders* (Graziano, McNamara, Geffken, & Reid, 2013) such as mood and anxiety disorders. Moreover, school going ADHD children widely show comorbid cognitive deficit and learning disabilities (E. Klimkeit, Rinehart, & Bradshaw, 2010; E. I. Klimkeit, Mattingley, Sheppard, Lee, & Bradshaw, 2005).

Studies focusing on the subgroups of children with ADHD and comorbid disorders report that, these children present with a more severe and heterogeneous clinical phenotype which is associated with additional psychological, emotional and social problems (Spencer, 2006). Further, in around 30-60% of ADHD childhood cases, ADHD related and comorbid symptoms persist into adulthood (Faraone et al., 2000), and

these translate into academic, occupational and social failures. Finally, it is also believed that ADHD comorbidity spectrum varies with age (Thome & Reddy, 2009). For example, untreated adult ADHD subjects are believed to be at a higher risk for developing antisocial personality traits and other psychiatric disorders, including, depression, addictive behaviors (substance abuse and other), risk-taking behaviors, and criminal offences (Biederman, Monuteaux, et al., 2006; Leslie & Wolraich, 2007; Molina et al., 2009).

4.3.2 ADHD and Comorbid Somatic Diagnoses

In addition, ADHD is comorbid with somatic disorders, such as, sleep problems, obesity (Cortese, Konofal, Dalla Bernardina, Mouren, & Lecendreux, 2008; Cortese et al., 2005; Cortese, Konofal, Yateman, Mouren, & Lecendreux, 2006; Cortese & Morcillo Penalver, 2010; Konofal, Lecendreux, & Cortese, 2010), and other medical disorders (for details Table 1.2). However, given the scope of this dissertation, we will discuss only the comorbidity between ADHD and obesity.

4.3.2.1 ADHD and Obesity and/or weight gain problems

ADHD has been consistently associated with *obesity* (Cortese & Morcillo Penalver, 2010). However, not much work has been done to better understand the underpinnings of this *comorbidity with obesity*.

Previous studies have shown that, obese children referred for obesity treatment present with a higher than expected prevalence of ADHD (reviewed by (Kalarchian & Marcus, 2012)). Additionally, subjects with ADHD were shown to be heavier than

expected and had a higher propensity towards weight gain. In support of these observations, data from community samples also documents associations between ADHD and obesity. More specifically, results from the National Survey of Children's Health (N=62,887 aged 5 to 17) reported that, subjects with ADHD not using active medication were ~1.5 times more likely to be overweight compared to controls (Waring & Lapane, 2008). Similarly, in a cross-sectional, nationally representative German sample of 2,863 parents and their children aged 11 to 17, associations were observed between overweight status and ADHD diagnosis (Erhart et al., 2012). Results showed that, the prevalence of ADHD was significantly higher for overweight or obese (7%) compared to both normal weight (3.5%) and underweight (4.9%) children. Also, after controlling for potential confounders (age, gender, and socio-economic status) overweight or obese children were twice as likely to have an ADHD diagnosis. Conversely, children with ADHD had an odds ratio of 1.9 for overweight/obesity status. In summary, these observations suggest that, children with ADHD may be at a higher risk for becoming overweight whereas overweight children may be highly predisposed for a diagnosis of ADHD.

Cross-sectional studies investigating the mechanism underlying the comorbidity between ADHD and problems with eating and weight gain have reported findings that may help in understanding the underpinnings of this association. More specifically, in a French clinical sample (N=99) of severely obese adolescents (aged 12 to 17 years) (Cortese et al., 2007), bulimic behaviours were significantly associated with ADHD symptoms. Also, a significant association between bulimic behaviours and ADHD index score (which measures symptoms of inattention, impulsivity and hyperactivity taken together) and a lack of association with the hyperactivity-impulsivity subscale (which

contains only one item on impulsivity) was observed on the Conners Parent Rating Scale. Hence, it was suggested that the association between ADHD symptoms and bulimic behaviours may be accounted for by impulsivity and inattention rather than hyperactivity (Cortese, et al., 2007).

Table 1.2.Health and environmental conditions that may be differentiated from or comorbid with ADHD

Mental health conditions

- 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

Cognitive dysfunction and learning disabilities

- Disorders of mathematics
- Disorders of Reading
- Disorders of written expression

Medical disorders

- Obesity
- Sleep 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

Environmental conditions

- 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 (Biederman, Newcorn, & Sprich, 1991; Culpepper, 2006; Greydanus, et al., 2007; Grizenko, Bhat, Schwartz, Ter-Stepanian, & Joober, 2006; Leslie & Wolraich, 2007; Spencer, 2006)

4.4 CARE AND TREATMENT OF ADHD

4.4.1 Pharmacological treatment

ADHD symptoms may be ameliorated by the use of pharmacological agents such as stimulants and non-stimulant medications (Curatolo, D'Agati, & Moavero, 2010). Interestingly both of these medications result in increasing the synaptic levels of catecholamines, namely dopamine (DA) and norepinephrine (NE), within the brain of children with ADHD.

4.4.1.1 Stimulant medications

Consistent results from studies conducted over the last five decades provide evidence that, methylphenidate (MPH) and dextroamphetamine (D-AMPH) are both effective in the treatment of ADHD (Wilens, 2008). Both of these drugs act by increasing the DA levels within the synapse, which in turn facilitates signaling to the post-synaptic neurons. More specifically, stimulants, such as MPH and D-AMPH, act by blocking the DAT and norepinephrine transporter (NET), and thus block the re-uptake of DA and norepinephrine leading to an increase in synaptic levels of both DA and NE

neurotransmitters (Zetterstrom, Sharp, Collin, & Ungerstedt, 1988). Additionally, D-AMPH also facilitates the release of DA and NE into extra-neuronal spaces and inhibits the catabolic activity of monoamine oxidase (MAO) (Kuczenski & Segal, 1975), an enzyme responsible for the catabolism of catecholamines.

4.4.1.2 Non-stimulant medications

Non-stimulant pharmaco-therapies are also important in the treatment of ADHD. These include medications such as atomoxetine (ATX), which selectively inhibit the re-uptake of synaptic DA in the prefrontal cortex (PFC) and NE and in turn increases synaptic levels of DA and NE within the PFC (Del Campo, Chamberlain, Sahakian, & Robbins, 2011). This change in synaptic catecholamine levels results, in improvement of neurocognitive performance, and amelioration of behavioural symptoms associated with ADHD (Del Campo, Muller, & Sahakian, 2012). In addition to ATX, clonidine and more recently guanfacine, two related non-stimulant medications, have shown to be effective in reducing ADHD symptoms (Sallee, Lyne, Wigal, & McGough, 2009). These drugs are selective alpha2A adrenergic receptor agonist and stimulate postsynaptic alpha2A adrenergic receptors which are highly concentrated in the PFC (Curatolo, et al., 2010). This action in turn facilitates signaling within the PFC resulting in improvement in ADHD symptoms (Sagvolden, 2006; Strange, 2008).

4.4.2 Psychological therapies and other interventions

In addition to pharmacological interventions, psychological therapies (Pelham, Wheeler, & Chronis, 1998) have also shown to be beneficial for ADHD symptoms. These

include psycho-educational input, behavioural therapy, cognitive behavioural therapy (CBT), interpersonal psychotherapy (IPT), family therapy, school-based interventions, and social skills training. Further, these therapies encourage the development of coping strategies for managing the behavioural disturbances of ADHD (Ramsay, 2007, 2011; Taylor et al., 2004). Ideally, multimodal therapeutic approaches involving medications and behavioural therapies are recommended for the treatment for ADHD (Taylor, et al., 2004). However, in certain circumstances such as parents and clinicians having reservations about medication use (Berger, Dor, Nevo, & Goldzweig, 2008), or in children experiencing severe adverse effects on sleep, appetite, or growth (Graham et al., 2011), psychological therapies and other interventions are highly efficacious (Sonuga-Barke et al., 2013). With regards to other support interventions, occupational, speech and language therapies have shown to be beneficial in the development of individual with specific difficulties in these domains.

5. NEUROPSYCHOLOGICAL MODEL OF ADHD

5.1 NEUROPSYCHOLOGICAL THEORIES

It is widely accepted that children with ADHD display deficiencies in attention to detail and maintenance of attention over a length of time (sustained attention). These subjects also exhibit high variability in performance during assigned task (Kebir & Joober, 2011). During the past four decades, researchers have proposed diverse theories in an attempt to identify and explain the mechanisms pertinent to the understanding of these impairments in subjects with ADHD.

Pioneering work by Douglas et al., in the early 70's showed that subjects with ADHD display problems associated with sustained attention and impulse control (Douglas, 1972). Likewise, many other theories focusing exclusively on the cognitive and behavioural aspects of ADHD have emerged since then. These include; executive dysfunction (Pennington & Ozonoff, 1996; Stahl & Pry, 2005); behavioural inhibition deficit (R. A. Barkley, 1997a, 1997b); deregulated arousal/activation (Sergeant, 2000); and delay aversion (Sonuga-Barke, 2002) models.

5.1.1 Executive Dysfunction Model

In the mid 1990's, Pennington and Ozonoff (1996) proposed the executive function deficit (EFD) theory of ADHD, which is widely accepted in ADHD (Pennington & Ozonoff, 1996). Executive functions (EFs) are neurocognitive processes needed to maintain an appropriate problem solving set to attain a future goal (Welsh & Pennington, 1988). Subjects with ADHD display deficits of EFs, motor inhibition and cognition. Supporting this model, a recent meta-analytic review of 83 studies showed that subjects with ADHD (total N=3734) exhibited significant impairment on EF tasks such as response inhibition, vigilance, working memory, and planning (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). These empirical findings provide credence to the EFD hypothesis.

However, this model fails to explain the comorbidity existing between ADHD and other developmental disorders, such as, dyslexia. More recently, multiple deficit models (Pennington, 2006) have been proposed as an extension of this earlier single core model

which promises to explain the complexity of ADHD, its risk and protective factors, and the comorbidities associated with ADHD.

5.1.2 Behavioural Inhibition Model

Work from Barkley (1997) helped in developing a unifying theory of ADHD (R. A. Barkley, 1997b). According to this model, behavioral inhibition deficits, i.e. dysfunctional suppression of behaviour (Nigg, 2006) is the primordial deficit in subjects with ADHD. These deficits in behavioural inhibition are believed to affect other functional abilities such as suppression of irrelevant responses, resisting external interference and performing complex sequences of response i.e. deficits in executive function. These in turn, may affect higher order abilities such as working memory, self-regulation of affect, motivation and arousal; internalization of speech; reconstitution and motor control; fluency and syntax. Furthermore, dysfunctional behavioural inhibition is proposed to influence the motor system leading to executive dysfunction, which in turn would affect motor control (R. A. Barkley, 1997b).

5.1.3 Cognitive-energetic Model

Sergeant (2000) suggested the Cognitive-energetic model of ADHD which focuses on the energy state of the affected children, and links motor behaviour (energy states) to deficits in EFs (Livesey, Keen, Rouse, & White, 2006; Sergeant, 2000). More specifically this model proposes that, deficiencies in inhibition are dependent on the energy state of individuals and also on the effectiveness of information processing. The information processing takes place across three main levels; “Process”: a computational

mechanism consisting of encoding, searching, decision making and motor organization, "State": comprising of energetic pools such as effort, arousal and activation; and "Management/evaluation": linked with planning, monitoring detection and correction of error.

5.1.4 Dual-pathway Model

Edmund Sonuga-Barke (2002) in light of his experimental findings debated that Barkley's unified model of ADHD failed in explaining the heterogeneous nature of ADHD. Alternatively, he proposed the "Dual-pathway Model" (Sonuga-Barke, 2002) which assumes that children with ADHD express a different motivational style i.e. they are motivated to avert delay (Antrop et al., 2006). Furthermore, this model implicates two distinct pathways involved in the executive and reward circuits, in ADHD (Sonuga-Barke, 2002). More specifically, 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 disengagement, whereas behavioural dysregulation leads to behavioral manifestation of ADHD. Alternatively, dysregulation of the motivational style pathway (reward circuits) exhibits as "delay aversion", which mediates the behavioural manifestations of ADHD symptoms and thus affects the task engagement.

5.2 ENDOPHENOTYPES AND ADHD

Genetic epidemiological studies support the relevance of decomposing ADHD into several behavioural dimensions (endophenotypes) as each is likely to have its own

etiological/genetic determinants (Hudziak et al., 1998; Martin, Scourfield, & McGuffin, 2002; Sherman, McGue, & Iacono, 1997). Furthermore, genetic epidemiologists believe that this approach may have potential in unraveling the genetics of complex neuropsychiatric conditions (Carlson, Eberle, Kruglyak, & Nickerson, 2004; Castellanos & Tannock, 2002; Gottesman & Gould, 2003; Joober, Boksa, Benkelfat, & Rouleau, 2002). As eloquently formulated by Gottesman and Gould (2003), "It stands to reason that more optimally reduced measures of neuropsychiatric 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 are intermediate constructs that lie between genes and clinical symptoms (Castellanos & Tannock, 2002), and that, can be objectively measured, ideally in a robust and reliable fashion (a characteristic lacking in their associated diseases) (Flint & Munafo, 2007). Additionally, 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). Furthermore, genetic epidemiologists have defined certain criteria which need to be satisfied before certain traits can be used as endophenotypes in psychiatric genetic epidemiological research. More specifically, endophenotypes should possess attributes such as being simple, quantifiable, rare in the general population, stable over time, specific to the disorder, and potentially associated with genes that may be underlying the disorder (Waldman, 2005).

Investigating *endophenotypes in ADHD* may empower neuroscientists to study underlying neurobiological mechanisms and detect genetic risks relative to ADHD (Almasy & Blangero, 2001; Gottesman & Gould, 2003) as these endophenotypes may share one or more of the same genetic risk variants as ADHD syndrome. In this connection, Castellanos and Tannock (2002), in a selective review, concluded that, endophenotypes which are investigated in genetic studies of ADHD should be solidly grounded in the neurosciences. They further proposed three endophenotypes; a specific abnormality in reward-related circuitry that leads to shortened delay gradients; deficits in temporal processing that result in high intra-subject inter-trial variability; and deficits in working memory, as most compelling quantitative traits worth considering during investigations aiming to uncover the causes of ADHD (Castellanos & Tannock, 2002). Likewise, others have proposed a number of potential endophenotypes that may be grouped in three broad categories, namely neuropsychological, neuroimaging, and electrophysiological endophenotypes (Doyle, Faraone, et al., 2005).

6 ADHD – AN ETIOLOGICALLY COMPLEX DISORDER

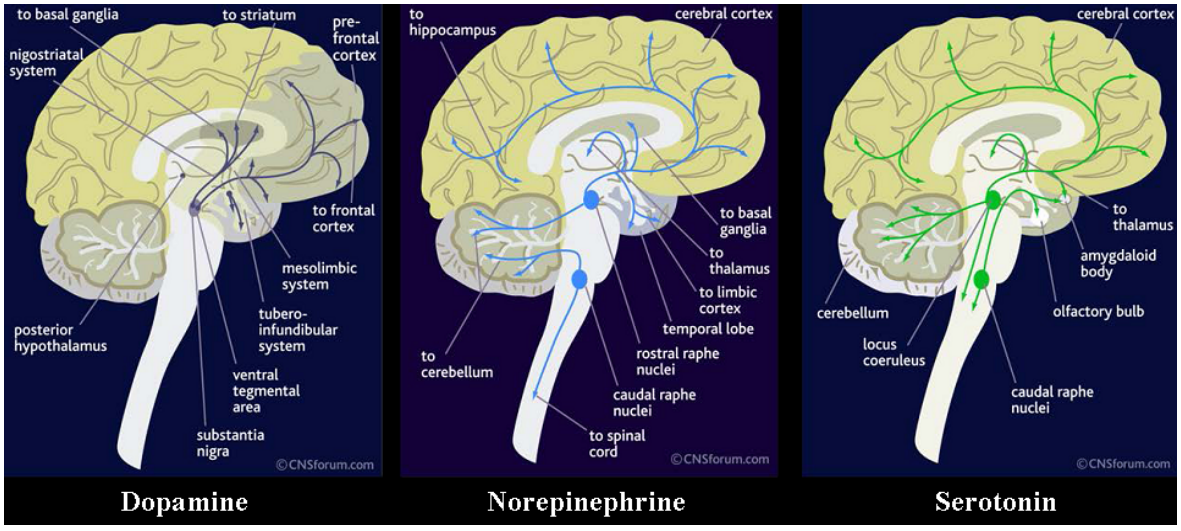
6.1 PATHOPHYSIOLOGY OF ADHD

To date the pathophysiology of ADHD is unclear and there is not yet a unifying theory. However, there are a number of complementing models attempting to explain the origins of this complex disorder.

6.1.1 Neurochemical hypothesis

Amongst the different proposed theories of ADHD pathophysiology, the most compelling is the *"Neurochemical hypothesis (Biological factors) of ADHD"* which proposes that several neurotransmitter systems (NTS) within the brain may be implicated in ADHD. Amongst these NTS, three major neurochemical pathways (NCP), namely dopamine (DA), norepinephrine (NE) and serotonin (5-HT) pathways (Aman, Roberts, & Pennington, 1998; Durston, 2003; Faraone et al., 1995; Sagvolden & Sergeant, 1998) (Figure 1.2) are believed to be important in the pathophysiology of ADHD. However, given that, 5-HT has been implicated in ADHD using evidence derived mainly from animal model studies of ADHD (Kostrzewa, Brus, Kalbfleisch, Perry, & Fuller, 1994; Marx, 1999; Volkow, Gatley, Fowler, Wang, & Swanson, 2000), this pathway will not be discussed further.

Figure 1.2 Major Neurochemical Brain Pathways believed to be involved in ADHD pathophysiology (Adapted from CNS forum, Lundbeck Institute website)



6.1.1.1 Dopamine and ADHD

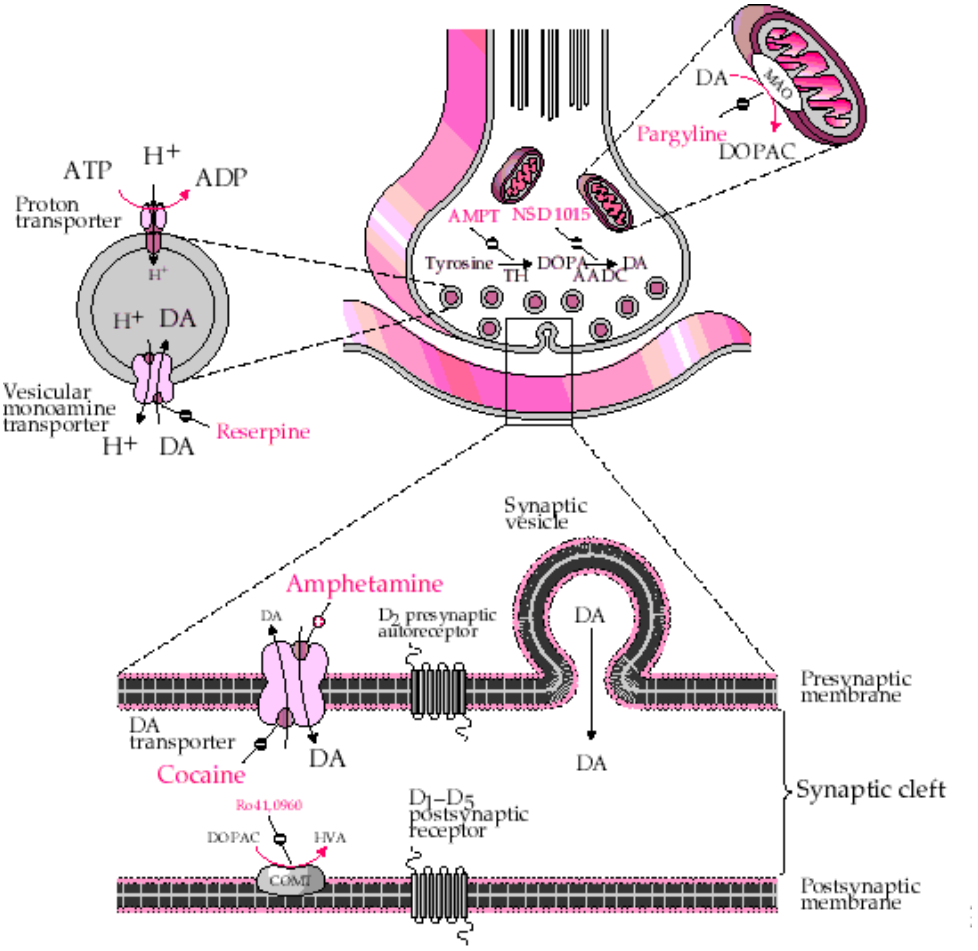
DA is a neurotransmitter involved in the modulation of motor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation (Tzschentke, 2001; Wu, Xiao, Sun, Zou, & Zhu, 2012). Clinical pharmacological studies show an amelioration of behavioural symptoms of ADHD secondary to the use of stimulants (Arnold et al., 1997), which increase DA synaptic concentrations in subjects with ADHD.

Similarly, in animal models of ADHD generated by developing lesions within the DA systems (Sagvolden, 2000; Schneider, Sun, & Roeltgen, 1994; Shaywitz, Klopper, & Gordon, 1978) or by introducing specific genetic alterations within the DA pathways (Genro, Kieling, Rohde, & Hutz, 2010), the use of stimulants results in decreased hyperactivity.

Furthermore, neuroimaging studies also support the role of dysregulated DA brain system in ADHD. More specifically, structural brain imaging studies show overall reduction in brain volume, especially in DA innervated structures, including, the caudate nucleus and globus pallidus (Castellanos & Tannock, 2002; Kieling, Goncalves, Tannock, & Castellanos, 2008). Also, functional neuroimaging studies report decreased activation of the DA pathway (Durston, 2003), encompassing both the neuro-cognitive and reward systems. Finally, candidate genes studies in ADHD subjects report associations between ADHD phenotypes and genetic variants of DA system genes, such as, Catechol-o-methyltransferase *(COMT)*, dopamine transporter (*DAT1*), and DA receptor 4 (*DRD4*) genes (Choudhry, Sengupta, Thakur, et al., 2012; Kambeitz, Romanos, & Ettinger, 2013; Tan-Kam et al., 2013). All of these findings when taken

together suggest an integral role of dysregulated DA brain system in the pathogenesis of ADHD (Arnsten, 2000).

Figure 1.3 An overview of the dopamine pathway to highlight the site of action of MPH (*http://www.chemistry.emory.edu/justice/chem190j/images/fig8.01.gif)*



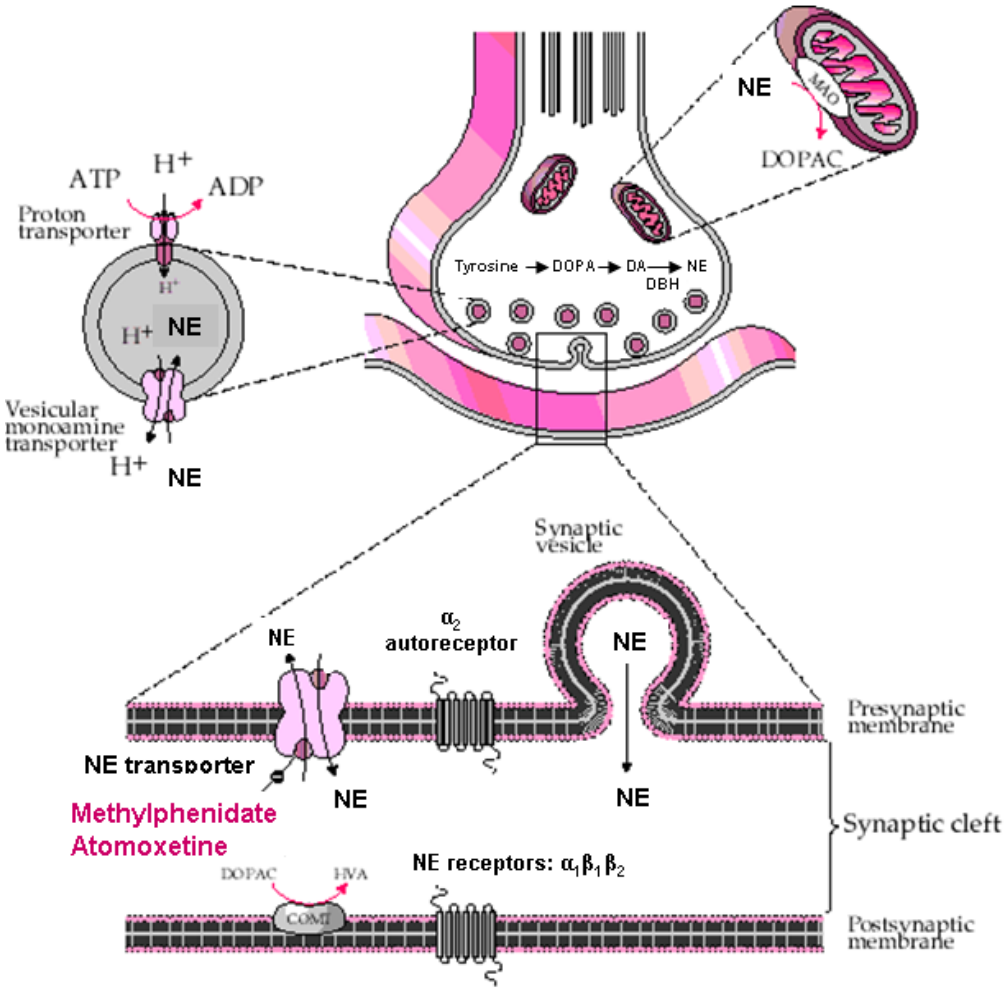
Dopamine (DA) is synthesized by the stepwise conversion of tyrosine to dihyroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (TH), and the subsequent conversion of DOPA to DA by aromatic amino acid decarboxylase (AADC). DA is packaged in small synaptic vesicles, and is released in response to a nerve impulse, when it binds to postsynaptic DA receptors: D_1, D_2, D_3, D_4, D_5. The synaptic concentration of DA is modulated via reuptake by the DA transporter; methylphenidate blocks the reuptake of DA by blocking the DA transporter. Additionally, DA may be inactivated by the enzyme COMT (Catechol-O-methyltransferase), which converts catecholamines into homovanillic acid (HVA). Excess DA that is not packaged into vesicles is degraded by monoamine oxidase (MAO), a mitochondrial bound protein

6.1.1.2 Norepinephrine and ADHD

NE is another important brain catecholamine which is considered to be a major player in the pathophysiology of ADHD (Biederman & Spencer, 2002). It is essential in the modulation of cognitive processes, including attention, alertness and vigilance (Hahn, Robertson, & Blakely, 2003; Jasmin et al., 2002; Ordway et al., 2005; Svensson, 1987). Previous studies show that, noradrenergic projections originating from NE nuclei (locus ceruleus) are found to be abundant within the PFC. PFC is a brain region vital for the modulation of attention and EFs. Consistent findings suggest that, low NE levels within the PFC result in poor self-control, decreased concentration, and greater motor activity (E. Klimkeit, et al., 2010) while restoration of NE levels decreases distractibility and improves cognitive function.

Similarly, clinical pharmacological intervention in subjects with ADHD with a selective NE reuptake inhibitor, ATX (increasing synaptic NE and DA levels), results in amelioration of behavioural symptoms of ADHD (Del Campo, et al., 2011). Finally, candidate genes studies in ADHD subjects report of associations between ADHD related phenotypes and genetic variants of the NE transporter gene (*SLC6A2*) (Sengupta, et al., 2012; Thakur, et al., 2012a). Given these findings it is plausible to consider a pivotal role of dysregulated noradrenergic systems, in addition to dampened DA systems, in the pathophysiology of ADHD.

Figure 1.4 An overview of the norepinephrine pathway



Norepinephrine (NE) is synthesized in identical steps as DA, starting from the substrate tyrosine. However, noradrenergic neurons contain an additional enzyme, dopamine β-hydroxylase (DBH), which converts dopamine to norepinephrine. Four main subtypes of adrenergic receptors have been identified: α_1, α_2, β_1, and β_2. As in the case of DA, the synaptic concentration of NE is modulated via reuptake by the NE transporter (methylphenidate also blocks the NE transporter), and by hydrolysis via COMT. Excess intracellular NE is degraded by MAO.

6.2 ETIOLOGIES OF ADHD

6.2.1 Genetic Factors

6.2.1.1 Behaviour Genetic Studies

6.2.1.1.1 Family Studies

Family studies have irrevocably shown that, there is a higher prevalence of ADHD psychopathology in the families of subjects with ADHD; this finding gives credence to the hereditary basis for ADHD syndrome. More specifically, 10% to 35% of the first degree relatives of subjects with ADHD have ADHD psychopathology. Amongst these family members, siblings are at the highest risk, (approximately 32%) to develop ADHD (Biederman, Faraone, Keenan, & Tsuang, 1991; Biederman, Faraone, & Lapey, 1992; Nigg, 2006; Pauls, 1991; Welner, Welner, Stewart, Palkes, & Wish, 1977). Furthermore, offspring's born to subjects with ADHD, are at a high risk of 40% to 57% to suffer from symptoms of ADHD (R. A. Barkley, Murphy, & Fischer, 2008; Biederman et al., 1995).

Additionally, family studies of ADHD also suggest that, ADHD comorbid with CD may be a familial subtype of ADHD as they are distinct from ADHD alone. More specifically, in the relatives of children diagnosed only with ADHD, the rates of hyperactive symptoms associated with ADHD are high (Biederman, Faraone, et al., 1991). Conversely, in case of ADHD subjects comorbid with CD, the parents and other relatives showed conduct problems, substance abuse, and depression (August & Stewart,

1983; Biederman, Faraone, et al., 1991; Faraone & Biederman, 1997; Faraone, Biederman, Mennin, Russell, & Tsuang, 1998; Lahey et al., 1988; Waschbusch, 2002).

These results point towards the genetic complexity and heterogeneity of ADHD syndrome. Furthermore, these also identify the use of comorbidity as a strategy to index more distinct and genetically homogenous subgroups of ADHD subjects to understand the genetic underpinnings of ADHD.

6.2.1.1.2 Adoption Studies

Earlier studies reported that, compared to adoptive parents, biological parents of hyperactive children showed higher rates of hyperactivity (Cantwell, 1975; Morrison & Stewart, 1973). Furthermore, adopted away boys had a greater chance of developing ADHD if one of their biological parents had been previously judged delinquent or had a criminal conviction (Cadoret & Stewart, 1991). Likewise, work by van den Oord (1994) investigating biologically related and unrelated pairs of international adoptees showed a strong genetic component (47% of the total variance) for Attention Problems as indexed by the Child Behavior Checklist (CBCL) (van den Oord, Boomsma, & Verhulst, 1994). Finally, exploring prevalence of ADHD in parents amongst ADHD children living with their biological parents to those living with adopted families to a control group showed, an elevated prevalence of ADHD amongst biological parents of ADHD children (18% vs. 6% vs. 3% respectively) compared to others (Sprich, Biederman, Crawford, Mundy, & Faraone, 2000).

6.2.1.1.3 Twin Studies

"Twin studies" by virtue of their design and methodology have played an influential role in elucidating important factors implicated in the complex pathophysiology of ADHD. More specifically, these studies have helped in computing the proportions of variance in "traits" influenced by multiple factors. These include, genetic factors (heritability), shared environment factors (common environmental factors shared by twin siblings in a family), and non-shared environment factors (unique environmental factors specific to one child in a family) (Plomin, DeFries, McClearn, & Rutter, 1997). Results from these twin models of ADHD have provided consistent evidence supporting the importance of a genetic contribution in ADHD (Nigg, 2006; Nikolas & Burt, 2010).

Earlier twin research in ADHD focused on the likelihood of twin siblings sharing the same disorder and/or associated symptom phenotype (concordance). These studies demonstrated a higher concordance for ADHD related symptoms (including, hyperactivity and inattention) between monozygotic (MZ) compared to dizygotic twins (DZ) (M. O'Connor, Foch, Sherry, & Plomin, 1980; Willerman, 1973). Further, in some twin samples (Heffron, Martin, & Welsh, 1984; Lopez, 1965) the concordance for hyperactivity within MZ twins was 100% and far less for DZ twin for the same phenotype (Heffron, Martin, & Welsh, 1984; Lopez, 1965). These earlier studies also showed that, concordance for ADHD (as a disorder) within MZ twins was high (ranging between 76-81 %) whereas in DZ twins it was low (ranging between 0-29 %) (Gilger, Pennington, & DeFries, 1992; Sherman, et al., 1997).

Interestingly, more recent twin studies in ADHD have attempted to compute heritability and contribution of environmental factors to ADHD (Nigg, 2006; Nikolas & Burt, 2010). More specifically, these studies report of a high degree of heritability for ADHD (ranges from 0.75 to 0.91 (Levy, Hay, McStephen, Wood, & Waldman, 1997)) with the average heritability being approximately 0.73 (Levy, Hay, & Bennett, 2006). Additionally, these studies show that, the effect of shared environmental factors on ADHD associated traits is negligible (Burt, Larsson, Lichtenstein, & Klump, 2012) and accounts for 0% to 5% of individual differences in ADHD traits. In contrast, results from similar twin ADHD studies suggest that, approximately 9 - 20% of the variance in ADHD symptoms (hyperactivity, impulsivity, and inattention) may be attributed to unique or non-shared environmental factors (Nigg, 2006; Nikolas & Burt, 2010). Further, the unique or non shared environmental factors believed to be associated with ADHD may include, factors associated with individual's social environment and other biological factors experienced by individual that are non-genetic in origin. For example, these can be biological hazards, neurological injuries and even unique parent-child interactions.

6.2.1.2 Molecular Genetics studies

6.2.1.2.1 Linkage studies

Linkage studies are conducted in families that have multiple affected individuals within several generations of their lineage. These studies allow identification of a genetic marker that is always inherited by only those who are affected by the disease. Identification of such a marker which co-segregates with the disorder points towards a

locus likely to contain risk genes for that disorder (Purper-Ouakil, Ramoz, Lepagnol-Bestel, Gorwood, & Simonneau, 2011).

With regards to ADHD, chromosomal regions (such as 5p13, 14q12, and 17p11) have been implicated with the disorder using affected sib-pairs and extended pedigrees. Furthermore, recently a meta-analysis (Zhou et al., 2008) of previous seven ADHD linkage studies (Arcos-Burgos et al., 2004; Asherson, 2004; Bakker et al., 2003; Faraone et al., 2008; Fisher et al., 2002; Hebebrand et al., 2006; Ogdie et al., 2004; Romanos et al., 2008) was done. This meta-analysis identified ten chromosomal regions with linkage signals. Amongst these chromosomal regions, a genome-wide significant linkage (P_{SR}=0.00034, P_{OR}=0.04) was identified on chromosome region from 16q23.1 to the q terminal (Zhou, et al., 2008). Strikingly, no previous candidate gene association study had identified a gene within this region. Additionally, this meta-analysis also reported nine other genomic regions (chromosomes 5, 6, 7, 8, 9, 15, 16, 17) which showed nominal or suggestive evidence of linkage (Zhou, et al., 2008).

6.2.1.2.2 Candidate gene association studies

Candidate gene association studies (CGAS), either family-based or case-control are both based on *a priori* biological hypotheses. In these studies, candidate genes are carefully chosen on the basis of their possible mechanistic implication in the pathophysiology of the disorder (Purper-Ouakil, et al., 2011) e.g. ADHD. Family-based association studies investigate an over-transmission of the risk allele from parents to affected offspring (proband) and case-control studies compare frequencies of genetic variants in both controls and affected probands (Purper-Ouakil, et al., 2011).

A focused review of previous CGAS in ADHD shows that candidate genes in these association studies are selected by targeting important brain systems, such as, dopaminergic, noradrenergic, and serotonergic systems. This gene selection strategy is influenced by the idea that psychostimulants treat ADHD symptoms by acting on brain pathways, increasing synaptic catecholamine levels which in turn ameliorate ADHD symptom severity. Interestingly, it is noteworthy that, after numerous CGAS in ADHD, we still can not implicate specific genes with certainty as a significant contributor in the etiology of ADHD (Franke et al., 2012; Franke, Neale, & Faraone, 2009). However, results from a recent meta-analysis of CGAS in ADHD show that, DA transporter (*SLC6A3/DAT1*), DA receptor D4 (*DRD4*), DA receptor D5 (*DRD5*), serotonin transporter (*SLC6A4/5HTT*), serotonin receptor 1B (*HTR1B*), and synaptosomal-associated protein 25 (*SNAP-25*) may be implicated in ADHD (Gizer, Ficks, & Waldman, 2009).

6.2.1.2.3 Genome-Wide Association Studies (GWAS) of ADHD

Genome-Wide Association Studies (GWAS) are a specialized form of genetic association studies as they do not require *a priori* biological hypotheses(Altmuller, Palmer, Fischer, Scherb, & Wjst, 2001). In these studies, the entire genome is scanned, and a large number of genetic variants (usually >100,000 SNPs) are tested amongst two groups of participants: subjects with the disease (cases) and similar subjects without the disease (controls). The GWAS design promises to identify potential genetic markers associated with the disorder (Hindorff et al., 2009), such as ADHD.

Till now, five GWAS have been conducted in ADHD (Lasky-Su et al., 2008; Lesch et al., 2008; Mick et al., 2010; Neale et al., 2008; Neale et al., 2010) and these GWAS report of 85 top-ranked ADHD candidate genes ($p<0.0001$). However, none of these genetic findings have passed the GWAS significance threshold (10^{-7}). Contrastingly, GWAS conducted in other neuropsychiatric disorders, such as schizophrenia, autism, and Alzheimer's disease have been successful, and their results have identified small effect genetic variants. With regards to the current negative ADHD GWAS findings, it is believed that future studies with much larger sample sizes, more homogenous phenotypes, and stratified subgroups of the disorder would be able to identify alleles associated with ADHD.

6.2.1.2.4 Copy Number Variants (CNVs) in ADHD

Copy number variants (CNVs) are large, rare duplications or deletions within the human genome that may span a single gene or multiple genes (Langley et al., 2011). In different ADHD cohorts, genome-wide analysis of CNVs (Elia et al., 2012; Lesch et al., 2011; Williams et al., 2012; Williams et al., 2010) show overrepresentation of CNVs affecting glutamatergic neurotransmission genes (Elia, et al., 2012; Williams, et al., 2012; Williams, et al., 2010)). Furthermore, these studies also implicate duplications at 15q13.3 as a novel risk factor for ADHD (Elia, et al., 2012; Williams, et al., 2012; Williams, et al., 2010). Finally, results from CNV GWAS suggest that rare structural variations may offer another alternative in detecting putative candidate genes which may be implicated in the etiology of ADHD.

6.2.2 Environmental Factors

Epidemiological studies have associated a number of environmental risk factors (ERFs) with ADHD. These ERFs are categorized them on the basis of the developmental period during which they impart influence on the development of offspring, namely prenatal, perinatal, and postnatal factors (Latimer et al., 2012). Additionally, these studies have also shown that, ERFs occurring during critical periods of fetal development result in significantly detrimental effect on the neurodevelopment of the offspring (Banerjee, Middleton, & Faraone, 2007). Likewise, these studies also suggest that, earlier ERF exposure results in widespread negative developmental consequences for the offspring (Tremblay, 2010).

6.2.2.1 Prenatal risk factors

A review of studies focusing on prenatal development reported that some prenatal ERFs (PERFs) may be implicated in the pathophysiology of ADHD syndrome and related phenotypes. These include, maternal smoking, alcohol consumption, illicit drug abuse, poor diet, stress and anxiety during pregnancy (Froehlich et al., 2011; Latimer, et al., 2012; Purper-Ouakil, et al., 2011).

6.2.2.1.1 Maternal smoking during pregnancy (MSDP) and ADHD

Maternal smoking during pregnancy (MSDP) is a highly prevalent but preventable behavior. In North America, 10-25% women report smoking during pregnancy (Ernst, Moolchan, & Robinson, 2001; Millar & Hill, 2004). Additionally, empirical evidence associates MSDP to varied adverse effects on pre- and postnatal

growth, which in turn are believed to result in cognitive dysfunction and behavioral problems, in children (Banerjee, et al., 2007). In support of this idea, MSDP is consistently linked with ADHD (OR = 2.39) (Linnet et al., 2003). Also, in a case-control study, MSDP resulted in an increased risk (2.7-fold) for ADHD (Milberger, Biederman, Faraone, Chen, & Jones, 1996). In addition, researchers suggest of a dose-response relationship between MSDP and hyperactivity (OR 1.30; 1.08–1.58) (Kotimaa et al., 2003). Finally, MSDP exposure in children results, in lower scores on arithmetic and spelling tasks (Batstra, Hadders-Algra, & Neeleman, 2003), lower IQ scores (Milberger, Biederman, Faraone, & Jones, 1998), as well as deficits in verbal learning. Further, these children also show, deficits in problem solving, and a slower response in eye-hand coordination compared to unexposed children (Cornelius, Ryan, Day, Goldschmidt, & Willford, 2001).

Recently, some researchers have suggested that the association between MSDP and ADHD related phenotype might be mediated by genetic factors. Indeed, this idea is supported by the fact that, there is high comorbidity between smoking behavior and ADHD (McClernon & Kollins, 2008). Additionally, it is believed that this association is predominantly due to shared genetic risks (Obel et al., 2011; Thapar et al., 2009). In summary, MSDP is believed to be implicated in ADHD and related phenotypes. Furthermore, it is suggested that there exist a possible gene – environment interplay between MSDP and ADHD risk genes.

6.2.2.1.2 Maternal exposure to stress during pregnancy (MESDP) and ADHD

Studies exploring effects of environmental risk factors on child development suggest that *prenatal stress* is associated with negative outcomes in children. Supporting this idea, epidemiological data shows that, prenatal stress results in increased rate of spontaneous abortions, fetal malformations, and preterm birth (Hedegaard, Henriksen, Secher, Hatch, & Sabroe, 1996). Further, offspring born to mothers stressed during pregnancy showed worsened intellectual and language abilities (Laplante et al., 2004). Additionally, in a large-scale community study, prenatal stress was linked with childhood externalizing problems in offspring (T. G. O'Connor, Heron, Golding, & Glover, 2003).

Likewise, studies investigating the effects of prenatal stress in children with ADHD suggest that, *prenatal stress* results in negative outcomes, in these children (Grizenko et al., 2012). More specifically, ADHD children born to mothers experiencing moderate and severe stress during pregnancy display more severe ADHD symptoms compared to those ADHD children which are born to mothers experiencing no or minimal prenatal stress (Grizenko, Shayan, Polotskaia, Ter-Stepanian, & Joober, 2008). Similarly, in a large Danish cohort (N=1,015,912) using maternal bereavement as a proxy for stress and controlling for several perinatal and maternal confounders it was shown that, only boys born to bereaved mothers (who had experienced unexpected death of a child or spouse) had a 72% increased risk of ADHD (Li, Olsen, Vestergaard, & Obel, 2010). Also, in another study which investigated the impact of maternal self-perceived distress (SPD) during pregnancy on offspring ADHD showed that, in 992 mother–child pairs from a prospective, longitudinal German sample, SPD during pregnancy was related

to offspring ADHD (Martini, Knappe, Beesdo-Baum, Lieb, & Wittchen, 2010). Alternatively, more recent findings suggest that, the association between maternal stress during pregnancy and ADHD symptomatology may be mediated by certain genetic factors (Choudhry, Sengupta, Grizenko, et al., 2012; Grizenko, et al., 2012).

6.2.2.2 Obstetrical complications as risk factors

A growing body of evidence suggests that ADHD may be associated with obstetrical (pregnancy and delivery) complications (OCs). These include, preterm birth, eclampsia, fetal postmaturity and distress, duration of labor, and antepartum hemorrhage (Ben Amor, et al., 2005).

6.2.2.3 Postnatal and infancy risk factors

In addition, postnatal and infancy risk factors have been suggested to play a role in ADHD. More specifically, *postnatal physical factors* including neonatal anoxia, seizures, traumatic brain injury, lead exposure, food additives, and dietary deficiencies may be implicated in ADHD and related phenotypes (Burnstein, 1992; Galera et al., 2011; Pineda et al., 2007). Additionally, *postnatal social and relational factors* including social adversity, hostile parenting, and parental psychopathology (Deault, 2010; Rodriguez et al., 2009; St Sauver et al., 2004) may also cause ADHD.

6.2.3 Gene and Environment Interplay in ADHD

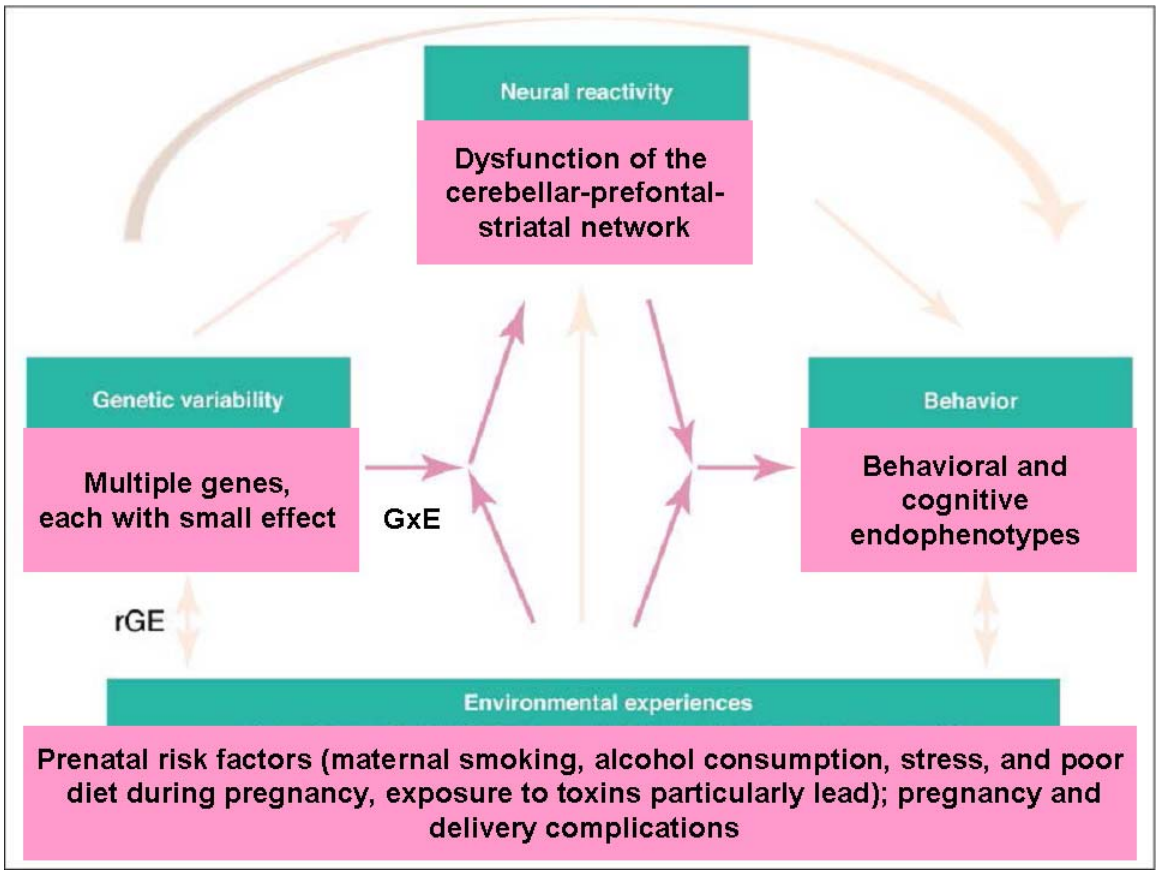
It is now well accepted that, the etiology of ADHD is complex and both genetic and environmental factors are implicated in ADHD. Furthermore, it is also firmly

believed that, investigating genes and environment interplay is essential in better understanding the pathogenesis of ADHD. More specifically, recently it has been suggested that, genetic and environmental factors, as well as the gene-environment interplay (G-E), may be implicated in the etiology of ADHD (Hyde, Bogdan, & Hariri, 2011). Gene-environment interplay includes both gene-environment interaction (GxE) and gene by environment correlation (rGE) (Knopik, Heath, Bucholz, Madden, & Waldron, 2009). More specifically, GxE proposes that the genotype of the individual modulates the sensitivity or response to a specific environmental exposure (Moffitt, Caspi, & Rutter, 2005). Moreover, rGE occurs when the genotype of the individual affects the likelihood of individual's exposure to a particular environment, thus individual's can somewhat shape and select their own environments through their behavior (Caspi & Moffitt, 2006). Recent work by Hyde et al (2011) proposes a model (Figure 1.5) which attempts to implicate genetic and environmental factors, as well as the gene-environment interplay (G-E) in the etiology of ADHD (Hyde, et al., 2011).

A review of ADHD literature identifies some studies that have explored GxE interactions in ADHD. These propose that, GxE interactions may be instrumental in helping researcher further their understanding of the phenotypic complexity of ADHD (Banerjee, et al., 2007). In a cohort of children exposed to MSDP, the 480-bp *DAT1* risk allele was associated with symptoms of hyperactivity and impulsivity. Also, in a twin model, carriers of the *DAT1* 440 allele were 2.9 times more likely to be diagnosed with ADHD combined subtype if they were exposed to MSDP (Neuman et al., 2007). Likewise, in another study, carriers of the risk variant of the *COMT* gene showed conduct disorder symptoms in ADHD only when they when they had low birth weight (Thapar et

al., 2005). Additionally, in a cohort of children exposed to maternal alcohol consumption during pregnancy, the *DAT1* haplotype was strongly associated with ADHD (Brookes et al., 2006). In summary, all these studies point toward the fact that, exploring GxE interactions in ADHD may help to understand the clinical phenotypic complexity, and the etiological heterogeneity in ADHD.

Figure 1.5: Depiction of the multifactorial etiology of ADHD (Adapted from (Hyde, et al., 2011))



7. SUMMARY

In summary, it is well accepted that, ADHD is a clinically heterogeneous disorder with a complex etiology. Given its high heritability, genetic factors are believed to play a significant role in its etiology. In addition, environmental risk factors are also implicated in ADHD.

The use of behavioral and neurocognitive *"endophenotypes"* may reduce the *"clinical heterogeneity"* of ADHD. Additionally, the *"etiological complexity"* associated with ADHD may be reduced by stratifying children based on exposure to major environmental factors implicated in ADHD such as maternal smoking and stress during pregnancy. This approach may be helpful in identifying a more homogenous subgroup of children with ADHD, which in turn may facilitate identification of genetic risk factors that show differential association with ADHD relevant endophenotypes. Moreover, both the *clinical heterogeneity* and *etiological complexity* associated with ADHD may be reduced by using *"comorbidity"*. Because, ADHD and its comorbid disorders, such as obesity, smoking behavior, etc are believed to share common genetic and environmental factors.

HYPOTHESIS

Our central hypothesis is that the complex genetic underpinnings of ADHD can be unraveled by using multiple strategies to dissect the clinical and etiological heterogeneity of ADHD. More specifically, the *clinical heterogeneity* may be reduced by examining clinically relevant endophenotypes and also by indexing a more homogenous subset of subjects by studying ADHD comorbid somatic disorders, such as, Obesity. Furthermore, the *etiological complexity* may be reduced by stratifying children based on exposure to major environmental factors implicated in ADHD, such as, maternal smoking during pregnancy and maternal stress during pregnancy.

OBJECTIVES

1. ***Use of Endophenotypes to investigate genetics of ADHD***

- To test the association between single nucleotide polymorphisms (SNPs) in *COMT* gene and executive functioning phenotype, in children with ADHD.

2. Use of Environmental factors to investigate genetics of ADHD

- To test the association between single nucleotide polymorphisms (SNPs) in *LPHN3* gene and ADHD in two groups of children stratified based on maternal exposure to stress during pregnancy and maternal smoking during pregnancy.

3. Use of Co-morbidity (obesity) to investigate genetics of ADHD

- To investigate the relation between body weight and clinical and behavioural characteristics of children diagnosed with ADHD.
- To examine the role of self regulation deficits in the relationship between body weight and ADHD in children.
- To investigate the possible association between *FTO* gene SNP *rs8050136* and ADHD in two groups of children stratified based on maternal smoking during pregnancy.

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CHAPTER 2

Study design

OVERVIEW

The current PhD research work is a subset of the "Clinical and Pharmacogenetic Study of Attention Deficit with Hyperactivity Disorder (ADHD)" (a clinical pharmaco-genetic study registered in ClinicalTrial.gov database # NCT00483106). It is a two-week double-blind, placebo-controlled, crossover randomized trial of methylphenidate (MPH) conducted under the supervision of Drs Ridha Joober and Natalie Grizenko at the Douglas Mental Health University Institute.

In this chapter, we will be presenting an overview of each of the methods used in this trial.

STUDY CONTEXT

After the completion of the baseline evaluations, children with ADHD either receive one week of placebo or 0.5mg/kg of MPH in a b.i.d dose, and these are then crossed over during the second week. The response to treatment (MPH) is evaluated by examining the change scores obtained by ADHD children on different cognitive, emotional and motor assessments conducted in the lab. In addition, therapeutic response is further assessed by examining improvement on the Conners' scales as assessed by parents at home and teachers at school.

RECRUITMENT OF SUBJECTS WITH ADHD

Children with ADHD were referred to the ADHD clinic at the Douglas Mental Health University Institute (DMHUI) in Montreal, by schools, community social

workers, family doctors, pediatricians, and child psychiatry outpatient clinics. The research protocol for the study was approved by the Research Ethics Board of the DMHUI. During the recruitment process, all details pertaining to the study were explained to the parents of children with ADHD who provided written informed consent on behalf of their children. Additionally, children with ADHD gave their verbal assent to participate in the project.

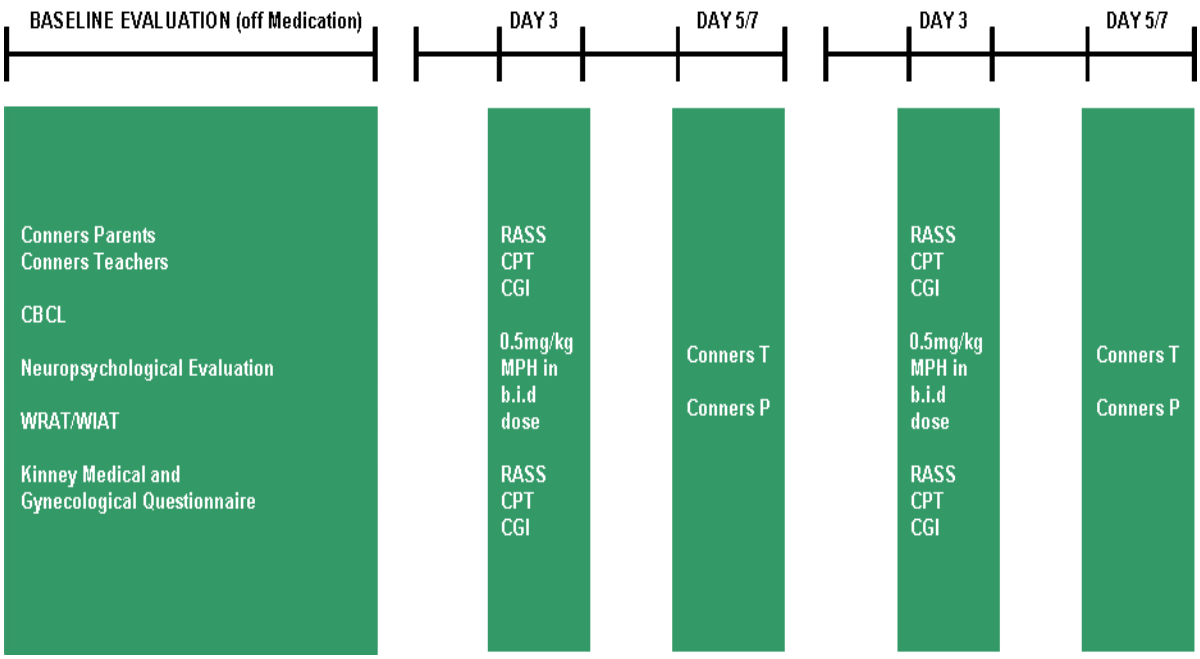
INCLUSION CRITERIA

1- Age: 6-12 years old

2- Diagnosis of ADHD (based on DSM-IV criteria), made by an psychiatrists (RJ or NG) based on:

- Clinical interview of the child and at least one parent
- Structured interview with parents using the Diagnostic Interview Schedule for Children version IV (DISC-IV, parental report)
- Evaluation of behavior in school by teacher (including the Conners' Global Index (CGI) -Teacher version), and at home by parents (CGI-Parents). Moreover, at least one CGI score either Parents or Teachers should be 65 or over.

Figure 2.1: Timeline of the two-week double-blind, placebo-controlled crossover trial of methylphenidate (MPH).



EXCLUSION CRITERIA

1- Previous history of mental retardation with an IQ less than or equal to 70 as measured by the Wechsler Intelligence Scale for Children-III (WISC-III)

2- Previous history of autism, Tourette's syndrome, pervasive developmental disorder or psychosis

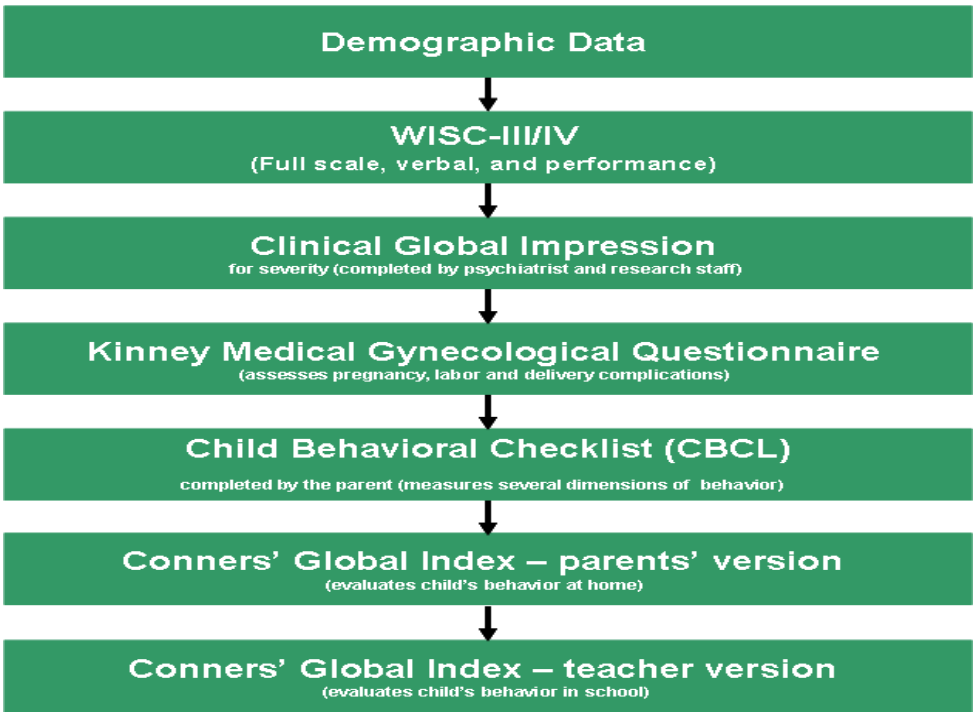
3- Any major medical condition or impairment that would prevent the child to complete testing during the study.

4- Concurrent treatment with any other medication except for methylphenidate (MPH) (in particular, patients receiving anti-epilepsy drugs were excluded)

PRE-BASELINE & BASELINE EVALUATIONS

A complete scheme of the pre-baseline and baseline evaluations conducted in the current study is presented in Figure 2.1. More specifically, all the child participants underwent a clinical assessment and a clinical diagnosis of ADHD (based on DSM-IV criteria) and its associated comorbid disorders was established by psychiatrists.

Figure 2.2 Outline of pre-baseline and baseline evaluations conducted in study participants



After this clinical assessment, demographic data on the family and child characteristics were collected. During the baseline evaluation period, participants were off medication. Further, they underwent a number of clinical and behavioral assessments. Moreover, behavioral profiles of children were assessed by the clinical research staff using, the Clinical Global Impression for severity (CGI-severity), by parents using the Clinical Behavioural Check List (CBCL) and the Conner's Global Index for parents (Conners'-P), and by teachers using the Conner's Global Index for teachers (Conners'-T). Finally, pre-, peri- and postnatal environmental events were scored using the Kinney Medical and Gynecological Questionnaire.

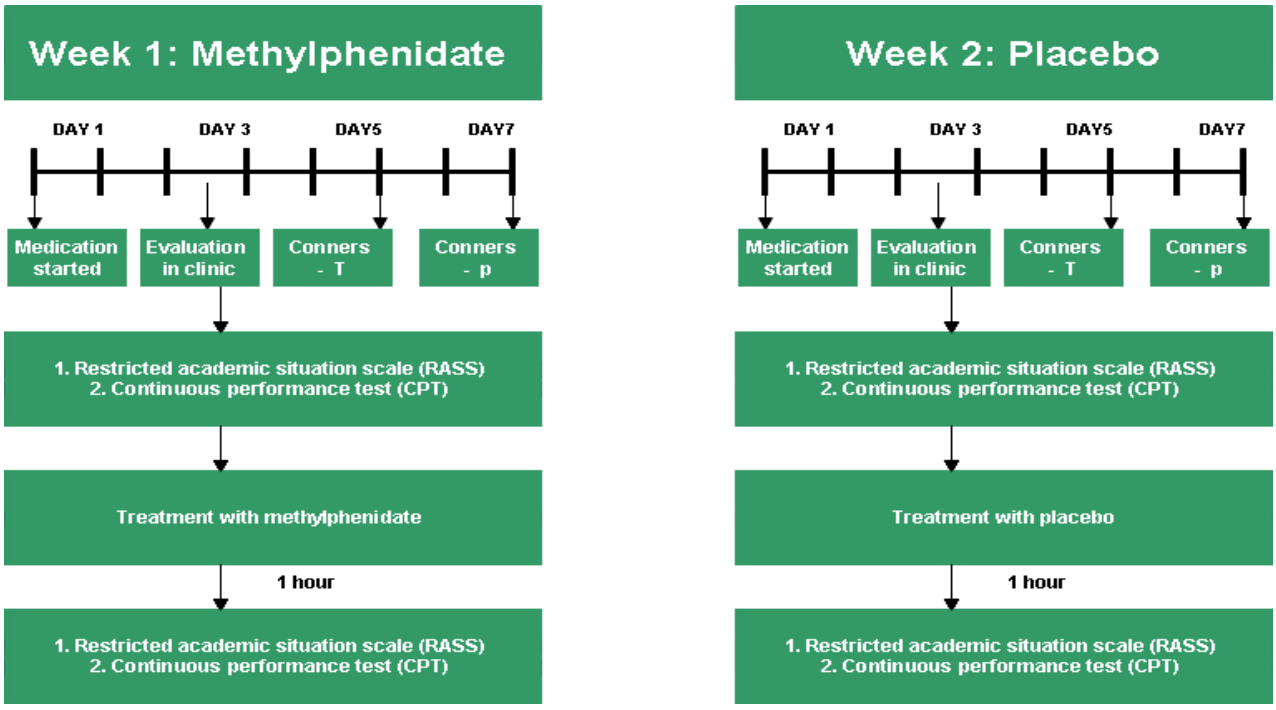
EVALUATION OF BEHAVIORAL AND THERAPEUTIC RESPONSE TO METHYLPHENIDATE

After the completion of the baseline evaluations, each child with ADHD received either MPH or placebo, each for a period of 7 days, in a randomized, double-blind sequence. Treatments (MPH or placebo) were placed in colored gelatin capsules. These were prepared by a clinical pharmacist who was not otherwise actively involved in the study. The capsules were sealed in individual, daily packets in order to help ensure accurate administration of medication. MPH was prescribed to children with ADHD in a divided b.i.d. dose (0.5 mg/kg/day; in the morning before school and at noon).

More over, on the 3rd day of each treatment week, each child was evaluated twice in the clinic (RASS, CPT and SOPT). More specifically, once before taking the

medication and then a second time 60 minutes after taking the medication. In addition, medication was administered for each child daily at the same dose and time over the treatment period. Further, the clinical research staff completed the CGI for severity of illness and improvement based on their observation during the testing day and parental reports. Additionally, therapeutic responses were collected from teachers (Conners'-T) and parents (Conners'-P) on the 5th and the 7th day respectively by a research assistant. The schematic presented in Figure 2.3 is representative of a child participant receiving MPH in the first week followed by placebo in the following week.

Figure 2.3: Description of behavioral measures and neurocognitive tasks administered during the two-week trial



EVALUATION OF COGNITIVE, EMOTIONAL AND MOTOR FUNCTION

COGNITIVE AND PSYCHOMETRIC EVALUATION

Wechsler Intelligence Scale (WISC)

Trained psychologist administered the WISC-III (Wechsler, 1991) and WISC-IV to evaluate the children's general cognitive ability. However, if the child participant had already undergone a WISC evaluation within the past 12 month period, than, the previous assessment data was used in the study. The WISC provides a standardized Full Scale IQ (FSIQ) which comprises of two subscales Verbal IQ (VIQ) and Performance IQ (PIQ) scores.

Wisconsin Card Sorting Test (WCST)

Abstraction and concept formation of ADHD children was evaluated by means of a computerized version of the WCST (Heaton, Chelune, Talley, Kay, & Curtiss, 1993). More specifically, in this task, children are asked to sort through the cards based on three different criterion i.e. colour, number, and shape of signs presented on the cards. After each trial, the child participant receives feedback on whether he/she achieved a correct or incorrect match. As the task progresses, the matching criterion changes (after ten consecutive correct matches), this change is referred to as "completing a category". After

this change, the child participant has to identify the new matching criterion using the feedback (correct/incorrect) given to him/her (reviewed in detail in (Taerk et al., 2004)).

In this study, we collected data for 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 EFs, they are not independent.

Finger Windows (FW)

Finger Windows (FW) is a subtest of the Wide Range Assessment of Memory and Learning (WRAML). Moreover, in this subtest, the participant is asked to repeat the sequential placement of a pencil into a series of holes on a plastic card as conducted by the examiner (Sheslow, 1990).

Self-Ordered Pointing Task (SOPT)

The SOPT was used to evaluate visual working memory ((Petrides & Milner, 1982); reviewed by (Taerk, et al., 2004)). In this task, a series of matrices of 6, 8, 10, and 12 images are presented to every child participant. Further, the child is asked to select (by pointing) one different image on each page. Errors occur when ever the child participant points to images previously selected on the preceding pages. Each set is presented to the child participant three times. Successful performance on this task involves working memory as well as planning and monitoring skills (reviewed in detail in (Taerk, et al.,

2004)). Previous studies, have reported significant differences in performance between ADHD children and normal controls on the SOPT ((Shue & Douglas, 1992); reviewed by (Taerk, et al., 2004)). As the SOPT, involves four levels of difficulty, data was collected for 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.

Tower of London (TOL)

Planning capacity of ADHD children was evaluated using the TOL (Shallice, 1982). More specifically, this test is extensively used to assess deficits in planning and problem solving aspects of executive functioning. Moreover, the validity and reliability of the TOL has been reported in previous studies. In addition, standardized administration and scoring procedures for the TOL have been developed for pediatric populations ((Sergeant, Geurts, & Oosterlaan, 2002; Shallice, 1982); reviewed in detail by (Taerk, et al., 2004)). The TOL test is composed of three colored beads placed on three rods, and there are 36 X 36 pairs of possible configurations. The goal of this task is to reach a target configuration with a minimal number of moves. Finally, TOL involves twelve levels of task difficulty, thus in this study, we collected data for 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.

Continuous Performance Test (CPT)

The most widely used computerized test of attention is the CPT (C. K. Conners, Epstein, Angold, & Klaric, 2003). It measures a person's sustained and selective

attention, impulsivity (C. Conners, 1999), response inhibition and executive control (Homack & Riccio, 2006). More specifically, in this test, the child is instructed to press the space bar or mouse whenever they see any letter except for the letter "X". Moreover, each letter is displayed on the computer screen for only 250 milliseconds and, the time interval between each presentation is called an inter-stimulus intervals (ISIs), which varies during, the test (1, 2 and 4 seconds) (reviewed by (Thakur, 2012)). Further, the test structure consists of 6 blocks and 3 sub-blocks, each containing 20 trials (letter presentations), with varying presentation order of the different ISIs (reviewed by (Thakur, 2012)).

Amongst the different CPT indices, the omission errors (OE) and commission errors (CE) are most often associated with ADHD (Epstein et al., 2003). OE occur when the child fails to respond to the target sequence, and these are a measure of "vigilance/sustained attention". Whereas, CE occur when the child responds to a sequence other than the target sequence (i.e. presses the spacebar when the letter “x” appears), and these are a measure of “response inhibition” (the ability to withhold a pre-potent response) (reviewed by (Thakur, 2012)).

MOTIVATIONAL STYLE EVALUATION

Restricted Academic Situation Scale (RASS)

The Restricted Academic Situation Scale (RASS) (Barkley, 1990) was used to observe and record the child's engagement in an assigned independent academic task (a set of math problems) in the presence of potential distractions, with no adult supervision

((Fischer & Newby, 1998); reviewed in detail by (Choudhry et al., 2013)). Task engagement/disengagement, is a distinct trait of ADHD (Gupta & Kar, 2009; Karama et al., 2009). Further, it is also a good predictor of the child's motivation during a monotonous and repetitive task. In this study, the RASS assessment was conducted in a specialized room within the clinic equipped with a worktable, a chair, an intercom, and some toys. The child participant was given a set of math problems at the current grade and instructed to complete as many as possible. After the instructions, the instructor then left the room and assessed the child's behaviour from behind a one-way mirror over a 15 minute period. All behavioural events were recorded at 30-second intervals according to five categories: “off-task”, “playing with objects”, “out of seat”, “vocalizing”, and “fidgeting” (further details in (Choudhry, et al., 2013))..

Choice delay task (CDT)

Motivational style was further evaluated by using the choice delay task (CDT). This test is specifically designed to assess the ADHD children's aversion to delay (Sonuga-Barke, Taylor, & Heptinstall, 1992; Sonuga-Barke, Taylor, Sembi, & Smith, 1992). In the CDT, the child repetitively (20 trials) chose between two reward paradigms. More specifically, a large reward of 2 points (exchanged for 7 or 10 cents) associated with a large period of delay (30 sec), and a smaller reward of 1 point (exchanged for 5 cents) associated with a smaller period of delay (2 sec). However, once the participant chose a reward paradigm, he/she cannot switch back to the alternative reward paradigm until the next trial ((Sonuga-Barke, Taylor, & Heptinstall, 1992; Sonuga-Barke, Taylor, Sembi, et al., 1992); further details in (Choudhry, et al., 2013)).

MOTOR ACTIVITY EVALUATION

Actigraphy

The overall motor activity of children with ADHD was evaluated on the day of testing by "actigraphy". This assessment requires the use of a small electronic device (Actiwatch®), which is worn on the non dominant hand, and is sensitive to acceleration. It records the subject's movements each 30 seconds and expresses it as motor activity counts. During the motor activity assessment, ADHD subjects put on the Actiwatch® in the morning and kept it until the end of their testing (in the early afternoon). Moreover, using this device, the average motor activity was calculated and considered in the analyses as reflective of the overall motor activity of the child (review by (Choudhry, et al., 2013)).

SYNOPSIS OF ASSESSMENTS

For each neurocognitive, motivational style, and behavioural assessment or evaluation carried out in the 2 week trial, a T-score, and/or total score, and/or standard score was obtained. In some assessments, a higher score was indicative of better behavior and/or performance while, in other evaluations, a lower score is indicated of improved behavior and/or performance. More specifically, IQ (average score 100) was assessed by using the WISC-III/IV and a standard score was obtained. Moreover, if the child had an IQ score above 100 this meant that said the child had better cognitive abilities. Likewise, both the Wisconsin Card Sorting Test (WCST) and the Tower of London (TOL) calculated standard scores with an average of 100, and a higher score indicated better set-

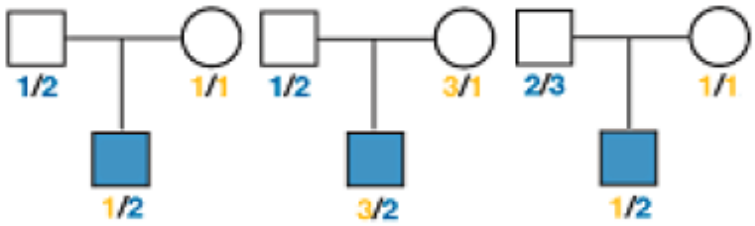
shifting and planning abilities respectively. Similarly, Finger Windows (FW) subtest provided a scale scores range from 1-19 and a higher score was considered better.

Alternatively, the Child Behavioral Checklist (CBCL) (average = 50; normal = 50-64; borderline = 65-69; problematic > 70), the Conners' Global Index-Parents (Conners'-P) and Teachers (Conners'-T) (average = 50; problematic > 65), and the Continuous Performance Test (CPT) (average = 50) yielded T-scores. Moreover, a higher score in these assessments was indicative of worse cognition and behaviour. Finally, the Clinical Global Impression (CGI), the Restricted Academic Situation Scale (RASS), and the Self-Ordered Pointing Task (SOPT) tabulated non standardized scores and a higher score was indicative of worse cognition and behaviour.

FAMILY-BASED ASSOCIATION TESTS

Family-based association tests (FBAT) were used in Chapters 3,4 and 7 of this thesis (Laird, Horvath, & Xu, 2000). More specifically, single SNP and haplotype tests of association were performed to investigate the association between selected markers with ADHD diagnosis and other quantitative phenotypes pertinent to ADHD. Initially, the analyses were conducted with the total sample. However, afterwards stratification by maternal stress during pregnancy and maternal smoking during pregnancy (yes/no) was done. Finally, offsets used in the FBAT analysis were based on average scores found in the population (e.g. 50 in the case of CBCL T-scores)

Figure 2.4: Depiction of allele transmission from parents to offspring (Adapted from (Thakur, 2012)).



FBAT test is based on the principle that, if an allele is associated with an abnormal level of a trait, it is expected to be transmitted more frequently than what is expected by chance, from parents to the child presenting an abnormal level of that trait (Laird, et al., 2000). Thus, if the outcome of the test is positive then this result is indicative of the presence of both allelic association and linkage. Furthermore, the over- or under-transmission from parent to affected offspring for each specific allele/haplotype is determined using the Transmission Disequilibrium Test (TDT) (Laird, et al., 2000).

Finally, the use of FBAT offers two major advantages over case/control association studies. More specifically, FBAT are not affected by population stratification, and these have higher statistical power (Haldar & Ghosh, 2011). Furthermore, as the non-transmitted parental alleles are themselves the control alleles in FBAT, this reduces other sources of bias, such as socioeconomic status.

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Taerk, E., Grizenko, N., Ben Amor, L., Lageix, P., Mbekou, V., Deguzman, R., et al. (2004). Catechol-O-methyltransferase (COMT) Val108/158 Met polymorphism does not modulate executive function in children with ADHD. *BMC Med Genet, 5*, 30.

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CHAPTER 3

Catechol-O-Methyltransferase Gene and Executive Function in Children with ADHD

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PREFACE

One of the difficulties in identifying potential genetic association between ADHD and candidate genes may be the fact that ADHD is heterogeneous in its clinical expression. Supporting this idea, genetic epidemiologists now believed that, the DSM-IV definition of ADHD, although clinically useful, may not be valid from a genetic point of view. In order to address this problem, epidemiological research has underscored the importance of breaking down the disorder into component "endophenotypes". Endophenotypes are more proximal to the biological etiology of the disorder, and are genetically less complex than the disorder as a whole. Amongst the different *endophenotypes*, the *neuropsychological endophenotypes* that index deficits in executive function may be particularly relevant for candidate gene association studies in ADHD because children with ADHD widely exhibit deficits in executive functioning, which in turn, translate into behavioral deviances observed in ADHD. Moreover, these neuropsychological tasks are known to activate the prefrontal cortex (PFC) and basal ganglia, brain regions which have been, previously implicated in ADHD. Furthermore, performance in these tasks is believed to be modulated by brain dopaminergic systems, which are thought to be integral to the pathophysiology of ADHD. Based on these observations, we decided to explore a candidate gene previously investigated in ADHD. *Catechol-O-Methyltransferase (COMT)* gene codes for the COMT enzyme that is chiefly responsible for the metabolism of catecholamines, including DA with in the PFC. Thus, strong *apriori* evidence suggests that the COMT gene is an interesting candidate for genetic studies of ADHD.

In this chapter, we employed a strategy, namely the use of *"endophenotypes"*, to identify candidate genes associated with ADHD. More specifically, a large sample of children with ADHD (N=445) were recruited and possible associations between COMT gene polymorphisms (SNPs; rs6269, rs4633, rs4818, and rs4680) and executive functioning phenotypes were tested using family-based association testing (fBAT) and quantitative trait analyses after adjusting for a number of important confounders. Significant associations were observed between neurocognitive endophenotypes and high risk alleles and haplotypes. However, after correction for multiple testing only one significant effect was observed between rs6269 (intronic variant) and the number of categories completed (a measure of concept formation ability) on the WCST. This suggests that *COMT* gene may be tentatively implicated in modulating EF in children with ADHD.

In summary, this study illustrates the importance of using endophenotypes as a means to reduce the clinical heterogeneity of ADHD. Also, it emphasizes that; future candidate genes studies investigating potential gene-phenotype association in ADHD should select candidate genes believed to be involved in the neurobiology of ADHD by apriori evidence. The use of these strategies will help researchers to elucidate the different pathways to ADHD and unravel its complex genetic architecture.

ABSTRACT

Objective: To examine the association between functional haplotypes in the *catechol-o-methyltransferase (COMT)* gene and ADHD diagnosis, and executive function (EF) in children with ADHD. **Method:** *COMT* single nucleotide polymorphism (SNPs; rs6269, rs4633, rs4818, and rs4680) were genotyped in 445 ADHD children. EF was assessed using Wisconsin Card Sorting Test (WCST), Tower of London, and self-ordered pointing task. *COMT* haplotypes were tested for association using family-based association testing (fBAT) and quantitative trait analyses. **Results:** fBAT analysis showed no association between *COMT* alleles/haplotypes and ADHD diagnosis and EF parameters. Using ANCOVA in the Caucasian only sample, significant associations between *COMT* haplotypes, and WCST indices were observed. However, after correction for multiple testing, the only significant effect observed was between rs6269 and the number of categories completed (a measure of concept formation ability) on the WCST, $F(1,285) = 8.92$, $p = .003$. **Conclusion:** These results tentatively implicate *COMT* gene in modulating EF in children with ADHD. *(J. of Att. Dis. 2012; XX(X) 1-XX)*

Keywords

COMT, ADHD, gene, executive functions, haplotypes, ANCOVA, fBAT

INTRODUCTION

ADHD is a common childhood disorder, with 5% worldwide prevalence (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). It is believed that the behavioral symptoms displayed by ADHD children are a result of underlying deficits in executive function (EF; (Barkley, 2010; Welsh & Pennington, 1988; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). EF encapsulates the neurocognitive (NC) processes important for goal-directed behaviors, including planning, sustained attention, cognitive flexibility, working memory, and response inhibition. EF is mediated by the prefrontal cortex (PFC) and its connections to subcortical loci (Tekin & Cummings, 2002).

Several studies have reported the association between EF and candidate genes implicated in the dopamine (DA) neurotransmission pathway (Barnes, Dean, Nandam, O'Connell, & Bellgrove, 2011). The *catechol-o-methyltransferase gene* (*COMT*; located on chromosome 22q11.2) has been of particular interest, given its role in the clearance of DA and norepinephrine (NE) in the PFC, and the paucity of the DA transporter (DAT) in this brain region (Chen et al., 2004; Lachman et al., 1996). COMT encodes two distinct isoforms of the protein resulting from alternative splicing: soluble COMT (S-COMT), present in the peripheral nervous system, and membrane bound COMT (MB-COMT), abundant in the brain (Tenhunen et al., 1994). Within Exon 4 of the *COMT* gene, a common single nucleotide polymorphism (SNP; C**G**TG vs. C**A**TG) results in the presence of methionine or valine at codon 108 (in S-*COMT*) or codon 158 (in MB-*COMT*). The valine allele at position 108/158 has been shown to have higher stability and

approximately two- to fourfold higher activity than the met variant (Chen, et al., 2004; Lachman, et al., 1996).

The *Val108/158Met* polymorphism has been implicated in the modulation of EF in normal controls and in patients with various mental disorders (Bilder et al., 2002; Goldberg et al., 2003), although, a recent meta-analysis concluded that *Val108/158Met* polymorphism does not show an association with EF (J. H. Barnett, Scoriels, & Munafo, 2008).

Evidence suggests that in addition to *Val108/158Met*, other SNPs within the gene may modulate *COMT* activity (Diatchenko et al., 2005). Specifically, a number of *COMT* haplotypes formed by four SNPs (*rs6269* in the P1 promoter, *rs4633* in Exon 3, *rs4818* and *rs4680—Val108/158Met—*in Exon 4) encode mRNAs with alternative secondary structures displaying differential levels of protein expression (Nackley et al., 2006). The three major haplotypes are designated as *high functionality* (GCGGval), *average functionality* (ATCAmet), and *low functionality* haplotypes (ACCGval; (Nackley, et al., 2006)Nackley et al., 2006). Compared with the GCGGval, the ACCGval haplotype shows an 18- to 25-fold decrease in enzymatic activity, paralleled by reduced protein levels. This effect is believed to be mediated by ACCGval haplotype being less efficiently translated.

We (Taerk et al., 2004) and others (Bellgrove et al., 2005; Mills et al., 2004) have previously investigated the effect of *Val108/158Met* on neuropsychological phenotype in ADHD, but results are mixed (Kebir, Tabbane, Sengupta, & Joober, 2009). Given the importance of other SNPs and haplotypes in modulating the *COMT* function, we

investigated their role in modulating executive functioning in ADHD. The main objective of this study was to examine the association of *COMT* haplotypes with (a) ADHD diagnosis and (b) performance on neuropsychological tasks in these children.

METHOD

PARTICIPANTS, STUDY PROCEDURES, AND ETHICS

A total of 445 ADHD children (345 boys and 100 girls), ages 6 to 12 (M = 9.05; SD = 1.86), were recruited from the Disruptive Behavior Disorders Program (DBDP) and the children outpatient clinic at the Douglas Mental Health University Institute. In all, 118 children were included in a previous study (Taerk, et al., 2004) that explored only the Val/Met polymorphism. All of the participants were referred to these specialized care facilities by school teachers, community social workers, and pediatricians. The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Children with ADHD and their parents were explained the study in detail, and they provided their assent and written consent to participate. Children included in this study met *Diagnostic and Statistical Manual of Mental Disorders* (APA, 1994) diagnosis criteria for ADHD. A comprehensive clinical evaluation was used to establish the diagnosis of ADHD. Details about diagnostic assessments and procedures have been described in detail elsewhere (Grizenko, Bhat, Schwartz, Ter-Stepanian, & Joober, 2006; Taerk, et al., 2004). Children were excluded from this study if they had an IQ less than 70 on the Wechsler Intelligence Scale for Children–III/IV (WISC-III or WISC-IV) and/or if they had an earlier diagnosis of Tourette syndrome, pervasive developmental disorder, and psychosis.

Among the total sample of affected ADHD children, 78.1% were male, 86.9% were of Caucasian ethnicity, and 28.9% belonged to families with an annual income of less than CN$20,000. In all, 52.8% met *DSM-IV* criteria for the combined subtype, whereas 37.3% and 9.9% were diagnosed with the inattentive and hyperactive subtypes respectively.

A total of 38.8% were previously receiving medication for their ADHD symptoms. Among comorbid disorders, 40.4% had oppositional defiant disorder, 21.7% had conduct disorder (CD), and 44.1% had an anxiety disorder. NC task performance was evaluated using Wisconsin Card Sorting Test (WCST), Tower of London (TOL), and self-ordered pointing task (SOPT). The details regarding NC task assessments, and procedures, have been described in detail earlier (Karama et al., 2008; Taerk, et al., 2004). All NC assessments were completed while the children were not taking any medication. In cases, where children were on medication prior to their inclusion in the study, these assessments were carried out at the end of a 1-week washout period.

GENOTYPING PROCEDURES

Blood and/or saliva samples were collected from each child participating in this study, as well as from parents and siblings, whenever possible. The study included 380 nuclear families having one or more children with a *DSM-IV* diagnosis of ADHD. Of the 380, 184 were trios with information from both parents, 18 were trios with two affected children, 49 were trios with information from one parent and one or more unaffected siblings, 115 were duos including the proband and one parent, while 14 were families with two affected siblings and one parent. The Val108/158Met polymorphism of the

COMT gene was genotyped using a polymerase chain reaction (PCR)–based method as previously described (Karama, et al., 2008; Taerk, et al., 2004). The three other SNPs (rs6269, rs4633, and rs4818) were genotyped at Genome Quebec using Sequenom iPlex Gold technology. The genotypes of the *COMT* SNPs did not depart from Hardy-Weinberg equilibrium ($p = .366$, $p = .367$, $p = .362$, $p = .080$, respectively).

STATISTICAL METHODS

Marker-to-marker linkage disequilibrium (LD) was measured using D′ statistics between each pairwise combination of all four *COMT* SNPs by Haploview V3.32 (www.broad.mit.edu/mpg/haploview/). Haploview was used to create a graphical representation of LD structure (Barrett, Fry, Maller, & Daly, 2005). UNPHASED 3.1.4 was used to generate *COMT* haplotypes for ADHD probands and their siblings from unphased genotypes (Dudbridge, 2008).

To investigate association of *COMT* SNPs with ADHD diagnosis and quantitative phenotypes, family-based tests of association (examining transmission disequilibrium of a specific allele/haplotype from parent to affected offspring) were performed on data from cases and family members using the family-based association testing (fBAT) statistical package (version 2.0.3; (Laird, Horvath, & Xu, 2000)). All the analyses were performed under the assumption of an additive model, with a null hypothesis of no linkage and no association. In addition, the effect of *COMT* alleles and haplotypes on cognitive and neuropsychological task performances in children with ADHD was compared between various genotype and diplotype groups. The effect of *COMT* alleles were investigated using a dominant model for each SNP; that is, children with ADHD

were separated into two main groups, one group consisting of participants carrying at least one copy of the dominant allele and the other group consisting of participants carrying both copies of the recessive allele—rs6269: A+ = AA + AG (*n* = 317), and GG (*n* = 68); rs4633: T+ = TT + CT (*n* = 275), and CC (*n* = 106); rs4818: C+ = CC + CG (*n* = 315), and GG (*n* = 62); and rs4680 (Val108/158Met*):* Gval+ = GvalGval + GvalAmet (*n* = 296), and AmetAmet = (*n* = 82). Furthermore, as previously proposed by Diatchenko et al. (2005), the effect of *COMT* haplotypes (*n* = 358) were investigated by comparing the following diplotype groups (three homozygotes and three heterozygotes): GCGGval/GCGGval (*n* = 61), GCGGval/ATCAmet (*n* = 150), GCGGval/ACCGval (*n* = 26), ATCAmet/ATCAmet (*n* = 79), ACCGval/ATCAmet (*n* = 35), and ACCGval/

ACCGval (*n* = 7).

The sample was restricted to Caucasians only for this analysis to limit the effects of population stratification. Chi-square statistics and ANOVA were used to compare *COMT* diplotype group differences for clinical characteristics in the current sample. Furthermore, ANCOVA and MANCOVA with *COMT* diplotype or allelic group as independent factor, and child performance on different EFs parameters test scores (WCST: total errors, perseverative errors, non-perseverative errors, number of categories completed, trials to complete first category; TOL: total score, total correct in first trial score; and SOPT: total errors) as dependent factor were also conducted. In these analyses, gender, age, IQ, clinical subtypes of ADHD, prior history of treatment with psychostimulants, and presence of CD were used as covariates. These factors are believed to have a potential confounding effect on the outcome of genetic association studies in

ADHD investigating neurocognition phenotypes (Banaschewski, Becker, Scherag, Franke, & Coghill, 2010; Kebir, et al., 2009; Mick & Faraone, 2008). Main gene effects were further explored by post hoc pairwise comparisons using the Sidak method, and Bonferroni correction was used to control for multiplicity of testing. Given that we performed three statistical (two MANCOVA and one ANCOVA) tests for each of the four *COMT* SNPs in our study, the cutoff for significance was set at $p = .05/12 = .004$.

RESULTS

COMT HAPLOTYPES IN CHILDREN WITH ADHD AND THEIR FAMILIES

Employing Haploview to compute LD, the four *COMT* SNPs were shown to be in strong LD with each other forming a single haplotype block (Figures 3.1 and 3.2). The *COMT* haplotypes ATCAmet, GCGGval, and ACCGval had respective frequencies of 0.454, 0.391, and 0.119, which are in accordance with previously reported frequencies (Nackley, et al., 2006). These haplotypes were used to assign ADHD children to *COMT* diplotype groups.

FAMILY-BASED ASSOCIATION ANALYSIS RESULTS

The fBAT single SNP association analysis for the four *COMT* SNPs with ADHD diagnosis conducted using additive genetic model was not statistically significant for the four *COMT* SNPs (all $p > .05$). Furthermore, none of the haplotypes showed any statistically significant association with ADHD diagnosis (data not shown). Finally,

Quantitative trait disequilibrium test (QTDT) analyses exploring possible association of *COMT* single SNPs/haplotypes with EFs parameters showed no statistically significant associations (all $p > .05$) in both fBAT and Haplotype based association test (HBAT) analyses (data not shown).

QUANTITATIVE TRAIT ANALYSES RESULTS

COMT and clinical characteristics in children with ADHD.

The *COMT* diplotype groups were similar with regard to gender, age, and average household income, clinical characteristics, and comorbid disorders, such as oppositional defiant and anxiety disorders (Table 3.1). However, it is important to note that ACCGval/ACCGval group did not have any participant with CD. Consequently, CD was added as a covariate in our final ANCOVA and MANCOVA analyses.

COMT and NC characteristics in children with ADHD.

Several genotypes and diplotypes showed nominal significant associations with various cognitive measures derived from the WCST and the TOL tests (Tables 3.2 and 3.3, respectively). However, after correcting the results for multiple testing ($p < .004$), all the observed genotype and diplotype effects on WCST and TOL indices became nonsignificant, except the effect of rs6269's A allele on WCST's number of categories completed, $F(1,285) = 8.92$, $p = .003$.

DISCUSSION

Given the putative role of *COMT* in the modulation of DA levels in the PFC, many studies have focused on the *COMT* gene as a candidate gene to explore its association with neuropsychological phenotypes in psychiatric patients and unaffected controls. Most studies have focused on the *COMT* Val108/158Met polymorphism with regard to its effects on cognitive abilities reporting divergent findings. A recent meta-analysis reported lack of significant association between *COMT* Val108/158Met and cognitive tasks mediated by the frontal cortex (J. H. Barnett, et al., 2008).

The inconsistencies in the results of the *COMT* Val108/158Met polymorphism studies may be attributed, at least in part to the complex structure of the *COMT* gene. It has been suggested that there are additional genetic variations within the *COMT* gene that may interact with Val108/158Met to determine its biological effects of this gene (Meyer-Lindenberg et al., 2006; Shifman et al., 2002). Nackley et al. (2006) showed that different *COMT* haplotypes have different levels of protein expression possibly due to the alternating mRNA secondary structure (Nackley, et al., 2006).

Two studies to date have explored the effect of *COMT* haplotypes on attention and cognitive function in normal children (Jennifer H. Barnett, Heron, Goldman, Jones, & Xu, 2009; Voelker, Sheese, Rothbart, & Posner, 2009). Voelker et al. (2009) reported on 2-year-old children (n = 45) and found that variation in the *COMT* gene influenced performance on a task of attention, where individuals with the high functionality (GCGGval) haplotype (low DA levels) performed better in each category (Voelker, et al., 2009). In contrast, Barnett et al. (2009) assessed cognitive function of normal children

aged 8 (verbal inhibition assessments) and 10 years (working memory tasks), and reported that children with average functionality diplotypes (ATCAmet/ATCAmet or the GCGGval/ACCGval; moderate DA levels) tended to perform better than those with high functionality diplotypes (GCGGval/GCGGval or the GCGGval/ATCAmet; low DA levels) and the low functionality diplotypes (ACCGval/ATCAmet or the ACCGval/ACCGval; high DA levels; (Jennifer H. Barnett, et al., 2009)).

The present study is the largest (n = 445 ADHD participants) investigating the possible association between *COMT* haplotypes and ADHD diagnosis, and its effects on NC task performance. We used both family-based and quantitative trait analyses, and used a comprehensive neuropsychological evaluation battery developed for children testing EF parameters indexing PFC. The participants were not taking any psychostimulant medication for 1 week prior to the EF assessments. We also took into consideration potential factors that might confound the results of EF abilities, such as age, IQ, gender, clinical subtypes, comorbid disorders, and prior exposure to medication, and controlled for these confounders.

After correcting for multiple testing (significant p values < .004), we observed that rs6269 shows an association with number of categories completed (a measure of concept formation ability) on the WCST. More specifically, participants with the A+ (AA + AG) genotype completed less WCST categories compared with GG participants (p = .003), showing decreased concept formation ability. This finding is very interesting, given previous results suggesting that ADHD children demonstrate impairments in concept formation ability (Hong et al., 2010). Although the effect size of this association

is very small (partial η2 = .03) and explains only 3% of the variance in EF, it is comparable with previous publications in normal children (Jennifer H. Barnett, et al., 2009).

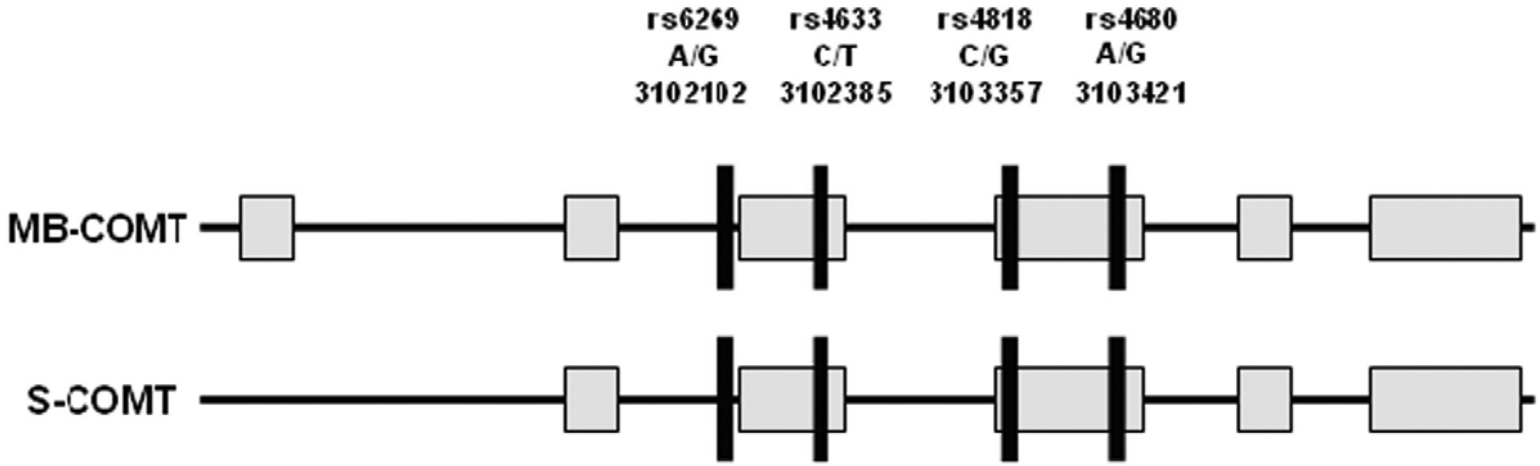
These results underscore the importance of examining functional haplotypes in *COMT*, going beyond the most extensively studied Val108/158Met (rs4680) polymorphism. The results of this present study are perfectly consistent with previous studies (including a recent meta-analysis) that conclude that there is no association between this SNP and PFC mediated cognitive function (J. H. Barnett, et al., 2008). They are also partially consistent with one of the only two studies examining these functional haplotypes (Voelker, et al., 2009), although differences in study design make a direct comparison difficult. In this study, children with the high functionality (GCGGval) haplotype performed better on assessments of attention. In the current study, children homozygous for this haplotype showed a trend (p = .04) for lowest trials to complete first on the WCST suggesting superior function than all the other haplotypes. These results are not conclusive in themselves. However, they suggest that further investigation is warranted to unravel the association between *COMT* haplotypes and PFC function in children with ADHD.

Family-based analyses did not show any significant association between the four alleles or their derived diplotypes and the various NC outcomes indexing EFs. Given the fact that family-based analyses include only patients with available parents who are heterozygous and in view of the putative small effect sizes of this locus on cognitive

functions reported in the literature, it is possible that the absence of association in the family-based analysis is due to reduced power to detect such effects.

To conclude, to our knowledge this is the largest study to date investigating the association of ADHD with the *COMT* haplotypes and the role of these haplotypes in modulating EF in children with ADHD. The results of this study suggest that the *COMT* haplotypes are not associated with ADHD diagnosis and that these haplotypes and their constituent SNP's alleles may influence neuropsychological task performance in children with ADHD. However, a replication in a larger sample and meta-analyses are needed to firmly confirm or rule out an effect size in the range that is now hypothesized for this locus in relation to EFs.

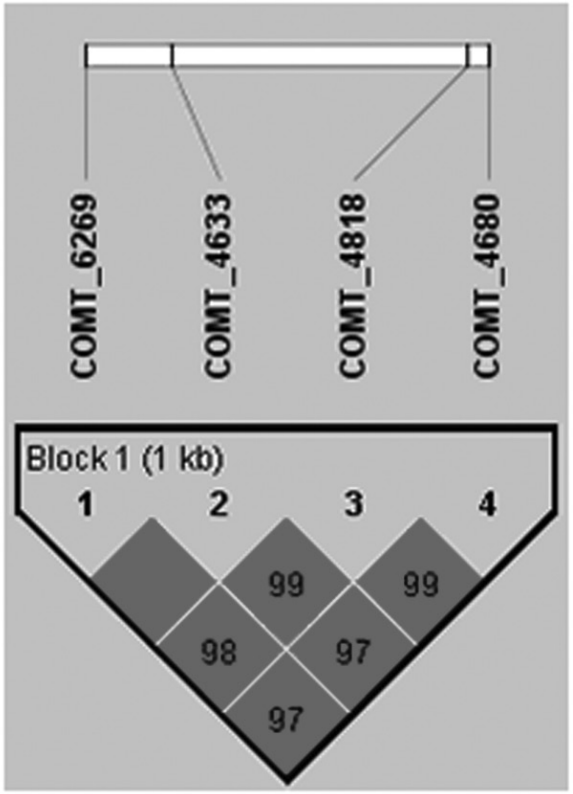
Figure 3.1. Haplotypes of the *COMT* gene in children with ADHD and their families



Haplotype	COMT SNPs				% Freq.	T:U	χ^2	*P*
LPS	G	C	G	G	0.391	71.0 : 64.0	0.37	0.54
APS	A	T	C	A	0.457	70.0 : 67.0	0.06	0.79
HPS	A	C	C	G	0.113	21.0 : 26.0	0.52	0.46

Note: MB-*COMT* = membrane-bound *catechol-o-methyltransferase*; S-*COMT* = soluble *catechol-o-methyltransferase*; Haplotype = *COMT* haplotypes, **%** Freq. = haplotype frequency in the data sample, T:U = transmitted vs. untransmitted ratio for haplotypes, $\chi 2$= chi-square statistic for association with ADHD diagnosis. Schematic representation of rs6269, rs4633, rs4818, and rs4680 in MB-*COMT* and S-*COMT* illustrating *COMT* genomic organization and single nucleotide polymorphism composition for the three haplotypes. *COMT* haplotypes (Nackley, et al., 2006) (Nackley et al., 2006) population percentage frequencies and results of multi-marker haplotype association tests with ADHD diagnosis for children with ADHD and their families.

Figure 3.2. Haplotype block structure of the *COMT* gene in children with ADHD and their families



LD Plot statistics

L1	L2	D'	LOD	r2
rs4633	rs4818	0.99	129.58	0.59
rs6269	rs4818	0.98	213.07	0.90
rs6269	rs4680	0.97	132.15	0.60
rs4633	rs4680	0.97	224.68	0.94
rs4818	rs4680	0.99	129.34	0.59

Note: *COMT = catechol-o-methyltransferase*; LD = linkage disequilibrium; LD plot statistics of rs6269, rs4633, rs4818, and rs4680; L1 = Locus 1, L2 = Locus 2, D'= D prime, a measure of pair-wise LD; logarithm of odds (LOD) = LOD score, *r*2 = goodness of fit. Haplotype block structure, as depicted by Haploview, is shown. Values for D' (×100) are shown, but those boxes with $D' <= 1$ are shaded in bright gray and are empty. Cells with $D' < 1$ are shades of gray.

Table 3.1. Demographic, Clinical, and Comorbid Characteristics of Caucasian Children With ADHD

	$GCGG_{val}$/ $GCGG_{val}$ (n=61)	***$GCGG_{val}$/ $ATCA_{met}$ (n=150)***	***$ATCA_{met}$/ $ATCA_{met}$ (n=79)***	***$GCGG_{val}$/ $ACCG_{val}$ (n=26)***	***$ACCG_{val}$/ $ACCG_{val}$ (n=7)***	***$ACCG_{val}$/ $ATCA_{met}$ (n=35)***	***Statistic & p-value***
Demographic characteristics							
Gender, M/F	45/16	117/33	65/14	21/5	4/3	25/10	χ^2=4.08, df=5, p=0.53
Age, yrs	9.10 (1.69)	9.06 (1.78)	9.33 (1.82)	9.03 (1.43)	8.08 (1.23)	8.55 (1.90)	$F_{5,357}$=1.49, p=0.21
Household income (% < $20,000 per yr)	29.70%	33.60%	32.10%	28.10%	10.00%	23.80%	χ^2=3.74, df=5, p=0.58
Clinical characteristics							
WISQ-III, full scale IQ	99.18 (11.69)	97.21 (13.61)	95.65 (13.24)	93.63 (11.76)	90.67 (16.00)	99.80 (15.66)	$F_{5,329}$=1.32, p=0.25
CBCL (total score)	70.25 (8.15)	69.59 (9.00)	69.01 (8.245)	67.65 (8.362)	63.86 (12.41)	67.89 (10.61)	$F_{5,350}$=1.04, p=0.39
CBCL (Attention problems score)	71.77 (10.22)	70.15 (9.74)	70.68 (9.97)	70.15 (8.40)	68.00 (9.57)	70.89 (11.03)	$F_{5,350}$=0.34, p=0.88
CBCL (externalisation score)	70.23 (8.91)	69.72 (10.41)	68.12 (10.29)	66.35 (9.12)	64.29 (10.38)	67.26 (12.17)	$F_{5,350}$=1.26, p=0.27
CBCL (internalisation score)	66.20 (9.48)	64.22 (10.12)	64.86 (9.51)	63.46 (10.88)	58.57 (12.88)	63.00 (10.74)	$F_{5,350}$=1.08, p=0.37
DISC-IV, Inattention Items	6.97 (2.28)	6.86 (2.33)	7.23 (1.85)	7.20 (1.38)	7.00 (1.73)	6.83 (2.69)	$F_{5,354}$=1.81, p=0.86
DISC-IV, Hyperactivity Items	5.85 (2.53)	5.75 (2.61)	5.59 (2.48)	4.96 (2.63)	5.29 (2.13)	5.80 (2.48)	$F_{5,354}$=0.53, p=0.74
DISC-IV, ADHD Subtype (C+H/I)	40/21	97/53	47/32	11/15	5/2	25/10	χ^2=6.81, df=5, p=0.23
Previously medicated (%)	23/37.70%	53/37.30%	32/42.70%	14/53.80%	1/14.30%	11/36.70%	χ^2=4.88, df=5, p=0.43
Comorbid disorders							
CD	12/20.00%	44/29.90%	15/19.00%	2/8.30%	0/0.00%	7/20.00%	χ^2=10.26, df=5, p=0.06
ODD	29/48.30%	61/40.90%	36/45.60%	11/45.80%	1/14.30%	14/40.00%	χ^2=3.70, df=5, p=0.58

AD	34/59.60%	63/47.40%	34/44.70%	7/33.30%	3/42.90%	11/33.30%	χ^2=7.89, df=5, p=0.16

Note: M = Male, F = Female. WISC-III = Wechsler Intelligence Scale for Children–III; CBCL = Child Behavioral Checklist; DISC-IV = Diagnostic Interview Schedule for Children–Fourth edition; C/I/H = Combined/Inattentive/Hyperactive; CD = conduct disorder; ODD = oppositional defiant disorder; AD = anxiety disorder. Values are mean (*SD*), counts, proportions unless otherwise indicated. Demographic, clinical, and comorbid characteristics were compared between these groups using the appropriate statistic depending on the nature of the data. Number of observations varied sometimes with regard to variables. High functionality haplotype = GCGGval, average functionality haplotype = ATCAmet, and low functionality haplotype = ACCGval (Nackley, et al., 2006).

Table 3.2. *COMT* Dominant Alleles and Neurocognitive Characteristics of Caucasian Children with ADHD

SNPs	***Dominant allele***	***Recessive allele***	***Statistic & p-value, Partial Eta Squared, observed power***
rs6269	***A+ (n=317)***	***GG (n=68)***	
WCST scores			
Total errors	44.84 (21.80)	37.44 (16.12)	$F_{1,285}$=5.15, **p=0.02****; 0.01; 0.61
Perseverative errors	21.28 (13.54)	17.46 (9.43)	$F_{1,285}$=3.43, p=0.06*; 0.01; 0.45
Non-Perseverative errors	23.56 (14.46)	19.98 (10.20)	$F_{1,285}$=2.17, p=0.14; 0.008; 0.31
number of categories completed	4.18 (1.80)	4.94 (1.28)	$F_{1,285}$=8.92, **p=0.003****; 0.03; 0.84
Trials to complete first category	25.95 (27.24)	16.19 (7.50)	$F_{1,285}$=6.23, **p=0.01****; 0.02; 0.70
SOPT scores			
Total errors	15.77 (7.91)	13.68 (6.26)	$F_{1,318}$=2.49, p=0.11; 0.008; 0.35
TOL scores			
Total score	105.78 (15.11)	109.63 (13.23)	$F_{1,278}$=2.87, p=0.09*; 0.01; 0.39
Total correct in 1st trial score	10.87 (1.26)	11.05 (0.99)	$F_{1,278}$=0.911, p=0.34; 0.003; 0.15
rs4633	***T+ (n=275)***	***CC (n=106)***	
WCST scores			
Total errors	44.26 (21.91)	41.58 (18.65)	$F_{1,282}$=2.26, p=0.13; 0.008; 0.32
Perseverative errors	20.86 (13.61)	19.73 (10.80)	$F_{1,282}$=1.16, p=0.28; 0.004; 0.18
Non-Perseverative errors	23.40 (14.42)	21.84 (12.50)	$F_{1,282}$=1.26, p=0.26; 0.004; 0.20
number of categories completed	4.23 (1.78)	4.55 (1.59)	$F_{1,282}$=3.66, p=0.057*; 0.01; 0.47
Trials to complete first category	24.86 (25.47)	21.17 (22.25)	$F_{1,282}$=1.77, p=0.18; 0.006; 0.26
SOPT scores			
Total errors	15.69 (7.94)	14.76 (6.96)	$F_{1,315}$=1.71, p=0.19; 0.005; 0.25
TOL scores			
Total score	105.37 (14.95)	109.04 (14.04)	$F_{1,275}$=4.15, **p=0.043****; 0.01; 0.52
Total correct in 1st trial score	10.86 (1.27)	11.02 (1.07)	$F_{1,275}$=2.52, p=0.11; 0.009; 0.35
rs4818	***C+ (n=315)***	***GG (n=62)***	
WCST scores			
Total errors	44.73 (21.86)	37.77 (16.70)	$F_{1,277}$=4.76, **p=0.03****; 0.01; 0.58
Perseverative errors	21.20 (13.57)	17.09 (9.43)	$F_{1,277}$=4.39, **p=0.03****; 0.01; 0.55
Non-Perseverative errors	23.53 (14.50)	20.68 (10.60)	$F_{1,277}$=1.27, p=0.26; 0.005; 0.20
number of categories completed	4.18 (1.80)	4.83 (1.32)	$F_{1,277}$=6.14, **p=0.01****; 0.02; 0.69
Trials to complete first category	25.92 (27.27)	16.60 (7.83)	$F_{1,277}$=5.39, **p=0.02****; 0.01; 0.63
SOPT scores			
Total errors	15.74 (7.94)	13.89 (6.36)	$F_{1,311}$=2.11, p=0.14; 0.007; 0.30
TOL scores			
Total score	105.57 (15.03)	110.39 (13.79)	$F_{1,271}$=3.91, **p=0.049****; 0.01; 0.50
Total correct in 1st trial score	10.86 (1.268)	11.08 (1.017)	$F_{1,271}$=1.62, p=0.20; 0.006; 0.24
rs4680 (Val158Met)	***G_{val}+ (n=296)***	***$A_{met}A_{met}$ (n=82)***	
WCST scores			
Total errors	42.72 (20.49)	45.67 (22.82)	$F_{1,281}$=2.01, p=0.15; 0.007; 0.29
Perseverative errors	19.67 (11.62)	22.91 (16.30)	$F_{1,281}$=4.81, **p=0.02****; 0.01; 0.59
Non-Perseverative errors	23.05 (13.95)	22.77 (14.08)	$F_{1,281}$=0.00, p=0.98; 0.00; 0.05
number of categories completed	4.38 (1.71)	4.13 (1.79)	$F_{1,281}$=2.19, p=0.14; 0.008; 0.31

Trials to complete first category	23.45 (23.67)	26.61 (29.83)	$F_{1,281}$=1.12, p=0.28; 0.004; 0.18
SOPT scores			
Total errors	15.36 (7.47)	15.55 (8.28)	$F_{1,312}$=0.59, p=0.44; 0.002; 0.12
TOL scores			
Total score	106.65 (14.61)	106.77 (14.96)	$F_{1,274}$=0.08, p=0.77; 0.00; 0.06
Total correct in 1st trial score	10.89 (1.22)	10.89 (1.20)	$F_{1,274}$=0.00, p=0.99; 0.00; 0.05

Note: *COMT = catechol-o-methyltransferase*; SNP = single nucleotide polymorphism; WCST Scores = Wisconsin Card Sorting Test scores; TOL scores = Tower of London scores; SOPT scores = self-ordered pointing task scores. Values are mean (*SD*) unless otherwise indicated. Quantitative traits were compared between these groups using the appropriate statistic depending on the nature of the data. Number of observations varied sometimes with regard to variables. A+ = AA + AG, T+ = TT + TC, C+ = CC + CG, Gval + = GvalGval + GvalAmet. **Significant effects. *Significant trends. *p* values < .05 in bold.

Table 3.3. *COMT* Haplotypes and Neurocognitive Characteristics of Caucasian Children With ADHD

	GCGG$_{val}$/ GCGG$_{val}$ (n=61)	***GCGG$_{val}$/ ATCA$_{met}$ (n=150)***	***ATCA$_{met}$/ ATCA$_{met}$ (n=79)***	***GCGG$_{val}$/ ACCG$_{val}$ (n=26)***	***ACCG$_{val}$/ ACCG$_{val}$ (n=7)***	***ACCG$_{val}$/ ATCA$_{met}$ (n=35)***	***Statistic & p-value, Partial Eta Squared, observed power***
WCST scores							
Total errors	38.47 (17.08)	43.47 (22.42)	45.59 (22.59)	47.00 (21.53)	55.33 (23.88)	42.62 (19.18)	$F_{5,263}$=1.06, p=0.37; 0.02; 0.37
Perseverative errors	18.04 (9.97)	19.91 (13.26)	22.56 (15.94)	24.95 (13.66)	21.17 (9.08)	20.15 (9.17)	$F_{5,263}$=1.36, p=0.23; 0.02; 0.48
Non-Perseverative errors	20.43 (10.53)	23.56 (15.07)	23.03 (14.14)	22.05 (11.41)	34.17 (26.56)	22.46 (11.97)	$F_{5,263}$=0.84, p=0.52; 0.01; 0.30
Number of categories Completed	4.78 (1.32)	4.32 (1.82)	4.16 (1.76)	4.05 (1.76)	2.83 (2.48)	4.19 (1.76)	$F_{5,263}$=1.48, p=0.19; 0.02; 0.51
Trials to complete first category	15.63 (6.96)	23.78 (23.92)	24.86 (27.71)	26.65 (27.00)	50.67 (57.36)	25.73 (23.70)	$F_{5,263}$=2.34, **p=0.04****; 0.04; 0.74
SOPT scores							
Total errors	14.33 (6.38)	14.92 (7.80)	15.11 (8.08)	15.95 (7.77)	18.00 (3.74)	17.10 (7.80)	$F_{5,292}$=0.28, p=0.91; 0.00; 0.12
TOL scores							
Total score	110.10 (13.54)	104.30 (15.32)	105.69 (14.39)	106.89 (17.35)	105.75 (2.98)	109.13 (14.32)	$F_{5,259}$=1.19, p=0.31; 0.02; 0.42
Total correct in 1st trial score	11.06 (1.00)	10.83 (1.36)	10.85 (1.22)	10.84 (1.38)	11.00 (0.81)	10.83 (0.98)	$F_{5,259}$=0.56, p=0.72; 0.01; 0.20

Note: *COMT = catechol-o-methyltransferase*; WCST Scores = Wisconsin Card Sorting Test Scores; SOPT scores = self-ordered pointing task scores; TOL scores = Tower of London scores. Values are mean (*SD*) unless otherwise indicated. Quantitative traits were compared between these groups using the appropriate statistic depending on the nature of the data. Number of observations varied sometimes with regard to variables. High functionality haplotype = GCGGval, average functionality haplotype = ATCAmet, and low functionality haplotype = ACCGval (Nackley, et al., 2006). **Significant effects.

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DECLARATION OF CONFLICTING INTERESTS

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CHAPTER 4

LPHN3 and attention-deficit/hyperactivity disorder: interaction with maternal stress during pregnancy

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PREFACE

Linkage studies using affected sib-pairs and extended pedigrees have implicated several chromosomal regions in ADHD. In particular, a recent meta-analysis identified 10 chromosomal regions with linkage signals. Fine mapping of one of these regions (4q13.2) led to the identification of the gene responsible for the linkage signal: *LPHN3,* which codes for Latrophilin 3. LPHN3 is a member of a newly-characterized family of G-protein coupled receptors, shown to be important for the regulated exocytosis of neurotransmitters, particularly norepinephrine (NE). It is highly regulated during postnatal brain development, with the highest levels observed immediately after birth. Further it has been associated with ADHD and its endophenotypes. In addition, the association with ADHD was consistently replicated in several different populations and an independent study. Thus, strong *apriori* evidence suggests that the *LPHN3* gene is an interesting candidate for genetic studies of ADHD.

In this chapter, we used another strategy, namely *"gene/environment interplay"*, to enhance capacity to identify genetic factors implicated in ADHD. We tested the association between six tag single-nucleotide polymorphisms (SNPs: rs2122643, rs1868790, rs6551665, rs1947274, rs6858066, and rs2345039) within the *LPHN3* gene and ADHD using family-based association testing (fBAT) in a large sample of children with ADHD (n = 380 families). Analysis based on stratification according to exposure to maternal smoking and maternal stress during pregnancy was used to investigate the effects of these environmental factors on genetic associations. This approach helped reveal a number of highly significant associations between tag SNPs (rs6551665,

rs1947274, rs6858066, rs2345039) within *LPHN3* and ADHD diagnosis, behavioral and cognitive measures relevant to ADHD and response to methylphenidate when the sample was stratified based on exposure to maternal stress during pregnancy. Thus, these results suggest that genetic variations in *LPHN3* may be an influential factor in the pathophysiology of ADHD, and also indicate possible interplay between genetics and *maternal stress during pregnancy*.

In summary, this study underscores the importance of the *"gene-environment interplay"* strategy in reducing the etiological heterogeneity of ADHD and identifying a more homogenous subgroup of children with ADHD. The use of stratification according to exposure to environmental risk factors will assist researchers in identifying genetic variants implicated in ADHD, which will help in turn elucidating pathways to the disorder.

ABSTRACT

Background: Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous behavioral disorder, complex both in etiology and clinical expression. Both genetic and environmental factors have been implicated, and it has been suggested that gene-environment interactions may play a pivotal role in the disorder. Recently, a significant association was reported between ADHD and *LPHN3* (which codes for latrophilin 3), and replicated in independent samples. **Methods:** We have examined the association between tag single nucleotide polymorphisms (SNPs) in *LPHN3* within the region previously implicated in ADHD. Family based association tests (FBAT) were conducted (n = 380 families) with the categorical diagnosis of ADHD, behavioral and cognitive phenotypes related to ADHD, and response to treatment (given a fixed dose of methylphenidate, 0.5 mg/day). Stratified FBAT analyses, based on maternal smoking and stress during pregnancy, was conducted. **Results:** Whereas limited association was observed in the total sample, highly significant interaction between four *LPHN3* tag SNPs (rs6551665, rs1947274, rs6858066, rs2345039) and maternal stress during pregnancy was noted. Analysis conducted in the sub-group of mothers exposed to minimal stress during pregnancy showed significant associations with ADHD, behavioral and cognitive dimensions related to ADHD, as well as treatment response. Although extensive association was observed with the candidate SNPs, the findings are partially inconsistent with previously published results with the opposite alleles over-transmitted in these studies. **Conclusions:** These results provide evidence for the interaction between a genetic and environmental factor independently shown to be associated with ADHD. If

confirmed in independent large studies, they may present a step forward in unraveling the complex etiology of ADHD.

Keywords

ADHD, *LPHN3*, maternal stress, environmental factors, GXE, genetic association

INTRODUCTION

Epidemiological studies have shown that prenatal exposure to various stressors (cigarette smoking, alcohol consumption, illicit drug use, exposure to stressful life events, and obstetrical complications) are associated with ADHD (Bhutta, Cleves, Casey, Cradock, & Anand, 2002; Knopik et al., 2005; Langley, Holmans, van den Bree, & Thapar, 2007; Langley, Rice, van den Bree, & Thapar, 2005; Thapar, Cooper, Jefferies, & Stergiakouli, 2011). Further, it has been proposed 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 (Thapar, et al., 2011; Wermter et al., 2010).

In a recent study, an association between polymorphisms within *latrophilin3* (*LPHN3*) and adult ADHD was reported (Arcos-Burgos, et al., 2010). LPHN3 is a member of the LPHN subfamily of G-protein coupled receptors (GPCRs) which have been shown to be important for the regulated exocytosis of neurotransmitters (particularly norepinephrine) (Davletov, et al., 1998; Rahman, et al., 1999; Silva, et al., 2009). LPHN3 is a brain-specific receptor, localized in the cerebral cortex, cerebellum, caudate nucleus, and amygdala. These include regions of the brain that have been shown to be important in ADHD (Aman, Roberts, & Pennington, 1998; Arnsten, 2009; Konrad & Eickhoff, 2010; Makris, Biederman, Monuteaux, & Seidman, 2009; Shaw & Rabin, 2009). LPHN3 has also been shown to be important in neurodegeneration following ischemia and hypoxia (Bin Sun, Ruan, Xu, & Yokota, 2002). Studies in the rat model have shown that expression of *LPHN3* is highly regulated during postnatal brain development, with the

highest levels observed immediately after birth (Kreienkamp, Zitzer, Gundelfinger, Richter, & Bockers, 2000).

The association between *LPHN3* and ADHD was first observed from fine-mapping of a region identified in a large, multi-generational linkage study conducted with a genetic isolate (Paisa population in Colombia) (Arcos-Burgos, et al., 2010). The initial finding was confirmed by the same group using both a case-control and family based design from five different populations (Arcos-Burgos, et al., 2010). The study reported an association both with ADHD as a disorder and with response to treatment with psychostimulants. Tag single nucleotide polymorphisms (SNPs) within the length of the gene were genotyped. A region delimited by SNPs rs1901223 and rs1355368, in the center of the gene encompassing exons 4 through 19, showed an association with ADHD in the initial study with the Paisa population. Meta-analysis of the seven independent replication samples confirmed the association with this region of *LPHN3*. Specifically, significant association was observed with the following SNPs: rs6551665 (OR = 1.23, 95% CI 1.09–1.37, p = 3.46 × 10−4), rs1947274 (OR = 1.23, 95% CI 1.09–1.38, p = 5.41 × 10−4), rs2345039 (OR = 1.21, 95% CI 1.08–1.35, p = 8.97 × 10−4).

A recent, independent case-control study in a Spanish population (Ribases, et al., 2011) confirmed the association between this region of *LPHN3* and adult ADHD. 43 tag SNPs were analyzed, and a three-marker haplotype (rs1868790-rs6813183-rs12503398), showed a highly significant association with combined type ADHD (global p-value = 8.3e–04, df = 3). Of note, a different set of tag SNPs (within the same region identified

earlier) showed an association with ADHD, while the original SNPs were not replicated in this study.

The objective of the current study is an investigation of five SNPs in the *LPHN3* gene, selected on the basis of their previous association with ADHD, with regard to: (a) behavioral and neurocognitive traits relevant for ADHD, (b) response of these behaviors to methylphenidate (MPH) as assessed in a double-blind, placebo-controlled, crossover trial, and (c) the effect of two important environmental risk factors, namely maternal smoking and stress during pregnancy in modulating the effect of this gene on behavioral outcomes and response to treatment. Given the complexity of ADHD, a detailed analysis of phenotypic traits that are driving the association with the disorder was conducted. Also, given the etiological complexity of ADHD, it was deemed important to investigate the interactions between this gene and environmental risk factors implicated in ADHD.

METHODS

PARTICIPANTS

Children with a diagnosis of ADHD, were recruited from the Disruptive Behaviour Disorders Program and the child psychiatry outpatient clinics at the Douglas Mental Health University Institute in Montreal. They were referred to these specialized care facilities by schools, community social workers, family doctors and pediatricians. The research protocol was approved by the Research Ethics Board of the Douglas Mental Health University Institute. Parents were explained the study and provided written consent. Children (affected child and unaffected siblings) were explained the study and

gave their assent to participate. The study included 380 nuclear families having one or more child with a DSM-IV diagnosis of ADHD. Details about diagnostic procedures have been described elsewhere (Grizenko et al., 2006). Briefly, the diagnosis was based on clinical interviews of the child and at least one parent, by a child psychiatrist. This clinical examination was supplemented with a structured clinical interview of parents using the Diagnostic Interview Schedule for Children-version IV, DISC-IV (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) and school reports including the Conners' Global Index-Teacher version questionnaire (Conners, Sitarenios, Parker, & Epstein, 1998a, 1998b). In the majority of cases, mothers were the primary informants. A child was excluded from the study if he/she had an IQ less than 70, and/or a diagnosis of Tourette syndrome, pervasive developmental disorder, and psychosis.

Of the total number of affected children, 78.3% were male and 85.5% were of Caucasian ethnicity. The affected children were between 6 and 12 years of age (mean = 9; SD = 1.8). 53.2% met DSM-IV criteria for the combined subtype, while 36.7% and 10.1% were diagnosed with the inattentive and hyperactive subtypes respectively. Among comorbid disorders, 40.4% had oppositional defiant disorder, 21.7% had conduct disorder, 44.1% had anxiety disorder (including phobias), and 8.3% had a mood disorder.

EVALUATIONS

In addition to the DSM-IV diagnosis of ADHD, clinically relevant dimensions of behavior i.e., child's behavior at home and in school were used as quantitative phenotypes in a quantitative trait loci (QTL) approach to complement the DSM-IV diagnosis of ADHD, as suggested by several researchers (Kuntsi, Rijsdijk, Ronald,

Asherson, & Plomin, 2005; Lasky-Su et al., 2008; Thapar, Langley, O'Donovan, & Owen, 2006). The behavior of the child at home was evaluated using the Conners' Global Index-Parents (Conners'-parents) (Conners, et al., 1998a, 1998b) as well as the Child Behaviour Checklist (CBCL) (Achenbach, 1991) while school behavior was assessed using the Conners Global Index-Teachers (Conners'-teachers) (Conners, et al., 1998a, 1998b). All these assessments were completed while the child was not taking any medication.

In addition to the clinical dimensions of ADHD, cognitive dimensions i.e., measures of executive function (EF), were included as quantitative traits in the genetic association analyses. EF encapsulates the range of cognitive abilities that are required for completing a given task, and include response inhibition, sustained attention, working memory, set-shifting, planning and organization. Deficits in EF are hypothesized to underlie the behavioral problems observed in ADHD (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). The strongest and most consistent impairments have been observed in spatial working memory, vigilance and response inhibition.

The battery of neuropsychological tests included the Wisconsin Card Sorting Test (WCST: measure of cognitive flexibility and set-shifting (Heaton, Chelune, Talley, Kay, & Curtiss, 1993)), Finger Windows (FW: visual-spatial working memory (Sheslow, 1990)), Tower of London test (TOL: planning, organization, and problem-solving capacity (Shallice, 1982)), Self-Ordered Pointing Task (SOPT: visual working memory, planning, and response inhibition (Petrides & Milner, 1982)), and Conners' Continuous Performance Test (CPT: measures attention, response inhibition, and impulse control

(Conners, 1994)). The WCST, TOL, SOPT, and CPT were performed as described (Gruber et al., 2007; Taerk et al., 2004). FW is a subtest of the Wide Range Assessment of Memory and Learning (WRAML). In this test, the child is required to repeat the sequential placement of a pencil into a series of holes on a plastic card, as conducted by the examiner.

When children were medicated prior to their inclusion in the study, the neuropsychological assessments were carried out at the end of a 1-week washout period to limit variability due to medication effects (Kebir, Tabbane, Sengupta, & Joober, 2009). In addition to the measures of EF, IQ (full scale, verbal, and performance IQ) was evaluated using the Wechsler Intelligence Scale (WISC; (Weschler, 1992)).

Obstetrical (pregnancy, delivery and perinatal) complications were assessed using the Kinney Medical and Gynecological Questionnaire and scored using the McNeil-Sjöström scale (McNeil, Cantor-Graae, & Sjostrom, 1994). Included in this questionnaire are assessments of smoking during the three trimesters of pregnancy. If the mother smoked at any time during the pregnancy (irrespective of duration), the child was coded as 'exposed'. During the interview, mothers were also asked to describe stressful life events experienced during the pregnancy. This information was used to score maternal stress levels from 1 to 4 based on the DSM-III and DSM-III-R axis IV scales (1 = no stress, 2 = mild, 3 = moderate, 4 = severe). Examples of mild stress include events such as arguments with friends, whereas moderate, severe stressors include separation, repeated physical or sexual abuse, imprisonment of a spouse, or death of a very close relative. Since information on environmental factors was collected retrospectively (and

may therefore be subject to recall bias), the information provided by the mother was corroborated with medical and obstetrical records as well as an independent interview with a second individual close to the mother (husband or other family member).

Response to treatment with MPH was assessed in a double-blind, placebo-controlled, within-subject (crossover) randomized control trial conducted over a 2-week period, as described (Grizenko, et al., 2006) (trial registration number: NCT00483106). Briefly, subjects received 1 week of treatment with placebo (PBO) and 1 week of treatment with 0.5 mg/kg of MPH in a divided b.i.d. dose (0.25 mg/kg, morning and noon), following a wash-out period. At the end of each week of treatment, the parents and teacher were asked to evaluate the behavior of the child using the Conners'-parents and Conners'-teachers respectively. These assessments were performed prior and after the administration of placebo and MPH. In addition, the clinical staff completed the Clinical Global Impression (CGI)-overall improvement based on their observation during half day of observation of overall behavior of the child while completing various tasks in the clinic.

GENETICS

The affected child, parents and unaffected siblings were invited to participate in the genetic component of the study. For each subject, DNA was extracted from a blood sample, a buccal swab or saliva sample, if the subject was only amenable to the latter. Of the 380 nuclear families that participated in the study, 184 were trios with information from both parents, 18 were trios with two affected children, 49 were trios with information from one parent and one or more unaffected sibling, 115 were duos including

the proband and one parent, while 14 were families with two affected siblings and one parent. While information on maternal smoking during pregnancy was available for all families, stress data was available for 328 of the 380 families.

SNP SELECTION AND GENOTYPING

A panel of six SNPs (rs2122643, rs1868790, rs6551665, rs1947274, rs6858066, and rs2345039) was genotyped using Sequenom iPlex Gold Technology (Ehrich, Bocker, & van den Boom, 2005). The SNPs were selected based on previous results showing that a region of *LPHN3*, between exons 4 through 19, was associated with ADHD (Arcos-Burgos, et al., 2010). The genotype distribution of the markers did not depart from Hardy Weinberg equilibrium ($p > 0.05$) (data not shown). An LD plot was constructed in Haploview v4.0 using genotype information downloaded from the HapMap Genome Browser (release#24) (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview). The default definition in Haploview was used to generate the plot. In this method, 95% confidence bounds on D' are generated for each pairwise comparison (Gabriel et al., 2002). A SNP block is formed if 95% of the informative comparisons are in strong LD with each other. Based on these criteria, there are 16 haplotype blocks in *LPHN3*. rs2122643, rs1868790, and rs2345039 are tag SNPs in blocks 8, 9, and 12 respectively, while rs6551665, rs1947274, rs6858066 are in haplotype block 11.

STATISTICS

Family based association tests (FBAT) were conducted using the FBAT statistical package (version 2.0.3 Harvard School of Public Health, Departments of Biostatistics and Environmental Health, Program for Population Genetics, Boston, MA, USA) (Laird, Horvath, & Xu, 2000). This test is based on the principal that, if a specific allele of G (or haplotype) is associated with an abnormal level of a trait, it is expected to be transmitted more frequently from parents to the child presenting an abnormal level of that trait. When this test is positive, it indicates the presence of both allelic association and linkage. Family based association analysis has two major advantages over population-based (case/control) association studies: it is not affected by population stratification, and it may have increased statistical power (Haldar & Ghosh, 2011). Further, because the non-transmitted parental alleles are the control alleles, this method controls for other possible sources of bias, such as socioeconomic status. All the analyses were performed under the assumption of an additive model, with a null hypothesis of no linkage and no association.

At the first level, FBAT analysis was conducted with the total sample. To test the interaction between environmental factors and genotype, subjects were then divided into groups: (a) based on maternal smoking during pregnancy; (b) based on maternal exposure to stressful life events during pregnancy (±moderate or severe stress during pregnancy). As this candidate gene showed replicated association with ADHD in previous studies, significance level was set at $p = 0.05$.

In a specific case of the FBAT (where both parents are known, and when the additive model is used), the Z^2 statistic can be considered equivalent to a χ^2TDT statistic

(N. Laird, personal communication). FBAT is an extension of McNemar's test used to calculate transmission disequilibrium in a pedigree, where χ^2TDT = (T − NT)2/(T + NT). T and NT denote the number of transmissions and non-transmissions of a specific allele from the parent to the affected offspring. To obtain an estimate of effect size, we applied this generalization and calculated the effect size Φ as for a Chi-squared test, where the following formula is used Φ = square root [χ^2/N (k − 1)], where N = sample number, and k = number of rows or columns or 2 in the McNemar's test. The number of informative families was used to calculate N. Effect sizes of 0.1, 0.3, and 0.5 are considered small, medium and large respectively (Supplementary Table 1).

RESULTS

In the total sample, significant association was observed with rs1868790 and rs6551665 when analysis was conducted with ADHD as a diagnostic entity (Table 1; Supplementary Table 1). Quantitative FBAT analysis with the total number of items (including inattention, hyperactivity and impulsivity items) on the DISC-IV (based on a clinical interview with parents), showed that the region of association was extended to include tag SNPs rs1947274 and rs6858066. One or more of the five tag SNPs (rs1868790, rs6551665, rs1947274, rs6858066, and rs2345039) showed significant association with behavioral assessment by parents (Conners'-parents and CBCL), IQ (full scale, as well as verbal and performance IQ), specific measures of EF (perseverative errors on the WCST, TOL total score, and CPT hit standard error block change), and treatment response (CGI-improvement) (Tables 1–3; Supplementary Table 1).

Most of the association was observed with the three tag SNPs in haplotype block 11 (rs6551665, rs1947274, rs6858066). The 'A' alleles of rs6551665 and rs1947274, and the 'G' allele of rs6858066 were over-transmitted to the higher scores on the DISC, Conners'-parents, and CBCL (total, as well as internalizing and externalizing factor scores) in the quantitative FBAT analysis, suggesting that these are risk alleles for the disorder. Exploratory analysis with the dimensional scores of the CBCL showed an association with different dimensions of ADHD. The strongest association was observed with somatic complaints. No association was observed with rs2122643 on any of the traits examined. Marginal association was observed with cognitive traits (full-scale and specifically performance IQ, perseverative errors on the WCST, Finger windows and Tower of London score) and treatment response (Clinical Global Impression-overall improvement). The effect sizes were small for each measure, ranging from 0.1 to 0.2 (Supplementary Table 1).

Stratified analysis, based on maternal stress during pregnancy, disentangled a highly significant GXE interaction with the *LPHN3* SNPs (Tables 1–3; Supplementary Table 1), with highly significant association observed in the 'no-stress' group. In contrast, a complete lack of association was noted in the 'stressed' group. The association between four tag SNPs (rs6551665, rs1947274, rs6858066, rs2345039) and the diagnosis of ADHD, behavioral and cognitive traits, as well as treatment response was highly significant in the group where mothers experienced mild or minimal stress during pregnancy.

The following measures showed the strongest associations: ADHD, number of ADHD items on DISC-IV, Conners'-parents and Conners'-teachers, each of the CBCL dimensions particularly delinquent and anxious/depressed behavior, full-scale and performance IQ, non-perseverative errors on the WCST, total score on the SOPT, and CPT overall index (which is a weighted sum of all the measures within the CPT). Highly significant association was also observed with CGI-improvement, and the evaluation of treatment response by parents (score on placebo week – score on MPH week). For each of these measures, medium to large effect sizes were obtained (0.3–0.5, Supplementary Table 1). The risk alleles (rs6551665-A, rs1947274-A, rs6858066-G, rs2345039-C) were over-transmitted to the higher scores (positive Z statistic) on the DISC, Conners'-parents and Conners'-teachers, and each of the CBCL dimensional scores suggesting that children with this genotype have a more severe clinical presentation (Supplementary Table 1). In terms of cognitive function, children with these risk alleles showed worse performance on the WCST, which measures cognitive flexibility and set-shifting. Here, the risk alleles showed an under-transmission (negative Z-score) to the higher scores (the higher standard scores imply a better performance on the test). Similarly, the risk alleles were associated with poorer performance on the SOPT and CPT. Since these are not standardized scores, the higher scores imply worse performance. These deficits in EF domains were observed even though the risk alleles showed an association with higher full-scale and performance IQ scores. Since most of the association was observed with the three tag SNPs in block 11, analysis was also conducted with the haplotype rs6551665- rs1947274-rs6858066 using FBAT (Supplementary Table 2). AAG appears to be the risk haplotype while GCA is the protective haplotype based on the respective

over-transmission and under-transmission to ADHD and each of the CBCL dimensional scores. The AAG haplotype also showed a significant association with the SOPT scores (worse cognitive function). In terms of treatment response, the risk haplotype AAG was associated with poor treatment response, while children having the GCA haplotype improved with treatment, as measured by the CGI-overall improvement scale. Using this scale, overall improvement in symptoms during the week of treatment with MPH (relative to the baseline state) is rated by the clinician on a 7-point scale with 1 = significantly improved to 7 = significantly worse. A negative Z-score (as observed with the GCA haplotype) therefore implies a significant improvement with treatment. This haplotype was also associated with a smaller change in the Conners'-Parent score after treatment. This is reasonable since it is associated with fewer behavioral problems at baseline. The significant GXE interaction with stress was in stark contrast to the lack of interaction with exposure to maternal smoking during pregnancy (Tables 1–3). Here, limited association with LPHN3 SNPs was evident in both groups (±maternal smoking during pregnancy).

DISCUSSION

The differential association between *LPHN3* tag SNPs and maternal stress during pregnancy provides evidence for the interaction between a genetic and environmental factor that have been shown to be independently associated with ADHD. ADHD is a Heterogeneous behavioral disorder, complex both in etiology and clinical expression. The GXE interaction with tag SNPs in *LPHN3* was noted with different dimensions of the disorder, including behavioral and cognitive phenotypes, as well as response to MPH

treatment. Conducted by independent evaluators in three environments (clinic, home and school), these evaluations represent different dimensions related to ADHD as a disorder.

In addition to the overall association with ADHD behavior, the *LPHN3* SNPs showed a strong association with full-scale IQ and specifically with performance IQ. Of the EF domains examined, a significant association was observed with the total score on the Self-Ordered Pointing Task, a measure of visual-spatial working memory, planning and response inhibition. This is interesting particularly in the light of meta-analysis results which showed that children with ADHD demonstrate significant impairment on all measures of EF, but the strongest and most consistent impairments were observed in spatial working memory, vigilance, and response inhibition (Willcutt, Pennington, Olson, Chhabildas, & Hulslander, 2005). Weaker associations with one or more of the tag SNPs was noted with non-perseverative errors on the Wisconsin Card Sorting Test, TOL score, and different dimensions on the CPT. However studies with larger sample sizes are required to clarify these associations. Finally, a significant association was observed between tag SNPs, rs6551655, and rs1947274, and treatment response as measured by the CGI-overall improvement and Conners'-parents difference score (total score on placebo week- total score on MPH week). This association was only noted in the no-stress group.

The result of this study suggests that stress during pregnancy may serve to delineate two pathways to the disorder, and the *LPHN3* association is particularly important in the low stress group. The genetic factors important when the mother is exposed to a high-stress environment remain to be elucidated.

An alternative explanation is that genetic influence on behavioral and cognitive outcome is accentuated in favorable environments. This hypothesis is derived from studies examining the relative importance of genetic and environmental factors on IQ, where epidemiological studies revealed an apparent contradiction (Turkheimer, 1991). In studies comparing IQ between adoptees and their biological versus adoptive parents, large genetic effects and small effect of environment were observed. In contrast, studies examining IQ of children rescued from poverty compared to their parents or impoverished siblings, showed a large effect of environment. This led to the hypothesis that the effect of family environment on cognitive ability is non-linear (Jensen, 1981; Scarr, 1981) and that genetic differences become more pronounced in adequate or enriched environments (Bronfenbrenner & Ceci, 1994). It may be reasonable to extrapolate this hypothesis to suggest that a high level of maternal stress during pregnancy leads to an impoverished environment for the developing fetus. Here then the effect of the environment far exceeds the influence of genetic factors in precipitating the disorder.

The sub-group of families where the mother experienced low to minimal stress during the pregnancy constituted 40% of the total sample, suggesting that the highly significant association is not driven by a few outliers. This sample was approximately comparable to the group where mothers experienced moderate or severe stress during pregnancy (Supplementary Table 1). However, it is possible that in this group, the contribution of *LPHN3* variants to the ADHD phenotype is smaller, based on the hypothesis discussed earlier. Due to the smaller effect size, a larger sample would be

required to detect an association. The possibility of Type II error in the subgroup of children exposed to maternal stress can therefore not be ruled out in the current study.

It is observed that the tag SNPs show an association with some ADHD dimensions and not with others. This is particularly stark in the different cognitive domains. A significant association is observed with the Self-Ordered Pointing Task, an assessment of visual working memory, planning, and response inhibition (Petrides & Milner, 1982) but not with the Finger windows (a different measure of visual-spatial working memory) (Sheslow, 1990). This may be due to either of two reasons: the variance of the phenotype may be different for each of the measures or the specific neurobehavioral pathway involved in each process may be different. Further work in large samples is required to tease apart these differences. The importance of testing genetic association not just with the categorical diagnosis of ADHD but with the component endophenotypes has been emphasized for complex behavioral disorders (Owen, O'Donovan, Thapar, & Craddock, 2011), and the detailed analysis presented in this paper is a step in that direction.

FBAT analysis uses the transmission disequilibrium test to examine the over-transmission of each allele from the parent to the affected offspring. The major advantage of using this method over case-control studies is that it is not prone to produce false-positives in the presence of hidden population stratification and admixture (Curtis & Sham, 1995; Laird, et al., 2000). In addition, simulation studies have shown that the family based design has higher power to detect an association compared to a case-control study with an equivalent sample size (Haldar & Ghosh, 2011). This study extends the

earlier case-control studies showing an association between *LPHN3* and childhood as well as adult ADHD (Arcos-Burgos, et al., 2010; R. Jain et al., 2011; Ribases, et al., 2011), and provides further insight into the etiological pathways involved.

Latrophilin3 is a member of the LPHN subfamily of G-protein coupled receptors (GPCRs) known as 'adhesion GPCRs' (Fredriksson, Lagerstrom, Lundin, & Schioth, 2003). The large, complex, N-terminal fragment of these receptors contains various motifs that have been implicated in cell adhesion. LPHN3 has been shown to be involved in regulated exocytosis of neurotransmitters and hormones. Small G proteins are highly enriched at synapses, playing a critical role for intracellular signaling (Lopez de Maturana & Sanchez-Pernaute, 2010). It has been shown that LPHN mediates Gaq/11-coupled signaling (Rahman, et al., 1999). Following the coupling, phospholipase C is activated, and intracellular Ca2+ stores are mobilized, with resultant exocytosis of norepinephrine. It has also been shown that UNC-13, a major presynaptic diacylglycerol receptor that is essential for vesicle mediated release of neurotransmitter, is involved in LPHN-dependent regulation of exocytosis (Brose, Rosenmund, & Rettig, 2000; Willson et al., 2004). The C-terminal region of LPHN has also been found to interact with synaptic scaffolding proteins of the ProSAP/SSTRIP/Shank family, which play the vital role of regulating the cytoskeletal architecture at synapses (Kreienkamp, Soltau, Richter, & Bockers, 2002; Kreienkamp, et al., 2000). It has been suggested that the interaction between LPHN and these scaffolding proteins affects the architecture of the cytoskeleton at the synapse, thereby controlling the vesicular fusion/docking process.

Norepinephrine (NE) and the related catecholamine dopamine (DA) have been considered to be major players in the pathophysiology of ADHD. The psychostimulants MPH and amphetamine, which are widely used for the treatment of ADHD, block the NE and DA transporters, resulting in increased synaptic concentration of the neurotransmitters (Krause, Krause, Dresel, la Fougere, & Ackenheil, 2006; Madras, Miller, & Fischman, 2005; Volkow et al., 2001). In addition, pharmacological agents that are selective for NE (including clonidine, guanfacine, desipramine, and atomoxetine) have been found to be effective in treating ADHD (Biederman & Spencer, 2002). Neuroimaging (Del, Chamberlain, Sahakian, & Robbins, 2011) and animal studies (Arnsten & Pliszka, 2011) have provided further evidence for the role of NE in ADHD. It is plausible therefore that polymorphisms that affect the structure of LPHN3 and/or transcription of the gene could affect signal transduction or vesicular trafficking, thereby affecting the concentration of NE at the synapse, with downstream effects on behavior and cognition. In vitro and in vivo studies will be critical to dissect the pathways involved going from gene to neurochemical function.

Although extensive association was observed with the candidate SNPs in *LPHN3*, the findings are partially inconsistent with previously published results (Arcos-Burgos, et al., 2010). Arcos-Burgos et al. reported the over-transmission of the G, C, and C alleles for rs6551665, rs1947274, and rs2345039 respectively. Even in the no-stress group where a highly significant association with these SNPs was observed, these alleles showed an under-transmission in our sample. This is not an uncommon occurrence in genetic studies of complex psychiatric disorders and remains to be clarified in independent samples. In the only other replication study with *LPHN3* conducted by an independent group

(Ribases, et al., 2011), it was not the original tag SNPs (Arcos-Burgos, et al., 2010), but others within the same region of the gene, that showed an association.

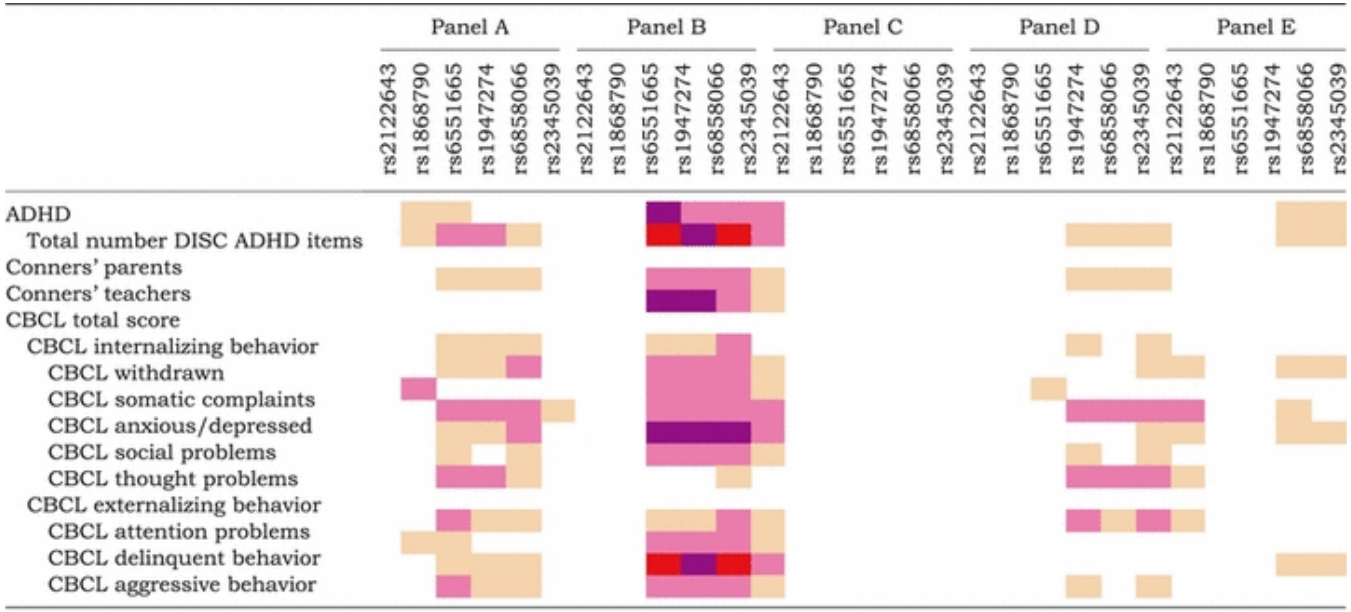
Because *LPHN3* has been previously associated with ADHD, the primary outcome result of this study (overall association with the disorder) was not corrected for multiple testing. The rest of the results were not corrected for multiple testing as they are considered exploratory and may provide insight into the association with the different dimensions of the disorder, and may help to inform related studies with ADHD. However it is important to note that even if the stringent Bonferroni correction was to be applied (6 SNPs X 5 exposure strata, $p = 0.002$), the association results in the low stress group would remain significant. Also, the extensive association observed with behaviors measured by different observers (parents, teachers, and clinical staff) and in different settings (school, home, clinic) suggests that these associations are unlikely to be chance findings.

It is also noted that the number of informative families in the groups with minimal versus severe stress during pregnancy, is comparable ($n = 63$ and 52 respectively at rs6551665). This reduces the risk that the differential association observed between the two groups could be an artifact driven by a small sample size in either one of these two groups. In addition, given that we used a within-family analysis, it is the non-transmitted alleles of parents that are used as controls, which reduce the possibility of other sources of bias (e.g., differences in socioeconomic status).

CONCLUSION

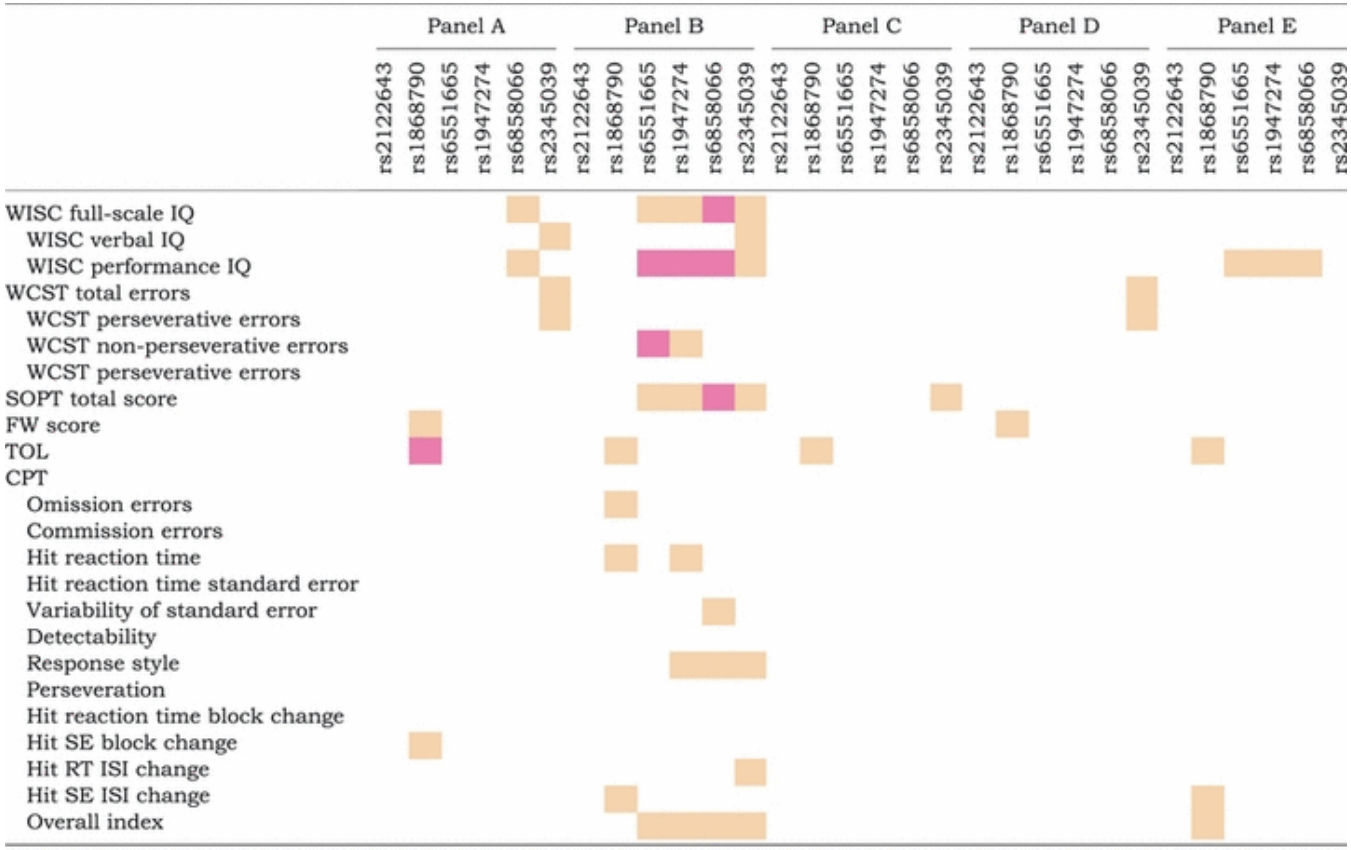
While confirmation from independent large studies is awaited, these results suggest that at least two independent pathways are important in ADHD. In the absence of moderate or severe stress, and irrespective of whether the mother smoked during pregnancy, *LPHN3* is at least one component important in precipitating the disorder. When the mother is exposed to moderate or extreme stressors during pregnancy, polymorphisms within *LPHN3* appear not to play a role in the disorder. It is likely that other pathways, yet to be elucidated, are more critical under these conditions. If confirmed, these results may present a step forward in unraveling the complex etiology of ADHD.

Table 4.1. Association of *LPHN3* with ADHD and behavioral traits



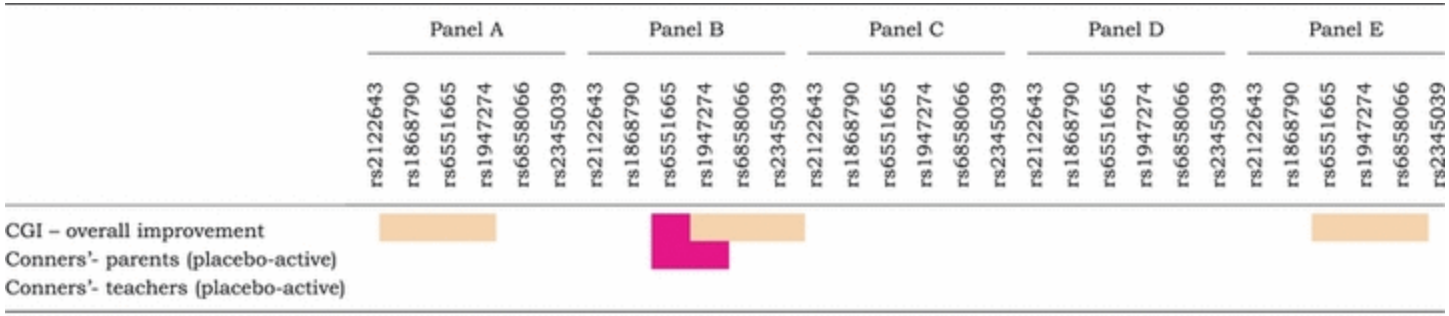
ADHD, Attention-deficit/hyperactivity disorder; CBCL, Child Behaviour Checklist.
Panel A: total sample.
Panels B, C: Stratification based on maternal stress during pregnancy (Panel B -Mild or minimal; Panel C -Moderate or severe).
Panels D, E: Stratification based on maternal smoking during pregnancy (Panel D -No; Panel E -Yes).
p-values are provided according to a color code as indicated by the appended scale:
0.01–0.05.
0.001–0.009.
0.0001–0.0009.
<0.0001.

Table 4.2. Association of *LPHN3* SNPs with cognitive traits



CPT, continuous performance test; FW, finger windows; SOPT, self-ordered pointing task; TOL, Tower of London test; WCST, Wisconsin Card Sorting Test; WISC, Wechsler Intelligence Scale.
Panel A: total sample.
Panels B, C: stratification based on maternal stress during pregnancy (Panel B -mild or minimal; Panel C -moderate or severe).
Panels D, E: Stratification based on maternal smoking during pregnancy (Panel D -no; Panel E -yes).

Table 4.3. Association of *LPHN3* SNPs with treatment response



CGI, clinical global impression.
Panel A: total sample.
Panels B, C: Stratification based on maternal stress during pregnancy (Panel B -mild or minimal; Panel C -moderate or severe).
Panels D, E: stratification based on maternal smoking during pregnancy (Panel D -no; Panel E -yes).

Table S1 Association of *LPHN3* with ADHD and behavioral traits in total sample and in families where mother smoked during pregnancy and experienced mild or minimal stress during pregnancy

File Name: JCPP_2551_sm_TableS1.xlsx

Web Link:

http://onlinelibrary.wiley.com.proxy1.library.mcgill.ca/store/10.1111/j.1469-7610.2012.02551.x/asset/supinfo/JCPP_2551_sm_TableS1.xlsx?v=1&s=a0de2ed9e6353398662cff92315c9e20ad3fb1a3

Table S2 Association of *LPHN3* SNPs (haplotypes) with ADHD relevant traits in total sample and in families where mother experienced mild or minimal stress during pregnancy

File Name: JCPP_2551_sm_TableS2.xlsx

Web Link:

http://onlinelibrary.wiley.com.proxy1.library.mcgill.ca/store/10.1111/j.1469-7610.2012.02551.x/asset/supinfo/JCPP_2551_sm_TableS2.xlsx?v=1&s=30a5a02490527828a3705a63b8e14f5e7b5b8248

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Key points

• Recently, a significant association was reported between *LPHN3* (which codes for latrophilin 3) and ADHD. A region in the center of the gene, encompassing exons 4 through 19, was shown to be associated with the disorder as well as response to treatment with psychostimulants

• In the current study, significant associations were observed between tag SNPs within *LPHN3* and ADHD, as well as quantitative behavioral and cognitive phenotypes.

• The major contribution of the paper is the finding that *LPHN3* shows a highly significant interaction with maternal stress during pregnancy. This interaction was observed with the disorder, behavioral and cognitive dimensions of the disorder, as well as response to treatment with methylphenidate.

• No interaction was observed with maternal smoking during pregnancy.

• If confirmed in independent studies, these results may present an important step forward in dissecting the etiology of ADHD, with important clinical implications for treatment.

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CHAPTER 5

Body Weight and ADHD: a comprehensive clinical and behavioral characterization

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PREFACE

ADHD is a complex disorder with a heterogeneous clinical expression, and it is highly comorbid with other psychiatric and somatic conditions. Amongst the comorbid somatic disorders, obesity and/or weight gain problems have been consistently associated with ADHD. More specifically, it is believed that, children with ADHD may be at a higher risk for being overweight; likewise, overweight children are highly predisposed for a diagnosis of ADHD. The underpinnings of this association are not well understood. However, some researchers believe that, there may be shared behavioral and neurobiological mechanisms underlying this association.

To date, no study has investigated the relation between body weight and clinical and behavioural characteristics of children diagnosed with ADHD. Thus, in this chapter, a sample of children with ADHD were stratified based on their weight status, and characterized with respect to several clinical and behavioural traits after adjusting for a number of socio-demographic confounders. Significant differences were observed among the three weight groups. More specifically, obese ADHD children were more likely to be diagnosed with the inattentive subtype of ADHD. Moreover, these children were more withdrawn, with less severe hyperactivity and less overall behavioral problems. In contrast, overweight children were predominantly diagnosed with the hyperactive subtype, exhibited more restless-impulsive behavior in the classroom but not in the home environment. These findings suggest that the association between weight deregulation and ADHD may help to understand the

phenotypic complexity of ADHD and the pathogenic pathways leading to weight gain in ADHD.

ABSTRACT

Background: Attention-Deficit/Hyperactivity Disorder (ADHD) is a complex and heterogeneous childhood disorder which often coexists with other psychiatric and somatic disorders. Recently, a link between ADHD and body weight disorders has been reported. The objective of this study is to investigate the relation between body weight/BMI and clinical/behavioural characteristics in children with ADHD. **Methods:** 290 ADHD children were stratified by weight status/BMI according to the WHO definition and compared with regard to their clinical/behavioral characteristics. All comparisons were adjusted for relevant socio-demographic characteristics. **Results:** Socioeconomic status, parental age at child birth, maternal smoking during pregnancy, and medication naivety status were significantly associated with weight/BMI in children with ADHD. After adjusting for these potential confounders, obese children were more likely to show withdrawn behaviors and to be diagnosed with the inattentive subtype of ADHD. They also showed less severe hyperactivity and overall behavioral problems. In contrast, overweight children were predominantly diagnosed with the hyperactive subtype, exhibited more restless-impulsive behavior in the classroom but not in the home environment. **Conclusions:** Our results suggest that differences in weight are associated with differential patterns of clinical characteristics in children with ADHD. Under the assumption that weight regulation and ADHD share some of their pathogenic pathways, these observations might help to understand these pathways and reduce the heterogeneity of ADHD.

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common childhood psychiatric disorder, characterized by severe and persistent inattention, hyperactivity, and impulsivity. It occurs in 8%–12% of the children (Faraone, et al., 2003) and often results in, social, academic, and vocational impairment (Biederman & Faraone, 2005). ADHD often coexists with other psychiatric (Rader, McCauley, & Callen, 2009) and somatic disorders (van den Heuvel, Starreveld, de Ru, Krauwer, & Versteegh, 2007), adding to the complex etiological and clinical heterogeneity of the disorder.

Like ADHD, childhood obesity is another serious healthcare problem, given its biopsychosocial ramifications. In the last few decades it has emerged as an epidemic in the developed world (Wang & Lobstein, 2006). Indeed, epidemiological data from the United States reports a three to four fold rise in obese children aged between 6-11 years (Ogden et al., 2006; Ogden, Carroll, & Flegal, 2008). Correspondingly, data from Germany suggests that 15% of its childhood and adolescent population is overweight (Kurth & Schaffrath Rosario, 2007).

Recently, it has been suggested that compared to controls, children with ADHD are more obese (Ptacek, Kuzelova, Paclt, Zukov, & Fischer, 2009a) and show higher propensity towards weight gain (reviewed by (Cortese & Vincenzi, 2012)). This tendency to gain weight in children with ADHD may be due to their disordered eating habits and an increased preference for the consumption of non-healthy foods. Indeed, studies examining disorganized eating behaviour in ADHD children have shown that these children tended to eat above the normal level, particularly at the beginning of the meal, and their preference for immediately available food was predicted by their parental ratings of impulsivity (Wilhelm et al., 2011). Furthermore,

a cross-sectional study of 1,799 Australian adolescents examining the effects of non-healthy foods consumption showed that the likelihood of an ADHD diagnosis increased in adolescents who either consumed higher amounts of fat, refined sugars, and sodium and/or ate less fiber, folate, and omega-3 fatty acids in their diet (Howard et al., 2011). Given these observations, it is considered plausible that ADHD and obesity may be linked, but the relationship between these complex disorders is not very well understood (Cortese & Vincenzi, 2012; Surman, Thomas, Aleardi, Pagano, & Biederman, 2006).

From a neuropsychological point of view, some theories have suggested that the link between ADHD and body weight dysregulation may be due to the impaired self-regulation that is shared between the two conditions. However, experimental data supporting these theories is very limited. Further, a recent study controlling for potential confounders didn't show any effect of BMI/weight on cognitive, emotional and motor characteristics in children with ADHD (Choudhry et al., 2013). Thus, it may be possible that, some other determinants link childhood obesity and ADHD.

At the behavioral level, studies have suggested that obesity is often associated with behaviors reminiscent of ADHD symptoms. For example, binge-eating, often observed in severely obese subjects (Hudson, Hiripi, Pope, & Kessler, 2007) and bulimia nervosa, is often associated with impulsivity, inattention and hyperactivity symptoms (Rosval et al., 2006). These behavioral traits are also reported in obese adults with abnormal eating behaviors (Davis, Zyzanski, Olson, Stange, & Horwitz, 2009). Conversely, it is believed that behavioral impulsivity, one of the cardinal symptoms of ADHD, could lead to excessive and impulsive eating in patients with ADHD (Cortese et al., 2007). These observations suggest that the neurobiological

pathways implicated in the regulation of behaviours relevant to ADHD may also be important determinants of eating behaviors and energy intake.

Studies investigating the possible relationship between obesity and ADHD or ADHD symptoms to date have reported diverse findings (Cortese & Vincenzi, 2012; Surman, et al., 2006). More specifically, clinical studies of extremely obese children and/or children with ADHD suggest a high prevalence of comorbidity between ADHD and obesity in children (Agranat-Meged et al., 2005; Braet, Claus, Verbeken, & Van Vlierberghe, 2007; Curtin, Bandini, Perrin, Tybor, & Must, 2005; Holtkamp et al., 2004; Hubel, Jass, Marcus, & Laessle, 2006; Ptacek, Kuzelova, Paclt, Zukov, & Fischer, 2009b). However, results from epidemiological studies are mixed, some suggesting a possible association between ADHD diagnosis and obesity (Erhart et al., 2012; Lam & Yang, 2007; Waring & Lapane, 2008), while others do not observe such an association (Drukker, Wojciechowski, Feron, Mengelers, & Van Os, 2009; Rojo, Ruiz, Dominguez, Calaf, & Livianos, 2006). Likewise, findings from longitudinal studies are discordant (Biederman, Spencer, Monuteaux, & Faraone, 2010; Graziano, Calkins, & Keane, 2010; Mustillo et al., 2003). These discrepancies may be due to the heterogeneity of the ADHD and the obesity phenotypes. Indeed, as ADHD has many subtypes (inattentive, hyperactive and the combined); it may be possible that these subtypes associate differently with overweight and/or obesity.

To the best of our knowledge, no study to date has explored an association between ADHD clinical traits and body weight in children with ADHD while controlling for potential confounders. Thus given this gap in the literature, the present study was conducted to investigate the relation between body weight and

behavioural/clinical characteristics of children diagnosed with ADHD using a detailed clinical/behavioural assessment battery and adjusting for potential confounders.

METHODS

SUBJECTS, STUDY PROCEDURES, AND ETHICS

Two hundred and ninety children with ADHD (215 males and 75 females), ages 6-12 [mean=9.15; SD=1.86], were recruited from the Disruptive Behaviour Disorders Program and the Douglas Mental Health University Institute (DMHUI) outpatient clinic. These ADHD children were also included in another study (Choudhry, et al., 2013) that, explored the effect of BMI/weight on self-regulation indices. All of these children were referred to these facilities by school teachers, pediatricians, and community social workers. The research protocol was approved by the Research Ethics Board of the DMHUI. Children with ADHD and their parents were explained the study procedures in detail, and provided verbal assent and written consent, respectively.

All children included in this study met DSM-IV diagnosis criteria for ADHD. A comprehensive clinical evaluation was used to establish the diagnosis of ADHD [see details in (Grizenko, Bhat, Schwartz, Ter-Stepanian, & Joober, 2006; Sengupta et al., 2012)]. Children were excluded from this study if they had an IQ less than 70 on the Wechsler Intelligence Scale for Children-III/IV (WISC-III or WISC-IV), Tourette syndrome, pervasive developmental disorder, or psychosis.

All the behavioral assessments were completed while the children were not taking any medication. In cases where children were on medication prior to their inclusion in the study, these assessments were carried out at the end of a one-week washout period.

CLINICAL AND BEHAVIORAL EVALUATION

The Child Behavior Checklist (CBCL), which assesses several behavioral dimensions of the child, was completed by the parents. The child's behavior at home and in the classroom environment were evaluated by parents and teachers using the Conners' Global Index for Parents and Teachers (CGI-P and CGI-T) respectively (Conners, 2003a, 2003b).

ANTHROPOMETRICS

All anthropometric measurements were taken by trained research assistants. Height of children with ADHD was measured using a wall mounted chart with the subjects being bare-footed. Similarly, body weight was measured using a doctor's clinical scale with subjects clad in regular casual attire and being bare-footed.

CALCULATION OF BODY MASS INDEX (BMI) AND DEFINITION OF WEIGHT CATEGORIES

Body mass index (BMI) is a simple index of weight-for-height which is used to classify overweight and obesity in normal and clinical samples. It is defined as "weight in kilograms divided by the square of the height in meters (kg/m^2)". BMI was initially calculated for all children with ADHD, and then it was converted to age- and gender-specific percentiles according to the criterion available in the World Health Organization (WHO) [website: http://www.who.int/en/]. The WHO defines "normal weight" as ranging between 3^{rd} to 84^{th} percentile, "overweight" as ranging between 85^{th} to 96^{th} percentile, and "obesity" as equal or greater than 97^{th} percentile.

STATISTICAL ANALYSES

Statistical analyses were performed with SPSS 15.0 version for Windows. Subjects were stratified into three groups according to the Weight/BMI classification as per WHO criterion. First, demographic and baseline characteristics of children with ADHD were compared between the three groups, namely normal (n = 168), overweight (n = 57), and obese (n = 59). Socio-demographic variables associated with BMI at a significance level of $p < 0.05$ were used as covariates in subsequent analyses. Second, clinical and behavioural outcome measures were compared between three weight/BMI groups using univariate ANOVA for continuous variables and chi-squared tests for categorical variables. Main effects were further explored by *Post Hoc* pairwise comparisons and the confidence intervals were adjusted using the Bonferroni correction.

RESULTS

As shown in Table 1, Socio-demographic characteristics of ADHD children stratified by BMI categories (normal vs. overweight vs. obese) showed that, the three weight groups were similar with regard to child's gender (% males), age, ethnicity (% white), and birth weight (gms). However, ADHD children within the normal weight (39.9%) group were significantly more likely to be previously using stimulant medication compared to the overweight (25.0%), and obese (20.3%) groups ($X^2 = 6.15$, df $= 2$, $p = 0.04$).

Exploration of differences in the family characteristics of ADHD children showed that, the three groups were similar with regard to Parental (mother and father) level of education, maternal alcohol consumption status during pregnancy, and child's adoption status. However, mother's ($F_{2,261} = 5.14$, $p = 0.006$) and father's

($F_{2,233} = 4.55$, $p = 0.01$) age at child birth, maternal smoking status during pregnancy (MSDP) ($F_{2,248} = 3.26$, $p = 0.04$), and annual family income ($X^2 = 7.84$, df $= 2$, $p = 0.01$) were significantly different amongst the three weight categories. These findings collectively suggest that overweight and obese ADHD children have a lower socioeconomic status (SES) compared to ADHD children with normal weight. All subsequent analyses were therefore conducted while controlling for aforementioned SES factors (parental age at child birth, annual family income, and MSDP) and prior history of treatment with psychostimulants.

As shown in Table 2, ADHD children within the obese group were significantly more likely to be diagnosed with the inattentive subtype (56.9%) compared to the overweight (35.1%), and normal weight (39.9%) groups ($X^2 = 6.77$, df = 2, p = 0.03). No between group differences with regard to comorbid disorders (oppositional defiant, conduct, anxiety, and depressive disorders) were detected (all $p>0.05$).

Obese ADHD children showed a less severe presentation in DISC clinical dimensions compared to the normal weight children (all pair-wise p-values comparing the normal weight group to the obese groups were ≤ 0.01). These differences were particularly marked for the total number of ADHD symptoms ($F_{2,215} = 3.97$, $p = 0.02$), a difference that is driven by less items of hyperactivity symptoms ($F_{2,215} = 4.04$, $p = 0.01$). Also, obese children were more withdrawn ($F_{2,201} = 6.07$, $p = 0.003$), and showed trends towards more Internalization ($F_{2,201} = 2.69$, $p = 0.07$) and social problems ($F_{2,201} = 2.49$, $p = 0.08$) compared to overweight ADHD children as measured by the CBCL.

Furthermore, with regards to the child's behavior in different environments, overweight children were significantly more restless-impulsive ($F_{2,194}$ = 2.99, p = 0.05), and may have more overall behavioral problems ($F_{2,194}$ = 2.66, p = 0.07) compared to obese ADHD children in a classroom setting as evaluated by CGI-T. However in a home environment, overweight children showed least restless-impulsive ($F_{2,181}$ = 3.52, p = 0.03), and overall behavioral problems ($F_{2,181}$ = 3.09, p = 0.04) compared to normal-weight children as evaluated by the CGI-P.

DISCUSSION

This study showed significant differences amongst normal, over-weight and obese categories in ADHD children with regards to their clinical profile, symptom severity and behavior in different environmental settings such as home and school. More specifically, obese children were mostly of inattentive subtype, while overweight children were predominantly hyperactive subtype. Obese ADHD children also had less severe hyperactivity symptoms translating into less severe total ADHD clinical presentation when compared to normal weight children with ADHD. Further, obese children with ADHD were more withdrawn, showed trends towards higher internalization and social problems compared to overweight children with ADHD as measured by the CBCL. In contrast, overweight children were more restless-impulsive, and showed trends towards more overall behavioural problems in a classroom setting compared to obese children with ADHD when assessed by CGI-T. Interestingly, overweight ADHD children showed least restless-impulsive, and overall behavioral problems compared to normal-weight children as evaluated by CGI-P within a home environment. These findings implied that certain domains of psychopathology may be differentially associated with weight categories in children with ADHD.

The current study has a number of strengths. This is the only large study examining the relations between weight status/BMI and symptom severity in children diagnosed with ADHD. Further, the clinical behavioural assessment is comprehensive, exploring several behavioural dimensions measured in different environments such as home and school by different raters. Also, all clinical/behavioural assessments were carried out while the children were not taking any medication (1 week washout period). Finally, the use of socio-demographic variables associated with BMI as covariates in the main analyses increases confidence in these results.

While the clinical/behavioural profile of obese ADHD children presented in this study is supported by previous clinical findings in obese subjects (Fleming, Levy, & Levitan, 2005), the association of overweight category with hyperactivity has not been shown before. More specifically, previous studies reported inattention in a clinical sample of severely obese subjects and 26.7% of these subjects displayed significant ADHD symptoms in both their child and adulthood (Fleming, et al., 2005). In addition, obese youth were described by their peers, teachers, and self-reports as more socially withdrawn (Zeller, Reiter-Purtill, & Ramey, 2008), and displaying significantly higher internalization problems and poorer social skills (Vila et al., 2004). The unexpected finding of the association of hyperactivity with the over-weight category in the ADHD children needs more clarification though literature is almost silent about it.

Nevertheless, some inferences are still possible regarding the differential classroom behavioral profiles exhibited by overweight and obese ADHD children in our study. Previous work hinted that weight-based stigmatization/bullying by peers

have a negative impact on psychological, behavioural and social characteristics ((Janssen, Craig, Boyce, & Pickett, 2004; Lumeng et al., 2010); reviewed by (Washington, 2011)). Peer bullying and/or teasing because of weight problems (Madowitz, Knatz, Maginot, Crow, & Boutelle, 2012) is shown to increase absenteeism and poor academic performance (Krukowski et al., 2009; Sigfusdottir, Kristjansson, & Allegrante, 2007), disruptive class-room behavior and acting out (Neumark-Sztainer, Story, & Faibisch, 1998; Zeller, et al., 2008), social withdrawal and poor self-esteem (Pesa, Syre, & Jones, 2000). Although, our study did not investigate absenteeism and/or academic performance, the disruptive classroom behavior (restlessness-impulsivity) in overweight children is consistent with above findings. However, the class-room behavior displayed by obese ADHD children in our study does not follow the trends seen in earlier studies probably since some variable such as; absenteeism and poor academic performance were not taken into consideration. We posit that this could be due to over-expression of isolation in obese children rather than disruptive behavior because of their physical limitation of not being able to respond to teasing as compared to overweight children. We, however, have not found studies favoring or discarding our hypothesis, and therefore, further clarifications are needed to understand the effect of school environment on psychological profile of overweight and obese ADHD children.

Interestingly, overweight and obese ADHD children in our study displayed less behavioural problems at home as assessed by CGI-P, this may be due to the absence of peer induced teasing or bullying. Moreover, the better home behaviour exhibited by these ADHD children may be explained by previous work exploring the involvement of ADHD children in sedentary activities. More specifically, it has been proposed that ADHD children spend more time at home involved in sedentary

activities such as playing video games and viewing television (Chan & Rabinowitz, 2006; Swing, Gentile, Anderson, & Walsh, 2010). Likewise, television viewing (Boone, Gordon-Larsen, Adair, & Popkin, 2007; O'Brien et al., 2007; Rey-Lopez, Vicente-Rodriguez, Biosca, & Moreno, 2008), electronic game playing and computer use (Granich, Rosenberg, Knuiman, & Timperio, 2010) has been associated with weight problems in children. Findings from CGI-P and CGI-T analyses, therefore, favored that school environment could be responsible for hyperactivity in overweight children, though more empirical evidence is needed to test related hypotheses.

Some limitations of this study also need to be considered. As mentioned earlier this study did not investigate absenteeism and/or academic performance with regards to body weight in children with ADHD. Future studies should address this issue, as this may help in understanding the class-room behaviour profiles exhibited by overweight and obese ADHD children. Furthermore, the moderating effects of physical activity patterns and eating habits/preferences on the relation between body weight and ADHD was also not examined due to lack of data. Given the link between physical activity, eating preferences, obesity and ADHD (Millichap & Yee, 2012; Souza, Barbosa Filho, Nogueira, & Azevedo Junior, 2011) these moderating effects need to be examined. Finally, our research design investigated BMI, which is a generalized measure of body mass, and lacked more direct and objective measures of obesity, such as underwater weighing, skin folds, etc.

In summary, the results of the current study suggest that, differences in weight categories are associated with differential patterns of clinical characteristics in children with ADHD. The above findings are of a preliminary nature and can serve as the basis for further research. For instance, work would be needed to understand the

social factors that might be contributing to dissecting clinical patterns between overweight and obese ADHD children. Under the assumption that weight deregulation and ADHD share some of their pathogenic pathways, social and biological, the proposed research might help to understand these pathways and reduce the heterogeneity of ADHD. Nonetheless, these findings do indicate that, care is needed in handling, overweight and obese ADHD children in the school environment in order to better control their disruptive or isolated behaviors with respect to their weight categories.

Table 5.1: Demographic and baseline characteristics of ADHD children stratified according to three BMI categories

	Normal-weight n = 168	Overweight n = 57	Obese n = 59	Test statistic, p-value and pair-wise comparison
Subject characteristics				
Gender (% males)	75.0%	66.7%	78.0%	$X^2 = 2.15$, df = 2, p = 0.34
Age	9.01 ± 1.86	9.08 ± 1.80	9.6 ± 1.86	$F_{2,281} = 2.23$, p = 0.10
Ethnicity (% white)	80.2%	71.9%	83.1%	$X^2 = 2.46$, df = 2, p = 0.29
Birth weight (gms)	3396.90 ± 711.62	3541 ± 814.951	3269.56 ± 521.03	$F_{2,126} = 1.04$, p = 0.35
Previous medication status (% yes)	36.1%	25.0%	20.3%	$X^2 = 6.15$, df = 2, ***p = 0.04,*** ┼ ╪
Family characteristics				
Mother's age at child's birth	29.51 ± 5.84	28.60 ± 7.10	26.58 ± 4.72	$F_{2,261} = 5.14$, ***p = 0.006,*** ╪
Mother's years of education	13.87 ± 3.28	13.27 ± 3.09	13.51 ± 2.53	$F_{2,245} = 0.77$, p = 0.46
Maternal alcohol during pregnancy (% yes)	24.0%	18.8%	16.7%	$X^2 = 1.52$, df = 2, p = 0.46
Maternal smoking during pregnancy (# of cigarettes per day)	2.43 ± 5.52	4.93 ± 7.73	3.71 ± 6.21	$F_{2,248} = 3.26$, ***p = 0.04,*** ┼
Was the child adopted? (% yes)	5.4%	3.6%	.0%	$X^2 = 3.39$, df = 2, p = 0.18
Father's years of education	13.14 ± 3.43	12.41 ± 3.59	12.32 ± 3.51	$F_{2,202} = 1.19$, p = 0.30
Father's age at child's birth	31.95 ± 6.03	30.61 ± 7.17	28.94 ± 5.26	$F_{2,233} = 4.55$, ***p = 0.01,*** ╪
Annual income status (% less than $20,000)	13.4%	29.1%	24.1%	$X^2 = 7.84$, df = 2, ***p = 0.01***

*Values are mean ± SD unless otherwise specified. Rt = right, lft = left, amb = ambidextrous, gms = grams, # = number. Significant Post Hoc tests: ┼ = normal vs. overweight, ╪ = normal vs. obese, ╫ = overweight vs. obese.

Table 5.2: Clinical characteristics of ADHD children stratified according to BMI categories

	Normal-weight n = 168	Overweight n = 57	Obese n = 59	Test statistic and p-value
DISC-IV ADHD items				
# inattention	7.09 ± 2.02	6.50 ± 2.69	6.57 ± 2.11	$F_{2,200} = 1.76$, p = 0.17
# hyperactivity	5.28 ± 2.64	5.28 ± 2.51	4.02 ± 2.79	$F_{2,200} = 3.61$, ***p = 0.03,*** ╪
# impulsivity	2.08 ± 1.03	1.95 ± 1.13	1.84 ± 1.11	$F_{2,200} = 0.94$, p = 0.39
# total ADHD	12.37 ± 3.64	11.85 ± 3.95	10.59 ± 3.96	$F_{2,200} = 3.91$, ***p = 0.02,*** ╪
ADHD Subtype, N (% yes)				
Combined	54.8%	49.1%	34.5%	$X^2 = 7.09$, df = 2, ***p = 0.03***
Inattentive	39.9%	35.1%	56.9%	$X^2 = 6.67$, df = 2, ***p =0.03***
Hyperactive	5.4%	15.8%	8.6%	$X^2 = 6.22$, df = 2, ***p =0.04***
DISC comorbidity, N (% yes)				
Oppositional defiant disorder	46.1%	37.5%	37.9%	$X^2 = 1.92$, df = 2, p = 0.38
Conduct disorder	9.7%	12.5%	15.5%	$X^2 = 1.50$, df = 2, p = 0.47
Anxiety disorder	43.6%	36.4%	40.4%	$X^2 = 0.91$, df = 2, p = 0.63
Depressive disorders	8.0%	3.6%	1.8%	$X^2 = 3.63$, df = 2, p = 0.16
CBCL (t scores)				
Total	66.23 ± 8.54	65.95 ± 9.51	68.98 ± 7.77	$F_{2,201} = 2.18$, p = 0.11
Internalization	62.78 ± 9.79	60.60 ± 10.04	65.39 ± 9.22	$F_{2,201} = 2.69$, *p = 0.07,* ╫
Externalization	64.75 ± 10.18	65.63 ± 11.37	67.09 ± 10.45	$F_{2,201} = 0.79$, p = 0.45
Withdrawn	61.07 ± 8.73	58.18 ± 6.72	64.61 ± 9.05	$F_{2,201} = 6.07$, ***p = 0.003,*** ┼ ╫
Somatic	58.52 ± 7.78	57.63 ± 6.78	61.09 ± 9.15	$F_{2,201} = 2.32$, p = 0.10
Social	63.50 ± 8.52	61.88 ± 9.16	65.39 ± 10.09	$F_{2,201} = 2.49$, *p = 0.08,* ╫
Conners scores				
ConnersP				
Emotional lability	63.08 ± 13.43	60.73 ± 13.48	64.12 ± 12.90	$F_{2,181} = 1.88$, p = 0.15
Restless-impulsive	72.99 ± 11.23	69.65 ± 10.26	70.44 ± 12.04	$F_{2,181} = 3.52$, ***p = 0.03,*** ┼
Total	71.64 ± 11.63	68.46 ± 10.75	70.02 ± 12.14	$F_{2,181} = 3.09$, ***p = 0.04,*** ┼
ConnersT				
Emotional lability	62.26 ± 16.58	68.28 ± 14.47	61.02 ± 14.72	$F_{2,194} = 1.59$, p = 0.20
Restless-impulsive	65.88 ± 10.40	71.54 ± 10.89	65.71 ± 12.60	$F_{2,194} = 2.99$, ***p = 0.05,*** ╫
Total	66.55 ± 12.61	72.82 ± 11.71	65.93 ± 13.45	$F_{2,194} = 2.66$, *p = 0.07,* ╫

*Values are mean ± SD unless otherwise specified. DISC-IVADHD items=ADHD number of items as assessed by the Diagnostic Interview Schedule for Children fourth edition; #=number. Significant Pairwise comparisons: ┼ = normal vs. overweight, ╪ = normal vs. obese, ╫ = overweight vs. obese. DISC comorbidity=ADHD comorbid disorders as assessed by the Diagnostic Interview Schedule for Children fourth edition. CBCL t scores=Child Behavioral Checklist T scores. ConnersP=Conners' Global Index for parents; ConnersT=Conners' Global Index for teachers.

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CHAPTER 6

Body weight and ADHD: examining the role of self-regulation

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PREFACE

In the previous chapter, we established that ADHD children within different weight categories displayed differential ADHD phenotype. Given the high comorbidity between ADHD and obesity/weight gain and previous theories suggesting that impaired self-regulation impacts both ADHD and obesity, we sought to investigate the relation between body weight/BMI and cognitive, emotional and motor characteristics in children with ADHD. To our knowledge, only one previous study to date has reported an association between body weight and executive functioning EF in children with ADHD, but it did not control for potential confounding factors, including childhood socioeconomic adversity. In this chapter, we addressed this potential gap in the literature and tested the hypothesis that self-regulation deficits may be modulating the association between obesity and ADHD in children. Furthermore, this study controlled for socioeconomic status, consisted of a large sample size and utilized a comprehensive assessment battery. The results of the study show that, both obese and overweight ADHD children exhibit significantly lower SES compared to normal weight ADHD children. Additionally, no significant differences were observed between the three groups with regards to their neurocognitive, emotional and motor profile. Thus, these results indicate that differences in weight/BMI are not accounted for by cognitive, motivational and motor profiles. However, socio-economic characteristics are strongly associated with overweight and obesity in ADHD children and may inform strategies aimed at promoting healthier weight.

ABSTRACT

Objective: Attention-Deficit/Hyperactivity Disorder (ADHD) is a complex and heterogeneous childhood disorder that often coexists with other psychiatric and somatic disorders. Recently, a link between ADHD and body weight dysregulation has been reported and often interpreted as impaired self-regulation that is shared between the two conditions. The objective of this study is to investigate the relation between body weight/BMI and cognitive, emotional and motor characteristics in children with ADHD. **Methods:** 284 ADHD children were stratified by weight status/BMI according to WHO classification and compared with regard to their neurocognitive characteristics, motivational style, and motor profile as assessed by a comprehensive battery of tests. All comparisons were adjusted for demographic characteristics of relevance including, socioeconomic status (SES). **Results:** Both Obese and overweight ADHD children exhibited significantly lower SES compared to normal weight ADHD children. No significant differences were observed between the three groups with regards to their neurocognitive, emotional and motor profile. **Conclusions:** Our findings provide evidence that differences in weight/BMI are not accounted for by cognitive, motivational and motor profiles. Socio-economic characteristics are strongly associated with overweight and obesity in ADHD children and may inform strategies aimed at promoting healthier weight.

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is an etiologically complex, highly heritable, common childhood psychiatric disorder with approximately 5% prevalence worldwide (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). It is characterized by age inappropriate patterns of severe and persistent inattention, hyperactivity, and impulsivity. ADHD often coexists with other psychiatric (Rader, McCauley, & Callen, 2009) and somatic disorders (van den Heuvel, Starreveld, de Ru, Krauwer, & Versteegh, 2007).

Recently, ADHD has been associated with body weight dysregulation (Cortese & Vincenzi, 2012; Surman, Randall, & Biederman, 2006). Indeed, it has been reported that ADHD subjects show higher than average body mass index standard deviation scores, and have significantly higher percentage of body fat and abdominal circumference compared to controls (Ptacek, Kuzelova, Paclt, Zukov, & Fischer, 2009a, 2009b). Conversely, obese subjects are more likely to present with attention problems (Agranat-Meged et al., 2005). In addition, some evidence suggests that patients with eating disorders tend to have attention problems akin to ADHD (Hudson, Hiripi, Pope, & Kessler, 2007; Rosval et al., 2006).

From a psychological point of view, both ADHD and childhood obesity have been conceptualized as disorders of impaired self-regulation (Francis & Susman, 2009; Graziano et al., 2011). Self-regulation is a psychological construct encapsulating the means by which individuals manage themselves in order to attain adaptative goals (R. A. Barkley, 2010). This construct implicates emotional and cognitive, particularly executive functions (EF), processes (S.D. Calkins, 2007; S. D.

Calkins & Fox, 2002). EF represent the neurocognitive processes important for goal-directed behaviours (Pennington & Ozonoff, 1996) including planning, sustained attention, cognitive flexibility, working memory, and response inhibition. Deficits in EF are believed to play an important role in ADHD. More specifically, ADHD children have shown significant impairments in different EF domains including response inhibition, vigilance, working memory, and planning (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Likewise, extremely obese individuals demonstrate significant differences in performances on tests of executive functioning such as planning, problem solving, and mental flexibility (Boeka & Lokken, 2008). Similarly, in comparison to their peers, obese children exhibit significant cognitive deficits in their attention shifting abilities (Cserjesi, Molnar, Luminet, & Lenard, 2007). Furthermore, in some studies, adults with elevated BMI display reduced cognitive performance (Gunstad et al., 2007). However, in a recent large longitudinal study, obesity indices were differentially associated with performance on neuropsychological tests; while global cognitive function, memory and language ability were found to be associated with obesity indices, attention and visuospatial ability showed the reverse trends (Gunstad, Lhotsky, Wendell, Ferrucci, & Zonderman, 2010).

In addition to EFs, self-regulation depends critically on motivational systems that may be dysregulated in both ADHD and obesity. In this regard, mesolimbic dopaminergic neurotransmission has been firmly implicated in the regulation of saliency of behavioral tasks (Bromberg-Martin, Matsumoto, & Hikosaka, 2010) and food stimuli (Ungless, 2004). It has been proposed that ADHD symptoms may be at least in part the consequence of reduced dopamine (DA) signaling in the mesolimbic system, resulting in reduced saliency of tasks (Volkow, Wang, Fowler, & Ding, 2005)

and aversion to delayed gratification (Sonuga-Barke, 2005). This emotional style may result in preferring immediate rewards and being less sensitive to reinforcement schedules (Luman, Oosterlaan, & Sergeant, 2005; Volkow et al., 2004), and consequently may contribute to abnormal eating habits (Davis, Levitan, Smith, Tweed, & Curtis, 2006) and promote inclination towards readily available non-healthy food in patients with ADHD. Similarly, pathological eating behavior (food addiction) in obese individuals has been conceptualized as a reaction compensating for a dampened DA reward system (Stice, Spoor, Bohon, & Small, 2008; Y. Wang, 2001). Under this scenario, excessive food intake may result in elevated dopamine activation in the mesolimbic pathway leading to a rewarding experience (X. F. Huang, Yu, Zavitsanou, Han, & Storlien, 2005; Kelley, 2004).

These neurocognitive/emotional parallels between ADHD and obesity are also supported by neuroimaging studies in ADHD and obese subjects (Mana, Paillere Martinot, & Martinot, 2010; Raji et al., 2010; Taki et al., 2008; Valera, Faraone, Murray, & Seidman, 2007). Further, it is also noticeable that psychostimulant medications, used to treat ADHD symptoms, also reduce appetite (Curtin, Bandini, Perrin, Tybor, & Must, 2005; Waring & Lapane, 2008). These observations taken together suggest that the neurobiological pathways implicated in the regulation of attention and motor control are also important determinants of energy intake and eating behaviors (Schweickert, Strober, & Moskowitz, 1997; Sokol, Gray, Goldstein, & Kaye, 1999). While the literature linking ADHD to body weight regulation is rich in neuropsychological hypotheses attempting to explain the link between these two complex disorders, the experimental data supporting these theories is very limited. To our knowledge, only one study reported an association between body weight and EF in children with ADHD (Graziano, et al., 2011). In addition, the association between

ADHD and body mass dysregulation could be due to many factors shared by these two conditions. Childhood socioeconomic adversity has been shown to impact both ADHD (Hjern, Weitoft, & Lindblad, 2010; Lasky-Su et al., 2007) and obesity (Orsi, Hale, & Lynch, 2011). More specifically, in a national cohort study with a sample of 7,960 children (aged 6-19 years), familial socioeconomic adversity factors including low maternal education level, single parent status and being recipient of social welfare predicted medicated status in school children with ADHD (Hjern, et al., 2010). Likewise, in developed countries, groups belonging to low socioeconomic status (SES) are more likely to be obese compared to other SES groups (Sobal & Stunkard, 1989; G. J. Wang et al., 2001; Y. Wang, Monteiro, & Popkin, 2002).

The present study aims at testing the hypothesis that self-regulation deficits are associated with obesity in children with ADHD. Further, our study controls for socioeconomic status, consists of a large sample size, and utilizes a comprehensive assessment battery.

METHODS

ETHICS STATEMENT

The research protocol was approved by the Research Ethics Board of the DMHUI. Children with ADHD and their parents were explained the study procedures in detail, and provided verbal assent and written consent respectively.

SUBJECTS AND STUDY PROCEDURE

Two hundred and eighty four children with ADHD (210 males and 74 females), ages 6-12 [mean=9.15; SD=1.86], were recruited from the severe disruptive

behaviours program and ADHD outpatient clinic at the Douglas Mental Health University Institute (DMHUI). These children were referred to the aforementioned secondary care mental health facilities from different sources including, school teachers, pediatricians, family physicians, and community social workers. Thus the children participating in the current study are reflective of the general ADHD population.

All children included in this study met DSM-IV diagnosis criteria for ADHD. A comprehensive clinical evaluation was used to establish the diagnosis of ADHD [see details in (Grizenko, Bhat, Schwartz, Ter-Stepanian, & Joober, 2006). Children were excluded from this study if they had an IQ less than 70 on the Wechsler Intelligence Scale for Children-III/IV (WISC-III or WISC-IV), Tourette syndrome, pervasive developmental disorder, or psychosis. These subjects were excluded from the study in-order to reduce the potential confounding of cognitive and weight by the afore-mentioned medical conditions, which have been associated with weight gain (Comings & Comings, 1990; Curtin, Anderson, Must, & Bandini, 2010; Olsson & Hulting, 2010) and cognitive anomalies (Bhattacharya & Klann, 2012; Geary, Hoard, & Hamson, 1999; Khalifa, Dalan, & Rydell, 2010; Reichenberg, 2005).

Among the total sample of affected ADHD children, 73.9% were male, and 18.0% belonged to families with an annual income of less than CN$20,000, 49.3% met *DSM-IV* criteria for the combined subtype, 42.3% were diagnosed with the inattentive and 8.1% with the hyperactive subtypes of ADHD. A total of 30.3% were previously receiving medication for their ADHD symptoms and the rest were medication naive. Among comorbid disorders, 41.9% had oppositional defiant disorder, 11.3% had conduct disorder (CD), and 40.1% had an anxiety disorder.

All the clinical and self-regulation assessments were completed while the children were not taking any medication. In cases where children were on medication prior to their inclusion in the study, these assessments were carried out at the end of a one-week washout period.

SELF-REGULATION EVALUATIONS

Neurocognitive performance

A comprehensive neuropsychological (NP) battery of tests specifically designed for children was used to study different executive function domains. Amongst the NP test battery, the Wechsler Intelligence Scale (WISC) (Weschler, 1992) evaluates the full scale (FS), verbal (V), and performance (P) IQ; the Wisconsin Card Sorting Test (WCST) (Heaton, Chelune, Talley, Kay, & Curtiss, 1993) assesses cognitive flexibility and set-shifting; the Finger Windows (FW) subtest (Sheslow, 1990) assesses visual-spatial working memory; the Tower of London (TOL) (Shallice, 1982) evaluates planning, organization, and problem-solving capacity; the Self-Ordered Pointing Task (SOPT) estimates working memory, planning, and response inhibition (Petrides & Milner, 1982); the Conners' Continuous Performance Test (CPT) (Conners, Epstein, Angold, & Klaric, 2003) measures attention, response inhibition, and impulse control; and the Stroop (colour and word) test evaluates cognitive flexibility, and resistance to interference from outside stimuli (Lansbergen, Kenemans, & van Engeland, 2007). The details regarding NC task assessments and procedures have been described in detail in a previous study (Thakur et al., 2013).

Motivational style

Motivational style was first evaluated by using the choice delay task (CDT), a test specifically designed to assess the ADHD children's aversion to delay (Sonuga-Barke, Taylor, & Heptinstall, 1992). In the CDT, the child repetitively (20 trials) chose between two reward paradigms; a large reward of 2 points (exchanged for 7 or 10 cents) associated with a large period of delay (30 sec), and a smaller reward of 1 point (exchanged for 5 cents) associated with a smaller period of delay (2 sec). However, once the participant chose a particular reward paradigm, he/she cannot switch back to the alternative reward paradigm until the next trial (Sonuga-Barke, et al., 1992).

In addition to the CDT, the Restricted Academic Situation Scale (RASS) (R. Barkley, 1990) was used to systematically observe and record the child's engagement in an assigned independent academic task (a set of math problems) in the presence of potential distractions, with no adult supervision (Fischer & Newby, 1998). Task engagement/disengagement, is a distinct trait of ADHD (Gupta & Kar, 2009; Karama et al., 2009) and it is also a good predictor of the child's motivation during a monotonous and repetitive task. The RASS assessment was conducted in a specialized room within the clinic equipped with a worktable, a chair, an intercom, and some toys. The child was given a set of math problems at current grade and instructed to complete as many as possible. The instructor then left the room and assessed the child's behaviour from behind a one-way mirror over a 15 minute time period. All behavioural events were recorded at 30-second intervals according to five categories: 'off-task', 'playing with objects', 'out of seat', 'vocalizing', and 'fidgeting'.

Motor activity

On the day of the testing, overall motor activity of children with ADHD was evaluated using actigraphy using a small electronic device (Actiwatch®) worn on the non-dominant hand, which is sensitive to acceleration. It records the subject's movements each 30 sec time period and expresses it as motor activity counts. Patients put on the Actiwatch® in the morning and kept it until the end of their testing in the early afternoon. The average motor activity was calculated and considered in the analyses as reflective of the overall motor activity of the child.

ANTHROPOMETRICS

All anthropometric measurements were taken by trained research assistants. Height of children with ADHD was measured using a wall-mounted chart with the subjects being bare-footed. Similarly, body weight was measured using a doctor's clinical scale with subjects clad in regular casual attire and being bare-footed.

CALCULATION OF BODY MASS INDEX (BMI) AND DEFINITION OF WEIGHT CATEGORIES

Body mass index (BMI) is a simple index of weight-for-height used to classify children into normal, overweight and obese categories. It is defined as "weight in kilograms divided by the square of the height in meters (kg/m^2)". BMI was calculated for all children with ADHD, and then converted to age- and gender-specific percentiles according to the criterion available in the World Health Organization (WHO) website (http://www.who.int/en/). The WHO defines "normal weight" as

ranging between 3rd to 84th percentile, "overweight" as ranging between 85th to 96th percentile, and "obesity" as equal or greater than 97th percentile.

STATISTICAL METHODS

Statistical analyses were performed with SPSS 15.0 version for Windows. Subjects were stratified into three groups according to the Weight/BMI classification as per WHO criterion. First, demographic and baseline characteristics of children with ADHD were compared between the three groups, namely normal (n = 168), overweight (n = 57), and obese (n = 59). Socio-demographic variables associated with BMI at a significance level of $p < 0.05$ were used as covariates in subsequent analyses. Second, neurocognitive, emotional and motor outcome measures were compared between three weight/BMI groups using univariate ANOVA for continuous variables and chi-squared tests for categorical variables. Main effects were further explored by Post Hoc pairwise comparisons and the Bonferroni correction was used to protect from type I error.

RESULTS

As shown in Table 1, the three groups of children were not different with regard to age, gender, handedness or birth weight. However, mothers and fathers of children in the obese and overweight groups were significantly younger at the birth of their children ($F_{2,261} = 5.14$, $p = 0.006$; $F_{2,233} = 4.55$, $p = 0.01$, respectively; all pair-wise p-values comparing the normal weight group to the other two groups were ≤ 0.01). In addition, compared to others, children in the overweight and obese groups belonged to a lower family income group ($X^2 = 7.84$, $df = 2$, $p = 0.01$), and were also

exposed to higher severity of maternal smoking during pregnancy (MSDP; # of cigarettes per day; $F_{2,248} = 3.26$, $p = 0.04$).

With regards to previous medication status, ADHD children within the obese group were significantly less likely to be previously on medication (20.3%) compared to subjects in the overweight (25.0%), and normal weight (36.1%) groups ($X^2 = 6.15$, $df = 2$, $p = 0.04$; Table 1). This finding is in line with previous data indicating that stimulant medication in ADHD subjects may result in weight loss due to suppression of appetite (Poulton & Cowell, 2003; Schertz, Adesman, Alfieri, & Bienkowski, 1996).

All subsequent analyses were conducted while controlling for aforementioned SES factors (parental age at child birth, family income status, and MSDP) and prior history of treatment with psychostimulants.

Additionally, as shown in Table 2, neurocognitive features as assessed by the WISC-III, WCST, TOL, SOPT, FW, CPT, and Stroop were not significantly different between the three groups (all $p > 0.05$). Further, no significant differences between the groups were found in emotional/motivational style as evaluated by the CDT and the RASS respectively (all $p > 0.05$; Table 3). Finally, the three groups were not significantly different with regards to motor activity as evaluated by actigraphy (all $p > 0.05$; Table 3).

DISCUSSION

It has been reported that children with ADHD have elevated risk for obesity in both epidemiological (Waring & Lapane, 2008) and clinical samples (Chen, Kim,

Houtrow, & Newacheck, 2010; Curtin, et al., 2005; Holtkamp et al., 2004). Given the well established health risks associated with childhood obesity (D. Y. Huang, Lanza, Wright-Volel, & Anglin, 2012; Li, Chen, Srinivasan, Xu, & Berenson, 2012), it is important to understand the socio-demographic, neuropsychological and emotional determinants of the relation between obesity and ADHD.

Different theoretical models have proposed that ADHD and body mass regulation disorders share common pathophysiological underpinnings. From a neuropsychological point of view, it is suggested that ADHD and obesity stem from impairments in individual's self-regulation (Francis & Susman, 2009; Graziano, et al., 2011). More specifically, deficits in Executive Functions (R. A. Barkley, 2010; Welsh & Pennington, 1988), dysfunctional motivational regulation systems (Sonuga-Barke, 2003) and aberrant goal directed motor activity regulated by brain dopamine systems are believed to be implicated in both disorders. Supporting this line of argument, neuroimaging data in ADHD and obese subjects report commonality in brain structural abnormalities, including in the frontal cortex (Mana, et al., 2010; Raji, et al., 2010; Taki, et al., 2008; Valera, et al., 2007), a locus considered to be important for self-regulation and EFs. In addition, ADHD subjects have been shown to have reduced DA receptor binding capacity in the hypothalamus, which controls for satiety and hunger (Volkow & Swanson, 2008).

The primary objective of the present study was to investigate the relation between body weight and cognitive, emotional, and motor activity characteristics in a large sample of children with ADHD. Results from our study do not identify statistically significant differences between weight groups (normal, over, and obese categories) in children with ADHD with respect to neurocognitive measures tapping

in executive functions, motivational style indices, and overall motor activity. These findings contrast with the results of the only other study examining the association between EF and weight in children with ADHD (Graziano, et al., 2011). More specifically, Graziano et al (2011) showed that children who performed poorly on a neuropsychological battery had greater BMI *z*-scores (overweight and obese) compared with children who performed better on the neuropsychological battery. Several factors may explain this discrepancy between the two studies. Compared to Graziano and colleagues, the current study has a much larger sample size (n=80 versus n=284), the participants have a narrower age range (4.5–18 years versus 6-12 years), and the neurocognitive assessment is more comprehensive in the present study. Unlike Graziano and colleagues, our participants were not taking any psychostimulant medication for at least one week prior to the neurocognitive assessments. Further, our statistical analysis model used parental (both mothers and fathers) age at child birth, family income status, MSDP, and prior history of treatment with psychostimulants as covariates to prevent potential confounding effects on the result outcomes, which was not the case in Graziano et al. study. In addition, the use of different neuropsychological tests indexing various EF domains in children with ADHD could also contribute to incongruent results. Contrary to Graziano et al. study where all cognitive domains were reduce into one single factor, in the present study; we elected to explore separately all performances assessed by various tests in order to identify any specific differences between the three groups. These analyses did not identify any significant differences, including in neuropsychological tests common to our study and Graziano and colleagues' work (Color-Word Interference Test).

The present study identified a strong association between socioeconomic (SE) characteristics and overweight and obesity status in children with ADHD, including

parental age at child birth, MSDP and annual family income. These results are in line with results of previous reports in children from the general population exploring the role of socioeconomic, geographic and environmental factors in influencing body weight gain and fat distribution (Orsi, et al., 2011). In developed countries, low SES strongly predicts obesity (Caballero, 2007; McLaren, 2007) and the largest increase in obesity is observed in individuals living within the defined range of poverty (Ogden, Carroll, Kit, & Flegal, 2012). Further, several lifestyle factors associated with obesity have a strong impact on children and adolescents belonging to low SES (Lieb, Snow, & DeBoer, 2009; Sanchez-Vaznaugh, Kawachi, Subramanian, Sanchez, & Acevedo-Garcia, 2008, 2009). For instance, younger children having limited accesses to healthy foods, recreational venues and safe housing are 20–60% more likely to be obese/overweight compared to others (Dunton, Berrigan, Ballard-Barbash, Graubard, & Atienza, 2009; Escaron, 2009; Oreskovic, Kuhlthau, Romm, & Perrin, 2009; Singh, Siahpush, & Kogan, 2010). Due to increased financial burden, low SES families may not be able to afford to pay for their children's involvement in any formal sport/recreation activities, and these children may have limited access to safer parks or recreational facilities because they reside in poor neighborhoods (Oliver & Hayes, 2005). This activity limitation prevents low SES child's engagement in a healthy, active lifestyle, and thus increases the chances of being overweight relative to their affluent high SES peers. Additionally, a diet with fruits and vegetables is highly recommended as part of a healthy lifestyle for growing children, but this may be less affordable for low SES families, resulting in poor nutrition and unhealthy weight gain. Consequently, it is plausible that lower SES is the main risk factor promoting overweight/obesity in children with ADHD.

Like ADHD (Biederman & Faraone, 2005; Linnet et al., 2003), weight gain/obesity is a multifaceted phenotype depending on complex interactions between genetic and environmental factors (Cortese & Vincenzi, 2012; Snyder et al., 2004). More research in larger independent samples is recommended to further explore the complex relations between gene-environment-obesity amongst children with ADHD.

Our study has a number of strengths. First, to our knowledge, this is the largest and most comprehensive study examining the relations between weight status/BMI and neurocognitive profiles, motivational status and motor activity in children with ADHD. In addition, all clinical, neurocognitive, motivational and motor activity assessments were carried out while the children were not taking any medication (1 week wash out period).

The main limitations of this study also need to be considered. This study could not investigate the moderating effects of physical activity patterns (Katz et al., 2010) and eating habits/preferences (Ebenegger et al., 2012) on the relation between body weight and ADHD due to unavailability of data. Future studies should address this issue, given the link between physical activity, eating preferences, obesity and ADHD. Further, our research design investigated BMI which is a generalized measure of body mass and lacked more direct and objective measures of obesity for example, underwater weighing, skin folds, etc. Finally, a link between altered sleep patterns, obesity and ADHD has been recently suggested (Cortese, Konofal, Dalla Bernardina, Mouren, & Lecendreux, 2008; Cortese et al., 2007), given the unavailability of the sleep data in this sample, we could not investigate this potential interaction.

To summarize, our results show that childhood obesity in ADHD is associated with specific socioeconomic characteristics but not associated with impairments in

self-regulation characteristics. These results do not support previous theories suggesting that impaired self-regulation promotes obesity in ADHD. Consequently, changing unhealthy life style amongst low SES children should receive more attention in future research, particularly those aiming at preventing childhood obesity amongst ADHD with children.

Table 6.1: Demographic and baseline characteristics of ADHD children stratified according to three BMI categories

	Normal-weight n = 168	Over-weight n = 57	Obese n = 59	Test statistic, p-value, post hoc comparison	Effect size (Cohen's *d*)	
					Normal-weight vs. Over-weight	Normal-weight vs. Obese
Subject characteristics						
Gender (% males)	75.0%	66.7%	78.0%	$X^2 = 2.15$, df = 2, p = 0.34		
Age	9.01 ± 1.86	9.08 ± 1.80	9.6 ± 1.86	$F_{2,281} = 2.23$, p = 0.10	-0.03	-0.31
Handedness (Rt/lft/amb.)	150/18/0	50/7/0	49/8/2	$X^2 = 8.15$, df = 4, *p = 0.08*		
Birth weight (gms)	3396.90 ± 711.62	3541 ± 814.951	3269.56 ± 521.03	$F_{2,126} = 1.04$, p = 0.35	-0.18	0.20
Previous medication status (% yes)	36.1%	25.0%	20.3%	$X^2 = 6.15$, df = 2, ***p = 0.04***		
Family characteristics						
Participating legal guardian (% mothers)	82.9%	82.1%	91.4%	$X^2 = 2.64$, df = 2, p = 0.26		
Mother's age at child's birth	29.51 ± 5.84	28.60 ± 7.10	26.58 ± 4.72	$F_{2,261} = 5.14$, ***p = 0.006,*** ╪	0.13	0.55
Mother's years of education	13.87 ± 3.28	13.27 ± 3.09	13.51 ± 2.53	$F_{2,245} = 0.77$, p = 0.46	0.18	0.12
Maternal alcohol during pregnancy (% yes)	24.0%	18.8%	16.7%	$X^2 = 1.52$, df = 2, p = 0.46		
Maternal smoking during pregnancy (# of cigarettes per day)	2.43 ± 5.52	4.93 ± 7.73	3.71 ± 6.21	$F_{2,248} = 3.26$, ***p = 0.04,*** ┼	-0.37	-0.21
Adopted (% yes)	5.4%	3.6%	0.0%	$X^2 = 3.39$, df = 2, p = 0.18		
Father's years of education	13.14 ± 3.43	12.41 ± 3.59	12.32 ± 3.51	$F_{2,202} = 1.19$, p = 0.30	0.20	0.23
Father's age at child's birth	31.95 ± 6.03	30.61 ± 7.17	28.94 ± 5.26	$F_{2,233} = 4.55$, ***p = 0.01,*** ╪	0.20	0.53
Annual family income (% less than $20,000)	13.4%	29.1%	24.1%	$X^2 = 7.84$, df = 2, ***p = 0.01***		

Values are mean ± SD unless otherwise specified. Rt = right, lft = left, amb = ambidextrous, gms = grams, # = number. Significant Post Hoc pairwise comparison nomenclature; ┼ *= normal vs. over-weight,* ╪ *= normal vs. obese,* ╫ *= over-weight vs. Obese.*

Table 6.2: Neurocognitive features of ADHD children stratified according to BMI categories

	Normal-weight n = 168	Over-weight n = 57	Obese n = 59	Test statistic and p-value	Effect size (Cohen's *d*)	
					Normal-weight vs. Over-weight	Normal-weight vs. Obese
Neurocognitive evaluation						
WISC-III						
verbal IQ	96.53 ± 13.53	97.37 ± 14.37	94.63 ± 14.61	$F_{2,185} = 1.27$, p= 0.28	-0.06	0.13
performance IQ	103.74 ± 13.79	101.16 ± 14.61	99.45 ± 15.97	$F_{2,185} = 0.83$, p = 0.43	0.18	0.28
full-scale IQ	96.81 ± 12.27	96.82 ± 13.47	93.82 ± 12.45	$F_{2,185} = 0.94$, p = 0.39		0.24
Wisconsin Card Sorting Test						
Perseverative errors (s score)	97.58 ± 13.23	99.37 ± 15.24	96.91 ± 10.90	$F_{2190} = 0.36$, p = 0.69	-0.00	0.05
Non-Perseverative errors (s score)	92.25 ± 15.90	96.94 ± 17.35	95.63 ± 15.04	$F_{2,190} = 1.56$, p = 0.21	-0.28	-0.21
Total errors (s score)	94.31 ± 14.50	96.86 ± 15.00	96.12 ± 12.98	$F_{2190} = 0.47$, p = 0.62	-0.17	-0.13
number of categories completed	4.14 ± 1.92	4.40 ± 2.06	4.30 ± 1.79	$F_{2,190} = 0.31$, p = 0.73	-0.13	-0.08
Failure to maintain set	1.48 ± 1.29	1.66 ± 1.51	1.86 ± 2.01	$F_{2,190} = 1.15$, p = 0.31	-0.12	-0.22
WRAML Finger Windows						
Standard score	9.74 ± 2.89	10.23 ± 3.25	9.68 ± 2.63	$F_{2,204} = 0.90$, p = 0.40	-0.15	0.02
Tower of London						
Standard Score	110.39 ± 15.49	112.11 ± 13.22	107.61 ± 18.40	$F_{2,182} = 0.97$, p = 0.37	-0.11	0.16
Self-Ordered Pointing Test						
Total score	16.98 ± 7.61	16.33 ± 7.13	15.77 ± 7.06	$F_{2,204} = 0.66$, p = 0.51	0.08	0.16
Stroop Test						
Word score (t score)	51.37 ± 9.15	51.69 ± 9.64	49.83 ± 7.36	$F_{2,172} = 0.36$, p = 0.69	-0.03	0.18
Color score (t score)	48.79 ± 6.62	48.41 ± 7.37	47.75 ± 5.42	$F_{2,172} = 0.20$, p = 0.81	0.05	0.17
Color-Word Score (t-Score)	44.73 ± 8.60	44.78 ± 7.60	44.15 ± 8.01	$F_{2,172} = 0.12$, p = 0.88	-0.006	0.06
Interference score (t score)	52.56 ± 8.50	52.13 ± 7.10	51.60 ± 7.49	$F_{2,172} = 0.06$, p = 0.93	0.05	0.11

Continuous Performance Test						0.06
Omissions (t-score)	58.42 ± 16.35	57.01 ± 15.67	57.35 ± 17.76	$F_{2,200} = 0.36$, p = 0.69	0.08	0.06
Commissions (t-score)	54.33 ± 8.24	55.24 ± 7.02	53.65 ± 8.45	$F_{2,200} = 0.59$, p = 0.55	-0.11	0.08
Hit response time (t-score)	53.93 ± 12.24	51.34 ± 11.08	53.88 ± 11.77	$F_{2,200} = 1.57$, p = 0.21	0.22	0.004
Hit response time standard error	59.61 ± 10.83	56.99 ± 10.51	58.20 ± 10.52	$F_{2,200} = 1.97$, p = 0.14	0.24	0.13
Variability of standard errors	58.24 ± 9.64	56.31 ± 9.10	57.11 ± 10.08	$F_{2,200} = 1.12$, p = 0.32	0.24	0.11
Response style (t-score)	53.36 ± 9.64	52.61 ± 9.74	51.62 ± 7.55	$F_{2,200} = 0.60$, p = 0.54	0.07	0.20
Perseveration	76.81 ± 39.39	76.04 ± 36.48	68.71 ± 36.01	$F_{2,200} = 0.74$, p = 0.47	0.02	0.21
Hit RT block change	52.90 ± 11.84	51.86 ± 13.12	49.98 ± 13.83	$F_{2,200} = 0.84$, p = 0.43	0.08	0.22
Hit SE block change	52.72 ± 9.59	52.21 ± 10.42	49.14 ± 11.84	$F_{2,200} = 1.59$, p = 0.20	0.05	0.33
Overall index	7.24 ± 10.08	4.70 ± 7.03	6.27 ± 9.86	$F_{2,200} = 1.92$, p = 0.14	0.29	0.09

**Values are mean ± SD unless otherwise specified. WISC-III=Wechsler Intelligence Scale for Children 3rd edition scores. S scores=standardized Scores.*

Table 6.3: Motivational style and Motor traits of ADHD children stratified according to BMI categories

	Normal-weight n = 168	Over-weight n = 57	Obese n = 59	Test statistic and p-value	Effect size (Cohen's *d*)	
					Normal-weight vs. Over-weight	Normal-weight vs. Obese
Motivational style evaluation						
Choice Delay Task						
Total score	28.98 ± 5.20	28.43 ± 5.61	28.98 ± 5.78	$F_{2,201} = 0.16$, p = 0.84	0.10	0.00
Task-engagement Traits						
RASS total score	55.22 ± 25.50	54.05 ± 26.79	50.83 ± 28.45	$F_{2,199} = 0.22$, p = 0.80	0.04	-0.57
RASS vocalization score	6.55 ± 7.48	6.33 ± 7.07	6.24 ± 8.41	$F_{2,199} = 0.32$, p = 0.96	0.03	0.03
RASS fidgeting score	12.84 ± 7.09	13.61 ± 8.47	14.32 ± 7.22	$F_{2,199} = 1.35$, p = 0.26	-0.09	-0.20
RASS off task score	15.02 ± 8.76	13.87 ± 9.83	13.37 ± 8.58	$F_{2,199} = 0.46$, p = 0.63	0.12	0.19
RASS plays with object score	14.75 ± 8.55	14.47 ± 9.01	12.97 ± 8.67	$F_{2,199} = 0.40$, p = 0.67	0.03	0.20
RASS out of seat score	5.97 ± 5.95	5.75 ± 6.44	3.97 ± 5.30	$F_{2,199} = 1.99$, p = 0.13	0.03	0.35
Motor activity evaluation						
Actigraphy testing						
Motor activity (Av. score)	85.23 ± 42.68	97.67 ± 47.95	81.76 ± 58.41	$F_{2,190} = 0.96$, p = 0.38	-0.27	0.06
Motor activity (SD. score)	93.33 ± 39.65	100.60 ± 40.67	86.57 ± 43.51	$F_{2,190} = 0.85$, p = 0.42	-0.18	0.16

**Values are mean ± SD unless otherwise specified. Scores=standardized Scores. RASS =Restricted Academic Situation Scale. Av. Scores = average score, SD. Scores = standard deviation score.*

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Declaration of Interests

RJ receives consultancy honorarium from Janssen Ortho and Pfizer Canada. All other authors deny any conflict of interest with respect to this study.

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CHAPTER 7

Association between obesity-related gene *FTO* and ADHD

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PREFACE

It has been suggested that, investigating associations between candidate genes identified in Genome-Wide Association Studies (GWAS) of ADHD and ADHD pertinent endophenotypes will help to further our understanding of the etiology of this complex disorder. However, as none of the investigated genes have passed the GWAS significance threshold (10^{-7}), a different approach for selecting candidate genes may be necessary to facilitate ADHD genetic studies. In line with this idea, some researchers have suggested that, it may be interesting to study genes identified in GWAS of psychiatric and somatic disorders comorbid with ADHD, as they may share behavioral and neurobiological mechanisms. Given that obesity/weight gain and ADHD are highly comorbid and clinically linked (as shown in chapter 3), we decided to investigate genes identified through GWAS of obesity. We selected rs8050136, a single nucleotide polymorphism (SNP) located in the 1st intron of the *FTO* gene located on chromosome 16 which has been most consistently associated with obesity/weight gain in both adults and children. Furthermore, given that the *FTO* gene has also been associated with smoking behaviour which is highly comorbid with ADHD, and that maternal smoking during pregnancy has been implicated in the etiology of ADHD, we further studied this SNP by stratifying our sample based on maternal smoking during pregnancy (MSDP) status.

More specifically, in this chapter, we conducted family-based association tests to study transmission of the risk allele of this SNP in relation to clinical diagnosis of ADHD, and a number of behavioral and neurocognitive phenotypes relevant to the disorder. By using comorbid disorders to investigate ADHD genetics, we identified a novel association

between the A* risk allele of rs8050136 and several traits relevant to ADHD in the total sample. These associations were stronger when the analysis was restricted to children who were not exposed to MSDP. Thus, these results suggest the involvement of the *FTO* SNP rs8050136 in modulating the risk for ADHD, particularly in those children who were not exposed to MSDP. If confirmed, this may explain, at least in part, the complex links between obesity and ADHD.

In summary, this study underscores the importance of using comorbidity as a strategy to reduce the clinical and etiological heterogeneity of ADHD and to help identify a more homogenous subgroup of children with ADHD. Also, it emphasizes that future ADHD genetic studies should select potential candidate genes which have been previously implicated in ADHD comorbid disorders by GWAS studies.

ABSTRACT

Objective: Attention-deficit/ hyperactivity disorder (ADHD) is an etiologically complex heterogeneous behavioral disorder. Several studies have reported that ADHD subjects are more likely to be over-weight/obese and that this comorbidity may be due to shared genetic factors. The objective of this study is to explore the association between ADHD and *FTO*, a gene strongly associated with obesity in genome-wide studies. **Design and Methods:** We selected one tag SNP (single nucleotide polymorphism, *rs8050136, risk allele A)* in the *FTO* gene and tested its association with ADHD. Family-based association tests (FBAT) were conducted with the categorical diagnosis of ADHD as well as behavioural and cognitive phenotypes related to ADHD. Further, stratified FBAT analyses based on maternal smoking during pregnancy (MSDP) status were conducted. **Results:** Statistically significant associations were observed between *rs8050136* and several of the traits tested in the total sample. These associations were stronger when the analysis was restricted to children who were not exposed to MSDP. **Conclusions:** These exploratory results suggest the involvement of the *FTO* SNP *rs8050136* in modulating the risk for ADHD, particularly in those children who were not exposed to MSDP. If confirmed, they may explain, at least in part, the complex links between obesity and ADHD.

Keywords

Environmental Factors, Genetics, Obesity, Psychopathology, Smoking, ADHD.

INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent and complex childhood disorder. ADHD has a strong genetic component (mean heritability 76%), and it has been suggested that multiple genes are involved in its etiology, each accounting for a small portion of increased risk (Biederman & Faraone, 2005). Additionally, environmental factors particularly maternal smoking during pregnancy (MSDP) is thought to play a role in the disorder (Linnet et al., 2003). Despite extensive research, results from genetic association studies have been difficult to replicate, which could be due to a high level of etiological heterogeneity of this disorder.

Recently, a link between neurobiological systems implicated in attention, motor control, appetite, and body mass regulation has been suggested (Cortese & Vincenzi, 2011). Further, it was reported that ADHD subjects have higher body mass index standard deviations scores (BMI-SDS), and higher percentage of body fat (Ptacek, Kuzelova, Paclt, Zukov, & Fischer, 2009) compared to controls.

Genome Wide Association Studies (GWAS) have strongly implicated several genes (Hebebrand, Volckmar, Knoll, & Hinney, 2010) in fat mass regulation and obesity. Among these, the *Fat Mass and Obesity* (*FTO*) gene located on chromosome 16 (Frayling et al., 2007), showed the strongest association with obesity and appears to be acting through the modulation of neurobiological systems. Indeed, the level of expression of this gene is highest in the brain (Frayling, et al., 2007). Further, a loss-of-function mutation of the *FTO* was associated with microcephaly, structural/functional brain abnormalities, and severe psychomotor delay (Boissel et al., 2009), whereas a

duplication of this gene due to 16q trisomy produces mental retardation, and ADHD (van den Berg et al., 2010). In the elderly, *FTO* has been associated with reduced brain volume (Ho et al., 2010), increased risk for Alzheimer's disease (Keller et al., 2011) and poorer performance in executive function domains (Benedict et al., 2011). In animals, a knockout of the *Fto* gene resulted in a phenotype with reduced body weight and fat, decreased motor activity and increased energy expenditure. Most interestingly, these effects were specifically related to an increased sympathetic (adrenaline and noradrenalin) tone in the central nervous system (Fischer et al., 2009).

Within *FTO*, a number of tag single nucleotide polymorphisms (SNPs) located in intron 1, have been associated with obesity (Frayling, et al., 2007). Among these, *rs8050136* (A/C) is in strong linkage disequilibrium (LD) with 5 other SNPs (r^2>=0.88) that were all associated with early onset obesity (Hinney et al., 2007). Interestingly, allele A of *rs9939609*, which is in complete LD with allele A of *rs8050136*, was found to be associated with lower alcohol consumption and cigarette smoking (Sobczyk-Kopciol et al., 2011).

Given the link between obesity and ADHD, the association of the *FTO* gene with obesity and behavioural phenotypes relevant for ADHD, and the implication of the *FTO* gene in brain development, we primarily investigated the association of *rs8050136* with ADHD and also explored the relation between *rs8050136* and ADHD related neurocognitive and behavioural phenotypes. Further, given the importance of MSDP in modulating risk for ADHD and the association between smoking and *rs9939609*, we also explored the association between *rs8050136* in children stratified with regard to MSDP.

METHODS AND PROCEDURES

SUBJECTS, STUDY PROCEDURES, AND ETHICS

Four hundred and fifty one ADHD children (349 boys and 102 girls), ages 6-12 [mean=9.05; SD=1.86], were recruited from the Disruptive Behaviour Disorders Program and the children outpatient clinic at the Douglas Mental Health University Institute. Children were referred to these specialized care facilities by schoolteachers, community social workers, and paediatricians. The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Children with ADHD and their parents were explained the study in detail, and they provided verbal assent and written consent respectively.

Children included in this study met DSM-IV diagnosis criteria for ADHD. A comprehensive clinical evaluation was used to establish the diagnosis of ADHD [see details in (Taerk et al., 2004)]. Briefly, ADHD diagnosis was based on clinical examination of the child and an interview of at least one of his or her parents, by a child psychiatrist (RJ or NG). In addition, a structured clinical interview with parents using the Diagnostic Interview Schedule for Children-version IV, DISC-IV was used to corroborate the diagnosis.

Children were excluded from this study if they had an IQ less than 70 on the Wechsler Intelligence Scale for Children-III/IV (WISC-III or WISC-IV), Tourette syndrome, pervasive developmental disorder, or psychosis. Amongst the total sample, 78.1% were male, 86.9% were of Caucasian ethnicity, and 28.9% belonged to families

with an annual income of less than CAD$ 20,000. 52.8% met DSM-IV criteria for the combined subtype, while 37.3% and 9.9% were diagnosed with the inattentive and hyperactive subtypes respectively. 38.8% were previously receiving medication for their ADHD symptoms. 2.1% were under-weight, 57.9% were of normal weight, 19.7% were over-weight, and 20.3% were obese as per BMI category according to WHO classification (this distribution of weight categories was not significantly different between the three genotype groups). Comorbid disorders such as oppositional defiant disorder (40.4%), conduct disorder (21.7%), anxiety disorder (44.1%), and mood disorder (8.3%) were present in proportions similar to those reported in previous studies. All the behavioural and neurocognitive assessments were completed while the children were not taking any medication. In cases, where children were on medication prior to their inclusion in the study, all clinical, behavioral, neurocognitive, and task-engagement assessments were carried out at the end of a one-week washout period.

CLINICAL AND BEHAVIORAL EVALUATION

Behaviours relevant to ADHD were assessed using several assessment tools for the purpose of quantitative genetic analyses as previously described (Grizenko et al., 2006). Briefly, the Child Behavior Checklist (CBCL), which assesses several behavioural dimensions of the child, was completed by the parents. Further, the child's behaviour in the home and classroom environment were evaluated by parents and teachers using the Conners' Global Index for Parents and Teachers (CGI-P and CGI-T) respectively.

NEUROCOGNITIVE EVALUATION

A neuropsychological (NP) battery of tests specially designed for children was used to study different executive function domains as described in a previous publication (Taerk, et al., 2004). The Wechsler Intelligence Scale (WISC) (Weschler, 1992) was used to evaluate the full scale (FS), verbal (V), and performance (P) IQ. The Wisconsin Card Sorting Test (WCST) (Heaton, Chelune, Talley, Kay, & Curtiss, 1993) was used to assess cognitive flexibility and set-shifting. Similarly, the Finger Windows subtest (Williams, Goldstein, & Minshew, 2006), was used to assess visual-spatial working memory, and the Tower of London (TOL) (Shallice, 1982) assessed planning, organization, and problem-solving capacity. Additionally, the Self-Ordered Pointing Task (SOPT) estimated working memory, planning, and response inhibition (Petrides & Milner, 1982). Finally, the Conners' Continuous Performance Test (CPT) (Conners, Epstein, Angold, & Klaric, 2003) was used to measure attention, response inhibition, and impulse control.

TASK-ENGAGEMENT EVALUATION

Task-oriented behaviour in children with ADHD was assessed within the clinic using the Restricted Academic Situation Scale (RASS) (Barkley, 1990). RASS is a specialized coding system developed for observing and recording the child's behaviour when he/she is assigned a task (a set of math problems in our study), during a simulated independent academic situation within a clinical setting. It assesses the child's ability for task engagement to regular, repetitive academic work in the presence of potential distractions, with no adult supervision, and has been conducted as described (Sengupta et al., 2008).

MATERNAL SMOKING DURING PREGNANCY EVALUATION

Obstetric complications, including pregnancy, delivery and perinatal complications were systematically assessed using the Kinney Medical Gynaecological Questionnaire and scored using the McNeil-Sjöström scale (Grizenko, Shayan, Polotskaia, Ter-Stepanian, & Joober, 2008). During this assessment, mothers were asked questions about smoking during the three trimesters of pregnancy. If mothers smoked during at least one trimester of their pregnancy, their children were coded as "exposed" (n=181), whereas, if mothers didn't smoke at all during pregnancy, their children were coded as "unexposed" (n=230).

GENETIC ANALYSES

The affected child and his/her family, including the parents and the unaffected siblings were invited to participate in the genetic component of the study. DNA was extracted for each participant, and his/her associated family member, using a blood sample, a buccal swab, or a saliva sample. The current study included a total of 380 nuclear families having one or more child with a DSM-IV diagnosis of ADHD. Of the 380, 184 were trios with information from both parents, 18 were trios with two affected children, 49 were trios with information from one parent and one or more unaffected sibling, 115 were duos including the proband and one parent, while 14 were families with two affected siblings and one parent. *rs8050136* SNP was genotyped using Sequenom iPlex Gold Technology (Ehrich, Bocker, & van den Boom, 2005), genotyping error was estimated using duplicates of 2 reference samples included in each plate. Further, genotypes for all the samples included in the study were read with 100% accuracy and

the genotype distribution (AA=13.4%, AC=48.7%, and CC=38.0%) of this marker did not depart from Hardy Weinberg equilibrium (P >0.05).

STATISTICAL METHODS

The Family based Association Test (FBAT) statistical package (version 2.0.3) (Laird, Horvath, & Xu, 2000) was used to examine the over-transmission of a specific allele from parent to affected offspring. Genetic association of *rs8050136* with ADHD diagnosis and behavioural, cognitive and task-engagement quantitative traits/phenotypes were examined. All the analyses were conducted under the assumption of an additive model, with the null hypothesis of no linkage and no association. At the first level, FBAT analysis was conducted with the total sample. However, given the results of earlier studies indicating that MSDP is the environmental risk factor most consistently associated with ADHD (Linnet, et al., 2003) and the fact that the *FTO* gene was associated with smoking behaviour, possible modulation of the association between the *FTO* genotype and ADHD related phenotypes were explored by stratifying participants into two groups based on MSDP status (± MSDP). At the time of submission this manuscript, this was, to our knowledge, the first study exploring possible association between *FTO* candidate gene and ADHD as a diagnostic entity, and given the previous literature indicating that mutations in this gene may cause attention and cognitive deficits, the significance level of association with ADHD as a diagnosis was set at P = 0.05 and not corrected for multiple testing.

RESULTS

In the total sample, *FTO SNP rs8050136* was marginally associated with ADHD diagnosis (P = 0.05) (Table 1). More specifically, the *A* allele (associated with obesity in weight studies) was under-transmitted from parents to the affected child. Further, exploratory quantitative FBAT analysis with the clinical, behavioural, IQ, EF, and task-engagement phenotypes revealed that the *A* allele of *rs8050136* was associated with better performance on the Finger windows total score (P =0.004), and the CPT reaction time (P =0.04) (Table 2). Likewise, concerning task-oriented behaviours the *A* allele of *rs8050136* was positively associated with RASS total score (P =0.01) (Table 3). However the *A* allele of *rs8050136* was not associated with BMI score (Table 4).

Additionally, stratified exploratory analysis, based on MSDP status indicated that the genetic associations of *FTO rs8050136* were significant only in the subgroup of patients who were not exposed to MSDP. For example, the association of *FTO rs8050136* improved by one order of magnitude with both ADHD diagnosis (P =0.008, Table 1) and task engagement RASS total score (P =0.001, Table 4) in the sub group of children who were not exposed to MSDP. More specifically, the *A* allele of *rs8050136* was significantly under-transmitted to ADHD children who were not exposed to MSDP and to those children with less severe behavioural and cognitive traits relevant for ADHD. FBAT –e option provided similar findings. Finally, stratified exploratory analysis based on ADHD subtype and medication naivety did not show any association between FTO SNP rs8050136 and ADHD (Supplementary Table 1 & 2).

DISCUSSION

The *FTO* gene has been consistently associated with the modulation of body mass (Tung & Yeo, 2011). Although, it may exert its effects on fat tissues through its effects on the adipocytes, (Wahlen, Sjolin, & Hoffstedt, 2008), there is strong evidence indicating that its effects are due to the modulation of brain pathways implicated in energy metabolism and food intake (Stratigopoulos & Leibel, 2010). Further, there is evidence that its effects may depend on its differential implication in various brain regions, and at different developmental stages (Farooqi, 2011). Indeed, it has been shown that mice lacking the *Fto* gene show hyperphagia, decreased locomotor activity, increased sympathetic tone (adrenaline and nor-adrenaline), and post-natal growth retardation (Fischer, et al., 2009). Interestingly, in humans, one case report suggests that over expression of *FTO* (16q11.2 – 16q13 duplication) results in a phenotype of ADHD associated with obesity and mental retardation (van den Berg, et al., 2010). Consistent with this result, it has been shown that experimental duplication or triplication of the *Fto* gene in mice results in an over expression of this gene and proportional increase in weight (Church et al., 2010). However, the literature is still unclear with regard to the effect of the risk alleles located in the first intron of the *FTO* gene on gene expression.

In this study we investigated the role of the tag SNP *rs8050136* within *FTO* in children with ADHD. Allele *A* of this polymorphism has been consistently associated with obesity. Additionally, we also explored the association between this gene and ADHD while stratifying children according to their exposure to MSDP. This latter analysis was motivated by a study suggesting that the obesity risk alleles within the *FTO*

gene are associated with lower risk for cigarette smoking (Sobczyk-Kopciol, et al., 2011). The stratification on the basis of MSDP was also motivated by data from our laboratory strongly suggesting that the groups of children exposed or not to MSDP may be different from a clinical (Thakur et al., 2013) and etiological points of view (Thakur, Sengupta, Grizenko, Choudhry, & Joober, 2012).

The main result of the study showed that the *A* allele of *FTO rs8050136* polymorphism is under transmitted to children with ADHD from their parents. Further, exploratory quantitative trait analysis showed that, *rs8050136 A* allele is associated with lower severity of ADHD and better functioning on tests measuring executive function, and task-engagement traits, but, not associated with BMI. These results were stronger on children who were not exposed to maternal smoking during pregnancy. While this paper was under review, Velders et al reported a study investigating the association between FTO and ADHD traits in a large cohort (n= 1718) of normally developing children. In line with our results, they observed that there was no association between *FTO rs9939609* and BMI in young children. More importantly, children with the *A* allele of *FTO rs9939609* were less likely to have symptoms of ADHD (OR = 0.74, = = 0.01) and showed more emotional control (OR = 0.64, p = 0.01) compared to children without the *A* allele (Velders et al., 2012). Given that *rs8050136* is in perfect linkage disequilibrium (LD) with *rs9939609* (r^2 = 1 and D'=1), the present study and the study reported recently by Velders et al. are highly convergent as they replicate the same results with regard to obesity and behavioural phenotypes in ADHD and in typically developing children.

Previous studies have significantly associated the A allele of *FTO rs8050136* polymorphism with obesity (Hinney, et al., 2007), thus we were expecting an over transmission of this allele to children affected with ADHD. Contrary to our expectation, this allele was under transmitted to affected children in this study. Nonetheless, given the fact that allele A is an intronic variant, it may be implicated in regulating the level of expression of the *FTO* gene, with an effect on gene expression that remains to be determined. In case this variant is associated with a lower expression of the *FTO* gene, its effects may partially mimic the knock-out of the *Fto* gene in mice. Under such scenario, children with ADHD who inherit this allele from their parents will be expected to have less severe psychopathology given that the knock out model of the *Fto* associates reduced motor activity and increased brain catecholamines (Fischer, et al., 2009). Indeed, since ADHD has been conceived as a disorder with lower brain catecholamines (including noradrenaline) (Biederman & Spencer, 1999), it is expected that higher level of catecholamines due to a partial loss of function associated with the A allele would explain a milder form of ADHD and better executive function. Also symptoms of ADHD are improved by the use of medication acting as selective norepinephrine-reuptake inhibitor, such as atomoxetine.

The current study also shows a stronger association of the *A* allele of *FTO rs8050136* polymorphism with ADHD relevant traits in the sub group of ADHD children whose mothers did not smoke during pregnancy, and this association was completely absent in those subject who were exposed to MSDP. These observations are consistent with a previous study showing that, homozygosity for the A allele of *rs9939609* (another *FTO* SNP in high LD with *rs8050136*) is associated with lower tobacco smoking

(Sobczyk-Kopciol, et al., 2011). Given the fact that ADHD is highly co-morbid with smoking, drug/substance-use, and alcohol abuse (reviewed by (Lee, Humphreys, Flory, Liu, & Glass, 2011)), it is possible that the A allele of *rs8050136* is indexing a group of children with ADHD whose mothers are less likely to smoke during pregnancy, and who have a milder form of ADHD. Interestingly, this is consistent with previous literature showing that children whose mother did not smoke during pregnancy have a milder form of psychopathology and better performances on executive function (Motlagh et al., 2011).

The finding that the A allele of *FTO rs8050136* polymorphism is not associated with BMI, is in contrast with results of previous GWAS (Scott et al., 2007). This result may be attributed to the relatively smaller number of subjects and the resulting lack of statistical power in our study to detect an effect on weight, although a recent study with a larger sample size reported results similar to our study (Velders, et al., 2012). In case the association with ADHD and cognitive traits reported here is true, this may suggest that the effect of the *FTO* gene on behavioural phenotypes is stronger than on the body weight phenotype during childhood, although this needs further confirmation in a larger sample of patients.

The primary outcome result of the current study (overall association with the disorder) was not corrected for multiple testing, because mutations in the *FTO* candidate gene have been previously associated with cognitive deficits (Benedict, et al., 2011), and neurocognitive deficits are believed to result in behavioural symptoms displayed by ADHD children (Swanson, 2003). However, given that the primary association investigated was with rs8050136 and ADHD as a diagnostic entity, even if the Bonferroni

correction was to be applied (1 SNP X three exposure strata, p = .05/3 = .016), the association results in the group where the mothers did not smoke during pregnancy, would remain significant. However, the remaining results which identify associations between *FTO* rs8050136 polymorphism and quantitative phenotypes were not corrected for multiple testing as they are considered exploratory. None the less, given the recent replication of our finding in an independent study (Velders, et al., 2012), these preliminary findings gain further validity and may help inform future genetic studies of the disorder. It is critical to have independent replication before definitive conclusions can be reached.

Finally, this is the first study investigating the association between *FTO* and ADHD, it employs a stringent family based association design, and explores several behavioural dimensions measured in different environment (home, school, and clinic) by different raters. The convergence of positive association along many of these dimensions and the robustness of the family based association study increases confidence in these results. Also, the selection of the genetic variants that were strongly associated with somatic phenotypes (obesity) implies that these variants may be functional. Nonetheless, further studies with bigger sample sizes are needed to confirm or negate these results. This is particularly true for the stratified analysis by MSDP. Indeed, the sub-group of children whose mother smoked during pregnancy is relatively small.

In conclusion, these results implicate *FTO* in the modulation of ADHD phenotype and suggest that this effect is more prominent in those children who were not exposed to

MSDP. If replicated in independent large samples they shed light on the physiopathology of ADHD and its possible link with obesity.

Table 7.1: FBAT output detailing the association between *rs8050136* and clinical and behavioural dimensions.

	Total Sample of ADHD children			ADHD children exposed to Maternal smoking during pregnancy			ADHD children not exposed to Maternal smoking during pregnancy		
Trait	**Family No.**	**Z statistic**	**P value**	**Family No.**	**Z statistic**	**P value**	**Family No.**	**Z statistic**	**P value**
ADHD	166	-1.947	0.051*	60	0.498	0.618	103	-2.63	0.008**
• Total number DISC ADHD items	171	-1.314	0.188	61	0.416	0.677	105	-1.749	0.080
Conners' Parents	168	-0.272	0.785	60	0.974	0.329	104	-1.064	0.287
Conners' Teachers	164	-1.009	0.313	59	0.677	0.498	101	-1.67	0.094
CBCL Total score	175	-0.769	0.441	62	0.626	0.531	108	-1.492	0.135
• CBCL Internalizing behaviour	173	-0.53	0.596	62	0.711	0.477	107	-1.251	0.210
• CBCL Externalizing behaviour	172	-0.57	0.568	61	0.747	0.455	106	-1.323	0.185
- CBCL Withdrawn	145	-1.06	0.289	58	0.724	0.469	82	-2.269	0.023*
- CBCL Somatic complaints	138	-0.826	0.408	50	0.254	0.799	82	-1.098	0.272
- CBCL Anxious/depressed	161	-0.807	0.419	59	0.823	0.410	97	-1.548	0.121
- CBCL Social problems	166	-0.466	0.641	61	1.392	0.164	99	-1.5	0.133
- CBCL Thought problems	154	-1.056	0.290	54	0.471	0.637	94	-1.752	0.079
- CBCL Attention problems	173	-1.43	0.152	62	0.502	0.615	106	-2.011	0.044*
- CBCL Delinquent behaviour	36	0.567	0.570	19	0.798	0.424	17	-0.174	0.861
- CBCL Aggressive behaviour	165	-0.748	0.454	61	0.814	0.415	99	-1.693	0.090

P values < 0.01 are highlighted with **, while trends for association (*P* values ≥ 0.01 and ≤ 0.05) are indicated with*. DISC-IV: Diagnostic Interview Schedule for Children-version IV. Conners' Teachers: Conners' Global Index-Teacher version questionnaire. Conners' Parents: Conners' Global Index-Parents version questionnaire. CBCL: Child Behaviour Checklist. Note: For the CBCL, a lower t-score is indicative of better behavior. FTO SNP *rs8050136* (A/C), allele frequency for the A allele = 0.384 and for the C allele = 0.616 in the total ADHD sample. Results in the table have been depicted for the *A* allele since this is considered the risk allele for obesity according to published GWAS.

Table 7.2: FBAT output detailing the association between *rs8050136* and cognitive endophenotypes.

	Total Sample of ADHD children			ADHD children exposed to Maternal smoking during pregnancy			ADHD children not exposed to Maternal smoking during pregnancy		
Trait	**Family No.**	**Z statistic**	**P value**	**Family No.**	**Z statistic**	**P value**	**Family No.**	**Z statistic**	**P value**
WISC IQ	155	1.444	0.148	54	1.138	0.255	97	0.541	0.588
• WISC Verbal IQ	150	0.676	0.499	54	-0.663	0.507	91	0.919	0.357
• WISC Performance IQ	148	0.84	0.401	53	1.368	0.171	90	-0.339	0.734
WCST Total errors standard score	152	0.025	0.980	55	-0.451	0.651	93	-0.231	0.817
WCST Perseverative responses	150	0.83	0.406	52	0.311	0.755	94	0.159	0.873
• WCST Perseverative errors	152	0.423	0.672	54	0.359	0.719	94	-0.455	0.649
• WCST Non-perseverative errors	151	-0.386	0.699	54	-0.755	0.450	93	0.153	0.878
FW Score	108	-2.83	0.004**	32	-0.319	0.749	73	-2.994	0.002**
SOPT score	168	-1.537	0.124	61	0.597	0.550	102	-2.342	0.019*
TOL score	139	-1.077	0.281	52	0.112	0.910	84	-1.664	0.096
CPT									
• Omission errors	142	-0.556	0.577	46	0.129	0.897	92	-0.003	0.997
• Commission errors	170	-0.98	0.327	61	-0.726	0.467	105	-0.736	0.461
• Attention score	170	-1.011	0.312	61	-1.286	0.198	105	-0.012	0.990
• Hit Reaction Time	169	-1.963	0.049*	60	-1.559	0.118	105	-1.062	0.288
• Perseveration score	142	-1.369	0.170	46	0.094	0.924	92	-2.214	0.026*
• Overall index	84	-0.068	0.945	39	0.25	0.802	40	0.22	0.825

P values < 0.01 are highlighted with **, while trends for association (*P* values ≥ 0.01 and ≤ 0.05) are indicated with*. WISC: Wechsler Intelligence Scale-version III or IV. SOPT: Self-Ordered Pointing Task. CPT: Conners' Continuous Performance Test. WCST: Wisconsin Card Sorting Test (measure of cognitive flexibility and set-shifting). TOL: Tower of London test (planning, organization, and problem-solving capacity). FW: Finger Windows (visual-spatial working memory). Note: for the WCST, FW, and TOL, a higher standard score is indicative of better performance; for the SOPT, a lower score is indicative of better performance; for the CPT, the t-scores are standard scores that use a mean of 50, where a high T-score (>= 60) indicates slow response speed. FTO SNP *rs8050136* (A/C), allele frequency for the A allele = 0.384 and for the C allele = 0.616 in the total ADHD sample. Results in the table have been depicted for the *A* allele since this is considered the risk allele for obesity according to published GWAS.

Table 7.3: FBAT output detailing the association between *rs8050136* and Task-engagement endophenotypes.

Trait	Total Sample of ADHD children			ADHD children exposed to Maternal smoking during pregnancy			ADHD children not exposed to Maternal smoking during pregnancy		
	Family No.	Z statistic	P value	Family No.	Z statistic	P value	Family No.	Z statistic	P value
RASS									
• Total score	172	-2.472	0.013*	62	-0.001	0.999	105	-3.128	0.001**
- Vocalization	104	-1.297	0.194	40	0.111	0.911	62	-1.723	0.084
- Fidgeting	167	-2.395	0.016*	59	-0.052	0.958	103	-2.833	0.004**
- Off-task	156	-2.451	0.014*	55	-0.058	0.953	96	-3.158	0.001**
- Plays with Object	150	-2.525	0.011*	55	-0.307	0.759	90	-3.053	0.002**
- Out of seat	132	-0.908	0.363	48	0.535	0.592	81	-1.402	0.161

P values < 0.01 are highlighted with **, while trends for association (*P* values ≥ 0.01 and ≤ 0.05) are indicated with*. RASS: Restricted Academic situation scale. FTO SNP *rs8050136* (A/C), allele frequency for the A allele = 0.384 and for the C allele = 0.616 in the total ADHD sample. Results in the table have been depicted for the *A* allele since this is considered the risk allele for obesity according to published GWAS.

Table 7.4: FBAT output detailing the association between *rs8050136* and BMI in ADHD children.

Trait	Total Sample of ADHD children			ADHD children exposed to Maternal smoking during pregnancy			ADHD children not exposed to Maternal smoking during pregnancy		
	Family No.	Z statistic	P value	Family No.	Z statistic	P value	Family No.	Z statistic	P value
BMI	84	-1.322	0.186	23	0.131	0.895	57	-1.915	0.055*

P values < 0.01 are highlighted with **, while trends for association (*P* values ≥ 0.01 and ≤ 0.05) are indicated with*. BMI: Body mass Index. FTO SNP *rs8050136* (A/C), allele frequency for the A allele = 0.384 and for the C allele = 0.616 in the total ADHD sample. Results in the table have been depicted for the *A* allele since this is considered the risk allele for obesity according to published GWAS.

Supplementary table 1: FBAT output detailing the association between *rs8050136* and ADHD diagnosis based on ADHD subtype.

	ADHD children – Combined/Hyperactive subtype			ADHD children – Inattentive subtype		
Trait	**Family No.**	**Z statistic**	**P value**	**Family No.**	**Z statistic**	**P value**
ADHD diagnosis	99	-1.04	0.295	68	-1.76	0.077

P values < 0.01 are highlighted with **, while trends for association (*P* values ≥ 0.01 and ≤ 0.05) are indicated with*.
FTO SNP *rs8050136* (A/C), allele frequency for the A allele = 0.384 and for the C allele = 0.616 in the total ADHD sample.
Results in the table have been depicted for the *A* allele since this is considered the risk allele for obesity according to published GWAS.

Supplementary table 2: FBAT output detailing the association between *rs8050136* and ADHD diagnosis based on previous exposure to stimulant medication.

	ADHD children previously exposed to stimulant medication			ADHD children not previously exposed to stimulant medication		
Trait	**Family No.**	**Z statistic**	**P value**	**Family No.**	**Z statistic**	**P value**
ADHD diagnosis	103	-156	0.117	58	-1.35	0.176

P values < 0.01 are highlighted with **, while trends for association (*P* values ≥ 0.01 and ≤ 0.05) are indicated with*.
FTO SNP *rs8050136* (A/C), allele frequency for the A allele = 0.384 and for the C allele = 0.616 in the total ADHD sample.
Results in the table have been depicted for the *A* allele since this is considered the risk allele for obesity according to published GWAS.

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Disclosure statement

Nothing to declare.

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CHAPTER 8

Discussion

SUMMARY

Attention-Deficit/Hyperactivity Disorder (ADHD) is complex, multifaceted neurodevelopmental disorder which is highly prevalent and affects nearly 8-12% of the general population (Faraone, Sergeant, Gillberg, & Biederman, 2003). Children with ADHD present with a persistent pattern of inattention, impulsiveness, hyperactivity and cognitive deficits. Further, the symptoms of ADHD are often aggravated by comorbid conditions including oppositional defiant disorder, conduct disorder, anxiety disorders, mood disorders and learning disabilities (Biederman, 2005). Due to the unrelenting symptoms of ADHD, children with the disorder experience problems at home and school. Moreover, these problems result into low academic achievement (Faraone, et al., 2003) and strained relationships with peers and family members. Childhood ADHD symptoms may persists into adulthood. Moreover, the symptoms of ADHD in adulthood may cause impairment in occupational and social spheres of a person's life, particularly when it is comorbid with substance abuse, antisocial behavior and criminality (Thapar, Harold, Rice, Langley, & O'Donovan, 2007). Given its chronic debilitations, ADHD has become a serious public health concern (Newlove-Delgado & Stein, 2012). More specifically, studies have shown that the mental health services needed to deal with the symptoms of ADHD places a burden on the health care system (Newlove-Delgado & Stein, 2012; Thapar, Langley, Asherson, & Gill, 2007; Verster & Cox, 2008). Additionally, the adverse consequences of ADHD impact not only the affected individual, but his/her colleagues, peers and family members, as well.

ADHD is a highly heritable disorder with an average heritability estimate of 76%, which points towards significant *genetic* contribution in the pathophysiology of ADHD (Biederman, 2005). However, deciphering the genetics of ADHD has been an uphill challenge for genetic researchers. ADHD is a composite of many complex behavioral dimensions/traits; each of these dimensions is believed to have a complex genetic structure. Moreover, it is believed that, multiple susceptibility genes contribute to the overall risk of ADHD, each with a small magnitude (Lander & Schork, 1994). This *polygenic etiology* presents a few challenges for genetic studies since different genes may result in the same phenotype, and each gene may have low *"penetrance"* so that all carriers do not develop the disorder (Chakravarti, 1999; Lander & Schork, 1994; N. Risch & Merikangas, 1996; N. J. Risch, 2000). *Environmental factors* and *gene-environment interplay* (G-E) further add to the complexity (Rutter & Silberg, 2002; Stergiakouli & Thapar, 2010) of ADHD.

Linkage studies using affected sib-pairs and extended pedigrees have implicated specific chromosomal regions in ADHD (Arcos-Burgos et al., 2002; Arcos-Burgos et al., 2004; Asherson et al., 2008; Bakker et al., 2003; Jain et al., 2007; Romanos et al., 2008; Sachdev, Chubukov, & Sokol, 1995). Further, a recent meta-analysis identified 10 chromosomal regions with linkage signals (Zhou et al., 2008). However, it has been noted that most findings were unique to individual studies, with lack of consistency across scans. In addition to linkage studies, a large number of *candidate-gene studies* have been conducted with ADHD. Furthermore, in these studies association has been tested using either the *case-control* or *family-based* design. More specifically, genes implicated in *dopamine (DA)*, *NE* and *serotonin (5-HT)* pathways have been selected as candidates based on direct (DA and NE) and indirect (5-HT) pharmacological evidence (Jain, et al., 2007). Results of these

candidate gene (Gizer, Ficks, & Waldman, 2009) studies have identified many genetic markers associated with ADHD. However, consistent replication of results for these candidate genes has been difficult (Neale, Medland, Ripke, Asherson, et al., 2010).

More recently, genetic researchers have directed efforts towards conducting *genome-wide association studies* (GWAS) of ADHD (Hendler et al., 2005; Lasky-Su et al., 2008; Lesch et al., 2008; Mick et al., 2010; Neale, Medland, Ripke, Anney, et al., 2010). This change in strategy is motivated by the idea that GWAS are effective in identifying small effect genetic variants, including those that are not located in or nearby genes. However, none of the GWAS in ADHD to-date have reported genes that passed the genome-wide significance threshold (10-7); which is in stark contrast to the results of GWAS of other complex traits, including somatic (Hinney et al., 2007) and behavioural disorders (Consortium, 2010; Ma et al., 2009). The negative ADHD GWAS results are believed to stem from the *genetic complexity* of the disorder. In line with this idea, the relative risk for ADHD in first-degree relatives (λ siblings) is much smaller ($\lambda = 5$) compared to the relative risk for schizophrenia and bipolar disorder ($\lambda = 10$), which is suggestive of genetic complexity. In order to facilitate future genetic studies, in dealing with, issues of genetic complexity, some researchers have proposed a different approach for selecting candidate genes in ADHD. More specifically, they have suggested that, it may be interesting to study genes identified in GWAS of ADHD comorbid psychiatric and somatic disorders as there may be shared behavioral and neurobiological mechanisms underlying these comorbidities. For example, Obesity/weight gain and ADHD are highly comorbid and clinically linked, and it is now believed that, the comorbidity among these two phenotypes could be due to shared genetic and environmental factors (Cortese et al., 2008; Cortese & Morcillo Penalver, 2010; Cortese & Vincenzi, 2012). Thus, the use

of *comorbidity* as a strategy may reduce the clinical and etiological heterogeneity of ADHD. In addition, this may help in identifying a more homogenous subgroup of children with ADHD which in turn would facilitate genetic studies of ADHD to elucidate pathways to the disorder.

In addition to genetics, environmental risk factors are also implicated in the pathophysiology of ADHD syndrome. Further, these factors are significant in ADHD susceptibility and account for approximately 30% of the variance in the ADHD phenotype. More specifically, epidemiological studies to date have proposed that, environmental risk factors occurring during critical periods of fetal development may result in significantly detrimental effect on the neurodevelopment of the offspring (Banerjee, Middleton, & Faraone, 2007) which may be critical to the emergence of symptoms associated with ADHD. Moreover, studies investigating environmental risk factors in ADHD suggest that, *maternal smoking during pregnancy* (Banerjee, et al., 2007) and *maternal exposure to stress during pregnancy* may be linked to many adverse effects on pre- and postnatal growth, which may translate into later in life poor cognitive ability and behavioral outcome in offspring. Thus, in light of these earlier findings, it may be pertinent to focus on studying these environmental risk factors in ADHD (Thapar, Harold, et al., 2007; Thapar, Langley, et al., 2007). Furthermore, given the complexity associated with ADHD, it is suggested that, *gene-environment interplay* strategy should be used to reduce the etiological heterogeneity of ADHD.

Adding to the already complex picture, ADHD present with another challenge, which is the *phenotypic heterogeneity* (ADHD subtypes) associated with the disorder. More specifically, ADHD has three clinical subtypes (hyperactive, inattentive, and

combined) which are categorized based on a multitude of clinical symptoms. With regards to this challenge, genetic epidemiologists believe that, considering the clinical definition of ADHD as per DSM IV in genetic studies of ADHD may conceal potential effects between specific ADHD dimensions and risk variants. In view of this, they have proposed a possible solution to this problem, which is exploring quantifiable intermediate constructs or "*endophenotypes"* (Gottesman & Gould, 2003) in genetic studies of ADHD because endophenotypes lie between genes and clinical symptoms (Castellanos & Tannock, 2002). Hence, studying endophenotypes in ADHD may be a worthwhile strategy to reduce phenotypic heterogeneity and may be helpful for genetic studies of ADHD in identifying risk variants associated with the disorder.

MAIN ASSUMPTIONS OF PhD RESEARCH

The main assumptions underlying the research work in this thesis are that:

(1) The *clinical heterogeneity* may be reduced by examining clinically relevant *"endophenotypes"* and also by indexing a more homogenous subset of subjects by studying *"ADHD comorbid disorders"*, such as, Obesity.

(2) The *etiological complexity* may be reduced by using *"gene/environment interplay"* i.e. stratifying children based on exposure to major environmental factors implicated in ADHD, such as, maternal smoking during pregnancy and maternal stress during pregnancy. Additionally, by investigating candidate genes which are firmly implicated in neurobiology of the ADHD (encoding key components of the DA, NE, and 5-HT pathways), and/or consistently shown to be involved in ADHD

(based on linkage studies, and candidate association studies), and/or are strongly associated with ADHD co-morbid disorders (as per GWAS).

We accomplished this task by collecting detailed clinical, behavioural, and neurocognitive assessments in three settings (clinic, home, and school) and environmental data and integrating it with extensive genetic information from a large sample and using *"quantitative trait analysis"* and *"family-based association test"* which are robust statistical methods to study gene-phenotype association.

First, we used *"endophenotypes"* approach to counter clinical heterogeneity of ADHD. This enabled us to report a tentative association between a well-known ADHD candidate gene (implicated in the DA systems within the PFC) and neurocognitive traits. Secondly, we utilized *"gene-environment interplay"* strategy to deal with etiological heterogeneity of ADHD and reported an association between an important ADHD candidate gene (identified through linkage and association) and traits relevant to ADHD in a group of children exposed to maternal stress during pregnancy. Finally, we employed *"comorbidity"* strategy to resolve issues of clinical and etiological heterogeneity of ADHD. More specifically, using this scheme, first, we comprehensively (behaviorally and clinically) characterized children with ADHD in relation to their BMI/weight categories. In addition, we reported that, self-regulation deficits are not implicated in the comorbidity between ADHD and Obesity. Furthermore, we identified a novel association between ADHD pertinent phenotypes and a polymorphism originally linked to Obesity by GWAS in a group of children not exposed to maternal smoking during pregnancy.

CATECHOL-O-METHYLTRANSFERASE GENE AND EXECUTIVE FUNCTION IN CHILDREN WITH ADHD

In chapter 3, our objective was to determine whether using *"endophenotypes"* as a strategy could reduce the clinical heterogeneity of ADHD. Additionally, selecting a potential candidate gene *"COMT"* believed to be involved in the neurobiology of ADHD by strong apriori biological evidence could facilitate investigation of gene-phenotype association in ADHD.

COMT Val108/158Met polymorphism, a functional SNP within the *COMT* gene had been previously associated with other cognitive dysfunction in disorders such as schizophrenia (Bilder et al., 2002; Goldberg et al., 2003). Yet, the role of *COMT* gene in ADHD is still debated because findings from all the previous *COMT* genetic studies in ADHD are mixed. One major reason for these inconsistent findings is that most studies investigated *COMT Val108/158Met* polymorphism in association with neurocognitive phenotype in ADHD (Bellgrove et al., 2005; Mills et al., 2004; Taerk et al., 2004). However, given the complex structure of the *COMT* gene, it is now proposed that, additional genetic variations within the *COMT* gene may interact with *Val108/158Met* to determine the biological effects of this gene (Meyer-Lindenberg et al., 2006; Shifman et al., 2002). Nackley et al. (2006) showed that different *COMT* haplotypes have different levels of protein expression possibly due to the alternating mRNA secondary structure (Nackley et al., 2006). Given the importance of other SNPs and haplotypes in modulating the *COMT* function, we investigated their role in modulating executive functioning in ADHD. Furthermore,

we examine the association of *COMT* haplotypes with (a) ADHD diagnosis and (b) performance on neuropsychological tasks in these children.

Family-based analyses in this study did not show any significant association between the four alleles or their derived diplotypes and ADHD diagnosis, and also the various neurocognitive outcomes indexing executive functions. However, the quantitative trait analyses results showed that the *COMT* haplotypes and their constituent SNP's (SNPs; rs6269, rs4633, rs4818, and rs4680) may influence neuropsychological task performance in children with ADHD. However, after correction for multiple testing, only one significant effect was observed between rs6269 and the number of categories completed (a measure of concept formation ability) on the WCST, suggesting that COMT gene may be tentatively implicated in modulating executive function, in children with ADHD.

In summary, our study did not lead to a strong evidence of association between COMT gene and ADHD in spite of the major efforts addressing the limitations of previous studies. Moreover, if there is an effect of COMT in ADHD, it may be very small and much larger samples sizes may be needed to reach firm conclusions.

LPHN3 AND ATTENTION-DEFICIT/HYPERACTIVITY DISORDER: INTERACTION WITH MATERNAL STRESS DURING PREGNANCY

LPHN3 gene is implicated in ADHD by linkage and association (Arcos-Burgos et al., 2010; Ribases et al., 2011). In addition, it codes for Latrophilin 3 (LPHN3), which is a brain specific receptor, important for the regulated exocytosis of neurotransmitters, particularly norepinephrine (NE). Given this strong *a priori* evidence, we considered *LPHN3* gene as an interesting candidate for genetic studies of ADHD.

Through the usage of family-based association tests in a large sample of children with ADHD (n = 380 families) exploring the association between *LPHN3* gene (alleles and haplotypes) and clinical, behavioral and neurocognitive traits, we identified limited associations in the total sample. However, highly significant interactions between four LPHN3 tag SNPs (rs6551665, rs1947274, rs6858066, rs2345039) and maternal stress during pregnancy was noted (Choudhry et al., 2012). More specifically, analysis conducted in the sub-group of mothers exposed to minimal stress during pregnancy showed significant associations with ADHD, behavioral and cognitive dimensions related to ADHD, as well as treatment response. Although extensive association was observed with the candidate SNPs, the findings are partially inconsistent with previously published results with the opposite alleles over-transmitted in these studies.

Given that much stronger association was only revealed after stratification based on exposure status to maternal stress during pregnancy, it is indeed possible that the effect of LPHN3 polymorphisms is masked in the total group when maternal stress during pregnancy is not taken into consideration.

BODY WEIGHT AND ADHD: A COMPREHENSIVE CLINICAL AND BEHAVIORAL CHARACTERIZATION

In chapter 5, our objective was to investigate the relation between body weight and clinical/behavioural characteristics of children diagnosed with ADHD. Although it is now established that a potential association does exist between Obesity/weight gain and ADHD, two comorbid phenotypes (Cortese & Vincenzi, 2012), the underpinnings of this association are not well understood. Some researchers believe that, there may be shared behavioral and neurobiological mechanisms underlying this association. However, to-date no study has explored an association between ADHD clinical traits and body weight in children with ADHD while controlling for potential confounders.

The results of the study showed that, the three weight status groups (normal, overweight, and obese) were significantly different from each other. More specifically, children with obese weight status were found to be more likely to be diagnosed with the inattentive subtype of ADHD and were more withdrawn, with less severe hyperactivity and less overall behavioral problems. In contrast, overweight children were predominantly diagnosed with the hyperactive subtype, exhibited more restless-impulsive behavior in the classroom but not in the home environment.

BODY WEIGHT AND ADHD: EXAMINING THE ROLE OF SELF-REGULATION

Previous theories suggest that self-regulation deficits impacts both ADHD and obesity and may be underlying this association. In chapter 6, our objective was to test the hypothesis that, self-regulation deficits are associated with obesity in children with ADHD.

The results of the current study show that, both obese and overweight ADHD children exhibited significantly lower SES compared to normal weight ADHD children. Additionally, no significant differences were observed between the three weight groups with regards to their neurocognitive, emotional and motor profile.

Findings from this study are of interest as these do not support previous theories suggesting that impaired self-regulation promotes obesity in ADHD. Morespecfically, these findings indicate that differences in weight/BMI are not accounted for by cognitive, motivational and motor profiles. However, socio-economic characteristics are strongly associated with overweight and obesity in ADHD children.

ASSOCIATION BETWEEN OBESITY-RELATED GENE *FTO* AND ADHD

In chapter 7, we investigated the association between ADHD diagnosis and ADHD pertinent phenotypes and one tag SNP (*rs8050136, risk allele A*) located in the *Fat Mass and Obesity* (*FTO*) gene (Frayling et al., 2007). The FTO gene showed the

strongest association with obesity in GWAS and appeared to be acting through the modulation of neurobiological systems. Additionally, as this gene was found to be associated with cigarette smoking (Sobczyk-Kopciol et al., 2011), we stratified the sample of ADHD affected children based on exposure to maternal smoking during pregnancy to explore effects of this environmental factor on the genetic associations.

By doing so, we identified a novel association between the A* risk allele of *rs8050136*, a SNP located in intron 1 of the *FTO* gene located on chromosome 16 (Frayling, et al., 2007) and several of the ADHD associated traits tested in the total sample. These associations were stronger when the analysis was restricted to children who were not exposed to MSDP (Choudhry et al., 2013). Thus, these findings suggest the involvement of the *FTO SNP rs8050136* in modulating the risk for ADHD, particularly in those children who were not exposed to MSDP. If confirmed, they may explain, at least in part, the complex links between obesity and ADHD.

STRENGTHS AND LIMITATIONS OF RESEARCH WORK

The research presented in this thesis has a number of strengths as it is a subset of one of the largest, double-blind, placebo-controlled study investigating ADHD children in the world, and the only one in Canada. First, all participants received a clinical diagnosis of ADHD by experienced psychiatrist (RJ & NG). Second, all participants underwent a medication washout period for at least 48 hours prior to testing day. Third, all child participants underwent comprehensive assessments utilizing validated scales in three different environments (home, school, and laboratory) by different observers (parents, teachers, and research staff). Fourth, all

EF tasks were administered in the morning time to maximize the standardization of the assessment and reduce the effect of fatigue on task performance. Fifth, comprehensive genetic and environmental profiling was done for every participant. Finally, all potential confounding factors believed to confound the results were controlled for during the analyses.

However, this study has some limitations which should be kept in mind when interpreting these results. First, the measure of MSDP and MESDP was based on retrospective mother reports. Second, we did not have detailed clinical information pertaining to familial somatic (weight problems) and mental health (ADHD). Third, with regards to the relation between body weight and ADHD, this study could not investigate, the moderating effects of physical activity patterns and eating habits/preferences due to lack of data. Finally, our research design investigated BMI, which is a generalized measure of body mass, and lacked more direct and objective measures of obesity, such as underwater weighing, skin folds, etc.

CONCLUSION

Results obtained in these studies support the idea that the use of multiple strategies in ADHD would facilitate identification of genetic variants implicated in the disorder. More specifically, examination of clinically relevant *"endophenotypes"* (neurocognitive phenotype) and investigation of *"ADHD comorbid disorders"* (Obesity) may reduce the *clinical heterogeneity* of ADHD. Similarly, the employment of *"gene/environment interplay"* as a strategy may increase sample homogeneity. In addition, the strategic selection of the candidate genes based on strong a priori biological relevance (encoding key components of the DA, NE, and 5-HT pathways),

consistent involvement in ADHD (based on linkage/candidate association studies), and replicated association with ADHD co-morbid disorders (as per GWAS) may help in the deciphering of the genetic *etiological complexity* of the disorder.

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