Dopamine transporter Genotype and Response to Methylphenidate Treatment and Side Effects in Children with ADHD

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ABSTRACT

Stimulant medications such as methylphenidate (MPH) are the most frequently used medication for ADHD. MPH acts primarily by blocking the dopamine transporter (DAT). About 70% of patients taking MPH show adequate therapeutic response, but the level of response to MPH vary from one child to the other. Similarly the level of MPH side effect varies from one child to the other and may be the cause of poor therapeutic adherence. Several studies have implicated the 3' untranslated region (UTR) variable number tandem repeat (VNTR) of the dopamine transporter gene (DAT1) in MPH therapeutic response and side effects.

This study presents the relation between response to and side effects induced by methylphenidate treatment in children with ADHD based on the dopamine transporter genotypes (3'UTR VNTR, rs6347 (exon9), rs8179029 (Int9), rs3836790 (Int8), rs463379 and rs460000). This study is based on an independent and larger (n=310) sample compared to a previous published paper exploring the association between 3'UTR VNTR and response to methylphenidate in children with ADHD (Joober et al., 2007) with a sample size of 150 patients. Results from this study revealed no association between the DAT1 genotypes and ADHD behavioral dimensions treatment response to methylphenidate However, we replicated the association between the DAT1 genotypes and MPH side effects.

Résumé

Les médicaments stimulants tels que le méthylphénidate (MPH) sont les médicaments les plus fréquemment utilisés pour le TDAH. MPH agit principalement en bloquant le transporteur de la dopamine (DAT). Environ 70% des patients prenant MPH spectacle réponse thérapeutique adéquate, mais le niveau de réponse à MPH varient d'un enfant à l'autre. De même, le niveau de l'effet de côté MPH varie d'un enfant à l'autre et peut être la cause de l'adhésion thérapeutique pauvres. Plusieurs études ont mis en cause la région 3 'non traduite (UTR) répétition en tandem de nombre variable (VNTR) du gène transporteur de la dopamine (DAT1) en réponse thérapeutique MPH et les effets secondaires.

Cette étude présente la relation entre l'intervention et les effets secondaires induits par le traitement de méthylphénidate chez les enfants atteints de TDAH basé sur les génotypes dopamine transporteurs (3'UTR VNTR, rs6347 (exon9), rs8179029 (Int9), rs3836790 (Int8), rs463379 et rs460000) . Cette étude est basée sur une (n = 310) échantillon indépendant et plus grand par rapport à un article publié précédente explorer l'association entre 3'UTR VNTR et la réponse au méthylphénidate chez les enfants atteints de TDAH (Joober et al., 2007) avec une taille de l'échantillon 150 patients. Les résultats de cette étude ont révélé aucune association entre les génotypes DAT1 et TDAH réponse au traitement des dimensions comportementales au méthylphénidate Cependant, nous avons reproduit l'association entre les génotypes DAT1 et les effets secondaires de MPH.

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LIST OF ABBREVIATIONS

Symbol	Abbreviations
ADHD	Attention-Deficit /Hyperactivity Disorder
AD	Anxiety Disorder
AMPH	Amphetamine
ANOVA	Analysis of variance
APA	American Psychological Association
CBCL	Child's Behavioral Check List
CD	Conduct Disorder
Conners'-P	Conner's Global Index-Parents
Conners'-T	Conner's Global Index-Teachers
DA	Dopamine
DAT	Dopamine Transporter Protein
DAT1	Dopamine Transporter Gene
DNA	Deoxyribonucleic Acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
EF	Executive Function
hDAT	Human dopamine transporter
HRR	Haplotype relative risk
IQ	Intelligence Quotient
MDD	Major Depressive Disorder
MPH	Methylphenidate
MR	Medication Response
MRI	Magnetic Resonance Imaging
NE	Norepinephrine
NET	Norepinephrine Transporter
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography

PFC	Prefrontal Cortex
RASS	Restricted Academic Situation Scale
SERS	Side Effects Rating Scale
SERT	Serotonin transporter
SLC6A3	Solute Carrier Family 6 member A3
SN	Substantia nigra
SNP	Single Nucleotide Polymorphism
SPECT	Single photon emission computed tomography
SPSS	Statistical Package for the Social Sciences
TDT	Transmission disequilibrium test
UTR	Untranslated Region
VNTR	Variable Number Tandem Repeats
VTA	Ventral Tegmental Area
WISC	Wechsler Intelligence Scale for Children

CHAPTER1

LITERATURE REVIEW

INTRODUCTION

1.1 ADHD Epidemiology and Clinical Presentation

Attention-Deficit/Hyperactivity Disorder (ADHD) is one of the most prevalent psychiatric disorders in school-aged children worldwide (Biederman, 2005).ADHD has a 6-9% prevalence rate (Dopheide & Pliszka, 2009). These estimates may vary depending on the diagnostic criteria used and the population demographics of the sample (Froehlich et al. 2007). ADHD is a major risk factor for educational failure, later antisocial and high risk behavior, and other psychopathology (Biederman et al., 2006; Devevensky & Japel, 2009). This is particularly true if ADHD is undiagnosed (Mannuzza, Klein & Moulton 2008) or untreated (Shaw et al. 2012). ADHD was once believed to be a childhood disorder that diminished with age, but 60% of childhood cases have persisted into adulthood (Biederman, Faraone, Spence & Wilens, 1993).

Diagnostic and Statistical Manual of Mental Disorders (DSM-V) of the American Psychological Association (APA 2013) defined ADHD by five essential diagnostic criteria. The five diagnostic criteria are as follows; (1) Persistent patterns of inattention and or hyperactivity-impulsivity that interferes with functioning or development, (2) Symptoms of inattention and or/hyperactivity-impulsivity are present before the age of 12, (3) Symptoms are present in more than one setting (4) Symptoms interfere with or reduce the quality of developmentally appropriate social, academic or occupational functioning and (5) Symptoms cannot be attributed to any other mental disorder (APA, 2013). ADHD is divided into three subtypes; predominantly inattentive subtypes which requires at least six or more inattention symptoms for the past 6 months, predominantly hyperactive-impulsive subtypes which requires six or more hyperactive impulsivity symptoms for the past 6 months and the combined subtypes which requires 6 or more symptoms with each of the inattentive and hyperactive-impulsive symptoms for the past 6 months (APA, 2013).

The DSM-V further categorizes ADHD cases by severity (APA 2013). A mild case of ADHD is described as one in which only minor academic, social or occupational impairments exist and few or no symptoms, in excess of those symptoms required to make the diagnosis, are present. A moderate case of ADHD is one in which symptoms and impairments fall between "mild and severe". ADHD case is said to be severe when a significant academic, social or occupational impairment exists and many symptoms, in excess to those symptoms required to make a diagnosis, are present or several symptoms are particularly severe (APA 2013).

1.1.1. Etiology of ADHD

The etiology of ADHD remains to be clearly identified. Numerous studies have revealed strong genetic and neurobiological foundations along with other contributing factors such as biological and environmental influences (Biederman, 2005). Family, twin and adoption studies have illustrated the nature of ADHD with a two to eightfold increase in the risk for ADHD in the parents and siblings of children with ADHD, with an average heritability of about 0.80

(Biederman, 2005; Faraone et al., 1992). The results have driven molecular genetics research to evaluate the genetic etiology of ADHD. Molecular genetics have implicated the 3' untranslated region VNTR (3'UTR) of the human dopamine transporter gene (DAT) as the genetic basis of ADHD (Faraone, Doyle, Mick, & Biederman, 2001). These findings support the suggested role of dopamine in the pathogenesis of ADHD. Relevant to DA catabolism, some studies found significant associations within the 10-repeat allele 3'UTR region DAT gene, although meta-analyses report either a weak association or no association (Faraone & Mick, 2010; Li et al., 2006; Yang et al., 2007). The inconsistencies may indicate that this allele is in partial linkage disequilibrium with another maker of ADHD, rather than directly conferring vulnerability for the disorder. The VNTR occurs at a non-coding site and thought to affect DAT expression, rather than

the amino acid sequence: the density of DAT binding sites for the 10-repeat polymorphism was found elevated by about 50% over the 9-repeat polymorphism (VanNess, Owens, & Kilts, 2005), which would be expected to increase synaptic DA clearance. Allelic variation in the DAT SLC6A3 gene has also been linked with response to stimulants, with subjects homozygous for the 9-repeat allele showing a diminished response (Stein et al;2005, Joober et al., 2007; Lott, Kim, Cook, & de Wit , 2004). Notably, elimination of the SCL6A3 gene in mice can produce hyperactive and impulsive behavior, which is reduced by stimulants (Gainet dinov et al., 1999; Giros, Jaber, Jones, Wightman, & Caron., 1999).

Furthermore, neurobiological, neuroimaging and neuropsychological studies established that ADHD arose from the dysfunction in pronto-subcortical pathways, which may be affected by dopaminergic and noradrenergic function (Biederman, 2005). The strongest evidence, supporting the role of dopaminergic dysfunction in the pathogenesis of ADHD, is the effectiveness of psychostimulant medication, to reduce the inattentive and hyperactive-impulsive symptoms of ADHD. Stimulant medication, such as methylphenidate, can reduce ADHD symptoms by inhibiting dopamine transporters and blocking dopamine and norepinephrine reuptake in the fronto-subcortical pathways (Elia et al., 1990).

Biological and environmental factors such as low birth weight, maternal weight status and smoking, alcohol exposure during pregnancy and psychosocial adversity may be attributed as risk factors for ADHD (Biederman et al., 1995; Mick, Biederman, &Prince, 2002). Psychosocial adversity refers to 6 family/environmental factors that are associated with ADHD and other childhood mental disorders: (1) Low socioeconomic status, (2) paternal criminality (3) Severe marital discord and family/ or family conflict, (4) large family size, (5) foster placement, and (6)

maternal mental disorder (Rutter, &Quinton, 1977). The likelihood of the diagnosis of ADHD increases as the number of the adversity factors increase (Biederman et al., 1995).

1.1.2 Neurological Bases of ADHD

Executive functioning. There has been developing concurrence that ADHD relates to deficits in executive functioning (EF). Even though several researchers speculate divergent cognitive functions surrounding EF, ADHD research uses a standard definition which is "those neuropsychological processes needed to sustain problem-solving toward a goal" (Wilcut, Doyle, Nig, Faraone, & Penington, 2005). It could also be described as, neurocognitve processes that facilitate future-oriented behavior by mediating planning, flexible use of strategies, impulse control, and organized search (Welsh, Penington, & Groiser, 1991; Wilcut et al., 2005). Wilcut et al.'s meta-analysis of 83 studies that administered EF measures to groups with ADHD found significant impairment on all EF tasks, with the strongest effects on measures of response inhibition, vigilance, working memory, and planning. Weaknesses in EF were not explained by intelligence, academic achievement, or symptoms of other disorders. Other reviews have found EF deficits regardless of gender or age (e.g., Seidman, 2006). Nevertheless, EF weakness does not account for all cases of ADHD. Some people with ADHD may have deficits in brain reward systems that are independent of EF impairments (Seidman, 2006).

Barkley's view of executive functioning. One of the most prominent ADHD researchers Rusel Barkley (2011b), suggests that ADHD is a disorder of self-regulation because the mental processes most often included in the construct of executive functioning are fundamentally those of self-regulation: inhibition, resistance to distraction, self-awareness, working memory, emotional self-control, and self-motivation.

Self-regulation and EF are both involved in goal-directed, future-oriented actions; they require sustaining actions over time to achieve one's goals; and they both include problem-solving. In his model, inattention and hyperactivity/impulsivity are the result of these underlying psychological deficits; in some sense, they are what remain in the absence of goal- directed behavior.

Barkley proposes that there are two forms of sustained attention, only one of which is affected by ADHD. The first is contingency-shaped attention, which is context- dependent. Absorption in video games exemplifies this type of attention. The person is focused and highly motivated to continue engaging in an activity that is rewarding and stimulating. The activity is challenging to motor reflexes, does not require significant cognitive input, and provides instant gratification (Barkley, 2006). The other form of sustained attention is goal-directed persistence, which is guided by internal motivation. Studying, which requires delayed gratification, is an example of this type of attention. People with ADHD are easily distracted by more stimulating events in these circumstances, leading to interference with task completion (Barkley, 2006; Pennington & Ozonoff, 1996). This distinction leads Barkley to assert that ADHD does not involve an attention deficit per se, but that difficulty with goal-directed persistence, which requires self-regulation, is one of the characteristics of the disorder.

Reward deficiency syndrome. Kenneth Blum was an early leader in developing a theory linking ADHD to other impulsive as well as addictive and compulsive disorders, including alcoholism, drug abuse, smoking, pathological gambling, and binge eating. The theory postulates a common genetic basis for all these disorders, the dopamine DRD2 A1 allele. Individuals with the A1 allele have fewer dopamine receptors in their brains, making it much more difficult for them to derive satisfaction from ordinary, everyday activities. This may translate into persistent cravings or stimulus-seeking behaviors of A1 carriers (Blum et al., 2008).

One study found that the A1 allele was present in 49% of children with ADHD, compared to 27% of controls (Comings, 2001). It was found in 60% of an adolescent group diagnosed as pathologically violent (Blum et al., 2008). This understanding of ADHD establishes a neurochemical link between ADHD and drug abuse as well as other negative outcomes and may account for the consistent group of subjects with ADHD symptoms who do not have EF deficits. Blum and other proponents of reward deficiency syndrome (RDS) give little attention to what factors determine whether a person's stimulation-seeking behaviors are destructive or, alternatively, if there are protective factors that lead to productive, creative, entrepreneurial expressions of RDS. Many researchers believe that personality traits are critical to the trajectory (positive or negative) of stimulus-seeking behavior (e.g., Farley, 1991, as cited in Kaplan, 2001).

1.1.3 Neuroanatomy in ADHD.

Given their role in EF, the prefrontal cortex, caudate nucleus, and corpus callosum are all implicated in ADHD. Several studies have compared frontal lobe volume in children with ADHD and controls (e.g., Almeida et al., 2010; Castellanos et al., 2002; Shaw et al., 2007) and all found a decrease in the volume of frontal lobe encephalic structures or cortical thickness. Furthermore, patients with frontal lobe damage report symptoms similar to ADHD, including hyperactivity, distractibility, and/or impulsivity (Anderson, 2008; Anderson, Jacobs, & Harvey, 2005; Eslinger, Biddle, Penington, & Page, 1999). The caudate nucleus is part of the basal ganglia, which receives signals from the prefrontal lobe necessary for the initiation of complex motor tasks. Studies have found the right caudate nucleus to be smaller in children with ADHD than controls (Castellanos et al., 2001; Castellanos, Giedd, Eckburg, & Marsh, 1994; Castellanos, Giedd, Marsh, & Hamburger, 1996; Tremols et al., 2008). Additional studies have found reduced caudate volume to be correlated with reduced inhibition and increased externalizing behaviors (Semrud-Clikeman et al., 2000).

1.1.4 Neurophysiology in ADHD.

The studies which have used positron-emission tomography (PET) and other imaging techniques to measure the cerebral blood flow of children with ADHD have repeatedly found decreased blood flow in the prefrontal cortex and caudate nucleus (Brandeis et al., 1998; Negoro et al., 2010; Öner, Öner, Aysev, Karuk, & İbis, 2005; Katya Rubia et al., 1999; Sieg, Gaffney, Preston, & Hellings, 1995). However, the reduction in blood flow is usually reversed with stimulant medication (Akay et al., 2006; Cho et al., 2007; Le et al., 2005; Weber, Lütschg, & Fahnenstich, 2007). It is important to note that many functional brain imaging studies generate relative rather than absolute data, making blood flow measurements better suited to research than diagnosis. Functional MRI (magnetic resonance imaging) scans can reveal localized brain activity while participants complete an EF-related task. Researchers have found that during tasks requiring response inhibition (a go/no-go task requiring subjects to press a button in response to visually presented stimuli but to avoid responding to a rare non target), the right inferior frontal area of the brain is activated. Such activation is noticeably reduced in children with ADHD (K. Rubia et al., 2000; Vaidya et al., 1998 Durston et al., 2003.). Durston et al. reported that children with ADHD activated other brain regions more than normally developing children. These regions were predominantly located in the dorsolateral prefrontal cortex and the posterior regions of the parietal and occipital cortex. Based on these findings, the authors hypothesize that these children may employ all these findings, the authors hypothesize that these children may employ alternative strategies involving greater reliance on working memory, vigilance, or sustained visual attention, respectively, to compensate for difficulties with inhibition (although the diversity of brain imaging research suggests that pinpointing such complex tasks to particular brain areas remains speculative).

1.1.5 Neurochemistry in ADHD.

Dopamine pathways appear to be the most frequently studied in relation to symptoms of ADHD. Dopamine has been found to modulate a wide variety of functions, including motivation, attention, learning, reward and operant conditioning (Schultz, 1997, 2001, 2006; Waelti, Dickinson, & Schultz, 2001). Studies measuring dopamine activity in children and adult ADHD subjects have generally found evidence of dopaminergic dysfunction. Data include PET measurements of dopamine receptor sites (Lou et al., 2004; Volkow, Wang, Newcorn, Telang, et al., 2007; Volkow et al., 2009);

PET and SPECT (single phantom emission computed tomography) measurements of the dopamine active transporter (DAT) (Dougherty et al., 1999; Krause, Dresel, Krause, Kung, & Tatsch, 2000; Volkow, Wang, Newcorn, Fowler, et al., 2007; Volkow et al., 2009); and measurements of homovanillic acid (HVA), the dopamine metabolite (Shaywitz, Cohen, & Bowers, 1977; Shekim, Javaid, Davis, & Bylund, 1983).

While the exact mechanisms of the dysfunction remain unclear, most researchers argue that many ADHD symptoms stem from abnormally low extracellular levels of dopamine (Volkow, Wang, Newcorn, Fowler, et al., 2007). The diversity of research findings, and the interrelatedness of different neurotransmitters, also points toward the involvement of the noradrenergic system. There is evidence of reciprocal interactions between the systems, and cyclic AMP blocks DAT as well as noradrenergic transporters (Viggiano, Ruocco, Arcieri, & Sadile, 2004). Dopamine may be more essential to sustaining attention, and norephenephrine may contribute more to executive functioning (Medscape Psychiatry, 2006). Treatment with amphetamines increases the synaptic concentration of dopamine by blocking presynaptic dopamine reuptake. Amphetamines also increase production of both dopamine and norepinephrine (Pliszka, 2005). Krause et al. (2000)

showed that four weeks of treatment with methylphenidate (e.g., Ritalin) reduced the DAT density in adult ADHD patients to the level of the control subjects. By reducing the density of transporters, more dopamine remains available in the synapse.

1.1.6 *Endophenotypes.*

Endophenotypes are intermediate phenotypes between diagnostic classifications and the underlying biological factors, representing quantitative and heritable traits that are found in unaffected relatives of the affected individuals (Almasy and Blangero, 2001, as cited in Nemoda et al., 2011). They help establish the trait-based genetic basis for psychiatric disorders. Analyzing reaction-time variability and accuracy during a Go/NoGo test, a European study found that unaffected siblings had intermediate scores between children with ADHD and controls. In the reward condition, reaction time was faster for ADHD children and their unaffected siblings than for controls, implicating a familial motivational dysfunction in ADHD (Uebel et al., 2010, as cited in Nemoda et al., 2011). Another study reported that children with ADHD and their siblings chose smaller, immediate rewards over larger, delayed rewards, confirming the heritability of delay aversion (Marco et al., 2009, as cited in Nemoda et al., 2011).

Endophenotypes research has also been conducted on dopamine genes. Loo et al. (2008, as cited in Nemoda et al., 2011) found that the 7R allele was associated with poor performance on intelligence measures, interference control, and working memory tasks, indicating the involvement of DRD4 in executive functioning. In a birth cohort study, the DRD4 7R allele and the DAT 10R allele predicted lower IQ, but only among those diagnosed with ADHD. ADHD participants with one allele or the other had lower IQs compared to those without either allele, and those with both alleles had the lowest IQs. One of the cohorts was followed up to age 26. The researchers found a

significant relationship between number of genotype risks (0, 1, or 2 alleles) and worse adult outcomes among children with ADHD. After controlling for IQ, the association reduced to non-significance, demonstrating a mediating effect of IQ on ADHD outcomes (Mill et al., 2006).

When a group of researchers used MRI neuroimaging, they found that children with ADHD who had the 7R allele had the thinnest right prefrontal cortex and posterior parietal cortex, followed by ADHD children without the allele. Children in the control group with the 7R allele had the next-thinnest cortex, followed by controls without the allele, demonstrating the gradations in expression of this gene (Shaw et al., 2007, as cited by Nemoda et al., 2011)

1.1.7 Sex differences in ADHD.

ADHD presentation is more common in males than in females during childhood (Froehlich et al., 2007). The ratio of boys to girls, diagnosed with ADHD, varies from 3:1 to 9:1 in community based samples and clinical samples respectively (APA, 2014; Skounti, Philalithis, & Galanakis, 2007). The large male dominance in ADHD may result from the under-diagnosis of females (Polanczyk & Rohde, 2007) because they may demonstrate less overt, disruptive behavior and more inattentive symptoms in comparison to males (Achenbach, 1991; Gaub & Carlson 1997). Teachers may tend to attend to troublemaking behavior and ignore inattentive behaviors, perhaps leading to an under identification of the inattentive presentation in schools (Gershon, 2002). In addition, males are at higher risk for comorbid behavioral disorders, such as Oppositional Defiant Disorders (ODD; Cortese, et al., 2008; Gaub & Carlson 1997; Gershon, 2002; Rucklidge, 2010). These behavioral problems may motivate referral to clinical diagnosis and can potentially explain part of the large male-to-female ratio in the childhood population with ADHD (Biederman, 2005).Some studies (Gaub & Carlson 1997) indicate that gender differences in the expression of ADHD may be the reason for the differences in the diagnostic prevalence of ADHD among males

and females. These gender differences in diagnosis and expression of ADHD do not appear to be genetic in basis. Smalley et al., 2000, investigated gender differences in the prevalence of ADHD among affected sibling pairs and their parents, and found that the differences observed were consistent with a model of inheritance in which girls require a greater loading of familial influences to develop ADHD.

1.1.8 ADHD and Comorbid Disorders

Several disorders are also comorbid with ADHD, compounding the impact the disorder can have on a child's life. Roughly 70% of children suffering from ADHD are believed to meet diagnostic criteria for other psychiatric disorders (Brown 2005). Comorbidity is so common among children with ADHD that it is classified as a "distinct clinical feature" of the disorder (Biederman 2005). Brown (2005) estimates that ADHD co-occurs most often with oppositional defiant disorder (in 40% of children; see also Barkley 2003), anxiety disorder (34%), conduct disorder (14%), tic disorders, depression, and bipolar disorder (Waldman et al. 2001) though figures tend to vary by type of ADHD present. Speech and language disorders as well as learning disabilities in areas like reading and spelling are also highly comorbid with ADHD (Lewis 2001, Stevenson 2001).

Perhaps most frightening for those suffering from ADHD is the risk of suffering from a "dented quality of life" (Al-Sharbati et al. 2005:264), including a greater risk of injuries and auto accidents, greater dependence on and abuse of alcohol and cigarettes, an increased likelihood of school failure or failure to graduate, greater chances of "falling out of the socially prescribed safety net" (Al-Sharbati et al. 2005:364), and a "diminished development of moral reasoning"
(Barkley 2003:81) (See also Williams and Taylor 2006). For those suffering from ADHD, the outcomes are rather grim; whether one outgrows the disorder or not, these differences in life outcomes are believed to persist (Barkley 2009: personal communication).

1.2 Dopamine

Dopamine (DA), a major neurotransmitter in the mammalian central nervous system, mediates a wide array of physiological functions including regulation of locomotor activity, cognitive processes, neuroendocrine secretion, and the control of motivated behaviors. It is biosynthesized from the amino acid tyrosine and stored in synaptic vesicles. In response to a presynaptic action potential, DA is released into the synapse by Ca2+-mediated exocytosis, where it binds to the dopamine receptors on the neighboring neuron and activates dopaminergic signal transduction pathways (Fig. 1). Dysfunctions in the DA system are believed to contribute to the development of several neurological and psychiatric conditions such as Parkinson's disease, depression, schizophrenia, attention deficit hyperactivity disorder (ADHD) and drug addiction (Schultz 2002; Arias-Carrion, Stamelou et al. 2010).

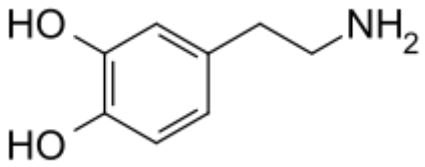

Figure 1: DOPAMINE (DA)

The neurotransmission of DA is terminated primarily by reuptake of DA into the presynaptic cell via the dopamine transporter (DAT). Thus, DAT plays a critical role in regulating the signal amplitude and duration of dopaminergic neurotransmission by mediating the reuptake of the transmitter from the extracellular space back into nerve terminals. DAT belongs to the neurotransmitter: sodium symporter (NSS) family also referred to as the SLC6 (solute carrier 6) family (Saier 1999), that includes transporters for norepinephrine, serotonin, glycine and GABA

(Amara and Kuhar 1993). These transporters utilize the transmembrane Na+ gradient as a driving force for transport of substrate across the plasma membrane. They are also characterized by co-transport of Cl-.

The reuptake process involves a conformational change in the transporter protein (Rudnick and Clark 1993). The mechanism of the translocation process includes at least three iconic states: outward-facing (open to outside), occluded, and inward-facing (open to inside) (Yamashita, Singh et al. 2005). The outward-facing transporter recognizes and binds Na+, Cl- and substrate. The binding of substrate and ions triggers the conformational change to an inward-facing conformation, allowing the release of the substrate and ions to the cytoplasm.

DAT is expressed almost exclusively in the areas of the brain with established dopaminergic circuitry: nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathway (Ciliax, Heilman et al. 1995; Ma, Ciliax et al. 1999). The mesolimbic and mesocortical pathways play key roles in reward assessment. Dopaminergic projections from the ventral tegmental area (VTA) to the limbic areas (nucleus accumbens, ventral striatum and amygdala) comprise the mesolimbic pathway, while projections from the VTA to the medial, prefrontal, cingulated and entorhinal cortex comprise the mesocortical pathway (Marsden 2006). The nigrostriatal pathway (substantia nigra to the caudate putamen) is associated with locomotor activity. The tuberoinfundibular pathway refers to a group of dopamine (DA) neurons in the arcuate nucleus of the hypothalamus that project to the median eminence and control prolactin secretion from the anterior pituitary gland (Weiner, R.I. & W.F. Ganong, 1978).

The DAT is a target of several clinically used drugs, including the psychostimulants methylphenidate, D-amphetamine, and modafinil and the antidepressant bupropion. In addition, the reinforcing and euphoric effects of the powerfully addictive psychostimulants methylphenidate

and d-methamphetamine ("crystal meth,"a vastly more potent analog of amphetamine, often administered in large doses by vaporization) are primarily mediated by interaction with the DAT (Mortensen and Amara 2003; Torres 2006). Such drugs bind to DAT and inhibit the reuptake of dopamine (Kuhar, Ritz et al. 1991). In addition to inhibiting uptake of extracellular dopamine, amphetamines also stimulate efflux of intracellular dopamine (Kahlig, Binda et al. 2005). The resultant accumulation of dopamine in the synaptic cleft potentiates neurotransmission of dopamine in those areas of the brain associated with reward and reinforcement. Consequently, the motor and reward pathways of the midbrain are activated, triggering the increased locomotor activity and euphoria associated with psychostimulant drug use.

Studies using mice lacking DAT demonstrated the importance of the transporter in psychostimulant action. DAT knockout mice display an attenuated response to methylphenidate and amphetamines and a reduced preference for methylphenidate under self-administration paradigms (Giros, Jaber et al. 1996). These mice, however, still self-administer methylphenidate, although more sessions were needed to meet self-administration criteria (Rocha, Fumagalli et al. 1998). The finding indicated that developmentally compensatory non-dopaminergic mechanisms can mediate methylphenidate-taking behavior in DAT-lacking animals. In particular, methylphenidate's interaction with the serotonin transporter (SERT) has been hypothesized to contribute to methylphenidate reward and reinforcement naturally or in compensation for the absence of DAT (Rocha, Fumagalli et al. 1998; Mateo, Budygin et al. 2004). Nevertheless, convincing preclinical evidence implicating specific activity at the DAT in methylphenidate dependence comes from the observation that the reinforcing effect of methylphenidate is lost in transgenic mice expressing a triple point-mutated DAT with preserved substrate translocation but little appreciable affinity for methylphenidate (Chen, Tilley et al. 2006). It is conceivable that

elevated basal dopaminergic tone in these mice causes adaptive changes, altering the response to methylphenidate; however, knockdown mutant mice with a ~ 90% reduction in DAT expression but with functionally unmodified DATs and elevated basal dopamine still exhibit robust, wildtype-like preference for methylphenidate. Interaction with the DAT is thus necessary for the reinforcing effects of methylphenidate in animals that carry the DAT (Tilley, Cagniard et al. 2007).

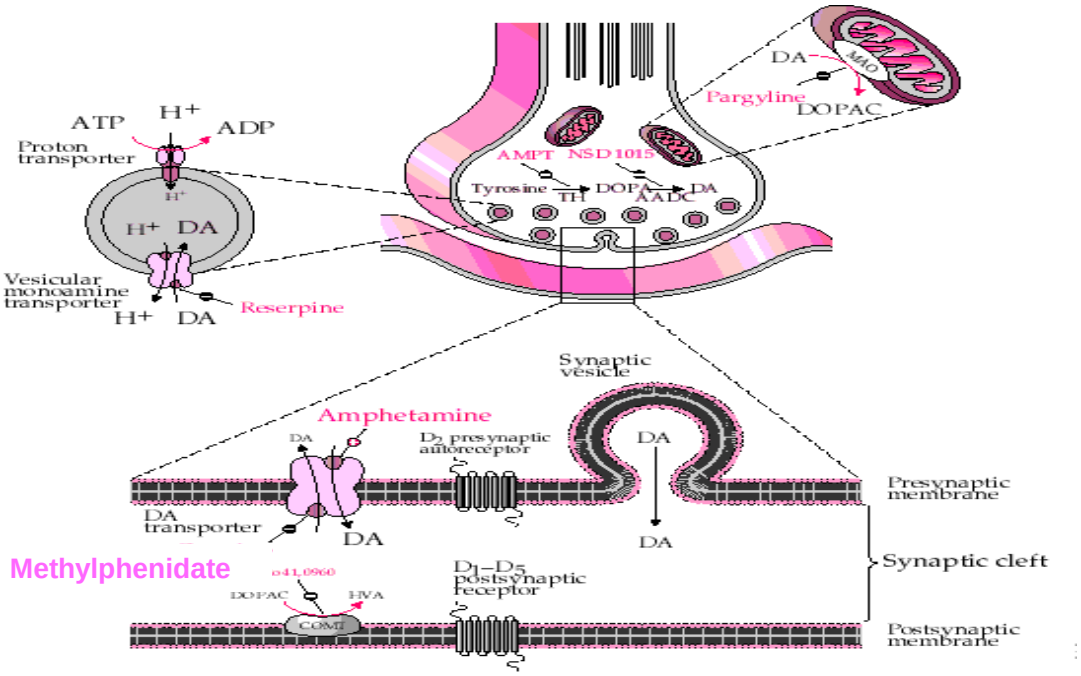

Figure 2. An overview of the dopamine pathway to highlight the site of action of methylphenidate . Neurotransmission is terminated primarily by reuptake of dopamine into the presynaptic neuron via the DAT. Methylphenidate blocks the dopamine reuptake and increases synaptic dopamine concentrations.

(*http://www.chemistry.emory.edu/justice/chem190j/images/fig8.01.gif).*

1.2.1 Role of Dopamine in ADHD

Though dopamine's association with specific ADHD symptoms (inattention versus hyperactivity-impulsivity) has not yet been clearly defined, there is strong empirical support for dopamine's role in the etiology of ADHD. Several studies now support the notion that individuals with ADHD have low levels of both tonic and phasic DA in the striatum (Sagvolden et al., 2005; Solanto, 2002). Both children and adults with ADHD have an excessive quantity of dopamine transporters (DAT's) in the striatum, thus decreasing the synaptic domapine level (see for review: Spencer et al., 2005).

Moreso, candidate gene studies of ADHD have shown that the DAT gene is associated with ADHD, with a modest effect size (Farone et al., 2005; see for review: Mick & Faraone, 2008). The most profound evidence comes from studies in which the effects of Methylphenidate (MPH) on cognitive functioning and symptoms of ADHD are assessed. MPH increases extracellular DA in the striatum and prefrontal cortex (PFC) by blocking DAT (Madras, Miller & Fischman, 2005). Because DA transporter density is highest in striatum (Madras et al., 2005), it has been supposed that the therapeutic manipulation of DA effects takes place primarily in striatum. MPH has consistently been shown to reduce ADHD symptoms (e.g., MTA cooperative group, 1999) and to improve performance on a number of cognitive skills such as response inhibition, response variability, and working memory (e.g., Langleben et al., 2006; Rubia et al., 2003; Scheres et al., 2003; Tannock et al., 1995).

1.2.2 Norepinephrine's role in ADHD

There is now mounting evidence for the role of Norepinephrine (NE) which is another neurotransmitter plays in ADHD, although it has not yet been studied as extensively as DA with regards to ADHD (Frank et al., 2007b). A primary confirmation to NE's role in ADHD is the effectiveness of Atomoxetine (ATX) in alleviating ADHD symptoms. ATX is a medication that blocks the NE transporter in the cortex, creating higher cortical levels of extracellular NE (Swanson et al., 2006).Because NE transporter density is highest in the prefrontal cortex (PFC) (Madras, Miller & Fischman, 2005), it has been supposed that the therapeutic manipulation of NE effects takes place primarily in the PFC (Frank et al., 2007a). Further, Sengupta et al. (2012) found a relationship between ADHD and an NE transporter gene. There has been little research on ATX effects on cognitive functioning. However, two studies have shown that it improves performance, as measured by a form of response inhibition, on the Stroop Color-Word Test (Faraone et al., 2005; Spencer et al., 1998

1.2.3 Dopamine transporter in ADHD

One of the most frequently studies ADHD genetic associations are with the DAT1 gene (Faraone et al., 2005). This gene codes for the dopamine transporter (DAT), the primary protein responsible for reuptake of dopamine from synaptic space in the striatum. DAT is expressed to some extent in the cortex where its primary role is likely reducing DA overflow in the extrasynaptic space (Cragg & Rice, 2004). Concentrations are magnitudes higher in striatum than cortex. (Ito, Takahashi, Arakawa, Takano, & Suhara, 2008).

DAT is a plasma membrane transporter with 12 transmembrane domains. Its primary activity is terminating the action of DA by reaccumulating the released molecules from the synapse back into

presynaptic terminals via coupling of DA to the movement of ions through the protein channel down the electrical gradient (Rudnick & Clark, 1993). The action of the synaptic DA is thereby terminated, and then is recycled back into active transporter by vesicular monoamine transporter (VMAT; Kimmel & Joyce, 2003). DAT helps to ensure the efficacy of the synapse by maintaining low neurotransmitter concentrations both in the active zone of the synapse and in extrasynaptic zones when there is volume overflow. In rodent DAT1 knockout models, DA levels are elevated and there are decreased rates of DA clearance (Gainetdinov, Jones, Fumagalli, Wightman, & Caron, 1998).

The gene which codes for DAT (DAT1) has been localized to chromosome 5pl5.3 and spans approximately 64Kbp. *DAT1* contains a number of polymorphisms across the gene, but the one that has been most widely studied in the context of ADHD and cognition is a variable number tandem repeat (VNTR) polymorphism in the 3'untranslated region (UTR). This VNTR contains a sequence of 40 base pairs that repeats anywhere from 3-11 times in the human form of the gene (Kang, Palmatier, & Kidd, 1999). The common forms are the 9-repeat (9R) and 10-repeat (10R) alleles, with the 10R allele more than twice as common as the 9R allele in some world populations (Kang, Palmatier, & Kidd, 1999). Since the polymorphism is in an untranslated region it cannot directly affect protein structure, but may affect protein expression level via regulation of mRNA structure or degradation (Fuke et al., 2001).

Of the many studies that have investigated the impact of this polymorphism on protein expression, a somewhat consistent picture is emerging, showing that carriage of the 9R allele results in lower expression of DAT (although alternative findings have been reported; see van Dyck et al., 2005) . This relationship has been found *in vivo* using PET (Heinz et al., 2000), *ex vivo* in cadaver tissue

(Mill, Asherson, Browes, D'Souza, & Craig, 2002), and in a well controlled *in vitro* model (VanNess, Owens, & Kilts, 2005).

Because the DAT protein is the primary mechanism by which DA is cleared from the striatal synapse, alterations in the expression of the protein would be expected to affect synaptic DA clearance rates and therefore receptor activity. Since the net effect of increased DA innervation of striatum on cortex seems to be excitation (see Carlsson et al., 2003 and also above section on Dopamine and ADHD), it would be expected that decreased expression or function of striatal DAT would result in cortical excitation. This notion has been supported by imaging genetics studies which consistently find increased cortical activity in carriers of the low-activity 9R allele (Bertolino et al., 2006; Bertolino et al., 2009; Schott et al., 2006).

The DAT1 gene is of particular interest in ADHD for several reasons. First, it is located in the 5pl3 region, close to one of only two regions identified in genome-wide studies in at least two independent samples (Hebebrand et al., 2006; Ogdie et al., 2003). Further, *DAT1* knockout mice show hyperactivity and insensitivity to psychostimulants (Giros, Jaber, Jones, Wightman, & Caron, 1996). As well, DAT density has been found to be altered in several studies of ADHD (J. Krause, 2008) with an early small study finding increases of up to 70% binding in striatum (Dougherty et al., 1999).

Effects of the *DAT1* gene variant on behavior have been rather inconsistent. (Rommelse et al., 2008). This has also been the case in terms of specific ADHD subtypes and symptom clusters (J.Krause, 2008). These findings are consistent with the overall picture of the gene in ADHD. It seems that there is good evidence for involvement of *DAT1* in the disorder, and in meta-analysis the association was significant, but the findings were somewhat weak, and both positive and negative findings have been published (Yang et al., 2007). The inconsistency is likely because any

single gene will have a very small effect in a disorder such as ADHD with many factors contributing to the expression of the disorder including interactions with many other risk genes, and pre- and post-natal environment.

A potentially greater confound in association studies is that they seek to link gene variation with a diagnosis that is based purely on a set of behavioral criteria, rather than to a biologically linked phenotype. A diagnosis such as ADHD which shows profound heterogeneity in phenotypic expression (Wahlstedt, Thorell, & Bohlin, 2009), is likely to represent multiple biological subtypes, each potentially associated with different gene combinations interacting in various ways with environmental effects.

1.2.5 ADHD and Psychostimulants

Psychostimulant medications such as methylphenidate amphetamines and mixed salts are the most commonly used in the treatment of ADHD (Vaughan et al., 2012). These drugs are known from animal (Dresel et al.1998) and human (Volkow et al., 2001) studies to primarily target DAT, but also have action at NET and possibly SERT. Orally delivered MPH blocks DAT in the striatum and increases extracellular DA (Volkow et al. 2001). Treatment of ADHD with psychostimulants has often been labeled paradoxical, since these drugs are known to increase motor activity in normal animals (Gainetdinov et al. 1999), yet effectively decrease these behaviors in children with ADHD and animal models of ADHD (Gainetdinov et al. 1999). Regardless, treatment with psychostimulants improves symptoms in most ADHD subjects (Swanson et al. 1993), but some subjects are refractive to treatment, or must discontinue use due to side effects such as insomnia and decreased appetite (Stein et al. 2003). These effects, along with a growing recognition of the role of NET proteins in DA clearance in the PFC (Gresch et al. 1995; Mazei et al. 2002), has led to the development of NET antagonists as nonstimulant pharmacological treatments for ADHD,

atomoxetine (Strattera®) being the first approved agent of this class (Corman et al. 2004). Both atomoxetine and MPH raise extracellular DA and NE levels in the rat PFC, whereas only MPH elevates DA in the striatum (reviewed in (Bymaster et al. 2002), consistent with the high levels of DAT but not NET in the latter region. Aside from regulation of DA terminals in the PFC, ADHD treatments may also modulate the activity of the DA cell bodies in the VTA that project to the PFC. Activity of DA neurons in the VTA can be modulated by DA levels, as well as by NE and NET blockers (Adell and Artigas 2004). Consistent with this idea, Choong and Shen (2004) found that decreased activity of DA VTA neurons in a rat model of fetal alcohol syndrome was normalized by systemic administration of MPH. They observed that increases in extracellular DA, not NE, mediated this response, which could be clinically relevant in ADHD, as MPH treatment has been reported to improve attention deficits in children with fetal alcohol syndrome (Choong and Shen 2004).

1.2.6 Association Studies of hDAT and ADHD

Association of the 7-repeat VNTR in *DRD4* with ADHD has been the most replicated finding, association of the 10-repeat VNTR in *DAT* with ADHD has been the second most replicated finding (reviewed in-(Demarion et al. 2003; Bobb et al. 2005). Some studies have examined whether the 3'VNTR of hDAT is associated/linked with childhood ADHD (Table 1). As shown in Table 1, the results have been mixed, with approximately half of the studies finding a positive linkage/association and the other half finding no evidence for linkage/association. This is not an uncommon occurrence in psychiatric genetics. Potential sources for replication failures include differences between studies in diagnosis criteria, diagnosis type or severity, ethnicity, sex, and study size.

Table 1.1. Results from association studies using the hDAT 3'VNTR and ADHD.

HRR: Haplotype relative risk, TDT: Transmission disequilibrium test

Study	N (Proband)	Ethnicity	Design	Association
Cook et al. 1995	50	82.4% Caucasian	HRR	Yes
Gill et al. 1997	40	Irish	HRR	Yes
Waldman et al. 1998	117	68% Caucasian	TDT	Yes
Daly et al. 1999	103	Irish	HRR	Yes
Palmer et al. 1999	209	80% Caucasians	TDT	No
Holmes et al. 2000	133	UK-Caucasians	Case /control	No
Todd et al. 2001	219	Missouri Twins	TDT	No
Curran et al. 2001	66	UK- Caucasians	TDT	Yes
Roman et al. 2001	81	86% European Brazilian	HRR	No
Barr et al. 2001	102	96% White European	TDT	No/Yes
Chen et al. 2003	110	Taiwanese	HRR	Yes
			TDT	Yes
			TRANSMIT	Yes
Muller Smith et al. 2003	105	94% Caucasians	Case/Control	No
Kustanovich et al. 2004	535	78% Caucasians	TDT	No
Qian et al. 2004	332	Han Chinese	Case/Control	Yes
	188		TDT	No
Bakkar et al, 2005	238	Dutch	TDT	No
Shang et al 2011	273	Chinese	TDT	No

1.2.7 *DAT1* 3'VNTR and Response to Methylphenidate

Since most pharmacological treatments for ADHD target DAT, it is conceivable that a functional polymorphism in DAT could impact treatment efficacy. Recent studies have attempted to determine whether DAT 3'VNTR status specifically plays a role in response to MPH (Table 2). Initially Cook et al. (1995) observed that homozygosity of the 10-repeat DAT 3'VNTR allele was correlated with poor MPH response in 30 African-American children with ADHD (Cook et al., 1995).

This result was replicated in a study of 50 Brazilian boys with ADHD, 30 homozygous for the 10-repeat allele and 20 with other genotypes, as 15/20 (75%) non-homozygotes demonstrated a greater than 50% improvement in basal scores after 30 days of MPH treatment, whereas only 14/30 (47%) 10/10 3'VNTR subjects exhibited a similar positive response, indicating a significant reduction in treatment efficacy in the 10/10 subjects (Roman et al. 2002). A poor response to MPH was also observed in a small study of Korean children with ADHD, in which only 2/7 (29%) of those with the 10/10 3'VNTR genotype had a favorable response after 8 weeks of treatment, whereas all four of the subjects without the 10/10 3'VNTR genotype responded favorably (Cheon et al. 2005).

A somewhat complimentary finding was recently reported, where homozygosity of the less frequent 9-repeat allele was associated with a poor response to methylphenidate (Stein et al. 2005, Joober et al.2007, Stein et al., 2014). Given the differences in findings between these studies, further work is needed, which preferably will utilize larger samples and take diagnostic subtype into account. Again, having neuroimaging data as a correlate to these studies may help to identify the underlying similarities and differences between these studies. For example, a study that reports a response to MPH treatment is associated with the 10/10 genotype may have subjects with a higher DAT binding level than a similarly conducted study that finds the 10/10 genotype does not improve MPH response. Additionally, since the functional relevance of the hDAT 3'VNTR is still unknown, there may be other variants in hDAT that alter its levels as well as the response to treatments such as methylphenidate. Thus, ADHD subjects at the extreme high or low end of DAT binding may be good candidates for the investigation of genetic variation in DAT that could contribute to ADHD.

1.2.8. Side effects of Methylphenidate in ADHD

A side effect also known as adverse effect, adverse event, or undesirable secondary effect is when a treatment goes beyond the desired effects and causes a problem (Barkley et al.1990). This varies for each patient and can depend on the following: general health of the patient, state of disease/disorder, age, weight and gender (Barkley et al.1990; Stein et al. 2003). Side effect rating scale (SERS) is comprised of 17 sides effects commonly associated with MPH treatment. Side effects are been ranked on 0-9 point scale from 0 absent, mild (score=1) to severe (score=9). Scores above 7 are usually considered to be severe. The 17 side effects of methylphenidate are, decreased appetite, insomnia, headaches, talk less, drowsiness, sadness, anxious, prone to crying, nightmares, stomachaches, stares a lot, uninterested, dizziness, irritability, euphoria bites finger, and tics,

(Ritalin side effects.drugs.com). While the common ones among them are, irritability, sadness, prone to crying, anxiety, decreased appetite, stomachaches and insomnia (Barkley et al.1990; Efron et al. 1997).

Table 1.2. Influence of hDAT 3 'VNTR on methylphenidate response in ADHD.

STUDY	**ETHNICITY**	**SAMPLE SIZE**	**STUDY DESIGN**	**RESULT**
Cook et al. 1995	82.4% Caucasian	50	Naturalistic	Significant (10/10)
Winsberg and Comings 1999	African-American	30	Naturalistic	Significant (10/10)
Roma et al., 2002	Brazilian	50	Naturalistic	Significant (10/10)
Kirley et al., 2003	Irish	180	Naturalistic	Significant (9/9)
Stein et al., 2005	89% Caucasian	47	Double blinded placebo controlled	Significant (9/9)
Cheon et al., 2005	Korean	11	Naturalistic	Significant (10/10)
Langley et al., 2005	British Descent	263	Naturalistic	Non-Significant
van der Meulen et al. 2005			Naturalistic	Non-Significant
McGough et al., 2006	59% Whites	81	Double blinded placebo controlled	Non-Significant
Zeni et al., 2005	Brazilian	111	Naturalistic	Non-Significant
Joober et al., 2007	90.7% Caucasian	150	Double blinded placebo controlled	Significant (9/9)
Purper-Ouakil et al., 2008	Meta-Analysis	475	5 Naturalistic out of 6	Significant (10/10)

Tharoor et al., 2008		243	Naturalistic	Non-Significant
McGough et al., 2009	73.2% Whites	82	Double blinded placebo controlled	Non-Significant
Froehlich et al., 2011	79% Caucasian	89	Double blinded placebo controlled	Significant (10/10)

CHAPTER 2

STUDY DESIGN

2.1 Overview

This present thesis is a subsection of a Pharmacogenetic Clinical trial of children (6-12 years) with Attention-Deficit/Hyperactivity Disorder (ADHD). This trial has a registration number NCT00483106 in the ClinicalTrial.gov database. A double-blinded, placebo-controlled, crossover randomized trial of methylphenidate (MPH) conducted at the Douglas Mental Health University Institute, under the supervision of Dr. Ridha Joober and Dr. Natalie Grizenko.

This chapter will be presenting an overview of the methods used in this trial.

2.2 Study design

As soon as the baseline evaluation is concluded, the children received either one week placebo or methylphenidate (0.5mg/kg) in a bid dose, which will then be crossed during the second week. Treatment response to methylphenidate is evaluated by examining the different scores which were been obtained by the children with ADHD in different cognitive, emotional and motor assessments conducted in the lab. This was also followed by assessment using the Conners' scales as evaluated by the parents and home and teachers at school.

Baseline	Week One		Week two	
Washout Period	Day 3	Day 5/7	Day 3	Day 5/7
Conners' Parents Conners' Teachers CBCL Neuropsychological Evaluation	RASS CPT CGI 0.5mg/kg MPH	 Conners'-T Conners'-P	RASS CPT CGI	 Conners'-T Conners'-P

WRAT/WIAT Kinney Medical and Gynecological Questionnaire	In b.i.d dose RASS CPT CGI	SERS-P	0.5mg/kg MPH in b.i.d dose RASS CPT CGI	SERS-P

Table 2.1: Timeline of the two-week double-blind, placebo-controlled crossover trial of MPH

2.3 Participants

Children who participated in the present study were recruited through the Disruptive Behavior Disorders Program (DBDP) and the child psychiatry outpatient clinic at the Douglas Mental Health Institute (DMHUI) in Montreal. They were all between 6-12 years of age. Children with symptoms of ADHD were referred from different sources such as schools, physicians and community care. All the subjects in this study met the DSM-IV criteria for ADHD symptoms which include inattention and/or hyperactivity-impulsiveness either in the home or school settings as determined by clinical interview which was conducted by a child psychiatrist. Written informed consent was provided by the parents and all the children verbally agreed to participate in the trial with MPH. Approval for the study was granted by the Research Ethic Board of Douglas Hospital.

Inclusion Criteria	Exclusion Criteria
✓ Age: 6-12 years ✓ Diagnosis of ADHD based on:	✓ Previous history of mental retardation with an IQ less than or equal to 70 measured by the WISC-III for children.

• Clinical interview of the child and at least one parent. • Structured interview with parents using DISC-IV, parental report. • Evaluation of behavior in different settings: 1) In school by teacher (Conners' Global Index (CGI) –Teacher. 2) At home by parents (CGI-Parents). N.B. at least one CGI score either Parents or Teachers should be 65 or above.	✓ Previous history of autism, Tourette's syndrome, pervasive developmental disorder or psychosis. ✓ Any major medical condition or impairment that would prevent the child to complete testing during the study. ✓ Concurrent treatment with any other psychostimulant medication except methylphenidate (MPH).

Table 2.2: Inclusion and exclusion criteria for study participants

2.4. Evaluation of children's behaviour and response to methylphenidate

After the baseline assessment, the children were randomly assigned to receive either 0.5mg/kg/day MPH or placebo in a divided BID dose for a period of one week after which they were crossed over the second week treatment accordingly. All treatments were prepared in identical gelatin capsules by a pharmacist who was not involved in the research project. Treatment (MPH and placebo) were packaged in individual blisters, which were clearly labeled and given to parents on the first day of the study. Blister packs were returned at the end of the two week study to verify the compliance to treatment. After the 5^{th} and 7^{th} day of each consecutive week a research assistant collects information for the therapeutic response assessments made by the teachers (Conners'-T) and parents (Conners'-P) accordingly.

2.5. Baseline assessment

At the time of the baseline assessment, the participants were off any psychostimulant medication. The baseline assessments include the following: (1) diagnosis of ADHD based on DSM-IV criteria, and its associated comorbid disorders; (2) collection of demographic data; (3) Full scale, verbal and performance IQ were measured by the Wechsler Intelligence Scale for Children-III (WISC-III). (4) Behavioral profiles of children were assessed by a psychiatrist and by research assistants using the Clinical Global Impression for severity (CGI-severity), by parents (CBCL, Conners'-P), and by teachers (Conners'-T).

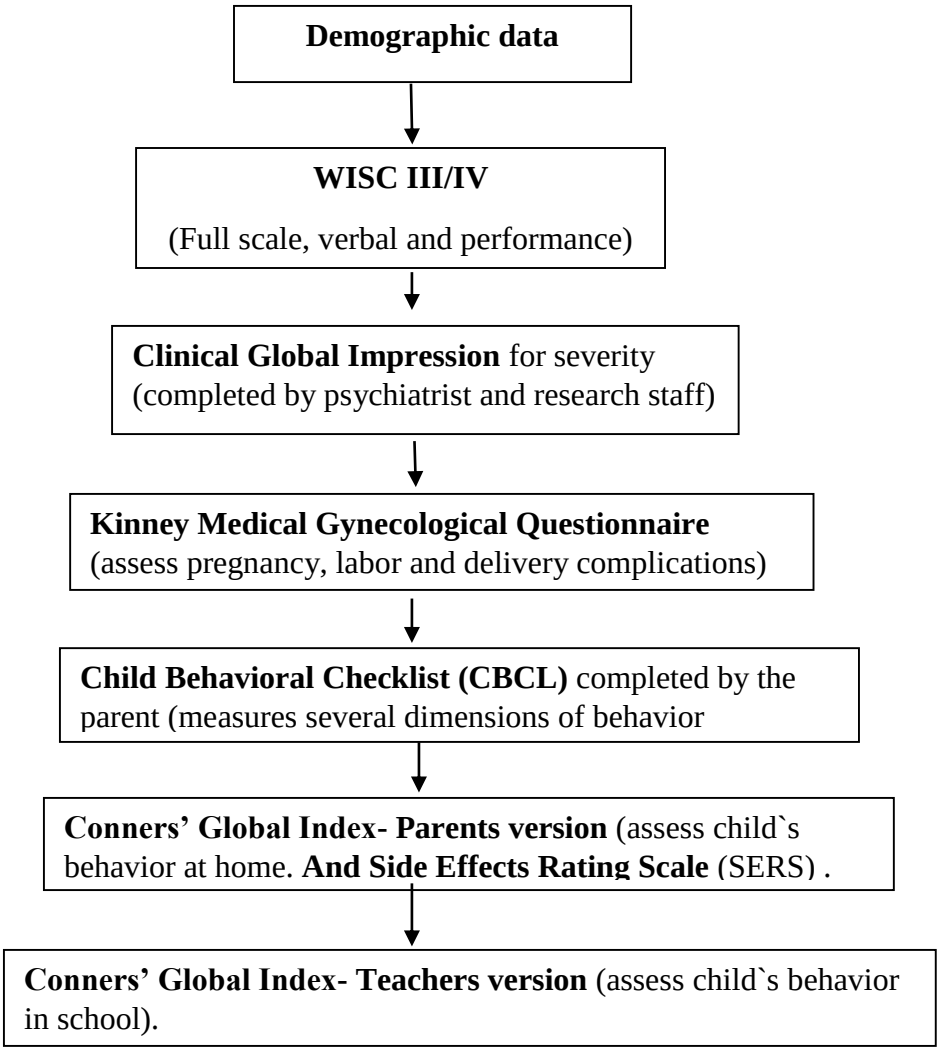

Table 2.3: Outline of baseline assessments conducted for the participants

2.5.1. Conners' Global Index (CGI).

The CGI is a widely used rating scale for assessing symptoms of ADHD and other psychopathology in children between 6-12 years of age. The CGI scale (CGI-Parents and Teachers) is comprised of 10 items representing the Hyperactivity Index of the original Conners' scale. Each of the items describes a behavior that is rated on a 4 point Linkert scale from 0 (not at all true) to 3 (very much true). The CGI parent also comprises of 2 factors which include: 'Emotional liability (EL) and 'Restless- impulsive (RI) behavior. Raw total and factor scores were transformed into normalized T-scores. A score of 65 or higher is considered to be clinically significant.

2.5.2. Child Behavior Checklist (CBCL)

This questionnaire was designed to be completed by parents regarding their child's functioning. It provides nationally norm-referenced T-scores and percentile scores in addition to raw scores for the following scales: aggressive behavior, anxious/depressed, attention problems, rule-breaking behavior, social problems, somatic complaints, thought problems, and withdrawn/depressed, activities competence, social competence, school competence, total competence, internalizing, externalizing, and total problems. In addition, there are six scales designed to reflect the DSM diagnostic categorization: affective problems, anxiety problems, somatic problems, attention deficit/hyperactivity problems, oppositional defiant problems, and conduct problems.

2.5.3. Side Effects Rating Scale (SERS)

SERS is a widely used rating scale to assess how children react to the medication (MPH). It is composed of 17 side effects commonly associated with MPH treatment. Side effects were ranked

on a 9-point scale from mild (score=1) to most severe (score=9). Scores above 7 were considered to be severe.

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Chapter 3

Dopamine transporter Genotypes and response to Methylphenidate Treatment and Side Effects in children with ADHD

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Preface

Variations in the dopamine transporter gene (*SLC6A3*) have been suggested to play a role in the pathogenesis of ADHD. The 3'UTR VNTR is one of the most studied polymorphisms in this gene. However, there have been inconsistencies in the finding, whereby some studies found a significant association between ADHD and the 10-repeat allele of the 3'UTR while some found an association with the 9-repeat allele or no association. Several studies also investigated the relation between genetic variants in this gene and therapeutic response to psychostimulant medications, with, here again, variable results.

This present chapter presents the results of a study exploring the relation between response to and side effects induced by methylphenidate treatment in children with ADHD based on the dopamine transporter genotypes (3'UTR VNTR, rs6347 (exon9), rs8179029 (Int9), rs3836790 (Int8), rs463379 and rs460000). This study is based on an independent and larger (n=310) sample compared to a previous published paper exploring the association between 3'UTR VNTR and response to methylphenidate in children with ADHD (Joober et al., 2007) with a sample size of 150 patients. Results from this study revealed no association between the DAT1 genotypes and ADHD behavioral dimensions treatment response to methylphenidate However, we replicated the association between the DAT1 genotypes and MPH side effects

Abstract

Background: Stimulant medications such as methylphenidate (MPH) are the most frequently used medication for ADHD. MPH acts primarily by blocking the dopamine transporter (DAT). About 70% of patients taking MPH show adequate therapeutic response, but the level of response to MPH vary from one child to the other. Similarly the level of MPH side effect varies from one child to the other and may be the cause of poor therapeutic adherence. Several studies have implicated the 3' untranslated region (UTR) variable number tandem repeat (VNTR) of the dopamine transporter gene (DAT1) in MPH therapeutic response and side effects. The aim of this study is to replicate (or refute) the association of the DAT1 genotypes with response to MPH treatment and side effects.

Methods: 310 Caucasian children with ADHD (6-12 years) were administered placebo and MPH (0.5mg/kg in a divided b.i.d dose), each in a 2-week in a double-blind placebo-controlled crossover trial. Therapeutic response was assessed using the Conner's Global index (CGI) Parents and Teachers, while side effects were assessed using the Barkley Side Effects Rating Scale (SERS).Children were genotyped for the 3'-untranslated region (3'UTR) variable number tandem repeat (VNTR), and the single nucleotide polymorphisms (SNPS) rs6347 (exon9), rs8179029 (Int9), rs3836790 (Int8), rs463379 and rs460000.

Results: No associations of DAT1 genotypes with ADHD behavioral dimensions or treatment response were observed. However we replicated a significant association between DAT1 genotypes and MPH side effects.

Conclusions: Results from this study revealed no association between the DAT1 genotypes response to methylphenidate or behavioral dimension of ADHD. An association with MPH side effects was however observed.

Keywords: ADHD; Dopamine transporter; Methylphenidate; Pharmacogenetics; DAT1 genotypes

1. INTRODUCTION

Although the prevalence of Attention-Deficit/Hyperactivity Disorder (ADHD) may vary depending on the diagnostic criteria used and the population demographics of the sample (Froehlich et al. 2007), it remains one of the most prevalent psychiatric disorders (6-9%) in school-aged children worldwide (Biederman, 2005). ADHD is a major risk factor for educational failure, later antisocial and high-risk behaviors, and other negative outcomes (Biederman et al., 2006; Devevensky & Japel, 2009). This is particularly true if ADHD is undiagnosed (Mannuzza, Klein & Moulton 2008) or untreated (Shaw et al. 2012).

Even though it is very well established that stimulant medications successfully improve symptoms of ADHD (Clinical practice guideline), there are still differences with regards to the dosages which are being administered and how long the effects last for (Greenhill et al.2001; Rapport et al., 1985; Wolraich et al., 2004). This variability maybe, at least in part due to individual factors that need to be identified to improve our capacity to individualize treatment for each child (Lowe et al., 2006). Numerous studies have investigated the role of genetics in predicting treatment response in patients with ADHD (McGough .2005; Lowe et al., 2006. Polanczyk et al., 2005). A large number of pharmacogenetic studies have investigated the role of a variable number tandem repeat (VNTR) polymorphism in the 3`untranslated region (UTR) of the human dopamine transporter gene (DAT1) in the pathogenesis of ADHD (Bellgrove et al., 2005). This is because methylphenidate acts primarily by blocking DAT and, thus increasing the synaptic dopamine concentration of dopamine (Volkow et al., 2002). However, there has been inconsistencies in the findings, whereby some studies implicated the 10-repeat in poor response to MPH (in ADHD susceptibility) (Cook et al., 1995 Winsberg and Comings 1999; Roma et al., 2002; Cheon et al., 2005; Froehlich et al.,

2011) while some studies implicated the 9-repeat allele in poor response to MPH(Kirley et al., 2003; Stein et al., 2005; Joober et al., 2007), while some found little or no association (Faraone & Mick, 2010; Li et al., 2006; Yang et al., 2007; Langley et al., 2005; van der Meulen et al., 2005; McGough et al., 2006; Zeni et al., 2005; Tharoor et al., 2008) (As shown in Table 3.1) .

The inconsistencies in the findings may be due to various factors including that small sample sizes, study design variations (open-label versus randomized controlled trials), outcome measurement differences, sample differences in demographic characteristics, and different dosing regimens . (Froehlich et al., 2010,. Stein et al., 2008. Polanczyk et al., 2008. Kieling et al., 2010). For instance, only four ADHD pharmacogenetic studies in school-age children have used randomized, placebo-controlled, double blinded design (Stein et al., 2005., Joober et al., 2007., McGough et al., 2009., Froehlich et al., 2011).Only one preschool (McGough et al., 2006) and three school-age (Joober et al., 2007., Cheon et al., 2005, Froehlich et al., 2011) trials have used the parent and teacher outcome ratings. Considering the fact that most children spend the period where stimulant blood levels is at the highest level with teachers rather than with parents and the association between genotype and psychostimulant response has shown different outcomes information in several studies (Joober et al., 2007., McGough et al., 2006), the inclusion of parent and teacher ratings is important in ADHD pharmacogenetics (Froehlich et al., 2010).

Even though about 70% of the patients exhibit therapeutic response, a lot of them had to discontinue treatment prematurely (Thiruchelvan et al. 2001; Schachar et al. 2002) due to some side effects caused by MPH (Stein at al. 2003; Barkley et al. 1990). Side effects varies from one patient to another. There are 17 known relatively common sides effects of MPH and the most common ones are decreased appetite, insomnia, stomachaches, anxious, prone to crying and

irritability (Goldman et al. 1998,; Marchei at al. 2010). Insomnia and decreased appetite are known to be dose-related while the remaining side effects appears not to be dose dependent (Tagaya. 2010; Stein et al., 2003; Greenhill et al. 2002). Some studies have reported that decreased appetite, insomnia, headaches and stomachaches increase in frequency and severity during treatment with MPH compared to placebo (Barkley et al. 1990; Pataki et al. 1993; Fine and Johnston. 1993; Fischer and Newby 1991).

In a previous report (Joober et al. 2007), we published the preliminary results of a registered double-blind placebo-controlled cross-over trial (ClinicalTrials.gov NCT00483106) indicating an interaction between the (SLC6A3) 40-bp 3'UTR VNTR polymorphism and treatment response. More specifically, children having the 9/9 genotype were found to be poor responders to MPH as assessed by the parents, Conners' Global Index (But not Teachers' Conners' Global Index), and also had more prominent side effects compared to the 2 other genotypes (9/10 and 10/10). In this present study we used an independent sample with a total of 310 patients compared to the previous published study where we had a sample size of 150. We used the same methodology of evaluation of ADHD and therapeutic response to MPH and re-investigated the role of DAT1 (SLC6A3), in modulating a two weeks therapeutic response to methylphenidate and its side effects. This study is unique compared to previous pharmacogenetic studies by its largest sample and design (doubled-blind placebo-controlled two weeks cross over trial) in school aged children with ADHD.

In addition to the 3'UTR VNTR polymorphism we also explored the role of five other single nucleotide polymorphisms (SNPs) namely: rs6347 (exon9), rs8179029 (Int9), rs3836790 (Int8), rs463379 and rs460000 in modulating short term therapeutic response to MPH in this extended sample of children with ADHD. These additional polymorphisms were selected because they have

been previously associated with increasing risk of ADHD (Brookes et al. 2006, Grunhage et al 2000; Vandenbergh et al 2000). Given these previous findings, it is possible that these SLC6A3 polymorphisms may also be implicated in the variability in response to MPH treatment in children with ADHD.

Thus, the main objective of this study is to replicate (or refute) the association of the DAT1 genotypes with response to MPH treatment and its side effects (0.5 mg/kg/day) in the largest sample using a rigorous methodology (2week double-blind placebo-controlled crossover trial)and multiple evaluators of response to MPH.

2. METHOD

2.1 Participants

The 310 Caucasian children (298 male and 72 female) who participated in the present study were recruited through the Disruptive Behavior Disorders Program (DBDP) and the child psychiatry outpatient clinic at the Douglas Mental Health Institute (DMHUI) in Montreal. They were all between 6-12 years of age. Children with symptoms of ADHD were referred from different sources such as schools, physicians and community services. All the subjects in this study met the DSM-IV criteria for ADHD symptoms which include inattention and/or hyperactivity-impulsiveness determined by clinical interview conducted by a child psychiatrist and completed by structural interview. Children were excluded from this study if their IQ is below 70 (third Edition; Wechsler 1991) or had evidence of Tourette syndrome or psychotic symptoms. Children were also excluded from the study if they were at the same time prescribed any medication other than MPH or had previous history of allergic reactions or tolerance to psychostimulants. Written informed consent was provided by the parents and all the children verbally agreed to participate in the trial with MPH. Approval for the study was granted by the Research Ethic Board of Douglas Hospital.

2.2 Study Design

Baseline assessment were made following a 2 weeks wash out period and 1 week before the start of the trial, while the children were not on any psychostimulant medication. Children who were previously on medication were invited to a washout period of at least one week before completing the baseline evaluation. The children were then randomly assigned to receive either 0.5mg/kg/day MPH or placebo in a divided BID dose for a period of one week after which they were crossed over the second week of treatment. All treatments were prepared in identical gelatin capsules by a pharmacist who was not involved in the research project. Treatment (MPH and placebo) were packaged in individual blisters, which were clearly labeled and given to parents on the first day of the study. Blister packs were returned at the end of the two week study to verify the compliance to treatment. At the end of each week, of treatment research assistant contacted the child's parents and teacher and asked them to fill the CGI-Parents and CGI-Teachers, respectively, taking into consideration the behavior of the child during the entire week of treatment (including weekends for parents).

Parents completed a Child Behavioral Checklist (Achenbach 1991) which assesses several behavioral domains and the Conners' Global Index for parents (CGI-Parents) (Conners' et al., 1998). The CGI parent is a widely used rating scale to assess symptoms of ADHD and other psychopathology in children between 6-12 years of age. The CGI scale is comprised of 10 items representing the Hyperactivity Index of the original Conners' scale. Each of the items describes a behavior that is rated on a 4 point Linkert scale from 0 (not at all true) to 3 (very much true). The CGI parent also comprises of 2 factors which include: 'Emotional liability (EL) and 'Restless-

impulsive (RI) behavior. Raw total and factor scores were transformed into normalized T-scores. A score of 65 or higher is considered to be clinically significant.

The teachers completed the CGI-teacher (Conners' et al., 1998) which is equivalent to CGI-parents and has the same metric characteristics. Parents also completed the side effect Rating Scale (Barkley et al., 1990), which is composed of 17 side effects commonly associated with MPH treatment. Side effects were ranked on a 9-point scale from mild (score=1) to most severe (score=9). Scores above 7 were considered to be severe.

2.3 Molecular Genetics

Blood or saliva samples were collected from each child participating in this study, as well as other available family members, including parents and siblings in order to extract DNA for the purpose of genetic analyses.The 3' UTR VNTR polymorphisms with-in the SLC6A3 gene were genotyped using methods as previously described (Joober et al. 2000; Brookes et al. 2006; Karama et al. 2008). For DAT I9 polymerase chain reaction (PCR) was carried out 20µl volume containing 9.4µl Milli-Q water, 2 µl 10x PCR buffer & 15Mm MgCL2, 4µl 5x Q-Solution, 0.4µl dNTPs (dATP, dCTP, dGTP, dTTP), 1µl primer Forward (10 pmol/µl) 1µl primer Reverse (10 pmol/µl), 0.2µl Taq polymerase (5U/µl), 2µl DNA (100ng/µl). Samples were amplified on a thermal cycler with an initial 94°C for 4 min step to heat activate the enzyme, 35 cycles containing a denaturing step at 94°C for 40s, an annealing step of 58°C for 40s, an extension step of 72°C for 30s and a final extension of 72°C for 10 mins, the Restriction enzyme used was TthIII. For DAT Exon9 the PCR was carried out at same volume as that of Int9 but the samples were amplified using an initial 94°C for 5 mins step to activate the enzyme, 35 cycles containing a denaturing step at 94°C for 30s, an annealing step of 64°C for 30s, an extension step of 72°C for 45s and a final extension of 72°C for 5 mins, the Restriction enzyme used was Dde I. For Int8 the PCR was carried out at a volume of

10µl containing 4.7µl Milli-Q water, 1µl 10x PCR buffer & 15Mm MgCL2, 2µl 5x Q-Solution, 0.2µl ants, 0.5µl primer Forward (10 pomp/µl) 0.5µl primer Reverse (10 pmol/µl), 0.1µl Taq polymerase (5U/µl), 1µl DNA (100ng/µl). Samples were amplified using an initial 94°C for 5 mins step to activate the enzyme, 30 cycles containing a denaturing step at 94°C for 30s, an annealing step of 60°C for 1 min, an extension step of 72°C for 1 min and a final extension of 72°C for 10 mins. Samples were loaded into the wells of the already casted agarose gel. And a minimum voltage was used to run the gel according to the size of the casted gel. Ethidium bromide was used as the fluorescent tag, which made the genotyped samples visible under the UV (ultra violet) light. Alleles were determined by comparison with molecular weight standards and control individuals with previously determined genotypes. Results were double-scored by two people to check for accuracy. SNPs rs463379 and rs460000 were genotyped at Genome Quebec center using Sequenom iPlex Gold technology.

3. STATISTICAL ANALYSIS

The effect of the SLC6A3 genotype on treatment response was tested using repeated measures analysis of variance (ANOVA), where the genotypes were the independent between subject factors and Conners' Global Index Parents (or Conners' Global Index Teachers) were within subject repeated factor(placebo vs. MPH). Main effect and any interactions were regarded as statistically significant when $p<0.008$, this is because we included 6 SNPs and therefore we did a Bonferroni multiple correction test. The same model of analysis was used to test the effects of genotypes on side effects. Significance for the side effects was set at $p=0.005$, accounting for multiple testing (0.05/17). Chi squared analysis or ANOVA was used to test for differences in the demographic and clinical between all genotypes groups in all the polymorphisms (3'UTR VNTR, rs6347 (exon9), rs8179029 (Int9), rs3836790 (Int8), rs463379 and rs460000).

4. RESULTS

Demographic and Clinical characteristics

The percentage of the ADHD subtypes for all the DAT1 genotype is shown in table 3.2.

The DAT1 genotype groups does not differ with regards to demographic and clinical characteristics except for some of the comorbidies, severity of behavioral problems assessed by the Child Behavioral Check List (CBCL) and Conners' Global Index Teachers at baseline as shown in tables 3.3A,3.3C, 3.3E and 3.3F respectively.

Effects of DAT1 polymorphisms on Conners' Global Index Parents

Using the repeated measures analysis of variance (ANOVA), none of the polymorphism revealed neither a significant genotype by treatment two-way interaction nor not significant main effect as shown in table 3.3.

Effects of DAT1 polymorphisms on Conners' Global Index Teachers

Using the repeated measures analysis of variance (ANOVA), none of the polymorphism revealed neither a significant genotype by treatment two-way interaction nor not significant main effect as shown in table 3.3.

Mean severity ratings for each of the 17 side effects for the DAT1 genotypes

The mean severity rating for each of the side effects were analyzed using repeated measures ANOVA analyzing treatment effect and treatment by genotype interaction. As shown in tables 3.4A-F it was observed that the side effects that significantly increased in severity with MPH treatment were anxious, prone to crying decreased appetite (treatment effect) insomnia and decreased appetite (treatment by genotype interaction) at $p< 0.005$. The side effects tic,

uninterested, dizziness, headaches and irritability also had significant changes at $p<0.05$ for treatment effect.

5. DISCUSSION AND CONCLUSION

Results from this study revealed no association between the DAT1 genotypes in the gene by treatment response to methylphenidate and behavioral dimension of children with ADHD. We did not replicate findings from our previous studies showing that children with the homozygous genotype for the less common 9-repeat allele displayed a poor response to MPH (according to the CGI Parent, but we replicated the findings based on the teachers report. The limitation between the previous study, other studies and this study is sample size and study design which this study is bridging some of the limitations.

The finding from this study is similar to other findings (McGough et al., 2009., Langley et al., 2005., Van der Meulen et al,. 2005, Bellgrove et al., 2008, Zeni et al., 2005, Kereszturi et al., 2008). While our previous our previous study (Joober et al., 2007), is similar to with other findings (Stein et al., 2005. Kirley et al., 2003).

In contrast the first ADHD pharmacogenetic study (Cook et al. 1995) and other findings (Cheon et al., 2005, Winsberg et al, 1999. Roman et al., 2002) reported an association between the homozygosity of the 10-repeat allele and poor response to MPH; however these studies were based on a small number of patients. More inconsistent findings appear to be emerging from placebo-controlled, prospective studies of children with ADHD. Nevertheless, most prior trials have depended on open-label or retrospective assessment, in which medication doses were not specified or were lower than those used in the community for optimal benefit (McGough .2005). Since the

effects of methylphenidate on ADHD symptoms often follow a linear dose-curve (Stein MA, et al., 2005) these lower doses might bias against finding significant treatment effects.

Current studies are also limited by the type of outcome measures used, as many studies depend on outcomes such as responders vs. nonresponders, which have limited power to detect effects compared with analyses of quantitative measures (Froehlich et al. 2010). In several cases, study results have differed depending on whether parents or teachers are the behavior-rating informants (McGough et al., 2006, Joober et al., 2007). Variation in sample size, composition and environmental exposure may also contribute to differences in ADHD pharmacogenetic study results. Modest sample sizes have limited statistical power to detect mild or moderate genetic effects. Another discrepancy in the findings is that pharmacogenetic effects may vary in different ethnic and racial groups. However, the promise of ADHD pharmacogenetics is far reaching, and includes the potential to develop individualized medication regimens that improve symptom response, lessen risk of adverse effects and increase long-term tolerability.

The MPH side effects result from this study reports significant association between the DAT1 genotypes and MPH. This is similar to previous studies (Barkley et al., 1990; Stein et al. 2003; Lee et al.2011) whereby insomnia and decreased appetite having the greater prevalence during treatment with MPH compared with placebo. More so, this study also reported significant effects of anxiousness and prone to crying with the children with 9-repeat allele displaying most of the side effects compared to the other 9/10 and 10-repeat. While the AA genotypes of the Ex9 and Int9 displayed more of the side effect compared to the AG and GG genotypes.

Only a few studies have investigated the side effects of MPH on children with ADHD. It will be plausible for further studies with same sample size or higher to investigate the DAT1 genotypes

and MPH side effects to see if the result will be replicated. As this might help clinicians to continue administering MPH to children with ADHD without hesitation.

We found significant effects for and externalization score and two comorbidities (CD and AD). Externalizing behavior problems is characterized by an under control of emotions which include difficulties with interpersonal relationships and rule breaking (Achenbach & Edelbrock, 1978; Hinshaw, 1992). Children diagnosed with ADHD are far more likely to exhibit externalizing problems than those without a diagnosis (Weis 2007) and these symptoms appear to increase the risk for greater severity of ADHD symptoms and higher rates of other negative outcomes (e.g delinquency, substance abuse ,school dys-function, suicidality) (Biederman , Newcorn , Sprich (1991); Becker, Luebbe, Langberg 2012).

CD comorbid with ADHD is a severe, persistent condition that has an earlier age at onset. (Biederman, J., et al., 1996 ; Hinshaw 1994) . Research shows that ADHD and CD represent two complex and distinct entities that are often associated. Children with these conditions without comorbidity present with different core symptoms and perform differently on objective measures for ADHD symptoms. Children with these comorbidities show the poorest outcome with each individual group. (Schachar, and Tannock, 1995). Researchers have attempted to understand the reasons for high comorbidity between ADHD and CD. They suggested several reasons which are as follows: that one disorder is a precursor to another; one disorder is a risk for developmental of the other; the disorders share the same related risk factors or there is a common underlying symptomatic basis for one or more of these behaviors. (Mannuzza, S., et al., 2004, Caron, and Rutter, 1991).

As many as 33% of children with ADHD have comorbid anxiety (MTA Cooperative Group 1999). The natural course of ADHD moves towards an internalization of the symptoms. As a result, the

emergence of anxiety may be a natural extension of ADHD. Individuals with the inattentive presentation have a stronger propensity for anxiety as they typically have internalizing temperaments. This is particularly true in females who may be highly sensitive and have more inattentive symptoms. However, having ADHD also exposes the individual to considerable negative situations and anxiety may be a compensation for environmental insults (i.e. in order to avoid conflict situations due to their impulsiveness, they use anxiety to create excessive internal control). Once anxiety develops, attention can be compromised.

Further research, likely involving multi-site collaborations to obtain larger samples, is clearly necessary before preliminary findings can be applied to contemporary clinical practice. Using the same study design and methodology will also be necessary in order to check for consistencies of result .Nevertheless, the promise of ADHD pharmacogenetics is far reaching, and includes the potential to develop individualized medication regimens that improve symptom response, lessen risk of adverse effects and increase long term tolerability.

Study's strengths and limitations

The outstanding strength of this current pharmacogenetic study is having the largest sample size for the dopamine transporter and therapeutic response in school aged children with ADHD. It also used the CGI-P and CGI-T in assessing the response to methylphenidate, rather than using only parents, which was done in most studies. Considering the fact that most children spend the period where stimulant blood levels is at the highest level while at school with the teachers.. Using a double-blind placebo controlled cross over design is also plausible instead of using the open-label or retrospective assessment, where medication doses are not been specified or were lower than those used in the community. A randomized control trials are the simplest and most powerful research design in which to evaluate the efficacy and effectiveness of intervention. The crossover

design increased precision in evaluation of the therapeutic response as it compares the effects of methylphenidate to placebo. This study also used quantitative measures to analyze the data to avoid the cut off points which has been practiced in previous studies as this could lead to biased results.

The findings for the side effects in this study was based on using a moderate dose of MPH and for a week period.

Table 3.1.Methylphenidate response in ADHD showing different designs, sample & results

STUDY	**ETHNICITY**	**SAMPLE SIZE**	**STUDY DESIGN**	**RESULT**
Cook et al. 1995	82.4% Caucasian	50	Naturalistic	Significant (10/10)
Winsberg and Comings 1999	African-American	30	Naturalistic	Significant (10/10)
Roma et al., 2002	Brazilian	50	Naturalistic	Significant (10/10)
Kirley et al., 2003	Irish	180	Naturalistic	Significant (9/9)
Stein et al., 2005	89% Caucasian	47	Double blinded placebo controlled	Significant (9/9)
Cheon et al., 2005	Korean	11	Naturalistic	Significant (10/10)
Langley et al., 2005	British Descent	263	Naturalistic	Non-Significant
van der Meulen et al., 2005			Naturalistic	Non-Significant
McGough et al., 2006	59% Whites	81	Double blinded placebo controlled	Non-Significant
Zeni et al., 2005	Brazilian	111	Naturalistic	Non-Significant

Joober et al., 2007	90.7% Caucasian	150	Double blinded placebo controlled	Significant (9/9)
Purper-Ouakil et al., 2008	Meta Analysis	475	5 Naturalistic out of 6	Significant (10/10)
Tharoor et al., 2008		243	Naturalistic	Non-Significant
McGough et al., 2009	73.2% Whites	82	Double blinded placebo controlled	Non-Significant
Froehlich et al., 2011	79% Caucasian	89	Double blinded	Significant (10/10)

Table 3.2 ADHD subtypes for the DAT1 genotypes in percentage

	SNPs					
	3'UTR VNTR	rs6347	rs8179029	rs3836790	rs460000	rs463379
Combined	51.9%	52.3%	52.1%	52.5%	51.4%	51.2%
Inattention	40.5%	40.3%	40.1%	40.1%	40.8%	41.0%
Hyperactive	7.8%	7.4%	7.8%	7.4%	7.8%	7.8%

Table 3.3. *DAT1* Genotypes showing Methylphenidate treatments response

SNPs	**Mean (SD)**			**Statistic &p-value Interaction**	**Statistic & p-value Main effect**
3' UTR VNTR	**9/9**	**9/10**	**10/10**		
Parents placebo week	62.17 (13.16)	64.86 (15.30)	61.96(12.86)	$F_{2,241}$=1.36,p=0.26	$F_{2,241}$=0.22,p=0.80
Parents Active medication week	56.22 (11.82)	56.96(12.99)	57.43 (11.82)		
Teachers placebo week	64.76(10.23)	64.82(14.02)	64.11(13.70)	$F_{2,255}$=0.70,p=0.50	$F_{2,255}$=1.30,p=0.27
Teachers Active medication week	55.73(10.80)	53.87(10.21)	55.44(11.82)		
rs6347	**AA**	**AG**	**GG**		
Parents placebo week	61.02(13.30)	64.94(14.04)	63.39(15.97)	$F_{2,248}$=1.31,p=0.27	$F_{2,248}$=0.15,p=0.86
Parents Active medication week	57.09(12.17)	57.08(12.89)	57.26(11.15)		

Teachers placebo week	64.70(13.61)	64.11(13.75)	65.69(13.84)	$F_{2,263}$=0.33,p=0.51	$F_{2,263}$=2.56,p=0.08
Teachers Active medication week	55.17(11.45)	53.42(10.34)	57.97(11.36)		
rs3836790	**2/2**	**2/3**	**3/3**		
Parents placebo week	62.26(13.56)	63.41(14.42)	62.74(13.95)	$F_{2,237}$=1.35,p=0.26	$F_{2,237}$=1.02,p=0.36
Parents Active medication week	53.37(11.91)	56.14(12.75)	58.13(11.82)		
Teachers placebo week	63.50(12.54)	63.87(13.72)	64.86(13.46)	$F_{2,254}$=0.39,p=0.67	$F_{2,254}$=2.29,p=0.16
Teachers Active medication week	55.50(11.44)	53.16(9.62)	55.55(11.92)		
rs8179029	**AA**	**AG**	**GG**		
Parents placebo week	62.48(13.66)	63.31(14.69)	67.55(13.60)	$F_{2,246}$=3.84,p=0.02	$F_{2,246}$=0.76,p=0.47
Parents Active medication week	58.59(12.15)	54.58(12.27)	56.64(11.60)		
Teachers placebo week	64.96(13.15)	63.97(14.13)	62.70(15.05)	$F_{2,263}$=1.12,p=0.33	$F_{2,263}$=1.90,p=0.15
Teachers Active medication week	55.76(11.57)	52.57(9.48)	54.90(12.55)		
rs463379	**CC**	**CG**	**GG**		
Parents placebo week	63.95(13.40)	61.20(14.77)	60.60(13.71)	$F_{2,232}$=0.14,p=0.87	$F_{2,232}$=0.34,p=0.72
Parents Active medication week	57.69(12.94)	56.20(11.30)	53.40(10.64)		
Teachers placebo week	65.76(13.57)	63.11(13.62)	60.36(17.06)	$F_{2,247}$=0.08,p=0.92	$F_{2,247}$=0.12,p=0.89
Teachers Active medication week	55.24(10.93)	53.73(11.32)	53.09(13.69)		
rs460000	**AA**	**AC**	**CC**		
Parents placebo week	60.60(13.71)	61.20(14.77)	64.63(13.62)	$F_{2,247}$=0.08,p=0.92	$F_{2,247}$=0.12,p=0.89
Parents Active medication week	53.40(10.64)	56.20(14.77)	57.77(12.92)		
Teachers placebo week	60.36(17.06)	63.11(13.62)	65.63(13.62)	$F_{2,248}$=0.12,p=0.89	$F_{2,248}$=0.08,p=0.92
Teachers Active medication week	53.09(13.69)	53.73(11.32)	55.18(10.92)		

Table3.3 A. Baseline characteristics of children with ADHD separated by their Genotype in the 3'UTR polymorphisms of the SLC6A3 gene.

	9/9 genotype(n=27)	9/10 genotype (n=126)	10/10 genotype(n=148)	Statistic and p-value
Males/Females(%Males)	24:34(88.9)	98:28(77.8)	108:40(73.0)	X^2=3.35,df=2,p=0.18
Age(years)	8.6(1.56)	8.94(1.84)	8.98(1.77)	$F_{2,300}$=0.51,p=0.59
Household income(%≤30,000$ per year)	14.8%	32.2%	31.9%	X^2=3.43,df=2,p=0.18
WISQ-III full scale IQ	93.88(13.37)	96.30(12.73)	95.49(12.28)	$F_{2,283}$=0.42,p=0.66
CGI Parent at baseline				
RI score	71.83(11.42)	73.79(11.70)	73.52(10.40)	$F_{2,267}$=0.31,p=0.73
EL score	63.25(13.44)	64.40(13.66)	62.97(13.59)	$F_{2,267}$=0.34,p=0.71
Total score	70.83(11.98)	72.51(12.09)	72.05(10.57)	$F_{2,267}$=0.22,p=0.80
CGI Teachers at baseline				
RI score	68.29(10.74)	69.78(11.44)	67.71(10.81)	$F_{2,284}$=1.14,p=0.32
EL score	60.42(13.24)	64.90(16.83)	63.06(16.29)	$F_{2,284}$=0.92,p=0.40
Total score	67.46(10.89)	70.10(12.98)	68.34(12.68)	$F_{2,284}$=0.84,p=0.44
CBCL				
Total score	69.33(8.98)	68.08(8.51)	68.13(7.44)	$F_{2,298}$=0.29,p=0.75
Attention score	71.63(11.17)	69.49(8.16)	71.54(9.28)	$F_{2,298}$=1.92,p=0.15
Externalization score	67.22(10.87)	67.52(10.36)	66.44(9.18)	$F_{2,298}$=0.42,p=0.66
Internalization score	67.56(8.99)	62.87(9.06)	63.94(9.89)	$F_{2,298}$=2.73,p=0.07
ADHD subtypes C/I/H	16/11/0	61/52/13	79/59/10	X^2=4.07,df=4,p=0.39
Comorbidity(%) with				
CD	22.1	13.5	3.4	X^2=14.18,df=2,p=**0.001**
ODD	34.6	50.8	47.6	X^2=2.27,df=2,p=0.32
AD	8.0	6.4	4.9	X^2=0.49,df=2,p=0.78
MD	12.0	4.8	2.1	X^2=5.54,df=2,p=0.06
Previously Medicated %	33.3	31.7	30	X^2=0.12,df=2,p=0.94

Table3.3 B. Baseline characteristics of children with ADHD separated by their Genotype in the rs3836790 polymorphisms of the SLC6A3 gene.

	2/2 genotype(n=20)	2/3 genotype (n=111)	3/3 genotype(n=165)	Statistic and p-value
Males/Females(%Males)	17:3(81.6)	88:23(79.3)	123:42(74.5)	X^2=1.06,df=2,p=0.59
Age(years)	8.62(1.65)	8.99(1.83)	8.97(1.75)	$F_{2,296}$=0.40,p=0.67
Household income(%≤30,000$ per year)	20.0%	35.6%	26.6%	X^2=3.37,df=2,p=0.19
WISQ-III full scale IQ	92.95(15.55)	96.48(12.47)	95.29(12.61)	$F_{2,277}$=0.74,p=0.48

CGI Parent at baseline				
RI score	73.95(12.16)	73.17(11.49)	73.52(10.69)	$F_{2,263}$=0.05,p=0.95
EL score	64.32(14.08)	63.65(13.73)	63.38(13.29)	$F_{2,263}$=0.04,p=0.96
Total score	72.79(12.72)	71.84(12.69)	72.14(10.80)	$F_{2,263}$=0.06,p=0.94
CGI Teachers at baseline				
RI score	66.42(11.51)	69.32(10.77)	68.53(11.29)	$F_{2,280}$=0.59,p=0.56
EL score	59.53(11.57)	64.10(16.25)	63.33(16.34)	$F_{2,280}$=0.66,p=0.52
Total score	65.68(11.21)	69.58(12.23)	68.94(12.94)	$F_{2,280}$=0.77,p=0.46
CBCL				
Total score	69.19(9.40)	67.59(8.90)	68.05(7.56)	$F_{2,295}$=0.36,p=0.70
Attention score	74.33(11.33)	69.73(8.40)	70.49(9.06)	$F_{2,295}$=2.30,p=0.10
Externalization score	66.90(11.46)	66.81(10.55)	66.73(9.22)	$F_{2,295}$=0.01,p=0.99
Internalization score	65.90(11.38)	62.66(9.64)	63.77(9.37)	$F_{2,295}$=1.14,p=0.32
ADHD subtypes C/I/H	11/8/2	64/41/8	83/70/12	X^2=1.05,df=4,p=0.90
Comorbidity(%) with				
CD	14.3	11.8	7.4	X^2=2.06,df=2,p=0.36
ODD	47.6	45.5	48.8	X^2=0.28,df=2,p=0.87
AD	10.5	4.6	5.6	X^2=1.09,df=2,p=0.58
MD	10.5	2.8	3.8	X^2=2.64,df=2,p=0.27
Previously Medicated %	23.3	32.4	30.9	X^2=0.61,df=2,p=0.74

Table3.3 C. Baseline characteristics of children with ADHD separated by their Genotype in the rs8179029 polymorphisms of the SLC6A3 gene.

	AA genotype(n=198)	**AG genotype (n=99)**	**GG genotype(n=12)**	**Statistic and p-value**
Males/Females(%Males)	146:52(73.7)	81:18(81.8)	9:3(75.0)	X^2=2.40,df=2,p=0.30
Age(years)	8.98(1.78)	8.93(1.76)	8.84(1.76)	$F_{2,308}$=0.04,p=0.96
Household income(%≤30,000$ per year)	29.5%	34.8%	16.7%	X^2=1.97,df=2,p=0.37
WISQ-III full scale IQ	95.13(12.21)	96.95(12.20)	91.50(16.95)	$F_{2,290}$=1.17,p=0.31
CGI Parent at baseline				
RI score	73.60(10.70)	72.89(11.51)	75.09(13.10)	$F_{2,273}$=0.25,p=0.78
EL score	63.68(13.22)	63.78(13.75)	64.36(17.01)	$F_{2,273}$=0.01,p=0.99
Total score	71.08(10.82)	72.96(11.93)	64.36(12.75)	$F_{2,274}$=0.81,p=0.45
CGI Teachers at baseline				
RI score	68.79(10.78)	68.66(11.24)	68.82(13.53)	$F_{2,291}$=0.01,p=0.96

EL score	63.57(15.96)	64.71(16.55)	56.64(15.48)	$F_{2,291}$=1.24,p=0.29
Total score	69.21(12.33)	69.23(12.81)	66.64(14.19)	$F_{2,291}$=0.22,p=0.80
CBCL				
Total score	68.01(7.86)	68.36(8.29)	71.83(9.67)	$F_{2,306}$=1.28,p=0.28
Attention score	70.71(9.02)	69.68(7.08)	78.50(12.73)	$F_{2,306}$=5.37,p=**0.005**
Externalization score	66.91(9.38)	66.90(10.93)	70.17(9.24)	$F_{2,306}$=0.63,p=0.54
Internalization score	63.43(9.74)	63.89(8.90)	69.42(10.76)	$F_{2,306}$=2.23,p=0.11
ADHD subtypes C/I/H	102/80/16	52/40/7	7/4/1	X^2=0.34,df=4,p=0.99
Comorbidity(%) with				
CD	7.2	15.3	8.3	X^2=4.88,df=2,p=0.09
ODD	47.2	44.9	66.7	X^2=2.20,df=2,p=0.36
AD	4.7	5.2	27.3	X^2=9.99,df=2,p=**0.007**
MD	3.6	5.2	9.1	X^2=0.99,df=2,p=0.61
Previously Medicated %	29.7	33.3	33.3	X^2=0.42,df=2,p=0.81

Table3.3 D. Baseline characteristics of children with ADHD separated by their Genotype in the rs6347 polymorphisms of the SLC6A3 gene.

	AA genotype(n=156)	**AG genotype (n=126)**	**GG genotype(n=36)**	**Statistic and p-value**
Males/Females(%Males)	111:37(75)	99:27(78.6)	28:8(77.8)	X^2=0.51,df=2,p=0.76
Age(years)	9.05(1.71)	9.01(1.86)	8.43(1.65)	$F_{2,309}$=1.86,p=0.16
Household income(%≤30,000$ per year)	31.7%	29.2%	30.3%	X^2=0.19,df=2,p=0.91
WISQ-III full scale IQ	94.94(13.37)	96.08(12.73)	95.57(12.28)	$F_{2,291}$=0.26,p=0.77
CGI Parent at baseline				
RI score	72.53(10.53)	74.15(11.38)	72.83(11.94)	$F_{2,274}$=0.65,p=0.52
EL score	62.72(13.57)	64.61(12.95)	63.44(15.70)	$F_{2,274}$=0.58,p=0.56
Total score	71.08(10.82)	72.96(11.93)	71.78(12.75)	$F_{2,274}$=0.81,p=0.45
CGI Teachers at baseline				
RI score	68.25(11.15)	68.71(11.10)	70.50(10.86)	$F_{2,292}$=0.56,p=0.57
EL score	63.63(16.05)	64.87(16.61)	60.50(15.03)	$F_{2,292}$=0.98,p=0.40
Total score	68.88(12.81)	69.38(12.66)	69.09(11.33)	$F_{2,292}$=0.05,p=0.95
CBCL				
Total score	67.60(8.08)	68.51(8.14)	68.86(8.78)	$F_{2,307}$=0.58,p=0.56
Attention score	70.03(8.48)	70.68(9.05)	71.94(10.81)	$F_{2,307}$=0.69,p=0.50
Externalization score	66.07(9.86)	67.52(10.13)	68.33(9.46)	$F_{2,307}$=1.14,p=0.32
Internalization score	63.58(9.87)	63.52(9.28)	64.61(9.95)	$F_{2,307}$=0.19,p=0.82
ADHD subtypes C/I/H	75/62/11	67/50/9	20/13/3	X^2=0.48,df=4,p=0.96
Comorbidity(%) with				
CD	7.5	12.5	8.6	X^2=2.18,df=2,p=0.34

ODD	42.5	50.4	54.3	X^2=2.53,df=2,p=0.28
AD	4.9	6.5	5.9	X^2=0.34,df=2,p=0.84
MD	3.5	4.5	5.9	X^2=0.56,df=2,p=0.76
Previously Medicated %	30.3	33.3	30.6	X^2=0.29,df=2,p=0.86

Table3.3 E. Baseline characteristics of children with ADHD separated by their Genotype in the rs460000 polymorphisms of the SLC6A3 gene.

	AA genotype(n=13)	**AC genotype (n=105)**	**CC genotype(n=176)**	**Statistic and p-value**
Males/Females(%Males)	6:7(46.2)	82:23(78.1)	136:40(77.30)	X^2=6.78,df=2,p=0.03
Age(years)	9.06(1.77)	9.17(1.88)	8.89(1.74)	$F_{2,293}$=0.78,p=0.46
Household income(%≤30,000$ per year)	33.3%	31.7%	28.6%	X^2=0.36,df=2,p=0.83
WISQ-III full scale IQ	96.62(14.87)	93.09(11.89)	96.74(12.74)	$F_{2,276}$=2.65,p=0.07
CGI Parent at baseline				
RI score	71.36(10.34)	71.74(11.54)	74.63(10.51)	$F_{2,258}$=2.27,p=0.11
EL score	59.64(12.06)	63.81(14.54)	64.28(13.06)	$F_{2,258}$=0.60,p=0.55
Total score	69.27(11.36)	71.00(12.37)	73.16(10.47)	$F_{2,258}$=1.47,p=0.23
CGI Teachers at baseline				
RI score	66.75(15.26)	67.35(10.82)	69.65(11.07)	$F_{2,276}$=1.49,p=0.23
EL score	56.75(16.99)	60.67(15.31)	66.26(16.43)	$F_{2,276}$=4.96,p=**0.008**
Total score	64.25(15.52)	66.96(12.08)	70.77(12.67)	$F_{2,276}$=3.78,p=0.02
CBCL				
Total score	63.15(9.49)	67.13(8.53)	69.38(7.51)	$F_{2,292}$=5.45,p=**0.005**
Attention score	67.00(11.02)	70.61(9.55)	71.20(8.5)	$F_{2,292}$=1.36,p=0.26
Externalization score	62.31(9.30)	65.40(9.50)	68.52(9.81)	$F_{2,292}$=5.07,p=**0.007**
Internalization score	60.31(12.24)	62.56(9.81)	64.83(9.06)	$F_{2,292}$=2.81,p=0.06
ADHD subtypes C/I/H	7/5/1	49/48/8	95/67/14	X^2=1.67,df=4,p=0.80
Comorbidity(%) with				
CD	0	5.8	13.1	X^2=5.25,df=2,p=0.07
ODD	50.0	47.6	47.4	X^2=0.03,df=2,p=0.99
AD	0	6.9	5.8	X^2=0.90,df=2,p=0.63
MD	8.3	3.0	8.3	X^2=1.17,df=2,p=0.56
Previously Medicated %	41.7	26.9	33.1	X^2=1.79,df=2,p=0.41

Table3.3 F. Baseline characteristics of children with ADHD separated by their Genotype in the rs463379 polymorphisms of the SLC6A3 gene.

	CC genotype(n=175)	**CG genotype (n=105)**	**GG genotype(n=13)**	**Statistic and p-value**
Males/Females(%Males)	136:39(77.7)	82:23(78.1)	6:7(46.2)	X^2=6.94,df=2,p=0.03
Age(years)	8.90(1.75)	9.17(1.88)	9.06(1.77)	$F_{2,292}$=0.75,p=0.47
Household income(%≤30,000$ per year)	29.6%	31.7%	33.3%	X^2=0.36,df=2,p=0.83
WISQ-III full scale IQ	96.63(12.69)	93.09(11.89)	96.62(14.86)	$F_{2,275}$=2.50,p=0.08
CGI Parent at baseline				
RI score	74.62(10.54)	71.74(11.54)	71.36(10.37)	$F_{2,257}$=2.25,p=0.11
EL score	64.19(13.05)	63.81(14.54)	59.64(12.07)	$F_{2,257}$=0.58,p=0.56
Total score	73.12(10.49)	71.00(12.37)	69.27(11.36)	$F_{2,257}$=1.41,p=0.24
CGI Teachers at baseline				
RI score	69.77(10.99)	67.35(10.82)	66.75(15.26)	$F_{2,275}$=1.67,p=0.19
EL score	66.39(16.39)	60.37(15.32)	56.75(16.70)	$F_{2,275}$=5.16,p=**0.006**
Total score	70.91(12.58)	66.96(12.08)	64.25(15.22)	$F_{2,275}$=4.04,p=0.02
CBCL				
Total score	69.34(7.50)	67.13(8.53)	63.15(9.49)	$F_{2,291}$=5.31,p=**0.005**
Attention score	71.17(8.52)	70.61(9.55)	67.00(11.02)	$F_{2,291}$=1.33,p=0.27
Externalization score	68.50(9.84)	65.40(9.50)	62.31(9.30)	$F_{2,291}$=5.01,p=**0.007**
Internalization score	64.75(9.02)	62.56(9.81)	60.31(12.24)	$F_{2,291}$=2.66,p=0.07
ADHD subtypes C/I/H	94/67/14	49/48/8	7/5/1	X^2=1.57,df=4,p=0.81
Comorbidity(%) with				
CD	13.2	5.8	0	X^2=5.31,df=2,p=**0.007**
ODD	47.1	47.6	50.0	X^2=0.04,df=2,p=0.98
AD	5.9	6.9	0	X^2=0.91,df=2,p=0.64
MD	5.3	3.0	8.3	X^2=1.17,df=2,p=0.56
Previously Medicated %	33.3	26.9	41.7	X^2=1.85,df=2,p=0.40

Note: Income was grouped into 2 categories: (1) Low < $30,000 CAD and (2) High > $30,000 CAD. WISC-full scale IQ = Wechsler Intelligence Scale for Children–III; Child Behavioral Checklist. CGI-P=Conners' Global Index Parents; CGI T= Conners' Global Index Teachers. 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.

* ODD = oppositional defiant disorder; CD = conduct disorder; AD= anxiety disorder; MD= mood disorder

* Anxiety disorder means having at least one of these disorders: social phobia, separation anxiety disorder, specific phobia, panic disorder, agoraphobia, generalized anxiety disorder, and selective mutism, post-traumatic stress disorder.

* Mood disorder means having at least one of these disorders: major depressive episode, dysthymic disorder, manic episode, and hypomanic episode.

Table 3.4 A. Mean severity rating for each side effects. 3'UTR VNTR

Side effects	Placebo mean±(SD)	MPH mean±(SD)	Treatment effect	P-value	Treatment by genotype interaction	P-value
Decreased Appetite	0.91±1.96	2.62±3.09	F=1.09	0.34	F=1.18	0.31
Insomnia	1.23±2.33	2.59±3.05	F=0.60	0.55	F=8.62	**0.001***
Headaches	0.66±1.78	1.24±2.28	F=1.79	0.17	F=0.26	0.77
Talk less	0.57±1.63	1.00±2.05	F=0.79	0.46	F=0.12	0.89
Drowsiness	0.36±1.33	0.46±1.38	F=0.24	0.79	F=1.03	0.36
Sadness	1.02±1.95	1.28±2.35	F=0.38	0.68	F=0.78	0.46
Anxious	1.49±2.23	1.44±2.27	F=5.69	**0.001***	F=1.48	0.25
Prone to crying	1.21±2.27	1.47±2.29	F=5.67	**0.002***	F=0.16	0.85
Nightmares	0.47±1.35	0.47±1.36	F=0.49	0.61	F=1.92	0.15
Stomachaches	0.75±1.88	1.09±2.16	F=2.96	0.74	F=0.31	0.75
Stares a lot	1.11±2.05	1.07±1.95	F=0.83	0.92	F=0.86	0.42
Uninterested	0.61±1.63	0.64±1.67	F=0.74	0.47	F=0.86	0.42
Bites fingernails	0.75±2.03	0.88±2.25	F=0.51	0.60	F=0.31	0.73
Dizziness	0.19±0.78	0.21±0.88	F=0.44	0.65	F=0.37	0.69
Irritable	2.49±2.94	2.45±2.83	F=0.54	0.59	F=0.22	0.80
Tics	0.60±1.67	0.71±1.87	F=4.01	0.02	F=0.16	0.86
Euphoria	1.18±2.23	1.10±2.11	F=0.18	0.84	F=0.01	0.99

Table 3.4 B. Mean severity rating for each side effects. Ex9

Side effects	Placebo mean±(SD)	MPH mean±(SD)	Treatment effect	P-value	Treatment by genotype interaction	P-value
Decreased Appetite	0.94±2.01	2.70±3.09	F=7.86	**0.002***	F=6.72	**0.003***
Insomnia	1.18±2.30	2.50±3.02	F=1.69	0.19	F=0.87	0.42
Headaches	0.66±1.77	1.29±2.32	F=0.14	0.87	F=0.96	0.39

Talk less	0.57±1.62	1.01±2.07	F=1.91	0.15	F=0.12	0.35
Drowsiness	0.34±1.29	0.44±1.36	F=1.11	0.33	F=0.85	0.43
Sadness	1.04±1.99	1.29±2.35	F=0.98	0.38	F=1.50	0.23
Anxious	1.47±2.32	1.50±2.33	F=0.95	0.77	F=0.91	0.40
Prone to crying	1.27±2.33	1.45±2.28	F=1.57	0.21	F=1.09	0.85
Nightmares	0.45±1.33	0.44±1.44	F=1.25	0.29	F=0.35	0.71
Stomachaches	0.73±1.86	1.07±2.13	F=0.88	0.42	F=0.08	0.92
Stares a lot	1.07±2.02	1.06±1.97	F=0.03	0.97	F=0.36	0.70
Uninterested	0.62±1.65	0.66±1.71	F=0.53	0.59	F=0.06	0.94
Bites fingernails	0.76±2.06	0.88±2.24	F=0.43	0.65	F=1.36	0.26
Dizziness	0.17±0.77	0.20±0.87	F=0.66	0.52	F=1.28	0.28
Irritable	2.47±2.95	2.46±2.82	F=0.26	0.23	F=0.23	0.79
Tics	0.62±1.73	0.66±1.79	F=1.45	0.32	F=2.29	0.10
Euphoria	1.21±2.25	1.11±2.15	F=0.55	0.58	F=0.67	0.52

Table 3.4 C. Mean severity rating for each side effects. Int 9

Side effects	**Placebo mean±(SD)**	**MPH mean±(SD)**	**Treatment effect**	**P-value**	**Treatment by genotype interaction**	**P-value**
Decreased Appetite	1.00±2.03	2.66±3.02	F=0.05	0.95	F=0.79	0.46
Insomnia	1.27±2.35	2.62±3.06	F=1.55	0.21	F=0.28	0.76
Headaches	0.67±1.77	1.28±2.27	F=2.62	0.08	F=3.09	0.04
Talk less	0.54±1.50	0.92±1.97	F=1.96	0.14	F=0.69	0.50
Drowsiness	0.34±1.25	0.37±1.23	F=2.04	0.13	F=3.23	0.04
Sadness	1.02±1.94	1.21±2.45	F=0.23	0.79	F=0.09	0.92
Anxious	2.67±2.90	1.47±2.30	F=6.59	**0.002***	F=3.64	0.03
Prone to crying	1.24±2.31	1.37±2.17	F=1.66	0.19	F=0.48	0.62
Nightmares	0.46±1.35	0.49±1.50	F=2.16	0.12	F=2.53	0.08
Stomachaches	0.77±1.91	1.18±2.17	F=0.56	0.57	F=0.26	0.77
Stares a lot	1.11±2.06	0.99±1.94	F=1.86	0.16	F=1.02	0.36
Uninterested	0.67±1.74	0.69±1.76	F=3.75	0.02	F=1.03	0.36
Bites fingernails	0.80±2.12	0.82±2.19	F=2.19	0.11	F=0.44	0.65
Dizziness	0.19±0.79	0.19±0.77	F=0.97	0.38	F=0.03	0.97
Irritable	2.46±2.97	2.44±2.84	F=0.84	0.43	F=0.80	0.45
Tics	0.69±1.84	0.58±1.65	F=3.36	0.03	F=1.70	0.18
Euphoria	1.16±2.20	1.26±2.21	F=0.59	0.56	F=0.27	0.76

Table 3.4 D. Mean severity rating for each side effects. Int 8

Side effects	**Placebo mean±(SD)**	**MPH mean±(SD)**	**Treatment effect**	**P-value**	**Treatment by genotype interaction**	**P-value**
Decreased Appetite	1.00±2.02	2.67±3.03	F=0.44	0.64	F=0.37	0.69
Insomnia	1.27±2.53	2.63±3.06	F=1.11	0.90	F=2.44	0.09

Headaches	0.70±1.80	1.32±2.30	F=0.53	0.59	F=2.96	0.05
Talk less	0.54±1.52	0.88±1.93	F=0.15	0.86	F=0.30	0.74
Drowsiness	0.34±1.27	0.38±1.25	F=0.83	0.44	F=3.18	0.04
Sadness	1.02±1.97	1.19±2.25	F=0.47	0.62	F=2.85	0.05
Anxious	1.48±2.32	1.51±2.34	F=2.62	0.08	F=2.06	0.13
Prone to crying	1.25±2.33	1.34±2.16	F=0.73	0.48	F=2.00	0.14
Nightmares	0.44±1.33	0.47±1.48	F=0.71	0.49	F=0.28	0.76
Stomachaches	0.79±1.92	1.20±2.20	F=0.84	0.43	F=0.46	0.96
Stares a lot	1.07±2.05	0.92±1.88	F=1.50	0.23	F=0.85	0.43
Uninterested	0.66±1.74	0.65±1.69	F=1.83	0.16	F=4.46	0.01
Bites fingernails	0.83±2.14	0.86±2.23	F=2.25	0.78	F=1.63	0.20
Dizziness	0.19±0.80	0.20±0.78	F=0.57	0.57	F=0.80	0.42
Irritable	2.46±2.99	2.43±2.82	F=1.61	0.20	F=1.74	0.17
Tics	0.58±1.63	0.69±1.84	F=0.84	0.43	F=1.87	0.16
Euphoria	1.16±2.19	1.15±2.22	F=0.51	0.60	F=0.96	0.38

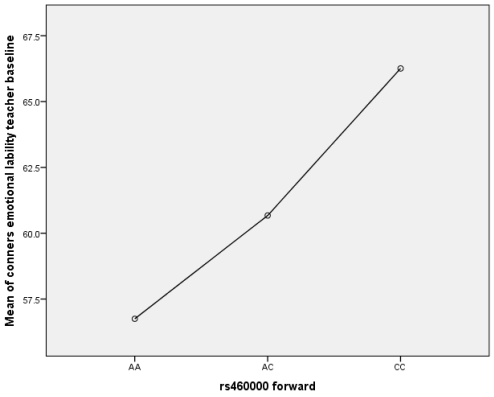
Table 3.4 E. Mean severity rating for each side effects. rs460000

Side effects	**Placebo mean±(SD)**	**MPH mean±(SD)**	**Treatment effect**	**P-value**	**Treatment by genotype interaction**	**P-value**
Decreased Appetite	0.96±2.04	2.72±3.10	F=0.49	0.62	F=0.46	0.63
Insomnia	1.14±2.29	2.47±3.04	F=0.92	0.40	F=0.33	0.72
Headaches	0.69±1.80	1.33±2.35	F=0.19	0.83	F=1.71	0.18
Talk less	0.58±1.66	1.01±2.09	F=1.19	0.15	F=0.33	0.72
Drowsiness	0.36±1.32	0.42±1.31	F=0.86	0.42	F=0.67	0.53
Sadness	1.05±2.02	1.29±2.36	F=0.40	0.67	F=1.17	0.31
Anxious	1.48±2.32	1.49±2.34	F=1.35	0.26	F=0.97	0.38
Prone to crying	1.27±2.34	1.45±2.29	F=0.06	0.94	F=0.12	0.89
Nightmares	0.46±1.35	0.45±1.46	F=2.24	0.11	F=0.04	0.96
Stomachaches	0.73±1.87	1.09±2.17	F=0.96	0.38	F=0.09	0.91
Stares a lot	1.08±2.03	1.06±1.98	F=1.94	0.15	F=0.33	0.72
Uninterested	0.61±1.64	0.65±1.72	F=1.75	0.17	F=2.54	0.08
Bites fingernails	0.77±2.09	0.86±2.22	F=0.82	0.44	F=0.17	0.85
Dizziness	0.18±0.78	0.20±0.87	F=6.28	0.006	F=1.47	0.23
Irritable	2.42±2.95	2.42±2.84	F=4.06	0.01	F=0.21	0.81
Tics	0.64±1.77	0.67±1.81	F=0.30	0.74	F=0.09	0.91
Euphoria	1.23±2.28	1.12±2.17	F=0.44	0.65	F=0.07	0.94

Table 3.4 F. Mean severity rating for each side effects. rs463379

Side effects	**Placebo mean±(SD)**	**MPH mean±(SD)**	**Treatment effect**	**P-value**	**Treatment by genotype interaction**	**P-value**
Decreased Appetite	1.03±2.06	2.69±3.03	F=0.17	0.85	F=1.02	0.36

Insomnia	1.20±2.33	2.55±3.06	F=1.04	0.35	F=0.56	0.57
Headaches	0.70±1.81	1.30±2.28	F=0.19	0.82	F=1.75	0.18
Talk less	0.55±1.53	0.95±2.01	F=0.29	0.75	F=1.30	0.28
Drowsiness	0.34±1.27	0.33±1.15	F=0.12	0.90	F=0.35	0.71
Sadness	1.01±1.94	1.19±2.24	F=0.99	0.37	F=1.58	0.21
Anxious	1.45±2.29	1.49±2.34	F=1.73	0.17	F=1.51	0.22
Prone to crying	1.22±2.31	1.30±2.14	F=0.58	0.56	F=0.12	0.90
Nightmares	0.46±1.35	0.49±1.51	F=1.84	0.16	F=0.05	0.96
Stomachaches	0.77±1.91	1.19±2.21	F=0.22	0.80	F=0.001	0.99
Stares a lot	1.07±2.03	0.96±1.92	F=0.31	0.74	F=0.02	0.98
Uninterested	0.67±1.73	0.71±1.79	F=1.98	0.14	F=1.22	0.30
Bites fingernails	0.80±2.15	0.79±2.15	F=1.80	0.17	F=0.19	0.82
Dizziness	0.18±0.80	0.16±0.70	F=2.39	0.09	F=1.79	0.17
Irritable	2.40±2.95	2.40±2.82	F=3.82	0.02	F=0.52	0.60
Tics	0.60±1.69	0.67±1.83	F=0.09	0.91	F=0.07	0.93
Euphoria	1.21±2.23	1.17±2.24	F=0.23	0.98	F=0.91	0.41



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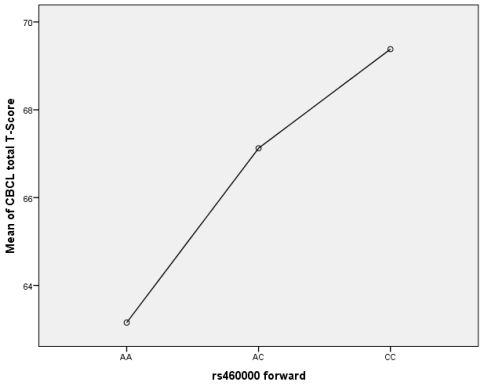

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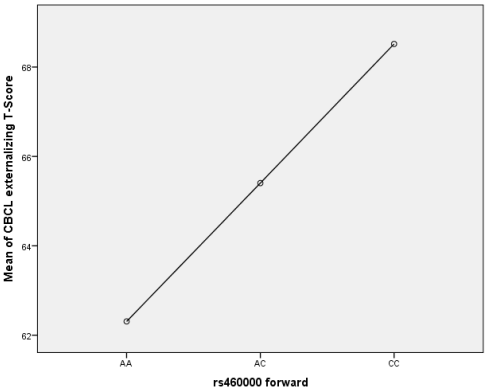

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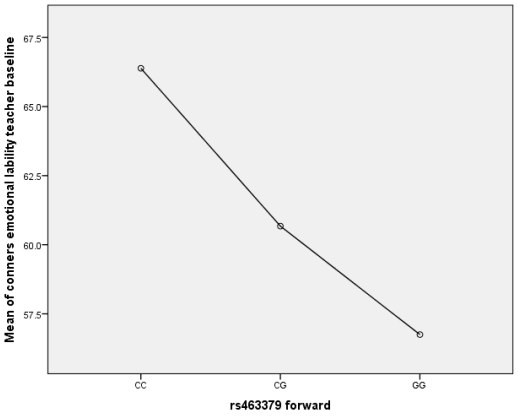

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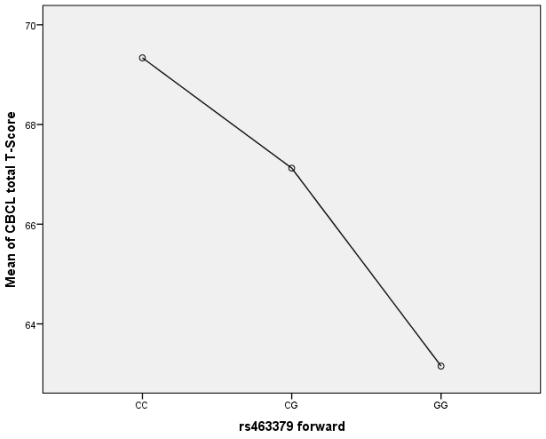

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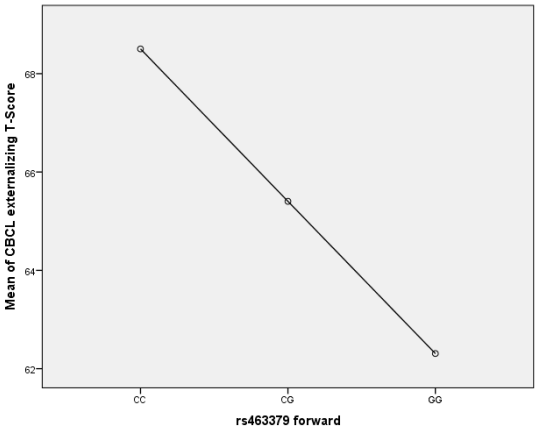

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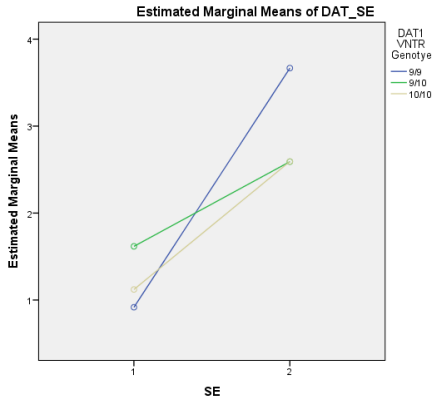

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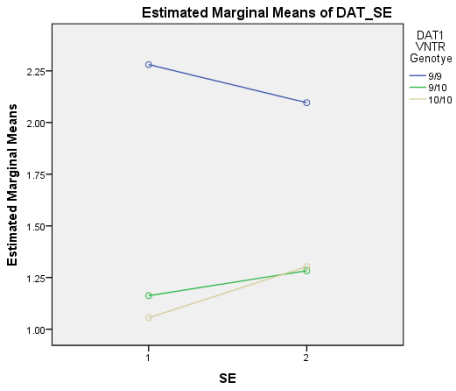
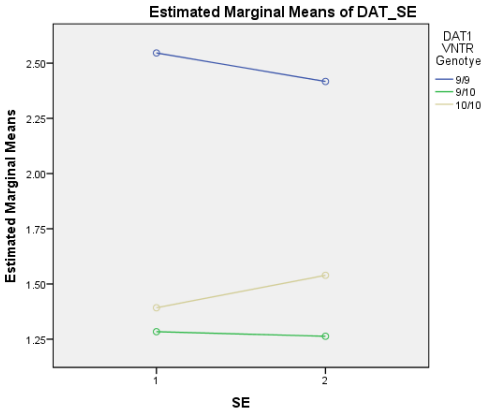


Covariates appearing in the model are evaluated at the following values: Child's age at baseline = 8.8871, Child's gender = 1.24

3H)



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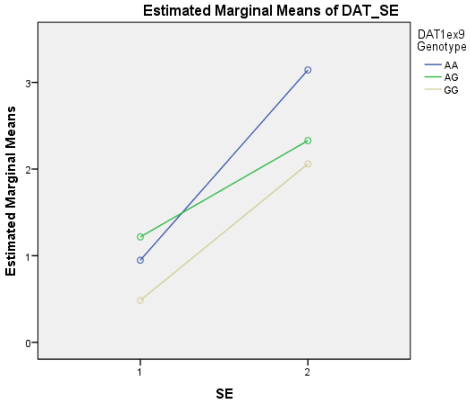
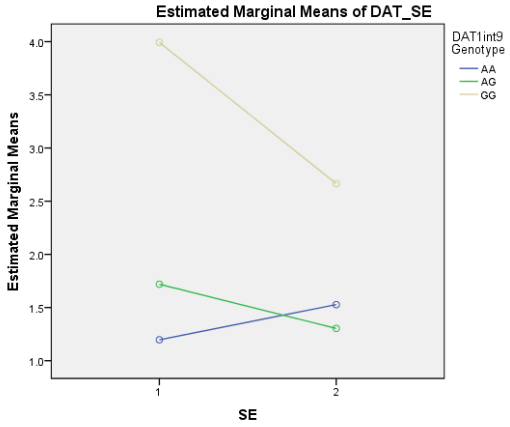


Covariates appearing in the model are evaluated at the following values: Child's age at baseline = 8.9418, Child's gender = 1.23

3J)



Covariates appearing in the model are evaluated at the following values: Child's gender = 1.24, Child's age at baseline = 8.9203

3K)

Figure.3A-3K.*DAT1* genotype groups showing significant association with some clinical, comorbidity and side effects. A) Showing significant effect of Conners' emotional liability

teachers at baseline B) showing significant CBCL total sore .C) showing significant CBCL externalization score. D). showing significant effect of Conners' emotional liability teachers at baseline .E) showing significant CBCL total score. F) Showing significant CBCL externalization score. G) Showing significant side effect insomnia. H). showing significant side effect prone to crying. I). showing significant side effect anxious. J). showing significant side effect decreased appetite. K). showing significant side effect anxious.

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CHAPTER 4

CONCLUSION

The pharmacogenetics research efforts of ADHD are known to be expanding worldwide. To date several promising findings which are related to prediction of treatment response and side effects have been reported, although the results have not been entirely consistent. Upcoming investigations should employ more standardized study designs while examining a wider range of stimulant and non-stimulant medications and a variety of outcome measures. Further future ADHD pharmacogenetic investigation may include studying polymorphisms in drug-metabolizing enzymes, as well as approaches that incorporate gene-gene interactions and effect modification by additional environmental exposures. More so, investigators have shown more interest in going beyond the studying of candidate genes by exploring whole-genome approaches.

Further research, likely involving multi-site collaborations to obtain larger samples, is clearly necessary before preliminary findings can be applied to contemporary clinical practice. Using the same study design and methodology will also be necessary in order to check for consistencies of result .Nevertheless, the promise of ADHD pharmacogenetics is far reaching, and includes the potential to develop individualized medication regimens that improve symptom response, lessen risk of adverse effects and increase long term tolerability.

The development of novel ADHD treatments may also help to provide an important clinical application for ADHD pharmacogenetics findings. Further knowledge of genes that predict ADHD treatment response might in the future, facilitate the development of more specific and efficacious medications for subsets of children with ADHD. Ultimately, it is hoped that pharmacogenetics research will allow clinicians to tailor individual treatment choices based on genotype.

Findings reported in this thesis could help to expand our understanding of therapeutic response and side effects of MPH that can be translated into an improved patient care and the clinical trials design.