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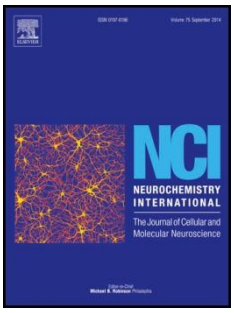

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Review article

Title: Does serotonin deficit mediate susceptibility to ADHD?

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Highlights

- Published neuroanatomical, pharmacological and genetic studies furnished evidences for involvement of serotonin in ADHD etiology
- Serotonin is mainly involved in hyperactive and impulsive domain of ADHD but perhaps not with inattention.
- serotonin plays an important role in the etiology of ADHD mainly *via* orbitofrontal-striatal circuitry
- Serotonin by its interaction with other monoaminergic system and environmental factors decipher additive effect of each individual 'small effects' in ADHD etiology

Abstract:

The onset of Attention-Deficit-Hyperactivity-Disorder (ADHD) in childhood is characterized by developmentally inappropriate levels of hyperactivity, impulsivity and inattention. A chronic deficit of serotonin (5-HT) at the synapse may trigger symptoms of ADHD. This review focuses on neuro-anatomical, experimental and clinical pharmacological evidence, as well as the genetic underpinnings of serotoninergic involvement in the etiology of ADHD. Neuro-anatomical investigations suggest that serotonin through the orbitofrontal-striatal circuitry may regulate behavioral domains of hyperactivity and impulsivity in ADHD. Studies from animal models of ADHD indicate intimate interplay between 5-HT

and dopaminergic neurotransmission. Selective serotonin re-uptake inhibitors, as also non-stimulant drugs acting on the 5-HT system are, however, clinically effective. They impart less severe side effects in patients with no risk of addiction. Oral administration of L-tryptophan, the amino acid precursor of 5-HT, significantly alleviates ADHD symptoms. Given the multifactorial nature of ADHD, candidate gene and genome-wide association studies have suggested that serotoninergic gene variants are associated with increased risk of ADHD with each locus individually exerting a modest effect on overall risk.

Key Words: Serotonin, ADHD, neuroanatomy, animal model, clinical pharmacology, genetics

List of Abbreviations

ADHD = Attention-Deficit-Hyperactivity-Disorder

DSM = Diagnostic and Statistical Manual of Mental Disorders

APA = American Psychiatric Association

ICD = International Classification of Disease

ADD = Attention-Deficit-Disorder

MPH = Methylphenidate

AMPH = Amphetamine

5-HT = Serotonin

SERT, 5-HTT, SLC6A4 = Serotonin Transporter

PFC = Pre-Frontal Cortex

DLPFC = dorsolateral PFC

IFC = inferior frontal cortex

OFC= orbitofrontal cortex

VMPFC = ventromedial PFC

5-HTR1A = Serotonin receptor 1A

5-HTR2A = Serotonin receptor 2A

BOLD = blood oxygen level dependent

fMRI = functional magnetic resonance imaging

5-HTR3A = Serotonin receptor 3A

DA = Dopamine

DAT-KO = Dopamine transporter knock out

SSRI = Selective Serotonin Reuptake Inhibitors

5-HTR4= Serotonin receptor 4

6-OHDA= 6-hydroxydopamine

5,7 DHT= 5,7-dihydroxytryptamine

5HTP= 5-hydroxy tryptophan

ATD = acute tryptophan depletion

DAT= dopamine transporter

5-CSRTT= 5-choice serial reaction time task

RTD = rapid tryptophan depletion

SHR = Spontaneously Hypertensive Rat

WKY= Wistar-Kyoto

SD = Sprague-dawley

NE= Norepinephrin

5HTT-KO = Serotonin transporter knock out

EDSS= Extra-dimensional Strategy Set-shifting

5-HTR1B= Serotonin receptor 1B

SDS = Steroid Sulfatase Deficient

NKR1= neurokinin-1 receptor

BrdU = 5-bromo-2'-deoxyuridine

HPLC = high performance liquid chromatography

5-HIAA = 5-hydroxyindoleacetic acid

NAcc = nucleus accumbens

TCAs = tricyclic antidepressants

SNRIs = serotonin-norepinephrine re-uptake inhibitors

FDA = food and drug administration

GRAS = generally recognized as safe

STin2= Serotonin transporter intron 2

VNTR = Variable nucleotide tandem repeat

5HTTLPR = Serotonintransporter linked polymorphic region

MZ = mono-zygotic

DZ = Di-zygotic

TDT= transmission disequilibrium test

SNPs = Single nucleotide polymorphisms

ASD = Autism Spectrum Disorder

IMAGE= International Multicentre ADHD Gene

TPH2= Tryptophan hydroxylase 2

GWLS= Genome-wide Linkage Scan

GSMA= Genome Scan Meta Analysis

CNVs = Copy Number Variants

PMEE= Positive Maternal Expressed Emotion

MDR = Multi Dimensional Regression

MAOA = Mono Amine Oxidase A

GPCR = G-Protein Coupled Receptor

LPHN3= latrophilin 3

ENIGMA = Enhancing NeuroImaging Genetics through Meta-Analysis

PGC = Psychiatric Genomics Consortium

1. Introduction

Attention-deficit-hyperactivity disorder (ADHD), a common neurobehavioral disorder of onset in childhood (Weyandt 2007) is characterized by developmentally inappropriate levels of hyperactivity, impulsivity in motor, emotional and social responses, a general lack of inhibition and pervasive inattention (DSM-V APA, 2013). In order to meet established criteria for clinical diagnosis, the symptoms must manifest in at least two different environmental domains (home, educational, social, or occupational) for a minimum of 6 months, have an onset before age 12 and cause significant behavioral impairment (DSM-V APA, 2013). Due to clinical heterogeneity and comorbidity, diagnostic criteria have been revised periodically and the prevalence of ADHD ranges from 2.2% -17.8% (Payton et al., 2001) with demographic and geographical variability (Rappley 2005). Worldwide the prevalence of ADHD is ~5.29% among school aged children (Polanczyk et al., 2007). ADHD is estimated to affect 5% children or adolescents and 2.5% adults (DSM-V APA, 2013).

ADHD occurs during childhood and adolescence; prospective studies clearly document the persistence of the disorder into adulthood in up to ~60% of affected children (Biederman et al., 1993, Spencer et al., 1998). Prospective studies, which followed subjects with ADHD from childhood to adulthood, have shown a reduction of hyperactive and impulsive symptoms over time with the strongest decline during adolescence while the inattentive symptoms persist into adulthood (Hart et al., 1995).

1.1 Origin of serotonergic hypothesis in ADHD

Candidate pathways for any disease may be identified based on effectiveness of pharmacological agents and their target molecules. In ADHD methylphenidate (MPH) and amphetamine (AMPH) are used as first line of treatment. These drugs mainly act on dopamine (DA) and noradrenalin (NA) system; serotonin (5-HT) is not their 'direct' target. Around 30% of ADHD cases are, however, 'non-responders' to first-line of treatment. Among responders improvement in ADHD symptoms is observed in only ~50% cases. MPH exposure before adulthood may lead to behavioral impairments in adulthood. In male pre-adolescence rats, fluoxetine (a 5-HT reuptake blocker) intake can normalize these behavioral impairments (Bolanos et al., 2008). These observations suggest the involvement of other neurotransmitter systems in ADHD etiology.

In neurocognitive models of ADHD, 'inhibition' is considered to be a core deficit (Barkley 1997). Serotonin influences behavioral inhibition and aversion (Crockett et al., 2009, Dayan and Huys 2009). Earlier, a decrease in blood 5-HT levels was reported among hyperactive and ADHD children (Coleman 1971, Spivak et al., 1999). Reduced binding of H^3 labeled imipramine (a non-selective 5-HT re-uptake inhibitor) to 5-HT uptake sites in ADHD children with comorbid conduct disorder has been reported (Stoff et al., 1996). Selective serotonin re-uptake inhibitors (SSRI) and Tri-cyclic-antidepressants (TCA) which act upon 5-HT system are second-line drug of choice for treating ADHD. These observations lead to the generation of serotonergic hypothesis of ADHD (Quist and Kennedy 2001). The hypothesis suggests that chronic deficiency in the available 5-HT level may contribute to the clinical symptoms of ADHD (Quist and Kennedy 2001). Oades et al., in 2008 have reported that in ADHD patients there is a 25% reduction in binding capacity of serotonin transporter (SLC6A4, SERT, 5-HTT). In recent years, clinical, neuro-anatomical, experimental and genetic aspects of ADHD have furnished evidence which supports a role of 5-HT in ADHD etiology. We review studies involving the serotonin system in ADHD. Gadow et al. (2013) have shown that the rs25531 polymorphism in the serotonin transporter promoter (5HTTLPR) is associated with increased risk of and susceptibility to both ADHD as well as ASD. Therefore, comorbidity of ADHD with ASD may be associated with pathophysiological changes of the serotonergic system.

2. Neuro-anatomical Evidences for Involvement of Serotonin System in ADHD

Neuroimaging data link ADHD with deficits in vital brain regions concerned with cognitive control, attention, executive function and motivation/reward function (De La Fuente et al., 2013, Makris et al., 2009). The brain-network underlying the pathophysiology is Pre-Frontal Cortex (PFC), dorsal anterior cingulate cortex, striatum and cerebellum (Curatolo et al., 2010). Till date, 16 neuroimaging studies in adult ADHD individuals have also shown structural and functional alteration in brain as well as network connectivity (Ramos-Quiroga et al., 2013, 2014). Structural connectomics studies have reported significantly reduced connectivity in the prefrontal-dominant circuitry which is related to in-attention and increased connectivity in the orbitofrontal-striatal circuitry which is related to hyperactivity/impulsivity (Wang et al., 2009). 5-HT plays a key role in the early development of human nervous system, which is different from its function in mature brain (Homberg et al., 2013). Serotonin synthesized by raphe nuclei is not the only source of 5-HT in placenta, it is additionally synthesized using

maternally derived tryptophan; this may be a crucial link between early genetic and environmental factors and their effect on brain maturation (Valesquez et al., 2013).

The PFC is the principal area of brain which regulates attention, cognitive control, emotion and motivation (Arnsten and Rubia 2012). The dorsolateral PFC (DLPFC) controls attention, planning and working memory, inferior frontal cortex (IFC) regulates cognitive functions, orbitofrontal cortex (OFC) and the ventromedial PFC (VMPFC) controls motivation and emotion (Arnsten & Rubia 2012). PFC contains a very large density of 5-HTR1A (inhibitory) and 5-HTR2A (excitatory) receptors (Puig et al., 2004). OFC is specifically dependent on 5-HT, and depletion of 5-HT from OFC significantly impairs OFC regulation of emotion, inhibition and of reversal learning (Clarke et al., 2007). The dorsomedial PFC is especially sensitive to effects of low tryptophan modulation (Evers et al. 2006). Moreover, Rubia et al. (2005) found a significant reduction of BOLD (blood oxygen level dependent) signal with tryptophan depletion in the right inferior and OFC during a go/no-go task (Rubia et al, 2005).

SSRIs are reported to have role in improvement of frontal control within fronto-limbic circuitries (Murphy 2010). Reward aversion circuitry, which is considered to be an integral part of ADHD neurobiology (Makris et al., 2009), is comprised of amygdale which, is highly innervated by serotonergic neurons. Using BOLD functional magnetic resonance imaging (fMRI), it was observed that differential excitability of the amygdale to emotional stimuli is driven by genetic variation in 5-HTT gene (Hariri *et al,* 2002). 5-HTT ablation affects the development of raphe-prefrontal network and somatosensory system (Homberg et al., 2013). There are a few positron emission tomography (PET) neuroimaging studies of 5HT and 5HTT levels relevant to ADHD. Karlsson et al., (2013), estimated Serotonin Transporter (SERT) density among 8 female ADHD patients, and reported lack of a significant link between SERT density and ADHD. Savli et al., (2012) have generated a normative range database of serotonergic system in different brain regions of healthy human volunteers using a Multi-Tracer PET study without focusing on ADHD pathology. PET studies on Rhesus monkeys have revealed that clinically relevant doses of Atomoxetine, the treatment drug for depression and ADHD, are capable of occupying both NET and SERT upto a range of >85% for SERT in a dose-dependent manner (Ding YS et al., 2014). 5-HTR3A controls neuronal migration and 5-HTR6 regulate dendritic differentiation (Engel et al., 2013). 5-HTR3 and reelin may control laminar and cellular identities of cortical areas involved in complex behavior (Homberg et al., 2013). Considering all these findings it is possible that serotonin plays an important role in the etiology of ADHD mainly *via* orbitofrontal-striatal circuitry.

3. Pharmacological Underpinnings of ADHD

3.1. Evidence from experimental animal models

A successful animal model of ADHD should possess "face validity" (phenotypic resemblance with human disease), "predictive validity" (similar drug response as of human) and "etiological validity" (similar type of behavioral effect resulting by gene mutation and environmental toxins). The underlying heterogeneity in ADHD has given rise to different animal models. Around nine of them have revealed serotonergic involvement in disease etiology.

3.1.1. Spontaneously Hypertensive Rat

Spontaneously Hypertensive Rats (SHRs) are the best characterized model of ADHD (Sagvolden et al., 2005). SHRs have 'face value' for ADHD as; they are hyperactive compared to Wistar-Kyoto (WKY) control rat strain (Russel et al., 1995). A variety of behavioral tests have also reported inattention and impulsivity in SHRs (Jentsch 2005; Bizot et al. 2007; Fox et al. 2008). As psychostimulants and guanfacine can ameliorate behavioral deficit in SHR, this model has predictive value (Sagvolden et al., 2006).

Brain 5-HT function is reported to be reduced in SHR (Stocker et al., 2003). In the chronic hypertensive rat model administration of fenfluramine (an SSRI) evoked less prolactin secretion (Stocker et al., 2003). Administration of citalopram, (an SSRI, 1-10 mg/kg) 1h before an elevated plus-maze test to the SHR promoted hypoactivity with increase in central 5-HT levels (Pollier et al., 2000). In MPH untreated rats, Roessner and associates have found a higher density of 5-HTT in striatum of SHR compared to WKY at day 50-90 (Roessner et al., 2009). In a recent study on juvenile (6 week old) male stroke-prone SHRs (SHRSP/Ezo), fluvoxamine significantly suppressed hyperactivity at high dose (30mg/kg) and in a low dose (3mg/kg) it was found to have effect on impulsive features (Hiraide et al., 2013). These findings in SHR model supports involvement of serotonin system in regulating hyperactivity, a core symptom of ADHD.

3.1.2. Neonatal 6-hydroxydopamine (6-OHDA) Lesioned Rat

This model has been created by selective chemical lesion of DA neurons using 6-OHDA in 5 day old rats (Shaywitz et al., 1976). When 6-OHDA was administered with desmethylimipramine (these preserve noradrenergic neurons), hyperactivity was observed 10-17 days after drug administration (Shaywitz et al., 1976). Inattention was also reported in the 6-OHDA lesioned model (Oke and Adams, 1978). Thus this model has 'face validity' in studies of ADHD. Drugs like AMPH and MPH can reduce

hyperactivity in 6-OHDA lesioned rat (Heffner and Seiden 1982; Archer et al. 2002) so, this model has 'predictive validity'. Though the primary target of this model was the DA system, discernable changes in 5-HT system were also reported (Stachowiak et al. 1984; Snyder et al. 1986; Bruno et al. 1987; Luthman et al. 1990 ; Zhang et al. 2002; Avale et al. 2004, Brus et al. 2004).

Tissue concentration of 5-HT and its metabolites, 5-HTT density, number 5-HT neurons, K^+ stimulated 5-HT release is increased in striatum of 6-OHDA lesioned rats (Stachowiak et al. 1984; Snyder et al. 1986; Bruno et al. 1987; Luthman et al. 1990, 1997; Zhang et al. 2002; Avale et al. 2004). In striatum and other DA denervated areas of 6-OHDA lesioned rat brain, expression of 5-HTR1 and 5-HTR2 found to be increased (Basura and Walker 1999).

The neurochemical imbalance responsible for hyperactivity in this model may derive from ~80% depletion in striatal DA level with concomitant ~70% increase in 5-HT level (Avale et al, 2004). Interestingly, normalization of elevated 5-HT level by depletion reversed hyperactivity to normal level in 6-OHDA lesioned rats but not in control rats (Avale et al, 2004).

Abolition of 5-HT hyperinnervation by administering (5,7-DHT) 10 weeks after 6-OHDA administration enhances hyperactivity in this model, compared to 6-OHDA alone (Kostrezewa et al., 1994). Like amphetamine, fenfluramine that releases 5-HT, and quipazine, a 5-HT receptor agonist, both can attenuate hyperlocomotion in these rats 5-HT receptor antagonist, mesulergine can block amphetamine-attenuated hyperlocomotion in this model (Heffner and Seiden 1982). SSRIs reported to reduce enhanced horizontal activity in 6-OHDA treated male Sprague-dawley (SD) rat (Davids et al., 2002). Moreover, the 5-HTR2 agonist, m-chlorophenylpiperazine (m-CPP), can reduce hyperactivity in 6-OHDA/5,7-DHT treated rat (Brus et al., 2004). All these findings suggest a complementary role for 5-HT and DA in mediating hyperactivity.

3.1.3. Dopamine Transporter Knock-Out (DAT-KO) Mice

DAT-KO mice are reported to be 3-5 times more hyperactive than control, with impaired learning ability and impulsivity (Gainetdinov et al. 1999, Li et al., 2010). As AMPH and MPH can reduce hyperactivity in this model, DAT-KO model is considered to have 'face validity' and 'predictive validity' as an animal model of ADHD.

In DAT-KO mice MPH can attenuate hyperactivity without affecting striatal DA level, so this calming effect is assumed to be imparted through the 5-HT or NE system (Gainetdinov et al. 1999).

Administration of fluoxetine (SSRI) or 5-HT precursors significantly attenuates hyperactivity in DAT-KO mice but nisoxatine (a NET inhibitor) does not (Gainetdinov et al. 1999). Treatment of DAT-KO mice with α-methyl-*p*-tyrosine (αMT, a tyrosine hydroxylase inhibitor) for 40 min results in ~80% depletion of extra-cellular DA with complete abolition of locomotion (Gainetdinov et al. 1999). Apomorphine (a DA-receptor agonist) could restore it but when apomorphine was given to fluoxetine treated DAT-KO, it could not restore locomotion (Gainetdinov et al. 1999). Although a 5-HTR2A antagonist, M100907 blocks hyperactivity in DAT-KO mice, it does not do so in control mice (Barr et al., 2004). This model supports the view that interaction between DA and 5-HT neurotransmission regulates locomotor activity.

3.1.4. Serotonin Transporter Knock-Out (5HTT-KO) rodents

Hypolocomotion studies in 5-HTT-KO mice (Kalueff et al., 2007) did not evidenced 'face validity' of this model but a candidate gene study regarding MPH treatment in ADHD has reported 5-HTT as an important gene in moderating treatment effects of MPH (McGough et al., 2009). Extracellular 5-HT level is 5-10 fold increased in striatum, cortex and substantia nigra of 5-HTT-KO mice (Mathews et al., 2004) suggests importance of this model in studying 5-HT effects in different behavioral paradigms

A reduced 5-HT level in 5-HTT-KO rodents resulted in improved performance in reversal learning (Brigman et al., 2010). A study by Nonkes et al., using Extra-dimensional Strategy Set-shifting (EDSS) task has reported HTT-/- rats showed improved strategy shifting and reduced latent inhibition, indicating that HTT has effects on attention and cognitive process (Nonkes et al., 2012).

3.1.5. Serotonin Receptor 1B (5-HTR1B) Knock-Out Mice

5-HTR1B-KO mice were created by homologous recombination and found to be hyperactive (Brunner and Hen, 1997). This model was considered as animal models of motor impulsivity (Brunner and Hen, 1997). So, this model has 'face validity' for ADHD. In a study with 5-HTR1B-KO male mice occurrence of dis-inhibition of impulsive behavior was reported (Bouwknecht et al., 2001). Recently, an *in vivo* study in transgenic 5-HTR1B (5-HTR1B$^{+/-}$, 5-HTR1B$^{-/-}$) and 5-HTT $^{-/-}$ mice have found that 5-HT clearance by the 5-HTT is under control of 5-HTR1B (Montanez et al., 2014).

Apart from these five animal models, 5-HT system is implicated in coloboma mutant mouse model, neurokinin-1 receptor KO (NKR1$^{-/-}$), 5-bromo-2'-deoxyuridine (BrdU)-exposed rat model and Steroid Sulfatase Deficient mice (SDS mice) models of ADHD. Coloboma mice exhibits hyperactivity, inattention and impulsivity (Bruno et al., 2007). The 5-HT system has not been studied in detail using

this model, although, decreased 5-HT release was reported in dorsal striatum of coloboma mice (Raber et al., 1997).

NKR$^{-/-}$ mice are hyperactive and respond to treatment with AMPH and MPH (Yan et al., 2009). In anesthesized NKR$^{-/-}$ mice, the firing rate of 5-HT neurons increased in dorsal raphe nucleus (Santarelli et al., 2001). Prenatal exposure to BrdU in SD rats causes marked hyperactivity in offspring (Kuwagata et al., 2004). These rats responded very well to the 5-HTR1A antagonist NAN190, with significant increase in 5-HT and its metabolite levels but 5-HT turnover ratio was found to be deceased in striatum of this model (Kuwagata et al., 2004).

Loss of function of X-linked STS gene gave rise to the SDS mouse model [39, X(Y)*O] of ADHD. SDS mice show hyperactivity, inattention, impulsivity. Significantly, high 5-HT level in striatum and hippocampus of SDS mice brain was reported (Trent et al., 2012). All these nine animal models of ADHD accentuate role of serotonin in ADHD.

3.2. Insights from clinical therapeutics and pharmacogenetics

3.2.1. Serotonin and its metabolite level in ADHD patients

Published studies have measured 5-HT or its metabolite levels in the peripheral system, under the assumption that in healthy individual, the peripheral system reflects CNS activity. The observations that blood 5-HT levels are deceased in hyperactive children lead to the serotonergic hypothesis of ADHD (Coleman 1971). Comparison of blood 5-HT levels among 11 hyperactive children and 11 controls, showed a significant decrease of 5-HT level in hyperactive cases (Bhagavan et al, 1975). No significant change in 5-HT levels of 49 hyperactive children was reported (Ferguson et al., 1981). ADD patients with normal intelligence exhibit hyperserotonimia resulting from lower level of plasma and protein-bound tryptophan and higher percentage of free tryptophan (Irwin et al, 1981). In contrast, decreased plasma 5-HT levels were reported in a sample of 35 severely affected ADHD children (Spivak et al, 1999).

Furthermore, Shetty and Chase have reported that the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) levels, measured in CSF of ADHD cases, is not different (Shetty and Chase 1976). However, another study showed 5-HIAA levels in CSF of 29 ADHD cases to be positively correlated with aggression (Castellanos et al, 1994). Interestingly, measurement of 5-HT utilization levels in 14 ADHD (DSM-III-R) children, among whom 93% were males with an average age of 9.8 yrs (ranging 6.5 to 14.3 yrs) shows a trend towards increase activity of 5-HT system (Oades et al., 1998). A retrospective study with 73 ADHD

children has reported whole blood 5-HT levels can indicate treatment efficiency of Pemoline. Patients with hyposerotonimia had diminished impulsivity and improved on attention task but those with higher 5-HT level did not improve on treatment with pemoline (Saul and Ashby 1986).

The reason behind this ambiguity of findings may be the age of ADHD patients, as in all of these studies samples were collected before or during adolescence, which is the time for most developmental changes (Oades et al., 2007). In these studies pharmacological profiles prior to measurement of neurotransmitter levels was not known; they may influence 5-HT levels significantly. There are no studies in adult ADHD individuals which may provide a better understanding of 5-HT levels in the etiology of ADHD, although in one study on ADHD children 5-HT metabolism is reportedly more active than DA metabolism (Oades 2002).

3.2.2. Stimulant medications acting on 5-HT system

Psychostimulants are used as primary line of treatment for ADHD. Methylphenidate (MPH, Ritalin) and amphetamine are drugs of choice (Swanson et al, 2003). MPH is primarily believed to block dopamine transporter (DAT) and nor-epinephrine transporter (NET) and it is reported that it has low affinity for 5-HTT (Gatley et al., 1996). Berger reported that a decrease in 5-HT level can cause hyperactivity and MPH acts by balancing 5-HT and DA tone (Berger 1999).

A study in male wistar adult rat, shows a significant increase in 5-HTT density in the medial frontal cortex, after 2 and 12 weeks latency of MPH treatment (20mg/kg, for 11 days) (Daniali et al., 2013). Microdialysis has shown that prior administration of methamphetamine (1mg/kg) potentiates MPH-induced locomotor activity in SD rats and methamphetamine was reported to increase extracellular 5-HT level in nucleus accumbens (NAcc) (Borycz et al., 2008).

Atypical signaling of NAcc to PFC may increase impulsive behavior and NAcc is important for reward processing (CostaDias et al., 2013). Additionally they have also reported that prior administration of fluoxetine (5-HT uptake blocker, 10mg/kg) also potentiates MPH- induced motor activity (Borycz et al., 2008). These findings indicate that increased 5-HT level may have important role in potentiating MPH activity. Using 5-HTR1B agonist and antagonist they have suggested a strong synergistic action of 5-HTR1B agonist and MPH (Borycz et al., 2008). The 5-HTR1B is an autoreceptor that can regulate 5-HT release (Bonanno et al., 1986), so it may be responsible for methamphetamine-induced increased 5-HT level, which by its tonic effect on DA, regulates motor activity induced by MPH. Markowitz et al., 2009 have reported that MPH can also act as an agonist at the 5-HTR1A. All these studies clearly

indicate that 5-HT has an important role in potentiating the effect of psychostimulants, though the exact mechanism(s) remains unclear.

3.2.3. Non-stimulant medicines acting on serotonin system

Stimulants are schedule II drugs and may be abused or misused among young and adolescents (Klein- Schwartz, 2002). Up to 60% of ADHD patients may have different disorders that are comorbid with ADHD symptoms (Biederman et al, 1991). Comorbid conditions such as anxiety, depression, and aggression cannot be treated properly with stimulants (Goez et al, 2007, Mohammadi and Akhondzadeh, 2007, Tannock et al, 1995). Nonstimulant medications including atomoxetine (selective NE reuptake inhibitor), TCAs, Bupropion (5-HTR1A agonist), Imipramine (a non selective 5-HT reuptake inhibitor) and the serotonin-norepinephrine re-uptake inhibitors (SNRIs, duloxetine, venlafaxine) are now being considered as second and third line treatments for ADHD (Park et al., 2014). TCAs function on 5-HT and NE reuptake inhibition (Pliszka et al., 2006) and usually show 60%-70% treatment efficacy within 2-6 weeks (Park et al., 2014). Sometimes TCAs are not well tolerated among ADHD patients as they may cause cardiac toxicity and they exhibit highly sedative and anticholinergic properties (Amitai and Frischer 2006).

A survey of published data shows that 13 studies have examined the effects of venlafaxine treatment in ADHD clinical samples as summarized in Table 1. Patients with comorbid disorders may benefit from a lower initial dose of venlafaxine which is slowly titrated to higher doses (Olvera et al, 1996). SNRIs increase extracellular 5-HT and NE levels in the PFC and striatum of SHR, but DA level increases only in PFC not in striatum (Higashino et al., 2014). A study using the SHR model reports that SNRIs can reduce the enhanced locomotion in the habituated open field task (Umehara et al., 2013). Duloxetine has higher affinity and more potent blockade of 5-HT and NE transporters *in vitro* and *in vivo* compared to venlafaxine (Bymaster et al., 2001). Continuous use of duloxetine, with no dose reduction, resulted in an ultimate decline of side effects (Mahmoudi-Gharaei et al., 2011). More studies with increased sample size and placebo controlled studies are required to decipher its role in ADHD treatment.

Insert Table 1 here →

Buspirone, which displays high affinity and selectivity for 5-HTR1A, has affinity for 5-HTR2A also (Sanghera et al., 1982). An open clinical trial of buspirone 0.5 mg/kg/day (range 15 to 30 mg/day) on 12 children with ADHD suggests its role in reducing hyperactivity, impulsivity (Malhotra and Santosh, 1998).

A double blind randomized clinical trial with MPH and buspirone indicates efficacy of buspirone over MPH in hyperactive and impulsive domains of ADHD (Davari-Ashtiani et al., 2010). In contrast, another study with MPH and buspirone has suggested that buspirone is less effective compared to MPH, but side effects like insomnia, headache and decreased appetite were significantly less in the buspirone group (Mohammadi et al., 2012). In the 2012 study all the patients were of ADHD combined subtype, but in 2010 study subtypes are not mentioned. It is possible that efficacy of buspirone is higher in hyperactive/impulsive subtype only.

3.2.4. Tryptophan depletion studies in ADHD

Tryptophan depletion, which causes reduction in brain 5-HT synthesis was found to impact aggression (vanPolier et al., 2014, Kotting et al., 2013, Zimmerman et al., 2013, Stadler et al., 2007) inattention (Zepf et al., 2010, Mette et al., 2013), behavioral inhibition (Zepf et al., 2008), impulsivity (Zepf et al., 2008) domains of ADHD. We have summarized findings on tryptophan depletion effects and ADHD in Table 2.

Insert Table 2 here →

The effectiveness of 5-HT and DA amino acid precursors have been studied in a clinical trial with 85 ADHD patients (aged 4-18 years) and ~77% of patients showed significant improvement (Hinz et al., 2011). Since 5-HT and DA amino acid precursors are generally recognized as safe (GRAS) by food and drug administration (FDA), no safety concerns are associated with this treatment (Hinz et al., 2011).

3.2.5. Pharmacogenetic Studies and Serotonergic Genes in ADHD

Pharmacogenetic studies of ADHD have primarily focused on the 5-HTT gene. McGough et. al., in a double-blind placebo controlled cross-over trial of 82 ADHD cases have studied MPH treatment effect by immediate MPH release, 3 times daily (McGough et. al., 2009). ADHD symptom response was predicted significantly by the STin2 VNTR polymorphism (p= 0.01) and math test outcomes, vegetative symptoms were predicted significantly by the 5-HTTLPR polymorphism (p = 0.003). Their study concluded that 5-HTT gene exerts moderate MPH treatment effect (McGough et. al., 2009). Studying modulated behavioral response of MPH (0.5 mg/ kg/day) in 157 ADHD patients Thakur et. al., have reported that patients with 's' or 'l_G' allele for 5-HTTLPR showed significant improvement in ADHD symptoms from placebo but not MPH treatment, whereas, 'l_A' allele carriers showed significant

improvement with MPH treatment (Thakur et. al., 2010). In an adult ADHD sample, there was no effect of the 5-HTTLPR or 3 SNPs of the 5-HTR1B gene on MPH treatment (Contini et. al., 2012).

The study by Wigging et. al., 2012 is noteworthy for detailing influence of 5-HTTLPR on default brain network connectivity and its development with age. Among 39 healthy children and adolescents individual with 's/s' genotype showed weaker connectivity in superior mFC compared to 'l_A/l_A' and 's/l_A' or 's/l_G' genotypes. Furthermore they have reported steepest age-related increase in connectivity within 'l_A/l_A' individuals, whereas,'s/s' individuals showed least age- related increase in neural connectivity (Wigging et. al., 2012). Consequently, the reports of 'no effect' of 5-HTTLPR polymorphism on MPH treatment in adult ADHD cases may derive from age dependent genotype interaction(s).

4. Behavioral Evidences for Involvement of Serotonin System in ADHD

4.1. Hyperactivity

It was thought previously that DA is the key regulator of hyperactivity but research findings from rodent (sections 3.1.2; 3.1.3 of this review) and hamster models suggest that 5-HT is also involved in hyperactivity (Takahasi et al, 2002, Kabuki et al, 2008, Baumann et al, 2011). Takahashi et al, using a microdialysis probe showed that infusion of 5-HT in rat hippocampus but not striatum, significantly increased motor activity (Takahashi et al, 2002). Using 5-HTR4 agonists and antagonists it has been shown that the hyperactivity resulting from 5-HT infusion is modulated by 5-HTR4 in the hippocampus (Takahashi et al, 2002).

Roborovskii hamsters are hyperactive and have been proposed as animal models for ADHD (Ikeda et al., 2014). The DA and 5-HT level in the whole brain of Roborovskii hamsters were significantly lower ($p<0.0005$) than those of the Djungurian, which supports an association of 5-HT level with hyperactivity (Kabuki et al, 2008). Administration of 5-hydroxy tryptophan (5-HTP) and amphetamine can reduce motor stimulant properties of amphetamine without altering DA releasing effect of amphetamine (Baumann et al, 2011). Taken together, these studies suggest that 5-HT exerts a regulatory role on hyperactivity.

4.2. Inattention

Inattention has been measured in animal models using continuous performance test (CPT) and 5-choice serial reaction time task (5-CSRTT; Robbins 2002). Tryptophan depletion studies in healthy men volunteers showed significant depletion in both free and total plasma level of tryptophan resulted in

improved attention (Gallagher et al, 2003). The impact of acute tryptophan depletion (ATD) on attention performance of adult male ADHD patients has been studied by orally administering a tryptophan-free amino acid mixture and carrying out a continuous performance test in the ATD group as well as control group on tryptophan balanced diet. Following a double blind study design it has been reported in ADHD-ATD group target/non-target discrimination ability and sustained attention is affected, which indicates that 5-HT neurotransmission is related to attention processing in adult ADHD (Mette et al., 2013).

4.3. Impulsivity

The expression of impulsivity involves different neural and psychological factors with major contributions from the brain DA and 5-HT systems (Dalley and Roiser et al., 2012). Soubrié has proposed that a common basis for a number of different behavioral effects associated with decreases in brain 5-HT levels was the disinhibition of behavior, which can be directly related to behavioral/psychological construct of impulsivity (Soubrie 1986). Impulsive individuals show high inclination for small instant rewards and delay aversion (Dalley and Roiser et al., 2012). Serotonin is reported to play pivotal role in relating behavioral inhibition with predictions of aversive result (Crockett et al., 2009).

5-HT may exert its effect on impulsivity by interacting with mesolimbic DA system (Robinson et al., 2008). Though amphetamine acts on DAT, the ability of amphetamine to reduce impulsivity on the delay discounting procedure is lost in brain serotonin-depleted rat (Helms et al., 2006) suggesting that interaction between DA and 5-HT systems is crucial in mediating and regulating impulsive behavior (Oades 2007,Winstanley et al., 2006). Studies on the 'impulsive response' using go/no go task and 5-CSRTT; Robbins 2002 have reported that para-chloroamphetamine (a 5-HT depleting stimulant)-treated rats showed impairment in go/no go task (Masaki et al., 2006). Global 5,7-di-hydroxytryptamine (5,7-DHT)-induced ablation of 5-HT increased impulsivity in a 5CSRTT variant (Winstanley et al., 2004). Using RTD technique it was demonstrated that changed 5-HT function leads to impulsiveness and increases susceptibility to aggressive behavior in patients (Zepf et al., 2008).

5. ADHD as a Multifactorial Disorder

ADHD was accepted among the most recognized genetic-based disorders in psychiatry (McGuffin et al., 2001), reported heritability from 0.75 to 0.90 (Stergiakouli et al., 2010). Different studies indicate that ADHD is influenced by multiple genetic pathways, several non-inherited factors and their interplay (gene-environment interactions).

5.1. Genetic Basis for ADHD

The genetic basis of ADHD was primarily built up by twin studies involving mono-zygotic (MZ) and di-zygotic (DZ). Studies have reported that phenotypic correlation in ADHD, is twice or more than twice in MZ twins compared to DZ twins (Levy et al., 1997, Bennett et al., 2006). A meta-analysis of twin studies in mental disorders have reported shared environment do not influence ADHD (Burt et al., 2009).

The reported relative risk of developing ADHD within the families with ADHD children varies between 4 and 5.4 for first-degree relatives (Biederman et al., 1990, Faraone et al., 2000). Reviewing adoption studies it has been found that hyperactive children resembled their biological parents more than they did their adoptive parents with respect to hyperactivity (van den Oord *et al.*, 1994, Sprich et al., 2000). By estimating sibling risk in ADHD-combined type patients it has been reported that risk of ADHD is nine-fold higher among siblings compared to general population (Chen et al., 2008). This supports findings from twin studies, suggesting genetic factors are primarily responsible for ADHD. Research to unveil the molecular genetic underpinnings in ADHD has mainly progressed through candidate gene studies, genome-wide linkage analysis, genome-wide association analysis, pharmacogenetic studies and gene-gene / gene-environment interaction analysis.

5.1.1. Candidate gene studies and serotonin system

Most published molecular genetic studies of ADHD during 1990-2008 have focused on candidate genes involving family-based or case-control studies with the goal of detecting association and/or linkage of genomic regions with ADHD. Based on the effectiveness of drug treatment, animal models and neuro-imaging studies, candidate gene polymorphisms have been selected mainly from DA, 5-HT and noradrenergic systems. Only those genes that have indicated significant pooled risk in three or more case-control or family-based association studies are described below.

5.1.1.1. Serotonin Transporter (SLC6A4, 5-HTT)

Functional changes in the 5-HTT cause modifications in the expression and/or function of most of the 5-HT receptors and also affect the synthesis, clearance and metabolism of serotonin (Murphy et al., 2008). Three polymorphisms of 5-HTT are well characterized: a in/del promoter polymorphism (5-HTTLPR), a VNTR polymorphism in intron-2 (STin2) and G689T, a SNP in

the 3′-untranslated region (Battersby et al., 1999). The 5-HTTLPR is described in terms of three alleles, designated 'long' allele with a SNP rs25531 (A/G) (l_A and l_G; comprising 16 repeats) or 'short' (s; comprising 14 repeats) (Parsey et al, 2006).

At least 5 groups reported positive association of the long (l) allele of the 5-HTTLPR with ADHD in family-based studies (Manor et al., 2001, Retz etal., 2002), as well as case-control analysis (Kopecková et al., 2008, Xu et al., 2008, Zoroglu et al., 2002). In contrast two case-control (Kopecková et al., 2008, Xu et al., 2008) and three family based association studies (Banerjee et al., 2006, Heiser et al., 2007, Wigg et al., 2006), have reported negative association. Interestingly, one study in Chinese Han population, have found a positive association of 's' allele with ADHD (Li et al., 2007). Considering 10 transmission disequilibrium test (TDT) and 9 case-control studies, meta analysis of 5-HTTLPR have reported modest but significant association with childhood ADHD (Fixed effects; p= 0.004 and random effects; p= 0.01, Gizer et al., 2009) with heterogeneity in effect size of all considered studies.

Landaas et al., in 2010 in a case-control study have reported a trend of over-representation of 's' allele (p=0.06) in ADHD cases (Landaas et al., 2010). Moreover, studying 7 tagged SNPs with 5-HTTLPR they have also found association (p=0.01) of rs140700. However, association was not replicated in pooled samples of 1454 cases and 1302 control individuals from Spain, Germany, Netherland and USA (Landaas et al., 2010), suggesting population-specific allelic distribution and association status.

Measurement of impulsive drive for immediate reward and delay aversion in 459 male children and adolescents, has revealed that 's' allele carrying individuals show more delay aversion, which links 's' allele to impulsivity (Sonuga-Barke et al., 2011). It is reported that l_A/l_A genotypes exhibit higher 5-HTT density in several brain regions compared to l_G/l_G, s/l_G or s/s genotypes (Willeit and Praschak-Rieder, 2010). A study in ADHD children with comorbid Autism Spectrum Disorder (ASD) reported, individuals having at least one copy of 'l_G' or 's' allele showed more severe symptoms of hyperactivity (p=0.001) and impulsivity (p=0.027) (Gadow et al., 2013).

A pathway- based association analysis taking DA, NE, 5-HT system candidate genes with quantitative measurements of ADHD symptoms shows significant association of the 5-HT pathway with hyperactive and impulsive symptoms of ADHD cases (871 individuals from 7 European countries and Israel) (Bralten et al., 2013). All these studies have reported association of 'l_G' and's' allele with hyperactivity and impulsivity which clearly suggests 5-HT system involvement in these two core

symptoms of ADHD. Regarding inconsistency of 'l' allele association status, discovery of tri-allelic nature of 5-HTTLPR may help to explain conflict of results and may lead to more accurate studies in the future.

STin2 VNTR

In three studies the STin2 polymorphism has been reported to be positively associated with ADHD (Banerjee et al., 2006, 2012; Zoroglu et al., 2002) but Langley *et al*. 2003 and Xu *et al.* 2005, 2008 have reported lack of association between STin2 polymorphism and ADHD. Meta analysis has not revealed significant association of STin2 VNTR with ADHD (Gizer et al., 2009).

G689T

The association of G689T with ADHD has been previously examined in Caucasian and Asian populations (Banerjee et al., 2009, Curran et al., 2005, Heiser et al., 2007, Kent *et al*. 2002, Wigg et al., 2006, Xu et al., 2005). In family-based studies Kent *et al.,* reported significant (p=0.04) association of the T689 allele with ADHD cases (Kent *et al*. 2002) which has not been confirmed by subsequent family-based (Banerjee et al., 2009, Heiser et al., 2007, Wigg et al., 2006, Xu et al., 2005) and case-control (Curran et al., 2005) studies.

5.1.1.2.Serotonin Receptor 1B(5-HTR1B)

Three association studies have identified G861 allele of synonymous G861C (rs6296) polymorphism within single exon of 5HT1B gene to be associated with increased risk of ADHD (Hawi et al., 2002, Quist et al., 2003, Smoller et al., 2005). Smoller *et al.,* 2005 examined 21 SNPs in 5HTR-1B and report that a haploblock comprising rs6296, rs6297, rs130060, rs6298, rs130058 and rs11568817 is linked with the inattentive subtype of ADHD. The findings were not, however, replicated in a subsequent study (Ickowicz *et al.,* 2007).

Meta analysis of 9 association studies have found significant association of 861G allele with ADHD (p=0.01), with no heterogeneity in study effect size (Gizer et al., 2009). Guimeraes et al., found significant over transmission of the 261G(rs11568817) /–161T(rs130058) /861G(rs6296) haplotype (P = 0.014). However, no association of 5-HTR1B polymorphisms was reported with ADHD-inattentive subtype (Guimeraes et al., 2009). We reported significant association of the C861 allele with ADHD cases from eastern India, with no association for rs130058, rs6298, rs11568817, rs6297 and rs6298 polymorphisms (Banerjee et al., 2012).

5.1.1.3.Tryptophan Hydroxylase 2 (TPH2)

TPH2 genes translate the rate limiting enzyme for 5-HT bio synthesis exclusively in the brain (Walther et al., 2003). Nine different studies have examined the association of TPH2 variants with ADHD (Sheehan et al., 2005, 2007, Brookes et al., 2006,Walitza et al., 2005, Manor et al., 2008, Johansson et al., 2010, Shim et al., 2010, Hsu et al., 2013, Park et al., 2013). They are summarized in Table 3.

Insert Table 3 →

Meta analysis of two SNPs in intron 5 of TPH2 (rs1843809 and rs1386493) indicates significant association with childhood ADHD (p=0.0002 and p=0.021, respectively) (Gizer et al., 2009). Using imaging-genetics approaches to investigate functional implications, the 'G' allele of rs4570625 and 'T' allele of rs11178997 reportedly causes reduction in pre-frontal brain function within adult ADHD patients and healthy controls (Baehne et al, 2008). Interestingly one study in 451 adult ADHD cases have reported over-representation of minor allele for rs10879345 (p=0.06) (Johansson et al., 2010). Scanning 14 tagged SNPs of TPH2 in a longitudinal twin study van Beijsterveldt et al, have reported no association with attentional problem (van Beijsterveldt et al., 2011).

5.1.1.4.Serotonin Receptor 2A (5-HTR2A)

This is the main excitatory receptor subtype among the G-Protein Coupled Receptors for serotonin (Cook et al., 1994). Two polymorphisms in the promoter region of 5-HTR2A (His452Tyr, -1438G>A) and a silent coding region variation (102T>C) have been studied extensively in ADHD (Quist et al., 2000, Zoroglu et al., 2003, Li et al., 2006, Guimaraes et al., 2007, Cho et al., 2012). Using TDT statistic, preferential transmission of 452Tyr allele to ADHD cases and no association with T102C has been reported (Quist et al., 2000). In contrast, Guimeraes et al., have reported preferential transmission of 452His allele to affected ADHD boys only (Guimaraes et al., 2007). The "no association status" of T102C was, however, replicated in a sample of 70 Turkish ADHD cases, and the 1438G/A polymorphism was also found to be negatively associated (Zoroglu et al., 2003). In contrast, in Korean ADHD families the 'C' allele of T102C is significantly associated (p=0.030) with ADHD (Cho et al., 2012). In adolescent ADHD cases the 1438G/A polymorphism is significantly (p=0.029) related to functional symptomatic reduction of ADHD (Li et al., 2006). However, a case-control study shows significant association of 5-HTR2A gene rs7984966 SNP with ADHD-C type in 188 adults and 263 children (Ribase et al., 2009). They have also reported 4 other SNPs (rs6561333, rs6561332, rs7997012 and rs7322347) to be associated with ADHD-C group in children and 2 SNPs (rs2770296, rs9534495) in adults (Ribase et al., 2009). These

inconsistencies of association status for 5-HTR2A polymorphisms may be due to different allelic distribution among population and clinical heterogeneity of ADHD samples genotyped.

5.1.2.Genome-wide Linkage Scans (GWLS) and the serotonin system in ADHD

GWLS utilized a collection of genetic markers across the genome and examined how those genetic markers segregate with the disease across multiple families. Linkage analysis, of families with multiple affected individuals, exploits within-family correlations between illness and the alternative sequences (alleles) of the markers that are closest to the disease-related gene(s). This approach does not rely on *a priori* hypotheses and may uncover novel genes and neuro-developmental processes that had not been previously considered. However, linkage study is much less powerful in complex disorders like ADHD, where risk variants have small effects and high genetic heterogeneity is present (Dawn and Barrett, 2005). To map ADHD susceptibility genes, to date there have been eleven GWLS (Smalley et al., 2002, Fisher et al., 2002, Bakker et al., 2003, Ogdie et al., 2003, Ogdie et al., 2004, Arcos-Burgos et al., 2004, Hebebrand et al., 2006, Romanos et al., 2008, Rommelse et al., 2008, Amin et al., 2009, Vegt et al., 2010) and one Genome Scan Meta Analysis (GSMA, Zhou et al., 2008). We have summarized the findings in Table 4.

It is noteworthy to mention that three studies in the Dutch population (Bakker et al, 2003, Amin et al, 2009 and Vegt et al, 2010) have reported chromosome 7p region to be linked to ADHD, which harbors the 5-HTR5A gene.

Using, eight neuropsychological ADHD endophenotypes as quantitative traits Rommelse et al., 2008 scanned 5407 autosomal SNPs, in 238 ADHD-C type probands and their siblings. They reported two significant genome-wide linkage signals at chromosome 2q21.1 for motor timing and 13q12.11 for digit span (executive/cognitive endophenotype). The 5-HTR2B gene is located in 2q36.3-37.1 region, while 5-HTR2A is located in 13q14-21(Table 4). This finding indicates towards linkage of serotonergic genes with ADHD endophenotype. By mapping serotonergic genes within all identified chromosomal regions (Table 4) we discerned 15, 5-HT receptor sub-type genes, TPH1, TPH2 and 5-HTT are located directly within linkage regions or located in nearby regions (indirect association). In GSMA chromosome 5 (contains 5-HTR1A, 5-HTR4) was identified with nominal linkage signal (Zhou et. al. 2008). Considering polygenic/multifactorial nature of ADHD (Martin et. al., 2014) each of the 5-HT system genes with 'small effect size' are linked with increased risk of ADHD.

Insert Table 4 here →

5.1.3.Genome-Wide Association Studies (GWAS) and Serotonergic Genes

GWAS have the potential to detect entirely novel associations where there is no *a priori* hypothesis. Studies can be done in case-control (from same ancestry), subjects with a range of values for a continuous trait and family based (The Psychiatric GWAS Consortotium, 2009). Till to date seven reported studies have followed the GWAS method to discover novel biological pathways and common variants for ADHD (Lasky Su et. al., 2008; Neale et. al., 2008; Neale et. al., 2010[a]; Neale et. al., 2010[b]; Mick et. al., 2010; Hinney et. al., 2011; Ebejer et. al., 2013).

Analyzing 438784 SNPs in 958 ADHD trios, Neale et. al., did not find any SNP to show genome wide significant association with ADHD (Neale et. al., 2008). Taking a combined approach of quantitative trait estimation and genetic association Lasky Su et. al., examined 429981 autosomal SNPs within 909 ADHD trios along with 6 quantitative phenotypes across 18 ADHD symptoms (Lasky Su et. al., 2008). The SNPs studied from 37 candidate genes reveal association probability values < 0.01 among 17 genes, 3 of those are from 5-HT system (5-HTR1E, 5-HTR2A and TPH2). After adjustment for multiple comparisons no SNPs achieved genome-wide significance (Lasky Su et. al., 2008).

Mick et al, have reported that 16 candidate genes, including 3 serotonergic genes (5-HTR1B, 5-HTR2A and 5-HTR2C) showed at least one significant association at $p < 0.05$ but, association became non-significant after multiple testing (Mick et. al., 2010). Including data from 4 projects Neale et. al., performed a meta-analysis considering 2064 trios, 896 cases and 2455 controls (Neale et. al., 2010[a]). They did not find any genome-wide significant association. Conversely, studying 2752 SNPs from 43 candidate genes, they have reported top 50 associated SNPs, rs11179003 of TPH2 with a 'p' value = 0.0006791 was one of these ADHD associated SNPs (Neale et. al., 2010[a]). Using 896 cases and 2455 controls from the same sample group as of previous study, case-control analysis did not reveal any genome-wide significant association (Neale et. al., 2010[b]). Ebejer et. al., performed GWAS using quantitative measures of ADHD sub-types and carried out meta-analysis with previously reported significant associations by Lasky Su et. al., 2008 and Neale et. al., 2010[a]. Both these analyses could not detect any genome-wide significant association (Ebejer et. al., 2013).

5.1.3.1.GWAS – Copy Number Variants (CNVs) and Serotonergic Genes

Failure of GWAS to map genome-wide significantly associated common variants in ADHD, gave rise to GWAS study with CNVs. Ramos-Quiroga et. al., performed the first whole genome CNVs study spanning 100 KB of human genome and reported CNVs are 1.33 times higher in ADHD cases (Ramos-Quiroga et. al., 2014). Scanning 146 candidate genes they have identified 21 genes, within which 8 were exclusive to ADHD cases which includes, 5-HTR1B (Ramos-Quiroga et. al., 2014).

5.2.Environmental Risks for ADHD

Environmental risks for ADHD may be prenatal, perinatal or postnatal in origin (Millichap 2008, Thapar et al., 2012). They are mentioned in Table 5.

Insert Table 5 here →

Only maternal smoking (OR=2.39, Langley et al., 2005) and preterm birth-low birth weight (RR=2.64, Aarnoudse-Moens et al, 2010) have shown consistent association with risk of ADHD.

5.3. Gene-Gene, Gene-Environment Interaction and Serotonergic Genes

Candidate gene studies of ADHD have identified several genes which accounts for only a small fraction of the heritable component. Unexplained 'dark heritability' of ADHD may be partially due to epistatic effects between genes and environment. Candidate genes each with 'small effect' might be by interacting with each other imposing an additive effect on ADHD etiology. In the last five years (2010-2014) several pharmacogenetic studies have been published with regard to gene x gene and gene x environment interactions (Bruxel et al., 2014).

5.3.1. Gene X Environment interaction and serotonin system

Animal studies have shown that malnutrition, maternal stress, infection, and toxic compounds (lead, bisphenol) influence prenatal brain development in circuits relevant to ADHD. Serotonergic deficiencies were reported in young adult rats exposed prenatally to bacterial lipopolysaccharide (Wang et al. 2009).

In children, exposure to polychlorinated bisphenyls (PCBs), lead and tobacco have been associated with ADHD symptoms (Cho et al. 2010; Eubig et al. 2010). Individual persons respond differently to environmental pathogens and factors depending on their genetic make-up. Most of the gene x environment studies in ADHD and comorbid disorders concentrated on the 5-HTTLPR

polymorphism (Sonuga-Barke et al., 2009, Nigg et al., 2010, Nikolas et al., 2010, Segurado et al., 2011, Bidwell et al., 2012, Wallis et al., 2012 and van der Meer et al., 2014).

One study in ADHD-C type children and adolescents, has reported that sensitivity to Positive Maternal Expressed Emotion (PMEE) is moderated by 5-HTTLPR (Sonuga-Barke et al., 2009). Nigg et al., have reported consistent evidence of positive interaction between 5-HTTLPR genotypes and psychosocial factors but no interaction with prenatal events (Nigg et al., 2010). Nikolas and associates have studied 5-HTTLPR interaction with appraisals of self-blame in relation to parents' marital dispute and reported that both high and low serotonergic activity may increase risk for ADHD interacting with psychosocial distress (Nikolas et al., 2010). Bidwell et al, have reported that 5-HTTLPR genotypes and hyperactive impulsive symptoms predict initial pleasant response for smoking (Bidwell et al., 2012). A recent study have reported's' allele carriers had significantly positive correlation between stress and ADHD severity (in hyperactive/impulsive dimension) compared to l/l genotype (vander Meer et al., 2014).

5.3.2.Gene X Gene interaction and serotonin system

Lack of replication, small effect size in genetic studies and underlying heterogeneity of ADHD indicates existence of genetics interactions between loci. Genetic factors which functions mainly through complex mechanisms, involves multiple genes and environmental factors; the effect might be missed if we study the gene individually without allowing for its potential interactions (Cordell et al., 2009).

A study in a dynamic minimal model of 5-HT function, suggests an epistatic interaction between pre-synaptic 5-HT system (Stoltenberg 2005). By pair-wise case-only interaction analysis Segurado et al have reported significant association between 5-HTT and 5-HTR1B (case-only test p=0.0045) (Segurado et al., 2011). In a family based association study using MDR statistics our group has reported significant interaction between risk alleles of STin2 (5-HTT) and risk allele of rs6296 (5-HTR1B) (Banerjee et al., 2012). Wargelius et al, have reported interaction between 5-HTTLPR-MAOA and MAOB (Wargelius et al., 2012). Their study have reported 's' allele for both 5-HTTLPR and VNTR of MAOA showed higher MAO-B activity in platelet within severe ADHD phenotype (Wargelius et al., 2012). MAO-A plays major role in degradation of 5-HT and NE whereas, MAO-B is important for DA degradation so, this reported interaction again points to inter-connected functions of 5-HT-NE and DA in relation to ADHD.

Following linkage strategy two studies have identified latrophilin 3 (LPHN3, a GPCR involved in cell adhesion and signal transduction) gene to be associated with ADHD (Arcos-Burgos et al., 2010; Ribases et al., 2011). Association studies have reported variants of LPHN3 are associated with response to stimulant medication (Arcos-Burgos et al., 2010). Interestingly, LPHN3 mutant mice showed changes in 5-HTT and 5-HTR2A gene expression and at 4-6 weeks of age null mutants showed increased 5-HT level in dorsal striatum with hyperactive phenotype (Wallis et al., 2012). Their observations pave the way to study LPHN3 gene interactions with serotonin system genes to explore ADHD etiology.

6. Concluding Remarks and Future Directions

In this review we have summarized findings which connect 5-HT system with ADHD using neuro-anatomical, clinical-pharmacological and genetic data. Most of the reports indicated towards involvement of 5-HT system in the hyperactive and impulsive component of ADHD but perhaps not with inattention. There seems to be little overlap between GWAS and reported linkage or candidate gene-based association study findings. To unveil complete etiology of ADHD combination studies using neuroimaging and genetics together termed as imaging genetics are emerging now (Wu et al., 2014). The Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium also has been formed to identify causative agents for ADHD (Thompson et al., 2014). Like ENIGMA if other consortia can be formed to combine Pharmacogenetic data with neuroimaging that may also be a promising way for ADHD research. Given high comorbidity in ADHD, the Psychiatric Genomics Consortium (PGC) was formed to study the common genetic pathways in 2007. GWAS study by PGC- cross disorder group in ASD, ADHD, bipolar disorder, major depressive disorder, and schizophrenia have identified four genome-wide significantly associated loci including 10q24, which harbor 5-HTR7 (10q21-q24) (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). It is noteworthy that IMAGE, ENIGMA and PGC consortiums concentrate on only on data from European and American populations. Female ADHD cases are reported to be nearly equal to men (Faraone 2000) and sex specific difference in neuropathology also evidenced in ADHD cases but, there is a dearth of published studies including female cases (Nussbaum 2012). Furthermore, it is possible that non-availability of serotonin at the synapse is due to metabolism via the Kynurenine pathway. However, there is currently no available experimental or clinical evidence for such involvement in ADHD. More studies are therefore warranted to decipher the interplay between glia and neurons in the etiology of ADHD.

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Table 1:Summary of clinical studies in human patients using SNRIs

Study	Drug	Demographics	Study Design	Dose	Result	Side Effect
Hedges D et al.,	Venlafaxine	18 adult patients,	Open trial	Started at	11 responders, 9	Nausea 50%, fatigue

1995		12 men 6 women, avg age 35 yrs		18.75mg/day	improved moderately or very much, 2 improved mildly and seven had side effects	33%, lower libido 17%, GI tract problem17%, Insomnia 17%, confusion 17%, neuromuscular problems 17%
Adler LA et al., 1995	Venlafaxine	16 adult with ADD	Open trial	25-225 mg/day for 8 weeks	In 12 patients ADD symptoms were 50% reduced	sedation, agitation nausea (within 4 patients in 1st week)
Pleak R and Gormly LJ, 1995	Venlafaxine	One 11 yrs girl		37.5 mg t.i.d- 100mg t.i.d	improved attention, reduced intrusiveness, less fidgetiness, diminished disorganization (after 6 weeks)	Increased BP with dose increase
Wilens T et al., 1995	Venlafaxine	Two adult of 45 yrs and 48 yrs		75 mg b.i.d with clonazepam 0.5 mg p.r.n and 18.75 mg t.i.d (2 weeks)	60% and 52% reduction in ADHD score	Not reported
Olvera RL et al., 1996	Venlafaxine	16 children (mean age 11.6 yrs.)	5 week open trial	1.4 mg/kg/day	Improved behavior in 7 subjects but not in cognitive symptoms	Not tolerable adverse effects in 4 cases
Findling RL et al., 1996	Venlafaxine	10 adults with ADHD	Open clinical trial for 8 weeks	37.5 mg b.i.d- 75 mg b.i.d	7 were responders showed significant reduction in ADHD symptoms	Mild side effects
Hornig-Rohan M and Amsterdam JD, 2002	Venlafaxine	17 adult ADD patient with MDD (5 women, 12 men), mean age (43 ± 15) yrs	8-12 week open trial of venlafaxin + TCAs+stimulants and bupropion	100-500mg/day	80% cases with only venlafaxine improved in ADD and MDD symptoms compared to 33% of stimulant treated	Mild nausea, insomnia
Carminati GG et al., 2006	Venlafaxine	3 patients (17 yrs boy, 23 yrs woman, 17 yrs girl) with autism and hyperactivity	Clinical trial with 18, 36 and 6 month follow-up	18.75mg/day	Improvement of symptoms even after follow up	
Mukaddes NM and Abali O, 2004	Venlafaxine	13 children and adolescent (mean age 9.9 ± 2.5 yrs.)	6 week open trial	18.75 mg/day - 56.25 mg/day	Significant reduction of total Conners parent score ($P < 0.002$)	somnolence (n = 2), stomachache (n = 2), and headache (n = 1)
Findling RL et al., 2007	Venlafaxine	38 cases (35 males, 5 females, ages- 5-17 yrs)	2 week open level out patient trial	0.5 mg/kg/day to 14 cases, 1.0 mg/kg/day to 13 cases and 2.0	Very much improved (3%), much improved (33%), minimally improved (36.4%), no change (21%), minimally worse	Headache (31.6%), Nausea (31.6%), Stomach ache (23.7%), Drowsy (23.7%), restlessness (23.7%), poor appetite (18.4%), Insomnia

				mg/kg/day to 11 cases	(3%), much worse (3%)	(18.4%), dizziness (15.8%), vomiting (15.8%)
Zarinara AR et al., 2010	Venlafaxine	38 patients (27 boys, 11 girls, ages 6-13 yrs); venlafaxin group; mean age 9.4; MPH group mean age 9.6	6-week, parallel group, randomized clinical trial, double blind	50 mg/day for <30 kg; 75 mg/day for >30 kg; MPH 20-30 mg/day	ARS-IV (Parent):score improved from ~31 to ~17; ARS-IV (Teacher): score improved from ~31 to ~18 in venlafaxin group. Difference between group was not significant.	In venlafaxin; abdominal pain (26%), somnolence (26%), restlessness (16%), headaches (16%), insomnia (11%)
Amiri S et al., 2012	Venlafaxine	20 cases on venlafaxin, 21 cases on placebo; mean age 30.5 yrs	Double blind 6 weeks trial	up to 225 mg/day	25% drop in ADHD index (measured by a self-report scale)	No serious adverse effects were reported
Tourjman SV and Bilodeau M 2009	Duloxetine	One 53 yrs man	Clinical trial after side effects of atomoxetine and MPH	60mg/day for 6 weeks	Significant reduction of ADHD symptoms	Nausea, fatigue, flushing, mild dizziness, lowered appetite at 1st week and amelioration of side effects after 6th week of treatment
Niederhofer H 2010	Duloxetine	2 male patient of ADHD-inattentive subtype, 16 and 19 yrs	Placebo duloxetine cross over trial	30 mg/day for 4 weeks	Mean ARS IV score decreased from 27.8 to 16.6	Mild sedation
Mahmoudi-Gharaei J et al., 2011	Duloxetine	17 cases ages 11-18 yrs	6 week open level	1st week 30mg/day then from 2nd week upto end 60mg/day	Significant improvement in inattention, hyperactivity subscale	Appetite decease 46%, dry mouth 30%, insomnia, somnolence, anxiety, nervousness
Bilodeau M et al., 2014	Duloxetine	30 adults aged 18-50 yrs	6 week double blind trial	60 mg/day	24 completed trial, CGI-improvement score increased, significant decrease in impulsivity	40% drop out due to side effects

ADD, attention deficit disorder; BP, blood pressure; MDD, major depressive disorder; ARS-IV, ADHD rating scale IV; CGI, clinical global impression; MPH, methylphenidate; t.i.d, thrice in day; b.i.d, twice in day

Table 2: Summary of Clinical Studies in Relation to Tryptophan Depletion and ADHD

Sample	Objective	Study Design	Findings	Ref
22 male adolescent ADHD patients	To study effect of RTD and 5HT depletion in CNS on aggression with	Double blind within subject cross over design with RTD and placebo	5-HT functioning in ADHD patients influences reactive aggression depending on aspects of trait-impulsivity.	Zepf FD et al., 2008

	respect to trait-impulsivity			
26 male children with ADHD, aged 9-15 yrs.	To study effect of RTD and 5HT depletion in CNS on reactive aggression in ADHD patients	Double blind within subject cross over design with RTD and BAL	Inverse correlation between 5HT level and reactive aggression. Age and intensity of attention do not have any impact on 5HT and aggression relationship	Stadler C et al., 2007
22 boys (age range: 9–15; mean: 10.9 yrs.), six of them had comorbid CD	To explore the effect of RTD and resulting brain 5HT synthesis reduction on behavioral inhibition using go/no go task	placebo-controlled double-blind within-subject crossover design	RTD caused significant increase in behavioral inhibition within highly aggressive group and lower rate of behavioral inhibition in low aggressive group.	Zepf FD et al., 2008
22 boys (age range: 9–15 yrs; mean: 10.9 yrs.), six of them had comorbid CD, Mean age 10.9 $\pm$1.8 yrs	To study effects of 5-HT turnover depletion By (RTD) on attentional performance	Double blind within subject cross over design with RTD and Trp-balanced Pla on two different days.	Attentional performance change depending on 5HT availability,	Zepf FD et al., 2010
20 male ADHD patients, mean age (30.25$\pm$9.37) yrs and 20 control, mean age 27.90$\pm$6.02 yrs	To study the effects of ATD on reactive aggression in adult patients with ADHD compared to healthy adult controls.	Double-blind within-subject crossover design with ATD and BAL	Lowered rates of reactive aggression were found in the ADHD group under ATD after low provocation (LP) compared to opposite effect in controls. Evidenced inverse association between trait impulsivity and ATD effect on aggression after LP	Zimmerman M et al., 2012
20 ADHD patients with risk of aggressive behavior	To investigate effect of ATD on reactive aggression and decision time in young people	Double blind within subject cross over	An effect of ATD on increased aggression after low provocation and non significant gender difference in decision time	Kotting WF et al., 2013
20 male adult patients with ADHD (age: M = 30.25 yrs, SD = 9.37 yrs) and 20 male control (age: M = 27.9 yrs, SD = 6.01 yrs)	To investigate 5HT depletion in CNS caused by ATD has any impact on attentional process or not, using target/non-target discrimination task	Double blind within subject cross over	In continuous performance test (CPT) reaction time deceased after ATD in ADHD group and increased in ATD-control group. Number of omission increased in ATD-ADHD group. Thus ATD affect target/non-target discrimination.	Mette C et al., 2013
15 young people with ADHD (7 boys, 8 girls), age 11.8$\pm$ 1.9 yrs	To study impact of brain 5HT depletion on physiological arousal using electrodermal activity in relation to reactive aggression	Double blind within subject repeated measures cross over	ATD was not associated with altered physiological arousal, as indexed by electrodermal activity (EDA). Baseline aggression was negatively correlated with the mean ATD effect on EDA.	vanPolier GG et al., 2014

RTD, rapid tryptophan depletion; BAL, tryptophan balanced control condition; CNS, central nervous system; ATD, acute tryptophan depletion (a process of diminishing central 5HT synthesis by orally administering TRP-free amino acid mixture); CD, conduct disorder

Table 3: Candidate gene studies for TPH2 and ADHD

Polymorphism	Location	Sample details	Test statistics	Findings	Ref
rs1843809 (T-G) rs1386497 (A-C)	Intron 5 Intron8	179 Irish nuclear families (ADHD-C 62.6%, inattentive 11.7%, hyperactive-impul 6.1%.)	TDT	'T' allele significantly transmitted more (p=0.0006) 'A' allele transmitted more (p=0.045)	Sheehan et al., 2005
rs4570625(G-T) rs11178997 (A-T)	Upstream regulatory region	103 families with 225 affected children (ADHD-C 70.7%, inattentive 25.8%, hyper-impul 3.6%)	TDT	'G' allele significantly transmitted more (p=0.049) 'T' allele transmitted more (p=0.033)	Walitza et al, 2005
rs11179027 rs1843809 (T-G)	Intronic Intron 5	142 ADHD and 139 control children	Case-control	Significant correlation of allele freq p=0.020, p=0.040	Park et al, 2013
rs1843809 (T-G) rs1386497 (A-C)	Intron 5 Intron8	107 families from UK origin	TDT	No significant association	Sheehan et al., 2007
rs4570625(G-T) rs11178997 (A-T)	Upstream regulatory region	312 ADHD cases from 282 families from Taiwan	TDT	No significant association	Hsu et al., 2013
rs4570625(G-T)	Upstream regulatory region	78 ADHD and 107 control from Korea	Case-control	No significant association	Shim et al., 2010
18 tagged SNPs		1,636 cases and 1,923 controls from IMpACT	Case-control	No significant association	Johansson et al., 2010
8SNPs, rs1386488(A-C), rs2220330(A-G), rs1386495(T-C), rs1386494(G-A), rs6582072(G-A), rs1386492(A-G), rs4760814(G-A), rs1386497(A-C)	All intronic	498 affected ones from 379 families	Family based association	Eight locus haplotype C-G-C-A-A-G-A-C found to be associated (49 transmission; 30 non-transmission) with ADHD-C type	Manor et al, 2008
Functional SNPs with MAF>0.05 and tagged SNPs ($r^2 \geq 0.8$)	5'UTR, exons, introns and 3'UTR	674 DSM-IV combined type probands with 808 siblings	TDT	Positive association with global P value 0.036; P-SUM from gene-wide test 0.1;	Brookes et al, 2006

TDT=Transmission disequilibrium Test; MAF=Minor allele frequency

Table 4: Genome-wide Linkage Studies of ADHD and Serotonergic Genes

Chromosome	Region	Sample Details	Score	5HT genes with Position	Ref
5	5p12	126 ASPs	LOD>1.5		Fisher et al., 2002
	5p(69cM)	164 ASPs	MLS=1.43[b]		Bakker et al, 2003

	5p13	308 ASPs	MLS=2.55		Ogdie et al., 2004
	5q33.3	16 Multigenerational Pedigrees	MLS=2.41	HTR1A; 5q11.2-q13, HTR4; 5q31-q33	Arcos-Burgos et al, 2004
	5p(17cM)	155 ASPs	MLS=2.59		Hebebrand et al., 2005
	5q13.1	8 Multigenerational Pedigrees.	MOD=2.82 (global)	HTR1A; 5q11.2-q13	Romanos et al., 2008
17	17p11	270 ASPs 308 ASPs 16 Multigenerational Pedigrees	MLS=2.98 MLS=3.63 MLS=2.83		Ogdie et al., 2003; Ogdie et al., 2004; Arcos-Burgos et al, 2004
	17p(D17S1308)	155 ASPs	LOD=1.39		Hebebrand et al., 2005
	17q12	238 ADHD-C cases, 112 affected 195 non affected siblings	LOD=2.34	5HTT, 17q11.2	Rommelse et al., 2008
16	16p13	126 ASPs 270 ASPs 308 ASPs	LOD>1.5 MLS=3.73 MLS=3.73		Fisher et al., 2002 Ogdie et al., 2003 Ogdie et al., 2004
7	7p13(70cM)	164 ASPs	MLS=3.04[a]		Bakker et al, 2003
	7p15.1-q31.33	Single family with 8 ADHD cases	LOD=2.1		Vegt et al., 2010
	7p (88cM)	155 ASPs	LOD-0.92		Hebebrand et al., 2005
	7q21.11	8 Multigenerational Pedigrees	MOD=3.14 (global)	HTR5A; 7q36.1	Romanos et al., 2008
9	9q33.3	164 ASPs	MLS=2.05[a]		Bakker et al, 2003
	9q (104cM)	155 ASPs	LOD=0.68		Hebebrand et al., 2005
	9q22 9q31.11-33.1	8 Multigenerational Pedigrees	MOD=1.39 MOD=1.50 (global)		Romanos et al., 2008
6	6q(166cM)	164 ASPs	MLS=1.19[a]	HTR1B; 6q13, HTR1E; 6q14-q15	Bakker et al, 2003
	6q12(89cM)	308 ASPs	MLS=3.3	HTR1B; 6q13, HTR1E; 6q14-q15	Ogdie et al., 2004
	6q(75cM)	155 ASPs	LOD=0.58	HTR1B; 6q13, HTR1E; 6q14-q15	Hebebrand et al., 2005
	6q22-23	8 Multigenerational Pedigrees	MOD=2.75 (global)	HTR1B; 6q13, HTR1E; 6q14-q15	Romanos et al., 2008
12	12q23(165cM)	126 ASPs	MLS=1.27	TPH2; 12q21.1	Fisher et al., 2002
	12q (166cM) 12q23.3	155 ASPs 238 ADHD-C cases, 112 affected 195 non affected siblings	LOD=2.10 LOD=2.63[c]	TPH2; 12q21.1	Hebebrand et al., 2005 Rommelse et al., 2008
	12p13.33	238 ADHD-C cases, 112 affected 195 non affected siblings	LOD=2.88[d]	HTR7P1; 12p13.1	Rommelse et al., 2008
4	4p16.3	164 ASPs	MLS=1.78		Bakker et al, 2003
	4q13.2	16 Multigenerational Pedigrees			Arcos-Burgos et al, 2004
	4q35.2	238 ADHD-C cases, 112 affected 195 non affected siblings	LOD=2.11[c]		Rommelse et al., 2008
15	15q15.1	164 ASPs	MLS=3.21		Bakker et al, 2003
	15q11.2-13.3	8 Multigenerational Pedigrees	MOD=3.68 (global)		Romanos et al., 2008
14	14q32.13	238 ADHD-C cases, 112	LOD=2.42[c]		Rommelse et al., 2008

		affected 195 non affected siblings			
	14q12	8 Multigenerational Pedigrees	MOD=4.17 (global)		Romanos et al., 2008
	14q11.2-22.3	Single family with 8 ADHD cases	LOD=2.08		Vegt et al., 2010
13	13q33.3	164 ASPs	MLS=1.91	HTR2A;13q14-q21	Bakker et al, 2003
	13q12.11	238 ADHD-C cases, 112 affected 195 non affected siblings	LOD=3.96[c]	HTR2A;13q14-q21	Rommelse et al., 2008
18	18q21-22	21 inbred patients	LOD>1.5		Amin et al., 2009
	18q11.2-13.3	8 Multigenerational Pedigrees	MOD=2.90 (global)		Romanos et al., 2008
10	10q26	126 ASPs	LOD>1.5	HTR7; 10q21-q24	Fisher et al., 2002
11	11q22 (113 cM) 11q (95 cM)	16 Multigenerational Pedigrees 155ASPs	NPL = 4.0 LOD = 0.41	TPH1; 11p15.3-p14, HTR3A; 11q23.1, HTR3B; 11q23.1	Arcos-Burgos et al, 2004 Hebebrand et al., 2005
2	2q35 2q21.1 2p25.1	8 Multigenerational Pedigrees 238 ADHD-C cases, 112 affected 195 non affected siblings	MOD = 2.98 (Global) LOD = 3.94[e] LOD = 2.20[e]	HTR2B; 2q36.3-q37.1	Romanos et al., 2008 Rommelse et al., 2008
1	1q25.1 1q25.3	8 Multigenerational Pedigrees	MOD = 4.17 MOD = 2.99 (Global)	5HTR1D; 1p36.3-p34.3, HTR6; 1p36-p35	Romanos et al., 2008
3	3p	238 ADHD-C cases, 112 affected 195 non affected siblings		HTR1F; 3p12, HTR3C' 3q27.1	Rommelse et al., 2008

[a]Narrow diagnostic criteria; [b]Broad diagnostic criteria; [c]quantitative trait is executive/cognitive task; [d]quantitative trait is combined score of executive/cognitive and motor task; [e]Quantitative trait is motor task

ASP=Affected Sibling Pair; LOD=log of odds; MOD=maximizes parametric LOD; MLS= Maximum multipoint LOD scores; 5HTT = Serotonin Transporter; TPH = Tryptophan Hydroxylase.

Table 5: Reported Environmental Risk Factors for ADHD

Type	Factors	Reference
Prenatal	Developmental cerebral abnormality	(Nopoulos et al., 2000)
	Alcohol in pregnancy	(Mick et al., 2002_a)
	Smoking in pregnancy	(Thapar et al., 1989)
	Maternal stress	(Rice et al., 2010)

	Drug use in pregnancy	(Linnet et al., 2003)
Perinatal	Prematurity, Obstetric complications	(Lou HC. 1996)
	Low birth weight	(Mick et al., $2002_{b,}$ Aarnoudse-Moens et al, 2009)
	Anoxic-ischemic encephalopathy	(Lou HC. 1996)
	Meningitis	(Millichap JG. 2008)
	Encephalitis	(Gau et al., 2008)
Postnatal	Viral meningitis	(Millichap JG. 2008)
	Encephalitis	(Gau et al., 2008)
	Cerebral trauma	(Catale et al., 2009)
	Lead, Polychlorinated biphenyls (PCBs)	(Eubig et al., 2010)
	Psychosocial adversity	
	Nutritional factors	(Lifford et al, 2008)
		(Pelsser et al., 2011)