ADHD Pharmacogenetics Across The Life Cycle:
New Findings and Perspectives

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Attention-deficit/hyperactivity disorder (ADHD) is a complex and heterogeneous disorder, affecting individuals across the life cycle. Although its etiology is not yet completely understood, genetics plays a substantial role. Pharmacological treatment is considered effective and safe for children and adults, but there is considerable inter-individual variability among patients regarding response to medication, required doses, and adverse events. We present here a systematic review of the literature on ADHD pharmacogenetics to provide a critical discussion of the existent findings, new approaches, limitations, and recommendations for future research. Our main findings are: first, the number of studies continues to grow, making ADHD one of the mental health areas with more pharmacogenetic studies. Second, there has been a focus shift on ADHD pharmacogenetic studies in the last years. There is an increasing number of studies assessing gene–gene and gene–environment interactions, using genome-wide association approaches, neuroimaging, and assessing pharmacokinetic properties. Third and most importantly, the heterogeneity in methodological strategies employed by different studies remains impressive. The question whether pharmacogenetics studies of ADHD will improve clinical management by shifting from trial-and-error approach to a pharmacological regimen that takes into account the individual variability remains unanswered.

How to Cite this Article:

Key words: ADHD; pharmacogenetics; medication response; stimulants; atomoxetine

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is characterized by persistent and age-inappropriate patterns of inattention, hyperactivity, and impulsivity [American Psychiatric Association, 2013]. It is one of the most common neurodevelopmental disorders affecting individuals across the life cycle. While the reported prevalence for school-aged children and adolescents is around 5.3–8.7% [Polanczyk et al., 2007a; Merikangas et al., 2010; Kessler et al., 2012; Willcutt, 2012], rates from 2.5% to 4.4% are described for adults [Kessler et al., 2006] reflecting late brain maturation in a
ADHD is a complex and heterogeneous disorder and its etiology is not yet completely understood. Despite evidences that environmental factors play an important role in its etiology, classical genetics studies support a strong genetic contribution for ADHD [Biederman and Faraone, 2005; Schmitt and Romanos, 2012]. Neurobiological studies suggest that ADHD is a frontal-striatum-cerebellum disorder, since these regions present lower maturation, volume, and activity in these patients [Castellanos et al., 2002; Cortese, 2012].

Also, neuropsychological data showed that ADHD children have behavioral inhibition failures and a poorer performance in cognitive and executive functions. These neuropsychological processes are related to frontostriatal circuits [Swanson et al., 1998]. Recent hypotheses have proposed that ADHD might also be the result from core deficits in inhibitory control to reward processes, which would lead to executive dysfunctions and impaired signaling of delayed reward, respectively [Nigg and Casey, 2005; Sonuga-Barke, 2005]. Functional magnetic resonance imaging (fMRI) studies have found decreased activation in ventral striatal area during delayed reward, respectively [Nigg and Casey, 2005; Sonuga-Barke, 2005]. It is possible that multiple neural pathways contribute to ADHD, so a dysfunction in any of these circuits could lead to symptoms and determine the heterogeneity seen in ADHD [Sonuga-Barke, 2005; Durston, 2003].

Pharmacotherapy has an essential role in the treatment of ADHD [Kornfield et al., 2013]. Many studies have documented the efficacy of stimulants (e.g., methylphenidate—MPH, amphetamines) and non-stimulants (atomoxetine) in reducing ADHD symptoms, as well as improving neuropsychological performance on measure of executive functions [Greenhill et al., 2002; Blum et al., 2011]. The alpha-2 agonists clonidine and guanfacine, which were developed and initially utilized as antihypertensive agents, also showed improvement in ADHD symptoms [Sallee et al., 2013]. Recently, the United States Food and Drug Administration (FDA) approved extended-release formulations of clonidine and guanfacine for ADHD children and adolescents [Intuniv, 2011].

The most recognized brain effect of MPH and a potential mechanism of action for ADHD symptom improvement is the dopamine transporter (DAT) blockade [Faraone and Mick, 2010]. However, it is known that MPH also blocks efficiently the norepinephrine transporter (NET) [Faraone and Mick, 2010; Hannestad et al., 2010]. Some studies have demonstrated MPH-induced improvement on prefrontal cortex functioning where NET density is higher. It was postulated that NET could transport dopamine in the prefrontal cortex because dopamine has a greater affinity for NET as compared with its affinity for DAT [Madras et al., 2005; Hannestad et al., 2010]. MPH also induces the increase in cortical cell excitability, which is mediated by activation of adrenergic alpha-2-A receptors (ADRA2A) [Andrews and Lavin, 2006]. MPH is currently prescribed either as immediate-release (IR) or extended-release (ER) formulations. An alternative to oral MPH administration is the MPH transdermal system that delivers racemic MPH, D,L-threo-MPH, through the skin [Anderson and Scott, 2006]. All of them determine good response in decreasing ADHD symptoms, although their pharmacokinetics are quite different [Faraone and Buitelaar, 2010].

An alternative treatment option is amphetamines. Their primary action is to increase synaptic concentration of neurotransmitters in the synaptic cleft. Amphetamines compete with the endogenous monoamines for transport into nerve terminals. Once inside the presynaptic terminal, amphetamines displace monoamines from the cytosolic pool, pumping neurotransmitter out of neurons into the synapse. The reuptake inhibition and probably inhibition of monoamine oxidase (MAO) also occur with this mechanism increasing synergically neurotransmitter concentrations in the synapse [Heal et al., 2013]. Like MPH, amphetamines have different formulations as IR, ER, and the prodrug lisdexamfetamine [Heal et al., 2013].

Non-stimulant medications, such as atomoxetine, are alternative psychopharmacological interventions for ADHD treatment. Atomoxetine is a highly specific NET inhibitor with adequate treatment efficacy [Michelson et al., 2001]. Although pharmacological agents are considered as effective and safe, there is considerable inter-individual variability among patients regarding response to medication, dosing effects, and occurrence of adverse events [Greenhill et al., 1996; Vaughan and Kratochvil, 2006]. Due to this variability, the treatment is often determined empirically in clinical practice through a gradual dosage titration and a trial-and-error approach.

Pharmacogenetic studies try to explain how individual genetic variability influences the pharmacokinetics and pharmacodynamics of the drug [Weinshilboum, 2003]. The term pharmacogenomics has been used to encompass studies investigating variations in network of genes and their relationship to medication response and to emphasize these insights to discover new therapeutic targets and optimize drug therapy [Evans and Johnson, 2001]. The great potential of ADHD pharmacogenetics (here used interchangeably with pharmacogenomics) for clinical application lies in predicting better medication choices, avoiding adverse effects, maximizing individual treatment outcomes and determining the most appropriate drug dosage. Early investigations on ADHD susceptibility genes were conducted based on data describing MPH action. Therefore, it has been hypothesized that polymorphisms in these same genes may also influence medication response in individual patients. Numerous initial studies focusing on dopaminergic genes have described evidence of genetic factors influencing MPH and atomoxetine response, as summarized in several reviews [Stein and McGough, 2008; Genro et al., 2010; Froehlich et al., 2010; Kieling et al., 2010; Polanczyk et al., 2010]. It is important to bear in mind that many factors (e.g., phenotypic presentation including ADHD types and comorbidities, neurocognitive processes, environment, and genetics determinants) combined in different ways contribute to ADHD phenotypic heterogeneity. Unfortunately, how this heterogeneity interferes on treatment response prediction and medication efficacy is not very clear yet. Moreover, the variance of the treatment response explained by pharmacogenetic data does not seem to be substantial [Polanczyk et al., 2010].

In this context, our aim is to present an updated review of the literature and to provide a critical discussion of the findings, new approaches, limitations, and recommendations for future research.
METHODS

Since our group has produced previous systematic reviews on ADHD pharmacogenomics in children [Kieling et al., 2010; Polanczyk et al., 2010] and adults [Contini et al., 2012], we aimed to update this previous work providing a more integrative lifespan perspective. Thus, we implemented a similar methodological procedure in this systematic review than the one in the previous studies, offering a sense of continuity for the readers. A systematic review of the literature was performed in MEDLINE via PubMed database (http://www.ncbi.nlm.nih.gov) and PsychINFO (http://www.apa.org/pubs/databases/psychinfo/index.aspx). We looked for original studies that have ADHD pharmacogenetics information published between January 2010 and September 2013 for children and adolescents and between February 2012 and September 2013 for adults. Relevant studies in children and adolescents published earlier than 2010 and in adults earlier than 2012 covered in our previous reviews are also mentioned here.

The search was limited to articles published in English. The following key-words were employed: attention-deficit/hyperactivity disorder or ADHD combined or ADHD, MPH response or stimulant response, MPH, atomoxetine, guanfacine, and lisdexamfetamine. In addition, a blanket search was conducted with only the term “Attention-Deficit/Hyperactivity Disorder” to ensure no relevant studies were missed. Next, an extensive review of the references of pertinent articles was carried out.

We selected articles that met the following inclusion criteria: (1) assessment of any medication indicated for ADHD treatment across the life cycle; (2) ADHD diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders—version IV (DSM-IV) or the International Classification of Diseases—10th Revision (ICD-10); (3) studies that used a candidate-gene or genome-wide association approach; (4) studies that defined response to medication in terms of symptoms improvement, evaluation of dose–response relationship, occurrence of adverse events, or objective parameters that reflect the central nervous system functioning (e.g., neuropsychological tests, cerebral blood flow, etc.).

The literature mining identified 90 publications. After reviewing the abstracts, we selected 33 articles and the full text of these publications and their reference lists were reviewed. Twenty-nine studies fulfilled the inclusion criteria and were included in this review. The lisdexamfetamine search did not result in any pharmacogenetic study.

RESULTS

Candidate Genes and Response to Medication

The majority of candidate genes investigated in pharmacogenetics studies are related to the catecholamine pathway, but neurodevelopmental genes and new genes involved in ADHD risk have been studied as well. Only one study evaluates atomoxetine as an ADHD medication. Investigations with amphetamine preparations or guanfacine were not found in the literature, and the remaining studies evaluate MPH treatment.

**DAT1.** The majority of studies on ADHD pharmacogenetics have focused on MPH response and the dopaminergic system. DAT inhibition by MPH in presynaptic neurons increases synaptic dopamine and neuronal signaling. This protein is distributed mainly in the striatum and nucleus accumbens, but also in the globus pallidus, cingulate cortex, thalamus, and the midbrain [Sasaki et al., 2012]. DAT represents the primary mechanism of dopamine regulation [Ciliax et al., 1999]. The gene that encodes DAT has been considered a good candidate for pharmacogenetics studies due to the major role of the transporter in stimulant’s action. The most studied polymorphism is the 40 base pair (bp) variable number of tandem repeats (VNTR) in the 3’ untranslated region at the dopamine transporter gene (*DAT1* or *SLC6A3*). In vitro studies suggested that DAT VNTR has a functional role, with the 10R being associated with higher expression levels of the transporter [Mill et al., 2002; Purper-Ouakil et al., 2008].

Previous reports have described decreased MPH responses in individuals homozygous with either 9-repeat allele (9R) [Stein et al., 2005; Joober et al., 2007] and 10-repeat allele (10R) [Winsberg and Comings, 1999; Roman et al., 2002b; Purper-Ouakil et al., 2008]. In agreement with these last studies, a recent investigation reported the association between *DAT1* VNTR and MPH response in 89 stimulant-naïve children with ADHD. Outcomes were assessed using the Vanderbilt ADHD Parent Rating Scales (VADPRS) and the Vanderbilt ADHD Teacher Rating Scales (VADTRS). The investigators reported that patients lacking the 10R allele showed a greater improvement across MPH doses compared to 10R carriers with effect sizes around 0.59–0.64. A reduction of 56% on hyperactive–impulsive scores at MPH dose of 2 mg/kg/day was observed in the patients without 10R compared to placebo ($P = 0.008$) [Froehlich et al., 2011]. The main finding of this study was a gene–dose interaction. However, this finding should be interpreted with caution because the sample is small and the significance level of the tests was adjusted for the number of outcomes assessed but not for the number of SNPs investigated. Nevertheless similar findings were previously described in an adult sample [Kooij et al., 2008].

Better responses were also reported for the homozygous 10R on MPH treatment [Kirley et al., 2003]. Recently, a longitudinal open label trial evaluated neurocognitive fuctions as response inhibition, planning and working memory in 108 stimulant-naïve boys with ADHD according to *DAT1* genotypes after 4, 8, and 24 weeks of treatment with 0.5 mg/kg/day dose, and 8 weeks after MPH withdrawal. Response inhibition was measured by the Continuous Performance Test II (CPT II), planning ability by the Tower of London (ToL) test, and working memory by N-back test. At 4 weeks, no difference on response inhibition improvement according to genotype was observed; planning ability was better in 10R homozygous subjects as compared to 9R carriers. At the working memory test, all children improved regardless of genotypes. After 8 weeks of treatment, 10R carriers had better response inhibition compared to 9R homozygous; the planning ability continued better in 10R homozygous compared to 9R carriers; these results did not change when working memory was considered. At 24 weeks, 10R carriers continued with better response inhibition; no difference among genotypes in planning ability was observed, and working memory was better in 10R homozygous. After MPH interruption, response inhibition worsened in all patients, but planning ability and working memory improvements were maintained. These results
presented a high power analysis (>0.80) [Pasini et al., 2013]. However, the interpretation of these results is difficult, because the authors analyzed dominant and recessive effects of the 10R allele according to the improvement trajectory between tasks.

No pharmacogenetic effect of the DAT1 VNTR was also reported in children [Langley et al., 2005; van der Meulen et al., 2005; Zeni et al., 2007; McGough et al., 2009] and in adults [Mick et al., 2006; Contini et al., 2010]. A recent meta-analysis demonstrated that there was no significant effect for the DAT1 VNTR on both MPH treatment response ($P > 0.5$) and specific symptom dimensions ($P > 0.2$). However, a subanalysis of naturalistic trials detected that 10R homozygous patients exhibited less improvement than individuals that are not 10R homozygous. In summary, DAT1 VNTR seems to be a non-reliable predictor of MPH treatment [Kambeitz et al., 2014]. Although DAT1 is an obvious candidate for MPH pharmacogenetics studies, findings available in the literature did not consistently suggest a role for this gene in the ADHD pharmacogenetics. Possible methodological explanations for spurious and conflicting findings like those found with DAT gene are explored in Table II. Moreover considering that the VNTR is probably non-functional and the complex architecture of DAT1 gene [Genro et al., 2008], other polymorphisms in this gene could account for these conflicting results.

**DRD4.** The dopamine receptor D4 (DRD4) gene is also frequently studied in ADHD pharmacogenetics, mainly a 48-bp VNTR polymorphism in the exon 3. The common variants are 2R, 4R, and 7R alleles. The 7R seems to be less responsive to dopamine, reducing the intracellular concentration of second messenger cyclic AMP [Asghari et al., 1995]. It is considered a risk allele for ADHD susceptibility [Gizer et al., 2009]. Froehlich et al. [2011] showed that 4R carriers have larger reductions on hyperactive-impulsive scores across MPH doses compared to other VNTR length allele carriers with effect sizes in the range of 0.40–0.61. Patients without the 4R showed 23% reductions in their hyperactive-impulsive scores compared to placebo, while 4R carriers reduced 40–49% ($P = 0.02$). Correcting findings for the number of polymorphisms evaluated, turned results not significant. However, there was a correction by the number of outcomes assessed ($\alpha = 0.025$) and the dose–gene interaction remained significant. This result is congruent with the expression of receptor enhanced by 4R and increased sensitivity to dopamine [Asghari et al., 1995; Schoots and Van Tol, 2003] and corroborates findings from a previous study [Cheon et al., 2007]. A recent study evaluated 114 Korean children with ADHD according to the presence of 4R homozygosity. The efficacy measure was defined by changes at Korean ADHD Ranting Scale (ADHD-RS), Clinical Global Impression Improvement (CGI-I) and Severity (CGI-S) from baseline to 8 weeks of treatment. The results were non-significant between genotypes [Ji et al., 2013]. Moreover, no effect was found in adult samples [Kooij et al., 2008; Contini et al., 2012]. Although there are some evidence to suggest a MPH greater responsiveness for 4R carriers, the available data are scarce and limited to children samples.

**NET1.** The noradrenergic system is implicated on the pathophysiology of ADHD based on the mechanism of MPH and on the fact that atomoxetine is a highly specific inhibitor of NET. Kim et al. [2010] conducted a study to evaluate the association between A-3081T (rs28386840) and G1287A (rs5569) single nucleotide polymorphisms (SNPs) at the norepinephrine transporter gene (NET1 or SLC6A2) and response to MPH treatment in Korean children with ADHD. The study enrolled 112 children and employed the Clinical Global Impression-Improvement (CGI-I) score and ADHD Rating Scale (ADHD-RS) as outcome measurement. The significance level was adjusted for outcomes and SNPs investigated at $\alpha = 0.01$. A trend for good response to MPH treatment in CGI-I was observed among 61.4% of T allele carriers compared to 37.9% of A allele homozygous for the A-3081T SNP ($P = 0.03$). However, authors did not find a significant gene over dose interaction effect for this SNP on MPH response. No significant association was found between response to medication and G1287A polymorphism. The power to detect differences at $\alpha = 0.01$ was low for this sample size [Kim et al., 2010]. These results may be explained by the presence of T allele being correlated with decreased promoter activity and, consequently, reduced levels of NET within the brain [Kim et al., 2006]. On the other hand, patients who are T allele carriers may present a specific ADHD subtype, where the etiology is closely related to NET action. This might explain the better pharmacological response in this group [Kim et al., 2010]. In the following year, the same group investigated five NET1 SNPs in a larger sample but they did not replicate the association between MPH response and NET1 polymorphisms [Lee et al., 2011]. No effect was found in an adult sample [Kooij et al., 2008]. All these results taken together suggest that NET1 influence on response to MPH is small. On the other hand, as most studies are from the same group, independent replication studies are clearly needed.

Yang et al. [2012] investigated whether NET1 gene influences atomoxetine response in 111 Chinese ADHD children. The response was defined as at least a decrease of 25% from baseline on the ADHD-RS total score. Remission was defined as each ADHD-RS item score $\leq 1$ at the end of the treatment. Their findings showed that rs3785143 in NET1 had a nominally significant association with responder status; the C allele was present in 77.1% of responders, whereas T allele was observed in 55.8% ($P = 0.005$). The significance was maintained after 5,000 permutations performed for multiple test correction ($P = 0.04$). Since this SNP is located within intron 1, it is possible that its function is responsible for NET1 transcription level or it is in linkage disequilibrium (LD) with a functional SNP. The rs2279805 at NET1 was significantly associated with the status of remission: 29.8% of individuals who were C allele carriers achieved remission versus 15.5% of T allele carriers ($P = 0.02$). However, the significance disappeared after multiple test correction [Yang et al., 2012]. This SNP is located in the sixth intron of NET1 and among the region of exons 4–9, which was associated with treatment response in another study [Ramoza et al., 2009]. Effect sizes for these SNPs were not estimated in that study.

Park et al. [2012b] examined the CPT in 53 children before and after MPH treatment. This test has been proposed as a potential ADHD endophenotype [Almasy and Blangero, 2001]. In that study, differences in baseline CPT measures and CPT post-treatment changes were assessed based on G1287A and A-3081T SNPs at NET1 gene. The corrected significance level was set at $P = 0.006$. Although no significant differences in SNP frequencies were observed according to baseline CPT measures, children with the G/G
genotype at G1287A showed a greater decrease in the mean omission error scores after MPH administration compared to A allele carriers ($P = 0.006$). The effect size of the gene was not evaluated. These results suggest improvement of attention after medication that is consistent with other studies showing that the G allele induced greater MPH response [Yang et al., 2004]. In addition, the T allele carriers at A-3081T showed a greater decrease in the mean commission errors scores compared to those A/A homozygous ($P = 0.007$), meaning improved impulsive behavior [Park et al., 2012b]. As mentioned before, T allele carriers may present lower NET levels within the brain, which might explain the better MPH response by having to block fewer NET to achieve response. Kim et al. [2013a] analyzed the association between neuropsychological measurements and G1287A and A-3081T SNPs at NET1 gene after MPH treatment. The study enrolled 101 ADHD children and employed Comprehensive Attention Test (CAT), which are visual and auditory selective attention task, sustained attention task, and flanker interference task; which measure the changes in response time variability. The corrected significance level was set at $\alpha = 0.0034$. No significant difference between the genotypes was found, but there was an additive effect of A allele at G1287A SNP in the auditory selective attention task at an uncorrected level ($P = 0.02$) [Kim et al., 2013a].

It is important to notice that these NET1 findings were derived from Asian samples. Different ethnic samples can present different genotype frequencies and this diversity may result in different medication response.

ADRA2A. The ADRA2A receptor is a central component of the noradrenergic system with a putative role on MPH action demonstrated in studies with animal models [Arnst and Dudley, 2005; Andrews and Lavin, 2006]. Previous pharmacogenetics studies with the adrenergic alpha 2A gene (ADRA2A) have demonstrated that G allele at SNP C-1291G (rs1800544) is associated with greater reduction of inattentive symptoms over time [Polanczyk et al., 2007b; da Silva et al., 2008]. Froehlich et al. [2011] also found a main effect of this ADRA2A genetic variant (C-1291G) on MPH response ($P = 0.003$). However, the G allele was associated with significantly higher rates of hyperactive-impulsive symptoms on placebo and across doses. The gene–dose interaction was below the threshold of statistical significance ($P = 0.03$) No association with inattentive improvements was found in that study. Yang et al. [2012] investigated a haplotype with two SNPs at ADRA2A (rs1800544 and rs553668) and described an association between GG haplotype and non-remission of ADHD symptoms with atomoxetine treatment, but the significance disappeared after multiple test correction. It is possible that GG haplotype may determine inadequate functioning of a2 receptor, which could lead to less improvement [Yang et al., 2012]. Kim et al. [2013a] showed the changes in mean response time variability increased additively with presence of G allele at MspI SNP for flanker interference task. The effect size was 0.09 based on Cohen’s $d$. For the sustained attention task, the additive effect of G allele was only detected at an uncorrected level [Kim et al., 2013a]. No effect of ADRA2A was found in an adult sample [Contini et al., 2011]. As mentioned earlier the potential reasons for this diversity of findings are described in Table II.

COMT. The catechol-O-methyltransferase enzyme catalyzes both dopamine and norepinephrine, especially in the prefrontal cortex. The most studied SNP at the catechol-O-methyltransferase gene (COMT) is a functional polymorphism, which leads to amino acid substitution valine to methionine (Val158Met—rs4680). The Met allele results in a threefold to fourfold reduction in enzyme activity [Lotta et al., 1995]. A study from our group tested the effect of the COMT gene in response to MPH on oppositional symptoms in 251 ADHD children and adolescents. In that investigation ADHD boys with at least one Met allele have a significantly higher improvement on oppositional defiant disorder symptoms over time than Val homozygous when they are treated with MPH [Salatino-Oliveira et al., 2011]. It has been hypothesized that comorbidity between ADHD and oppositional defiant disorder is due to shared genetic liability either operating directly or indirectly through gene–environment interactions [Nadder et al., 2002]. There are evidences that the high-activity COMT genotype in ADHD may play a role in the manifestation of antisocial behavior [Langley et al., 2010]. Moreover, COMT may play a regulator role in catecholamine balance created by MPH action in the prefrontal cortex [Chen et al., 2004]. Froehlich et al. [2011] evaluated the same SNP and showed that Val homozygous had greater improvement in hyperactive-impulsive symptoms with increasing doses when compared to other groups, but this finding did not achieve statistical significance. However, this pattern is consistent with previous pharmacogenetics studies that have suggested a positive association between Val allele and response to MPH [Cheon et al., 2008; Kereszterwi et al., 2008]. No effect was found in an adult sample [Contini et al., 2012]. COMT association with medication response may be restricted to some symptoms and further studies in children as well as in adults are warranted.

TPH2 and DBH. The tryptophan hydroxylase-2 (TPH2) is a brain-specific enzyme involved in serotonin biosynthesis, converting the amino-acid tryptophan to 5-hydroxytryptophan, which is further decarboxylated into serotonin [Walther et al., 2003]. Serotonin dysregulation has been related to impulsive and aggressive behaviors in children, and has thus been hypothesized to play a causal role in ADHD [Lucki, 1998]. The dopamine beta hydroxylase enzyme (DBH) catalyzes the conversion of dopamine (DA) into norepinephrine (NE) and is expressed within the secretory vesicle [Gaspar et al., 1989]. Since changes in both these catecholamine networks could be underlying ADHD pathophysiology, the genes that encode these two enzymes were considered candidate genes for ADHD susceptibility [Roman et al., 2002a]. These genes were investigated in an adult sample by Contini et al. [2012]. No significant association of either gene with response to MPH was reported. Given their function, these enzymes might interfere on responsiveness, but this was not demonstrated in that single study.

5-HTT. The potential role of serotonin in ADHD was addressed by Gainetdinov et al. [1999]. These investigators observed reduced hyperactivity after a selective serotonin reuptake blocker (fluoxetine) administration to mice whose DAT gene was knocked out. The serotonin transporter (5-HTT) is responsible for the presynaptic re-uptake of serotonin. A 44 bp insertion/deletion functional polymorphism in the 5-HTT gene promoter region (HTTLPR) leads to changes in expression of this transporter in humans. The basal activity of the long variant (L) is about threefold higher than the short (S) variant [Heils et al., 1996]. A functional SNP (A > G—rs25531) has also been identified within the LPR
region, located in the L allele, resulting in its division into two distinct alleles (L_A and L_C); the L_A variant is associated with 5-HTT mRNA higher levels while the L_C and S variants are associated with lower transcriptional activity [Lipsky et al., 2009].

Thakur et al. [2010] conducted a study to evaluate the association between 5-HTTLPR and behavior response to MPH. A total of 157 children with ADHD were assessed using Conners’ Global Index for parents and teachers (CGI-Parents and CGI-Teachers) during treatment with placebo and MPH. They identified an association between the triallelic 5-HTTLPR polymorphism and behavioral response to MPH assessed by CGI-Parents. L_A-L_A individuals presented significant improvement with MPH and responded minimally to placebo; homozygous children for the low expressing alleles (S + L_C = S’) responded significantly better to placebo and did not have additional improvement with MPH, whereas S’L_A genotype had an intermediate profile. No association between 5-HTTLPR polymorphism and therapeutic response as assessed by teachers was reported [Thakur et al., 2010]. These results showed that patients had significant behavioral response independently from the treatment with the active drug or placebo. These findings are difficult to integrate to previous results because it started as a comparative investigation (MPH vs. placebo), but it was decomposed in two naturalistic investigations in which the genetic effects were assessed independently. No effect was found in an adult sample with both 5-HTTLPR and serotonin HTR1B receptor gene (HTR1B) (rs11568817, rs6296, and 13212041) [Contini et al., 2012]. These negative results suggest that serotoninergic genes may not be involved with response to MPH either in children or adults, but more studies with different SNPs are needed.

**BDNF.** Given that ADHD is often regarded as a neurodevelopmental disorder, genes affecting neuroplasticity and neuronal development are a new set of candidate genes. One such gene is the brain-derived neurotrophic factor (BDNF), which is involved in several processes including differentiation and survival of dopaminergic and serotonergic neurons [Hyman et al., 1991; Henningsson et al., 2009]. The most studied BDNF SNP is Val66Met (rs6265). This polymorphism may impact intracellular trafficking and BDNF activity-dependent secretion, and might be related to ADHD pathophysiology [Kent et al., 2005]. MPH response analyses according to BDNF Val66Met polymorphism were reported in a prospective study. The significance level was set at $P = 0.0017$, because they evaluated seven SNPs (one in the BDNF and six from other genes) and four outcomes. The total sample comprised 102 ADHD children and response status was assessed by CGI-I scores and ADHD-RS. The results pointed to a higher proportion of symptom remission (95.2%) in Val/Val homozygous than in Met-carrier children (74.1%) ($P = 0.013$). Val allele homozygous were more frequently (81%) assessed as “not ill” or “very mild” according to CGI-I compared to 37% of Met allele carriers ($P = 0.0002$). Moreover, more than 50% reduction in ADHD-RS scores was observed in 95.2% of the Val allele homozygous patients and in 74.1% of Met allele carriers ($P = 0.018$) [Kim et al., 2011]. The Val/Val genotype has been related to increased activity-dependent secretion of BDNF [Flanagan et al., 2006]. Met allele carriers showed decreased volume in the dorsolateral prefrontal cortex and subcortical regions [Pezawas et al., 2004]. An explanation for better MPH response in ADHD children who are Val/Val homozygous might be due to a lower degree of brain anatomical deficit and functional impairment in these individuals [Kim et al., 2011].

**SNAP25.** Another neurodevelopmental gene is synaptosomal-associated protein 25 (SNAP-25), a neuron-specific protein implicated in exocytotic catecholamine release. Contini et al. [2012] evaluated two SNPs (rs3746544 and rs363020) on MPH response in 164 adults with ADHD and no effect was found. Although several evidences suggest a role for these genes is ADHD susceptibility, their role on response to medication remains to be determined.

**LPHN3.** Recently a new gene has been associated with ADHD pathogenesis, the latrophilin 3 gene (LPHN3). The association between markers at chromosome 4q13.2 (near LPHN3) and ADHD was first observed in a linkage study of a large multigenerational families in a population isolate, the Paisa from Colombia, where the prevalence of ADHD is high [Arcos-Burgos et al., 2002]. LPHN3 is a member of the LPHN subfamily of G-protein coupled receptors (GPCRs), which have been shown to be important for exocytosis of neurotransmitter regulation [Rahman et al., 1999; Linet’ska et al., 2002]. A subsequent study by Arcos-Burgos et al. [2010] examined extensively LPHN3; stimulant response was assessed in 240 children from a US sample. ADHD symptoms were rated on and off stimulant medication on the Strengths and Weaknesses of ADHD-Symptoms and Normal-Behavior (SWAN) scale. A significant association between response to stimulant medication and SNP marker rs6551665 was found both at the marker and haplotype level. Individuals with either one or two G alleles had a better response to medication in three out of nine questions which compose the inattentive dimension and one out of nine questions that integrated the hyperactive/impulsivity dimension of SWAN scale. When the authors analyzed scale full score, they found an association for the inattentive dimension, but not for the hyperactive/impulsivity dimension [Arcos-Burgos et al., 2010].

The effect of six SNPs within the LPHN3 gene on MPH response was evaluated in an independent sample of 416 children with ADHD. The main outcomes were evaluated with the Restricted Academic Situation Scale Task (RAST) and motor activity as measured by a hand held automatic device (Actiwatch) for hyperactivity dimension. Four SNPs (rs1947274, rs2345039, rs6551655, and rs6858066) have a significant effect in discriminating good responders from non-responders [Labbe et al., 2012]. These authors reported that the rs6858066 G allele confers both risk to ADHD and better treatment response. Similar findings were reported in an adult sample [Ribasés et al., 2011]. Labbe et al. [2012] also reported that the G allele of SNP rs6551665 is associated with poor response to treatment, whereas Arcos-Burgos et al. [2010] found an association with a better response with this same SNP. These authors do neither hypothesize a functional role for these SNPs nor for LPHN3 in MPH response. Since the first study found an association with inattentive dimension and the second one with the hyperactive dimension, it is possible that differences in sample subtype composition in terms of symptoms might be responsible for divergent results in LPHN3 pharmacogenetics studies. Inconsistent findings in many genes appear again as in previous reviews. The heterogeneity of outcomes measures and population stratification could explain some of the conflicting results. However, several studies corrected the analysis and demonstrated that most positive associations showed small effect sizes.
for the studied genes. Executive functions analyzed above are potentially useful as endophenotypes. They are hypothesized to be more suitable for detecting risk genes because they are genetically less complex by being etiologically closer to biological pathway leading from gene to behavior, diminishing phenotypic heterogeneity [Castellanos and Tannock, 2002].

**Gene–Gene Interactions Studies**

Investigations on individual genetic polymorphisms may not be the best approach for pharmacogenetic studies, since genetic factors might work primarily through a complex mechanism that involves multiple genes and, possibly, environmental factors. Thus, the effect of the gene/polymorphism might be missed if the single gene is examined without allowing for its potential interactions with these other unknown factors. One way to assess this relationship is conducting gene–gene interaction studies [Cordell, 2009].

Hong et al. [2012] investigated independent and interaction effects of DAT1, DRD4, ADRA2A, and NET1 genes on MPH response in 103 children with ADHD, using ADHD-RS and CGI-I scores as outcome measures of treatment response. The results suggest some independent positive findings for SNPs in NET1. For the G1287A polymorphism, the proportion of good responders was 30.2% for G/G homozygous children and 16.0% for A-allele carriers children (P = 0.08). Considering the A-3081T SNP, 28.9% of T-allele carriers were good responders when compared to 7.4% with the A/A genotype (P = 0.02). Considering the significance level set for the analyses after correction for multiple comparisons (α = 0.0083), multivariate logistic regression demonstrated effects for interactions between the DRD4 VNTR genotypes and those of either the ADRA2A DraI (rs555668) (P = 0.0004) or the NET1 A-3081T polymorphisms on MPH response (P = 0.01) (trend); significant interaction effects were also detected between the ADRA2A DraI polymorphisms genotypes and those of either the NET1 G1287A or the A-3081T polymorphisms (P = 0.0066 and 0.0003, respectively). In addition, trends for interaction effects were detected between the DAT1 VNTR genotypes and the NET1 A-3081T polymorphisms (P = 0.06); and between DRD4 VNTR genotypes and the ADRA2A C-1291G polymorphisms (P = 0.06). However, given the small group sizes produced by dividing the sample according to genotypes and response status, the chance of type II errors probably increased, negatively influencing the power of these analyses (Nagelkerke R² = 0.40) [Hong et al., 2012].

Jain et al. [2012] screened possible interacting regions within LPHN3 gene and performed a correlation subset analysis. The rs6551665 LPHN3 associated SNP showed an interaction effect with a haplotype harbored on 11q. This haplotype (rs666642–rs877137) encompasses the neuronal cell adhesion molecule 1 (NCAM1) and ankyrin repeat and kinase domain containing 1 (ANKK1) genes. This pharmacogenetic study evaluated 82 individuals with ADHD. The effect of LPHN3–11q interaction was examined using SWAN scores (questions 1–9 indicate inattentive symptoms and questions 10–18 indicate hyperactivity symptoms) during treatment. The results demonstrated a significant two locus model effect compared with a single locus effect for question 18 (P = 0.0036), when additive effects at LPHN3 and dominant effect at 11q are considered. The GG genotype at rs6551665 together with two copies of the susceptibility haplotype on 11q (G-G) were correlated with significant improvement of symptoms in question 18 after treatment, whereas the AA genotype at rs6551665 and fewer than two copies of 11q haplotype were correlated with poorer response. This result should be interpreted cautiously, as the analysis of one question cannot be extended to the full hyperactive dimension. Further investigations are necessary to elucidate the biological mechanism of gene products on 11q and LPHN3 to determine how this interaction plays [Jain et al., 2012].

Gene–gene interactions have been increasingly explored in ADHD susceptibility studies, and they are becoming more attractive and studies investigating these interactions have emerged in recent years in the pharmacogenetics field. It is important to notice that the small effect size of candidate genes studies reviewed here suggests that response to MPH is influenced by several different genes and they can interact with each other. However, in the studies addressed here no a priori hypotheses were clearly established or biological pathways were determined for these interactions.

**Genome-Wide Association Studies**

By scanning the whole genome and identifying promising areas, the genome-wide approach might be relevant to ADHD pharmacogenetic investigations. One of the first genome-wide association studies in pharmacogenetics evaluated response to transdermal MPH in 187 children with ADHD. No association at genome-wide statistical significance was observed. However, among the top findings, the glutamate receptor, metabotropic 7 gene (GRM7) and two SNPs within NET1 showed potential involvement in MPH response [Mick et al., 2008]. This possible association with NET1 is consistent with results previously described in the candidate gene studies detailed above.

Increase in blood pressure is a potential adverse effect of MPH treatment [Cortese et al., 2012]. Recently, Mick et al. [2011] conducted a genome-wide association analysis of blood pressure response to MPH in 140 children. SNPs (316,934) were available for analysis. Due to the small sample size for GWAS and a heterogeneous sample, they did not identify a genome-wide statistically significant association between MPH treatment and changes in blood pressure. Suggestive findings involved genes functionally related to blood pressure regulation and other cardiovascular phenotypes, as a SNP in K<sup>+</sup>-dependent Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (SLC24A3). A genetic enrichment analysis implicated five biological processes: FERM domains, immunoglobulin domains, the transmembrane region, channel activity and type-III fibronectins [Mick et al., 2011]. This set of genes may be involved in adverse effects and should be further explored.

To date, only two GWAS in ADHD pharmacogenetics have been applied to identify genetic variants associated with medication response or adverse effect. Both have failed to identify genes at the stringent genome-wide significance level. GWAS with larger samples are required to detect moderate or small effect of genes involved with treatment response and may contribute to discover
other molecular targets in less obvious pathways relate to ADHD pathophysiology.

Gene–Environment Interaction Studies

In addition to genetic factors, environmental contributions might influence phenotypic variability and also clinical aspects of the disorder, as severity and comorbid profile. Chazan et al. [2011] demonstrated that an adverse environment can predict a worse MPH response in ADHD children. In gene–environment studies, it has been hypothesized that genotypes modulate response to environmental risk factors and may play a pivotal role in the disorder. Grizenko et al. [2010] assessed differences between ADHD subtypes (combined/hyperactive vs. inattentive) taking into account genetics factors (DAT1 VNTR, DRD4 VNTR, and 5-HTTLPR), comorbidities, environments factors and response to MPH treatment in 371 children with ADHD. Treatment response was evaluated by Conners’ Global Impression Scale for parents and teacher (CGI-P and CGI-T, respectively). Comorbidity and stress during pregnancy were also assessed for each participant. Children with ADHD combined/hyperactive subtypes presented higher co-morbid rate of conduct disorder, higher frequency of L/L genotype for the 5-HTTLPR, good response to treatment and were exposed to moderate stress during their mothers’ pregnancy when compared to children with the inattentive subtype. No differences were found across subtypes for the DAT1 and DRD4 genes. Moreover, a combination of the L/L genotype and stress during pregnancy lead to an eight times higher risk of having combined/hyperactivity subtype, and this may be one of many possible mechanisms linking stress to genotype in ADHD development [Grizenko et al., 2010]. Notice that this study did not evaluate if genes influence medication response by itself. However, it demonstrated that ADHD subtypes might lead to different disorder outcomes concerning treatment response. Effects of genotypes, comorbidities, and environmental factors on disease may also differ among ADHD subtypes. These features must be considered in future pharmacogenetics studies. It has been demonstrated that hyperactive children respond more to pharmacotherapy, but the non-hyperactive ones need lower MPH doses to improve symptoms [Barkley et al., 1991; Stein et al., 2003]. The study of pooled samples comprising all subtypes could lead to heterogeneous results.

A study examined the role of SNPs in LPHN3 in MPH response, taking into account maternal smoking and stress during pregnancy in 132 nuclear families. Main outcomes were Conners’ Global Index for parents and teachers, and the clinical staff completed the Clinical Global Impression scale. Several association tests were conducted using each SNP separately. Since most of them were associated with the three tag SNPs (rs6551665–rs1947274–rs6858066), haplotype analysis was also performed. Considering the CGI-overall improvement scale, the risk haplotype AAG was associated with poor treatment response in the mild or minimal maternal stress group (P = 0.01), while the GCA haplotype have a better improvement with treatment (P = 0.03). However, considering Conners’ Global Index for parents and teacher, this statistical significance was not maintained. It is interesting to notice different association between CGI for parents and teachers as well as clinical response, possibly due to reference bias [Choudhry et al., 2012]. This finding is in agreement with the one from the first LPHN3 study where the G allele from the rs6551665 leads to a good response to MPH treatment [Arcos-Burgos et al., 2010]. However, it differs from Labbe et al. study [2012] results where the G allele at rs6858066 confers better response and G allele at rs6551665, poor response. Despite the small number of studies with LPHN3, it is possible that this gene influences clinical response only in favorable environments.

Thakur et al. [2012] conducted a study to evaluate the association between 30 tag SNPs within NET1 and response to MPH treatment in children with ADHD, with stratification based on maternal smoking during pregnancy. The study enrolled 475 children evaluated by Conners’ Global Index, Clinical Global Impression, and the Restricted Academic Situation Scale (RASS). The significance level after Bonferroni correction was set at α = 0.002. The authors observed that the T allele at rs36021 was associated with more behavioral and cognitive deficits in the subsample which mothers smoked during pregnancy. In response to MPH, the T allele was associated with greater improvement on the CGI (P = 0.001), Conners’ (P = 0.009), and RASS (P = 0.0003). On the other hand, in the sample in which mothers did not smoke during pregnancy, the C allele at rs3785152 was associated with significant improvement on CGI (P = 0.0005) and RASS task disengagement (P = 0.0003). As both SNPs are located within introns, they might be involved in gene regulation or be in LD with functional variants [Thakur et al., 2012]. It is interesting to note that the same rs36021 allele seems to interact with exposure to maternal smoking and leads to both worst behavioral and cognitive functions and better response to MPH treatment. It is possible to speculate that a severe case may present greater room for improvement than other cases, so the effects of pharmacotherapy may be easier to be detected in this group.

Environmental factors are involved in ADHD etiology and evidence demonstrates that these factors also influence gene associations. The studies reported here show that environment is an important feature to be considered and evaluated in pharmacogenetic studies as they may alter response to medication and gene effects. Also, studies considering G×E interactions may contribute to explore the controversial results from various genes.

Neuroimaging Studies

Neuroimaging measurements constitute endophenotypes of great interest and have already been applied in several studies to investigate neurobiological effects of risk genes in ADHD [Tost et al., 2012]. However, this methodology was employed in few pharmacogenomic studies. Szobot et al. [2011] evaluated ADHD risk alleles at DRD4 and DAT1 genes and striatal DAT occupancy after treatment with MPH. A total of 17 children with ADHD and substance use comorbidity underwent a single-photon emission computed tomography (SPECT) at baseline and after 3 weeks on MPH; the results showed that the combination of DRD4 7R allele and 10R homozygosity at DAT1 was significantly associated with a smaller DAT occupancy in caudate and putamen, bilaterally (P = 0.02–0.006). The R² of these analyses ranges from 0.50 to 0.56. These associations are lower if genotypes are not included. Striatal DAT occupancy was not significant for each genotype considered separately (P ≥ 0.08). Drug use had an independent
effect in all brain areas (except left putamen) and there was no significant MPH dose effect. It is possible to speculate that there is an additional effect of both DRD4 risk allele and risk genotype at DAT1, leading to a less efficient MPH occupancy [Szobot et al., 2011].

One study investigated MPH response-related hemodynamic changes according to SNAP-25 polymorphisms. The functional near-infrared spectroscopy (fNIRS) was evaluated in 16 children right-handed with ADHD on or off MPH with an interval of 24 hr. Outcomes assessed were the difference of oxyhemoglobin (HbO2) and deoxyhemoglobin (HHb) levels recorded during incongruent stimuli and neutral stimuli. They showed that Mnull (rs3746544) genotype at the SNAP-25 gene was significantly associated with HbO2 levels change in right prefrontal (P = 0.015) and HHb levels in left prefrontal (P = 0.033) regions with MPH treatment; mean left prefrontal HHb concentration increased during MPH use in patients with Mnull G-allele carriers, whereas it decreased in patients with T/T genotype. The right prefrontal HbO2 concentration increased in the T/T and decreased in the T/G or G/G group. The SNAP-25 Ddel polymorphism (rs10513132) was significantly associated with change of right prefrontal HHb concentration with MPH use (P < 0.001). HHb levels in right prefrontal increased with MPH treatment in the Ddel C-allele carriers and decreased in the T/T group. When both Ddel and Mnull genotypes were taken into account, the genotype status was significantly associated with right HHb concentration change (P = 0.003). Pairwise comparisons revealed that children with Ddel C-allele carriers and Mnull G-allele carriers had significantly different right HHb concentration changes when compared with children with Ddel T homozygous and Mnull T homozygous or Ddel T homozygous and Mnull G-allele carriers genotypes (P = 0.05 and < 0.001, respectively). They did not find association between genotype and treatment with behavioral performance. The blood flow cerebral increases during brain activation, but not all of oxygenized blood is used, so HbO2 increases and HHb decreases during sustained activation. Higher HbO2 levels and lower HHb levels might be related with neurovascular coupling and increased blood flow with HHb levels from activated brain region. Individual T homozygous in both SNPs did not increase HHb in right prefrontal, suggesting genotypes differently affect neurovascular coupling [Öner et al., 2011]. It is difficult to compare this study with other previous studies because they did not evaluate symptomatic response and comorbidity as well as did not split groups between responders or non-responders.

In another study, SPECT was performed in 37 drug-naïve ADHD children before and after treatment with MPH according to genotype for NET1 G1287A and A-3081T polymorphisms. The clinical evaluation relied on ADHD-IV and CGI-I scores. Before MPH treatment, no significant differences in cerebral perfusion were observed between children with different NET1 genotypes. After treatment, they found that children with G1287A G/G genotype showed more symptoms improvement as compared to A-allele carriers (P = 0.022). Hyperperfusion was observed in the right inferior temporal gyrus and left middle temporal gyrus of children with G/G genotypes compared to those without this genotype (P < 0.001 for both). No significant perfusion differences were observed in association with the A-3081T SNP. This finding should be interpreted with caution, as it was significant only at the uncorrected threshold, but it suggests that G1287A may contribute to an intermediate phenotype [Park et al., 2012a].

Although promising, the number of neuroimaging studies currently available is limited. The lack of uniformity of methodologies, outcomes investigated, and replication makes the interpretation of these results a difficult task.

**Drug Metabolism Genes and Adverse Events**

Besides the study of response to medication itself, adverse events have attracted great interest lately. The carboxylesterase 1 (CES1) gene encodes the main enzyme involved in MPH metabolism. In a naturalistic study, Bruxel et al. [2013] assessed 213 Brazilian children with ADHD based on −75 T > G CES1 (rs3815583) polymorphism. The primary outcome was appetite reduction, the most reported MPH adverse effect, measured by the Barkley Stimulant Side Effect Rating Scale. The G-allele carriers had worse appetite reduction scores than T/T homozygous over time of treatment (P = 0.03); G-allele carriers presented a 3.5 times higher risk to have the highest appetite reduction scores when compared to T allele homozygous (P = 0.009) with effect size based on Cohen’s d of 0.02. A trend effect of the daily mean MPH dose was also observed (P = 0.08). The SNP functionality is unknown, but since it is located at the 5’ UTR it could have an effect on gene regulation [Bruxel et al., 2013].

Cardiovascular adverse effect due to MPH treatment has also been reported. SNPs at norepinephrine genes (NET1 G1287A, NET1 A-3081T, ADR2A Del, ADR2A C-1291G) were investigated in 101 Korean children with ADHD. Electrocardiographic parameters (PR, QRS, AT intervals), heart rate (HR) resting, seated pulse, and blood pressure (BP) were evaluated. The data revealed that children with the ADR2A C-1291G C/C genotype showed an 18.5% increase in diastolic blood pressure (DBP) when compared to baseline, but children with the G/G or G/C genotype showed only a 0.2% decrease after MPH administration. Individuals with a NET1 A-3081T T/T genotype showed 12.5% increase in HR compared to baseline; whereas children with the A/T or A/A genotype showed a 3.5% and 2.5% increase after treatment, respectively [Cho et al., 2012]. Two previous studies reported that the sympathomimetic effects of norepinephrine activation by MPH along with activation of dopamine systems might lead to increases in systolic blood pressure (SBP), DBP, and HR at therapeutic doses [Volkow et al., 2003; Negray et al., 2009].

Recently, the relationship of stimulant side effects and dopamine receptor genes (DRD1 rs4532, DRD2 rs6277, DRD3 rs6280 DRD4 7932167, and COMT rs4680) was evaluated in 90 Caucasian children. The outcome was the modified Barkley Side-effect scale, which was reduced to three factors (nausea, social withdrawal, and irritability) using principal components analysis, which accounted for 48.2% of the variance. The allelic status was coded by presence of minor, heterozygous, and major homozygous. The main findings demonstrated a significant effect of the minor allele homozygous for DRD1 polymorphism (rs4532) with more severe side effect for withdrawal and a trend for nausea after Bonferroni correction. This SNP is located in the 5’ UTR and it may affect gene expression [Levy et al., 2013].

Another study examined the association between adverse effects due to MPH treatment and ABCB1 gene. It encoded efflux...
tions described that TT homozygous have nine times higher risks of adverse effects when compared to other genotypes ($P = 0.005$). A functional assay was also performed to determine if SNPs variability differ in MPH transport. It was observed that the $T$ allele reduced ABCR1 MPH-transporting activity across the cell membrane when compared to $A$ or $G$ allele. These results suggest that $T$ homozygous children could have reduced P-glycoprotein function, leading to more absorption due to diminished efflux activity and therefore more MPH bioavailability, resulting in severe adverse effects [Kim et al., 2013b].

Park et al. [2014] conducted a study to evaluate the association between adverse events of MPH and Neurotrophin 3 (NTF3) SNPs in 96 ADHD children. NTF3 is a neurotrophic factor that involved in the differentiation, development, maintenance, and survival of various kinds of neurons [Maness et al., 1994]. Two SNPs were selected, rs6332 and rs1805149, from tag SNPs of NTF3. The polymorphism rs6332 located in exon 3 was associated with ADHD susceptibility, intelligence, and selective attention deficit [Syed et al., 2007; Cho et al., 2010]. The gene seems to have a role in the pathophysiology of mood disorders [Duman, 2002]. Adverse events were assessed using the Barkley Stimulant Side Effect Rating Scale during 2 weeks of treatment. Principal components analysis was conducted to reduce the number of variables. Six factors were selected (emotionally, disengagement, aches/tics, over-focus/euphoria, sleep/appetite, dizziness/drowsiness), which accounted for 63.1% of variance. Each factor had more than one item of Barkley Rating Scale. The results demonstrated the $A/A$ homozygous at rs6332 presented the highest emotionally and over-focus/euphoria factor scores ($P = 0.042$ and $P = 0.045$, respectively). When the subjects were divided according to rare allele, individuals with the $A$ allele had statistically higher emotionally factor score compared to individuals without this allele. Also, they divided according to common allele and found that patients with the $G$ allele had lower over-focus/euphoria factor score compared to patients without the $A$ allele. The authors showed the emotional adverse event was associated with rs6332. So, they analyzed each item of emotional factor. Subjects $A/A$ homozygous showed the highest proneness to crying and nail biting scores compared to other genotypes. Subjects with $A$ allele showed lower nail biting compared to subjects without, respectively, allele. The rs1805149 SNP did not present any significant association. The results demonstrated that NTF3 SNP is associated with specific emotional side effects, corroborating with the possible role the NTF3 gene in emotional problems [Hock et al., 2010; Fernandess et al., 2010]. However, a limitation of this study is that the analyses were not corrected for multiple comparisons; no effect size of the SNP was presented [Park et al., 2014].

**INTEGRATIVE COMMENTS**

In recent years, two phenomena can be identified in ADHD pharmacogenetic field. First, the number of studies continues to grow, making ADHD one of the mental health areas with more pharmacogenetics studies. This was clearly exemplified in a previous review on pharmacogenetics of child psychiatric disorders. It identified only one study in autism pharmacogenetics and another one in major depressive disorder or anxiety disorder, in comparison to the 33 studies in ADHD pharmacogenetics [Polanczyk et al., 2010]. Second, and probably most important, there has been a change of focus concerning ADHD pharmacogenetic studies. There are a decreasing number of published studies targeting dopaminergic genes while more attention has been given to noradrenergic genes (see Table 1). This could be explained by the large amount of DAT1 and DRD4 studies without consistent genetic effects in clinical response. Moreover, as we stressed in our previous
<table>
<thead>
<tr>
<th>Refs.</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>Design</th>
<th>Sample characteristics</th>
<th>Medication, dose</th>
<th>Primary outcome measure</th>
<th>Primary results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Froehlich et al. [2011]</td>
<td>DAT1</td>
<td>3' UTR 40-bp VNTR</td>
<td>RCT, double-blind, multiple dose, placebo, 4w (n = 89)</td>
<td>Mean age: 8.13y, 73% boys, 79% Caucasian</td>
<td>MPH</td>
<td>Vanderbilt ADHD rating scales for parents and teachers</td>
<td>Children without 10R presented great improvement at hyperactivity symptoms</td>
</tr>
<tr>
<td></td>
<td>DRD4</td>
<td>Exon 3 48-bp VNTR</td>
<td></td>
<td></td>
<td></td>
<td>Mean maximum dose: 1.57 mg/kg/day</td>
<td>Children without 4R presented less improvement hyperactivity symptoms</td>
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<tr>
<td></td>
<td>COMT</td>
<td>rs4680 [Val158Met]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No association</td>
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<tr>
<td></td>
<td>ADRA2A</td>
<td>C-1291G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No association</td>
</tr>
<tr>
<td></td>
<td>DAT1</td>
<td>3' UTR 40-bp VNTR</td>
<td>Naturalistic, 1mo (n = 171)</td>
<td>Mean age: 35y</td>
<td>MPH-IR</td>
<td>Good response: reduction ≥ 30% at Swanson, Nolan, and Pelham Rating Scale version IV (SNAP-IV) and Clinical Global Impression-Severity (CGI-S): 1–2 points</td>
<td>No association</td>
</tr>
<tr>
<td></td>
<td>Intron 8</td>
<td>240-bp VNTR</td>
<td></td>
<td>52% male European–Brazilians</td>
<td>Minimal dose: 0.3 mg/kg/day</td>
<td></td>
<td>10R carrier showed great improvement in response inhibition</td>
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<tr>
<td></td>
<td></td>
<td>C-839T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10R homozygous presented great improvement in planning ability</td>
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<tr>
<td></td>
<td></td>
<td>3' UTR 40-bp VNTR</td>
<td>Clinical trial, 24w (n = 108)</td>
<td>Mean age: 9.9y, 71.2% male Korean</td>
<td>Short acting-MPH</td>
<td>Executive functions</td>
<td>10R homozygous showed great improvement in working memory</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Mean dose: 0.5 mg/kg/day</td>
<td></td>
<td>No association</td>
</tr>
<tr>
<td>Ji et al. [2013]</td>
<td>DRD4</td>
<td>Exon 3 48-bp VNTR</td>
<td>Case-control, 8w</td>
<td>Mean age: 9.6y, 71.2% male Korean</td>
<td>OROS-MPH</td>
<td>Clinical Global Impression-Improvement (CGI-I)</td>
<td>No association</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n = 114 cases and 84 controls</td>
<td>Minimal dose: 18 mg/day</td>
<td>Clinical Global Impression-Severity (CGI-S) ADHD-RS</td>
<td>Children T allele carriers presented better response;</td>
</tr>
<tr>
<td></td>
<td>NET1</td>
<td>A3081T [rs28386840]</td>
<td>Naturalistic, 8w (n = 112)</td>
<td>Mean age: 9.1y, 82% boys Korean</td>
<td>MPH</td>
<td>ADHD-RS good response: 1–2 points at Clinical Global Impression-Improvement (CGI-I) ADHD-RS</td>
<td>No association</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1287A [rs5569]</td>
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<tr>
<td>Refs.</td>
<td>Gene</td>
<td>Polymorphism</td>
<td>Design</td>
<td>Sample characteristics</td>
<td>Medication, dose</td>
<td>Primary outcome measure</td>
<td>Primary results</td>
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<tr>
<td>Lee et al. [2011]</td>
<td>NET1</td>
<td>rs2242446</td>
<td>Clinical trial, 8w (n = 112)</td>
<td>Mean age: 10.2, 83% boys Korean</td>
<td>OROS-MPH</td>
<td>Good response: 1–2 points at CGI-I and CGI-S; reduction ≥ 50% at ADHD-RS</td>
<td>No association</td>
</tr>
<tr>
<td>Yang et al. [2012]</td>
<td>NET1</td>
<td>rs3785143</td>
<td>Prospective, clinical trial, 8–12w (n = 111)</td>
<td>Mean age: 9.6y, 82.9% male Chinese</td>
<td>Atomoxetine</td>
<td>Response: reduction ≥ 25% ADHD-RS</td>
<td>C allele was the most presented in responders</td>
</tr>
<tr>
<td></td>
<td>NET1</td>
<td>rs2279805</td>
<td></td>
<td></td>
<td>Dose range 0.5–1.4 mg/kg/day</td>
<td>Remission: score ≤ 1 ADHD-RS</td>
<td>Individuals C allele carriers achieved remission</td>
</tr>
<tr>
<td></td>
<td>ADRA2A</td>
<td>rs1800544</td>
<td></td>
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<td></td>
<td></td>
<td>These SNPs in GG haplotype were associated with non-remission of symptoms</td>
</tr>
<tr>
<td>Park et al. [2012b]</td>
<td>NET1</td>
<td>A-3081T (rs28386840)</td>
<td>Clinical trial, 8w (n = 53)</td>
<td>Mean age: 8.9y, 84.9% male Korean</td>
<td>MPH</td>
<td>CPT</td>
<td>T-allele carriers showed a greater decrease in the mean commission errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1287A (rs5569)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Children G/G showed a greater decrease in the mean omission error scores</td>
</tr>
<tr>
<td>Contini et al. [2011]</td>
<td>ADRA2A</td>
<td>C -1291G</td>
<td>Naturalistic, 1mo (n = 165)</td>
<td>Mean age: 35y, 54.5% male European–Brazilians</td>
<td>MPH-IR</td>
<td>Good response: reduction ≥ 30% at SNAP-V and CGI-S: 1–2 points</td>
<td>No association</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G -262A</td>
<td></td>
<td></td>
<td>Minimal dose: 0.3 mg/kg/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim et al. [2013a]</td>
<td>NET1</td>
<td>G1287A (rs5569)</td>
<td>Prospective study, 12w (n = 101)</td>
<td>Mean age: 8.7y, 80% male Korean</td>
<td>OROS-MPH</td>
<td>Comprehensive Attention Test (CATT)</td>
<td>Additive effect of A allele in auditory selective attention task (uncorrected) on better response</td>
</tr>
<tr>
<td></td>
<td>ADRA2A</td>
<td>A-3081T (rs28386840)</td>
<td></td>
<td></td>
<td>Initial mean dose: 0.24 mg/kg/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs553668</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No association</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-1291G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Additive effect of G allele in flanker interference task on better response</td>
</tr>
<tr>
<td>Contini et al.</td>
<td>5-HTT</td>
<td>5-HTTLPR</td>
<td>Naturalistic, 1mo</td>
<td>Mean age: 35y, 54.3%</td>
<td>MPH-IR</td>
<td>Good response: reduction</td>
<td>No association</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Refs [2012]</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>Design</th>
<th>Sample characteristics</th>
<th>Medication, dose</th>
<th>Primary outcome measure</th>
<th>Primary results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salatino-Oliveira et al. [2011]</td>
<td>COMT</td>
<td>rs6296</td>
<td>Naturalistic, 3mo (n = 251)</td>
<td>Mean age: 9.5y, boys European–Brazilian</td>
<td>MPH</td>
<td>SNAP-IV</td>
<td>Met carriers showed greater improvements at ODD symptoms than Val homozygous</td>
</tr>
<tr>
<td>Thakur et al. [2010]</td>
<td>5HTT</td>
<td>5-HTTLPR</td>
<td>Placebo-controlled, double blind, 2w (n = 157)</td>
<td>Mean age: 9.0y, 83.4% male</td>
<td>MPH</td>
<td>Conner’s rating scale for parents and teachers</td>
<td>L&lt;sub&gt;A&lt;/sub&gt; showed improvement with MPH</td>
</tr>
<tr>
<td>Kim et al. [2011]</td>
<td>BDNF</td>
<td>rs6265 (Val66Met)</td>
<td>Prospective study, 12w (n = 102)</td>
<td>Mean age: 8.7y, 80.4% male</td>
<td>OROS-MPH</td>
<td>Good response: 1–2 points at CGI-I and CGI-S; reduction ( \geq 50% ) at ADHD-RS</td>
<td>Val homozygous showed greater improvement than Met carriers</td>
</tr>
<tr>
<td>Arcos-Burgos et al. [2010]</td>
<td>LPHN3</td>
<td>rs6551665</td>
<td>ON/OFF medication (n = 240)</td>
<td>Mean age: 12.0y, 70.8% male United States</td>
<td>Stimulant medication</td>
<td>SWAN</td>
<td>G allele carriers presented a greater response to medication</td>
</tr>
<tr>
<td>Labbe et al. [2012]</td>
<td>LPHN3</td>
<td>rs1947274</td>
<td>Placebo-controlled, double blind, 3w (n = 416)</td>
<td>Mean age: 9.0y, 76.7% male</td>
<td>MPH</td>
<td>RAST</td>
<td>G allele is associated with a poor response to treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2345039</td>
<td></td>
<td>85% Caucasian</td>
<td>0.5 mg/kg/day</td>
<td>Actiwatch</td>
<td>G allele was associated with greater response to treatment</td>
</tr>
</tbody>
</table>
revisions, ADHD pharmacogenetics needs to move to other directions. Future studies should address gene–gene and G×E interactions, and the discovery of new candidate genes through GWAS. Also, other outcome measures should be further explored, such as endophenotypes and neuroimaging data. Besides response to treatment, more studies should assess the pharmacokinetic properties of the drug in study and adverse events. There is a clear process of shift in this direction (Fig. 1). Since the amount of variance in treatment response explained by genes does not seem to be substantial based on findings available, this shift may open more promising paths for the ADHD pharmacogenetic field.

The genetic etiology of ADHD involves interactions among multiple genetic variants and environmental conditions [Nigg et al., 2010]. In this review, it was possible to note that many articles evaluate more than one gene. Two studies employed a gene–gene interaction approach and found an effect of the interaction in MPH response, but they did not provide clear biological plausibility for their findings. However, several new investigations continue to perform analyses for each SNP separately although assessing several SNPs, but without performing synergy or epistatic interactions tests among them. It is important to note that gene–gene interaction analyses need caution in the way of interpreting the findings. When all possible interactions are accounted for, the number of potential tests is very large and splitting samples according to many SNPs leads to a diminished power for analyses. Future studies will require larger samples sizes and a priori plausible biological hypothesis to address gene–gene interactions. Another very relevant question that remains open and should be further explored is whether genes involved in ADHD would be differentially expressed across the life cycle (childhood and adulthood) and how it could moderate treatment response, the results already published did not provide data that could explain the inconsistent findings in different developmental stages.

Environmental factors implicated in ADHD often involve exposure to adverse circumstances early in life. For example, the consequences of fetus exposure to the toxic effects of nicotine are well documented [Ernst et al., 2001; Blood-Siegfried and Rende, 2010]. Thus, it is not a surprise that the environment factors reported in the three gene–environment interaction studies described in this review were maternal smoking and maternal stress during pregnancy. The focus in ADHD pharmacogenetics is still how genes and environmental factors interact with one another in relation to drug response.

There has also been increasing interest in cognitive and neuroimaging endophenotypes. The first investigations produced encouraging results as the refinement of phenotypes that can be explained by genotypic differences in treatment outcome. In addition, this kind of study may provide means to investigate the biological mechanisms involved in the action of the medication used for the disorder. Neuroimaging methods are being applied to investigate neurobiological effects of risk genes in ADHD and they may provide an explanation on how gene variants and environmental influences can affect brain activities before and after stimulant administration.

A large portion of ADHD patients present comorbidities. It has been reported that comorbidity may moderate ADHD treatment response. For example, two studies demonstrated that ADHD children with comorbid oppositional defiant disorder have reduced or lack of response to treatment [Ghuman et al., 2007; Goez et al., 2007]. Therefore, it is essentially important that there are

<table>
<thead>
<tr>
<th>Study methodology</th>
<th>Methodological requirements</th>
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<tbody>
<tr>
<td>Randomized clinical trials</td>
<td>Clinically sound outcome measures with cut-off point defined previously from analyses</td>
</tr>
<tr>
<td>A priori definition of the hypotheses tested</td>
<td>Study design deposited in appropriate open access repositories (e.g., clinical trials.gov)</td>
</tr>
<tr>
<td>Standard range doses</td>
<td>Power assessment before analyses</td>
</tr>
<tr>
<td>Genetic targets with biological plausibility</td>
<td>Presentation of the effect sizes</td>
</tr>
<tr>
<td>Adjustments for the effects of comorbidity</td>
<td>Presentation of nominal significance and correction for multiple comparisons</td>
</tr>
<tr>
<td>Adjustment for potential confounders</td>
<td>Make available all other genetic targets already explored with the same sample [published and unpublished]</td>
</tr>
</tbody>
</table>

Despite correction for multiple comparisons might not be implemented considering these targets, it is also important to inform the number of previous associations analyses performed.

![FIG. 1. Number of pharmacogenetics studies according to different outcomes in attention-deficit/hyperactivity disorder in two reviews. Data for the period up to 2010 were taken from Kieling et al. [2010].](image-url)
some clinical strategies to manage frequently comorbid disorders with ADHD and to optimize outcomes [Shier et al., 2013]. On the other hand, the prevalence of these conditions leads to samples without statistical power to detect the influence of comorbidities in ADHD treatment [Polanczyk et al., 2008].

In this review, almost all studies that investigated MPH pharmacogenetics focused on genetic variants of drug targets, such as transporters and receptors and have emphasized symptom reduction. Five studies and one GWAS addressed tolerability during MPH treatment. These investigations suggest that the prediction of adverse events and medication tolerability would be a clinically relevant area of research, since adverse events are major impediments to long-term treatment adherence [McGough et al., 2006, 2009]. Surprisingly, little attention has been paid to the genetic variability of drug metabolism in the area of ADHD pharmacogenomics. Atomoxetine metabolism by CYP2D6 is well described but more pharmacogenetics studies are necessary to understand if there is room for CYP2D6 testing when using atomoxetine in the context of personalized medicine.

The fact that MPH doses were not associated with genotype in some studies cited in this review does not discard the possibility of association. In naturalistic studies, clinicians have incremented doses in each follow-up visit according to response and adverse events with initial and follow-up doses that, although not fixed, were supposed to target respectively around 0.3 and 0.7 mg/kg/day at a routine clinical practice. Hence, some studies designs are not adequate to investigate the association between genotype and MPH dose. Froehlich et al. [2011] investigated fixed-dose range and found that MPH response was dose dependent according to DAT1 VNTR.

Effect size in pharmacogenetics studies can provide the gene magnitude to predict a degree of variability in treatment response. According to data structure, the studies in this review described different effect size types. All of them showed that SNP’s have a small effect size and therefore they support the prediction that response to MPH is influenced by several different polymorphisms, each one exerting a small effect [Faraone and Mick, 2010], given that medications have large effect sizes in general [Spencer et al., 2000].

The heterogeneity in methodological strategies employed by different studies remains impressive. Differences encompass the design of the study (retrospective, naturalistic, open-label, placebo-controlled), response of treatment definition (25%, 30%, or 50% reduction of symptoms), scales used to assess response, information source (clinician, parents, and/or teachers reports), dose titration, duration of treatment, statistical analysis (multiple comparisons without correction), identification and control for relevant confounding factors, sample sizes, among others. However, one thing calls even more attention regarding methodological issues: very few if any of the investigations in the field clearly disclose if their research questions were set a priori of analyses and the number of other genes/SNP’s comparisons made besides the one with positive results or those presented in the article (e.g., authors ran uncountable association and only present those that are related to the biological plausibility of the manuscript). This lack of information opens a large avenue for Type I error. Thus, these methodological differences in the studies taken all together are likely to explain the inconsistent results and constitute a significant barrier to interpret non-convergent findings as failure to replicate findings [Polanczyk et al., 2008]. Investigations should employ more standardized study designs while examining wider dose ranges of both MPH and atomoxetine, as well as standardized outcome measures, because environmental factors, differences in personal styles in perceiving and reporting symptoms among teachers and parents, as well as the interaction between the observer and the child have an important impact on the child’s behaviors and the way of reporting them [Stein, 2004]. On the other hand, power assessment before beginning new studies will provide reliable findings, even though methodologies are different between several studies.

As ADHD diagnosis is based on operational criteria—DSM-5—standardized outcome measures of medication response are also required. Because the studies identified in this review used several different outcomes, it becomes difficult to compare them. In addition, the different study designs also account for the finding heterogeneity. Consistent results from different methodological strategies have been found in randomized clinical trials. The approach used to define genotype groups for analysis also represents a limitation in many studies, since rare genotypes are grouped together and the analyses take into account a dominant effect. When other ways of grouping genotypes, based on the presence of one or more alleles, were implemented, different results have emerged. The dosage of medication should be evaluated by standard range dose, which deduce better the pharmacogenetics effect than flexible dosage. Moreover, it would be interesting to study novel molecular targets as glutamate-related genes, which mediate intracellular signaling neuron pathway [Lesch et al., 2013]; or neurodevelopmental genes during medication treatment, since medicated children with ADHD did not differ from control patients in white matter volume of brain and unmedicated children with ADHD differ from control group [Castellanos et al., 2002]. Medication effect might influence these genes responsible for synaptic plasticity or brain development. Metabolism genes would be better understood if the medication plasma concentration levels could be monitored. It would help to understand how the medication clearance undergoes according to genotype and its influence on dosage medication.

Based on everything exposed above, it is possible that a proportion of the findings presented up to now are based on convenient and unsubstantiated strategies, focusing on significant statistical results. To improve the quality of research in the field, we suggest some recommendations for future studies which are listed in Table II. Since power is always a matter of attention in pharmacogenetic investigations, multi-sites studies with comparable methodology, allowing joint analyses, are extremely important [see Polanczyk et al., 2008].

To date, several studies have been dedicated to understand the genetic variants underlying interindividual variability in pharmacological parameters, as well as adverse effects and efficacy. Such information could improve treatment, by shifting from trial-and-error approach to a pharmacological regimen that takes into account the individual variability. Given the small effect of genetics variants studied so far, it is an open question whether, how, or when results from ADHD pharmacogenetics studies will be useful in clinical management.
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