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Progress and Promise of Attention-Deficit Hyperactivity Disorder Pharmacogenetics

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Abstract

One strategy for understanding variability in attention-deficit hyperactivity disorder (ADHD) medication response, and therefore redressing the current trial-and-error approach to ADHD medication management, is to identify genetic moderators of treatment. This article summarizes ADHD pharmacogenetic investigative efforts to date, which have primarily focused on short-term response to methylphenidate and largely been limited by modest sample sizes. The most well studied genes include the dopamine transporter and dopamine D₄ receptor, with additional genes that have been significantly associated with stimulant medication response including the adrenergic α_{2A} -receptor, catechol-O-methyltransferase, D₅ receptor, noradrenaline (norepinephrine) transporter protein 1 and synaptosomal-associated protein 25 kDa.

Unfortunately, results of current ADHD pharmacogenetic studies have not been entirely consistent, possibly due to differences in study design, medication dosing regimens and outcome measures. Future directions for ADHD pharmacogenetics investigations may include examination of drug-metabolizing enzymes and a wider range of stimulant and non-stimulant medications. In addition, researchers are increasingly interested in going beyond the individual candidate gene approach to investigate gene-gene interactions or pathways, effect modification by additional environmental exposures and whole genome approaches. Advancements in ADHD pharmacogenetics will be facilitated by multi-site collaborations to obtain larger sample sizes using standardized protocols. Although ADHD pharmacogenetic efforts are still in a relatively early stage, their potential clinical applications may include the development of treatment efficacy and adverse effect prediction algorithms that incorporate the interplay of genetic and environmental factors, as well as the development of novel ADHD treatments.

Attention-deficit hyperactivity disorder (ADHD) is a prevalent neurobehavioral disorder characterized by developmentally inappropriate difficulties sustaining attention, controlling impulses and modulating activity level. There is an increasing number of stimulant and non-stimulant medication options for ADHD, with numerous new compounds in development.[1] Abundant data indicate that stimulant medications, including methylphenidate and amphetamine preparations, improve symptoms of children with ADHD with generally large effect sizes.[2] However, the majority of children and adolescents with ADHD do not remain on medication consistently,[3] despite the persistence of symptoms and impairments. Even

among responders, there is marked variability in optimal dosage, duration of effect and tolerability. Moreover, in spite of robust acute symptom reduction from ADHD medications, evidence of long-term response and improvement in functioning among children who received treatment has been scant,[4] with evidence suggesting some improvements in outcome only recently emerging.[5,6] Furthermore, to date no factors have been identified that consistently predict medication response and optimal dosage in individuals with ADHD.[7] In the absence of these data, treatment is often determined empirically in clinical practice through a gradual dosage titration and a trial-and-error approach with different medication preparations.

ADHD is a highly heritable disorder, with current estimates suggesting that 60-80% of the phenotype variance is due to genetic factors.[8] The search for candidate genes associated with ADHD has been largely driven by the understanding that medications for the disorder have drug targets in the catecholamine system.[9] Conversely, it is likely that variability in individual drug response might be related to genetic factors. As a result, ADHD researchers have been increasingly interested in the potential to tailor and optimize patient therapies using pharmacogenetic and pharmacogenomic findings. This article reviews definitions, summarizes research findings to date, highlights areas in need of future investigation and discusses the potential clinical implications of pharmacogenetics and pharmacogenomics in the management of ADHD.

1. Overview of Pharmacogenetics and Pharmacogenomics

Pharmacogenetics is the study of genetic variability in medication response.[10] The guiding principle of pharmacogenetics is that, by studying individual candidate genes, susceptibility to adverse medication effects or failure of response can be linked to specific gene variants that affect drug-metabolizing enzymes, receptors or transporters.[11] In contrast to pharmacogenetics, pharmacogenomics is a more recent term, which broadly encompasses efforts to utilize the human genome to better understand and develop pharmacological treatments.[12] The hallmark of pharmacogenomics is the ability to study simultaneously the contribution to drug effects of many genes using genomic techniques, allowing massive parallel analysis of gene variations.[11] However, it should be noted that the terms pharmacogenetics and pharmacogenomics are often used interchangeably.[13]

Based on advances in molecular biology, such as the advent of high-throughput gene sequencing and the mapping of the human genome, pharmacogenetic and pharmacogenomic studies have the potential to inform individualized treatment decisions, and subsequently improve long-term patient outcomes.[14] The promise of ADHD pharmacogenetics is far reaching, and includes the potential to develop individualized medication regimens that improve symptom response, lessen risk of adverse events, increase long-term tolerability, and thus contribute to long-term treatment compliance and enhanced general effectiveness. However, despite considerable promise, ADHD pharmacogenetics is still a relatively young field, with further development necessary before research findings can be translated into clinical practice on a broad scale.

2. Genetic Studies of Attention-Deficit Hyperactivity Disorder (ADHD)

Etiology

Candidate genes in ADHD susceptibility studies have, in large part, been selected based on our understanding of the mechanism of action of stimulant medications.[9] ADHD stimulant medications are believed to exert their therapeutic effects by increasing the amount of dopamine and noradrenaline (norepinephrine) available in the neuronal synapse. Specific mechanisms of action include blockade of the dopamine and noradrenaline transporters (SLC6A3 and SLC6A2, respectively), inhibition of monoamine oxidase (which metabolizes

dopamine and noradrenaline) and enhanced release of the catecholamines from the presynaptic cell.[15] As a result, to date, ADHD etiology studies have focused on genes related to catecholamine pathways. Candidate genes associated with increased risk for ADHD based on pooled odds ratios across three or more studies are the dopamine receptors (DRD4 and DRD5), dopamine transporter (SLC6A3), dopa- β -hydroxylase (DBH), serotonin receptor (HTR1B), serotonin transporter (SLC6A4), and synaptosomal-associated protein 25 kDa (SNAP25).[9] Other genes of increasing interest in ADHD susceptibility studies include catechol-O-methyltransferase (COMT),[16,17] the adrenergic α_2A -receptor (ADRA2A)[18-20] and the noradrenaline transporter protein 1 (SLC6A2).[21,22]

3. ADHD Pharmacogenetic Research Studies

While knowledge about the presumed mechanisms of action of ADHD medications initially informed searches for polymorphisms related to increased risk for the disorder, these same polymorphisms are logical candidates to predict medication outcomes.[23] A number of preliminary studies suggest that candidate genes involved in catecholamine pathways influence individual patient responses to ADHD treatments (tables I and II), with the majority of studies examining predictors of methylphenidate response. Unfortunately, results from these reports are often contradictory, and the nature, magnitude and direction of purported genetic effects remain unclear. Variations in study design may be partially responsible for the disparate findings, since a recent meta-analysis of ADHD pharmacogenetics investigations determined that the design of the study (open-label studies vs. randomized controlled trials) was significantly associated with heterogeneity of results.[47] Concerns raised regarding retrospective, observational pharmacogenetic studies have included biased ascertainment of outcome,[48] as well as a tendency to underestimate environmental contributions and overestimate genetic effects.[49] Therefore, in this article, results of double-blind, placebo-controlled trials are presented separately from those of naturalistic studies for each genetic polymorphism.

This review first summarizes ADHD pharmacogenetics studies to date for SLC6A3 and DRD4, the most well studied genes. A review of findings for additional genes that have been significantly associated with stimulant medication effects on ADHD symptoms in at least one prior published study, including ADRA2A, COMT, DRD5, SLC6A2 and SNAP25, follows. Other genes that have been evaluated in at least one ADHD pharmacogenetic study but have shown no evidence of significant main effects are referenced in table I, including the dopamine D₂ receptor (DRD2), nicotinic acetylcholine α_4 -receptor (CHRNA4), serotonin 5-HT_{1B} and 5-HT_{2A} receptors (HTR1B, HTR2A), and serotonin transporter (SLC6A4). Articles and abstracts cited in this review were identified via a systematic review of the literature using the PubMed (<http://www.ncbi.nlm.nih.gov/sites>) and PsycINFO (<http://www.apa.org/psycinfo/>) databases. The following search terms were utilized: 'pharmacogen*' AND 'ADHD' OR 'attention-deficit' OR 'attention' OR 'hyperactiv*' OR 'methylphenidate' OR 'amphetamine' OR 'atomoxetine' OR 'stimulant' OR 'psychostimulant'. A review was also conducted of references from relevant papers ascertained during the database search.

3.1 Dopamine Transporter (SLC6A3)

Several lines of evidence support the choice of SLC6A3 as a candidate gene for ADHD treatment response. SLC6A3 encodes a presynaptic protein responsible for reuptake of dopamine from the synaptic cleft. It is a major site of action for methylphenidate and amphetamine, which bind to and inhibit SLC6A3, thereby increasing synaptic dopamine.[50, 51] An association between ADHD and the 10-repeat (480-base-pair) allele of a variable number tandem repeat in the 30-untranslated region of SLC6A3 was initially described in 1995 [52] and replicated in multiple, but not all, follow-up reports.[9] Numerous neuroimaging studies reveal that ADHD patients express increased dopamine transporter densities in striatal

regions,[53] and that individuals with the 10-repeat allele exhibit approximately 50% greater densities than other genotypes.[30] This suggests that ADHD medications that block the dopamine transporter might serve to attenuate the effects of underlying brain pathophysiology. Similarly, it has been hypothesized that functional polymorphisms in SLC6A3 may influence response to ADHD medications. For this reason, SLC6A3 has been the focus of the bulk of ADHD pharmacogenetics studies to date. Unfortunately, study design (e.g. prospective vs. retrospective, use of a control group), dosing method and regimen, and outcome assessments have varied widely from study to study. Below we summarize results from both prospective controlled studies and naturalistic ADHD pharmacogenetic studies.

3.1.1 Placebo-Controlled SLC6A3 Methylphenidate Studies in Children—Only three prospective, double-blind, placebo-controlled trials of SLC6A3 3'-untranslated region polymorphisms have been conducted in pediatric samples thus far. Stein et al.[27] found that the presence of one or two 10-repeat alleles was associated with higher rates of symptom reduction and reduced impairment (as rated by parents) in 47 children and adolescents treated with 18, 36 and 54 mg of osmotic release oral system (OROS) methylphenidate. Individuals homozygous for the less common 9-repeat allele demonstrated a nonlinear dose-response curve, had more stimulant-related adverse effects and remained more impaired during treatment. Similar findings were reported for the 9/9 genotype group in a double-blind, placebo-controlled trial of 159 children with ADHD conducted in Montreal by Joobar et al.[29] Although children with either the 9/10 or 10/10 genotypes displayed a significant positive response to methylphenidate 10 mg, the 9/9 genotype group displayed a negative response on parent symptom ratings but not teacher ratings. Finally, in a study of 81 American preschoolers with ADHD treated with methylphenidate, there were no genotype effects of SLC6A3 on a composite measure based on parent and teacher symptom ratings.[28] However, on parent ratings of ADHD symptoms, there was a negative effect for the homozygous 9-repeat genotype. Of note, the difference between parent and teacher ratings and the pharmacogenetic effects of SLC6A3 observed in the preschool study was similar to that reported by Joobar et al.[29] In summary, two of the three placebo controlled pediatric trials of SLC6A3 polymorphisms identified an improved methylphenidate response for 10-repeat homozygotes,[27,29] while 9-repeat homozygosity was associated with a diminished parent-rated medication response in all three studies.[27-29]

3.1.2 Naturalistic SLC6A3 Methylphenidate Studies in Children—Unlike the placebo-controlled studies discussed in section 3.1.1, the 11 naturalistic trials of the relationship between the SLC6A3 10-repeat allele and methylphenidate response published to date have not yielded consistent results.[26,30-39] In fact, only one of the naturalistic pediatric trials replicated the placebo-controlled pediatric trial findings, by identifying a link between the SLC6A3 10-repeat allele and improved methylphenidate response.[32] This analysis, based on parental retrospective reports in 119 Irish children, found that individuals with one or two copies of the 10-repeat allele were more likely to have improved methylphenidate response.[32] In this study, a linear relationship existed between the number of 10-repeats and degree of improvement.

In contrast, the SLC6A3 10-repeat allele has been associated with diminished methylphenidate response in four pediatric naturalistic pharmacogenetic studies,[30,31,33,38] including the first ever ADHD pharmacogenetic study. This report found a significant difference in response rate among 30 stimulant-naïve, African American youth based on the SLC6A3 genotype. Specifically, 86% of nonresponders were homozygous for the 10-repeat allele compared with 31% of responders ($\chi^2 = 6.9$; degrees of freedom [df] = 1; $p = 0.008$).[30] An additional study examined response in 50 European-Brazilian boys with ADHD who underwent open titration with methylphenidate up to 0.7 mg/kg/day:[31] medication response was defined as >50% reduction in baseline ADHD ratings, and individuals who failed to meet this threshold were

more likely to be homozygous for the 10-repeat allele (Fisher's exact test; one tailed, $p = 0.04$). A third study assessing ADHD symptom reduction in 11 Korean subjects found that only 27% of subjects homozygous for the 10-repeat allele met methylphenidate response criteria compared with 100% of subjects without this genotype ($\chi^2 = 5.2$; $df = 1$; $p = 0.06$).[33] Finally, a recent study of 141 French children also found that SLC6A3 10-repeat homozygotes were over-represented in the low methylphenidate response group, as defined by having a less than 2-point improvement in clinical global impression severity score (33% of 10/10 subjects compared with 16% of other genotypes [$\chi^2 = 5.8$; $df = 1$; $p = 0.02$]).[38]

No significant effect of the SLC6A3 10-repeat allele on methylphenidate response was documented in the remaining six uncontrolled studies of children with ADHD.[26,34-37,39] These included prospective, open-label samples of 82 Dutch children (treated with <0.6 mg/kg/day),[35] 122 Hungarian children (mean dosage 0.55 mg/kg/day),[26] 26 Irish children (mean dosage 0.6 mg/kg/day)[36] and 111 Brazilian youth (mean dosage 0.5 mg/kg/day), [37] as well as two retrospective analyses examining 168 youth in the UK[34] and 156 children from the US[39] (mean medication doses not specified).

3.1.3 Meta-Analysis of SLC6A3 Methylphenidate Studies in Children—Purper-Ouakil et al.[38] conducted a meta-analysis of SLC6A3 10-repeat effects on methylphenidate responder versus nonresponder status, combining the results of six studies for a total of 475 children. This meta-analysis found that individuals with the 10/10 genotype were less likely to show a moderate to good response to methylphenidate compared with those carrying other genotypes (mean odds ratio = 0.46; 95% CI 0.28, 0.76). However, given the above noted variability in SLC6A3 study results between pediatric placebo-controlled and naturalistic trials, it should be noted that five of the six studies in this meta-analysis utilized a naturalistic design. [38]

3.1.4 SLC6A3 Methylphenidate Studies in Adults—To date, two SLC6A3 pharmacogenetic studies - both of which were placebo-controlled, double-blind trials - have been conducted in adult samples.[45,46] The first reported no relationship between the SLC6A3 30-untranslated region genotype and response in 66 subjects titrated to a maximum methylphenidate dosage of 1.3 mg/kg/day.[45] However, the sample included only three individuals with the 9/9 genotype, limiting statistical power to detect an effect for this genotype group. The second study, which titrated methylphenidate dosages to a maximum of 1.0 mg/kg/day, found that carriers of a single SLC6A3 10-repeat allele were more likely to have a favorable medication response compared with 10-repeat homozygotes.[46] Of note, the single 9/9 homozygous patient was removed from this analysis.

3.1.5 SLC6A3 Haplotype Methylphenidate Studies—Because studies investigating the association between the SLC6A3 10-repeat allele and risk for ADHD have yielded inconsistent findings, increasing interest has centered around the potential effects of a SLC6A3 haplotype involving the 30-untranslated region 10-repeat allele and a 3-repeat allele of 30-base pair variable number tandem repeat in intron 8 (SLC6A3 10/3). Intriguingly, three studies of the SLC6A3 10/3 haplotype have all documented an association with ADHD.[54-56] Recently, the first pharmacogenetic study of the SLC6A3 10/3 haplotype was also conducted, but failed to document significant effects of SLC6A3 10/3 on methylphenidate response in a pediatric sample.[36] Study limitations included the relatively small sample size ($n = 26$) and the naturalistic study design.

3.1.6 SLC6A3 Amphetamine Studies—In one of the few pharmacogenetic studies not conducted with methylphenidate, amphetamine was administered to college students in a double-blind, placebo-controlled, crossover trial.[57] In this study, Lott et al.[57] reported that individuals with the SLC6A3 9/9 genotype were less able to 'feel' amphetamine effects relative

to other genotype groups. It should be noted that this was not an ADHD sample, and consisted of college student volunteers. Nonetheless, this finding is consistent with prior suggestions of a differential effect of stimulants on individuals homozygous for the 9-repeat relative to genotypes containing the 10-repeat.[27-29]

3.2 Dopamine D4 Receptor (DRD4)

DRD4 encodes a dopamine receptor that regulates dopamine synthesis and release, as well as the firing rate of dopamine neurons.[58] The association of the 7-repeat (48-base pair) variable number tandem repeat polymorphism in the coding region of DRD4 with ADHD is one of the most replicated findings in psychiatric genetics, yielding odds ratios ranging from 1.4 to 1.9.[9] In vitro studies indicate that the 7-repeat allelic variant is less responsive to dopamine, [59,60] suggesting a possible functional role for this polymorphism in stimulant medication response.

3.2.1 Placebo-Controlled DRD4 Methylphenidate Studies—Although no placebo-controlled DRD4 pharmacogenetic trials have been conducted thus far in a school-age cohort, prospective, double-blind, placebo-controlled trials have been conducted in an adult cohort [46] and a preschool cohort.[28] Kooij et al.[46] found no association between the 7-repeat allele and methylphenidate treatment response in their study of 42 adults with ADHD. Similarly, PATS (Preschool ADHD Treatment Study) did not identify significant effects for the 7-repeat allele in terms of symptom reduction or dose response on parent, teacher or parent-teacher composite behavior ratings.[28] However, PATS did document significant links between DRD4 variable number tandem repeat polymorphisms and adverse effects. Specifically, PATS participants homozygous for the 4-repeat allele were 3 times more likely to develop abnormal picking behaviors with methylphenidate treatment, while those with one or two copies of the 7-repeat allele were more than four times more likely to exhibit social withdrawal with increasing dose.

3.2.2 Naturalistic DRD4 Methylphenidate Studies—Findings in naturalistic DRD4 methylphenidate pharmacogenetic studies have been mixed. Thus far, consistent with the placebo-controlled trials, four of the nine naturalistic studies have found no significant link between methylphenidate symptom response and the 7-repeat allele, including a retrospective study of 159 US children,[39] and prospective, open-label studies of 30 US children,[30] 111 Brazilian children[37] and 122 Hungarian children.[26] However, a prospective, open-label study of 100 Turkish children showed that transmission of the 7-repeat allele was more likely in methylphenidate responders compared with nonresponders,[40] and a retrospective study of 82 Dutch children found a borderline significant ($p = 0.09$) association between the 7-repeat allele and better methylphenidate response.[35]

In contrast, three prospective, open-label studies suggest an association between the DRD4 7-repeat allele and diminished methylphenidate response,[41-43] consistent with prior indications that this variant encodes a dopamine receptor that is hyporesponsive to its agonist. [59,60] Specifically, Hamarman et al.[42] demonstrated, in 45 subjects, that those with at least one copy of the 7-repeat allele required higher doses of methylphenidate for optimal symptom reduction. In a study of 47 German subjects, Seeger et al.[41] found that children with at least one DRD4 7-repeat allele and homozygosity for the long allele of a serotonin transporter promoter polymorphism had significantly less improvement in functioning with methylphenidate treatment compared with those with other genotype combinations. Conversely, in a separate report on 83 Korean children, subjects who were homozygous for the DRD4 4-repeat polymorphism were much more likely to exhibit positive responses on parent and teacher behavioral ratings than those with other genotypes.[43] It should be noted that the 7-repeat genotype is extremely rare in Asian populations, and that children homozygous

for the 4-repeat in the above study had higher baseline ADHD symptoms than children with other genotypes.

3.2.3 DRD4 Atomoxetine Studies—One prior study has shown that children with at least one copy of the DRD4 4-repeat allele showed a trend towards improved response with atomoxetine.[61] In addition, improvement on the hyperactivity subscale of the ADHD Rating Scale was maximized in the absence of any 7-repeat variant.

3.3 Adrenergic α_2A -Receptor (ADRA2A)

ADRA2A encodes a noradrenaline autoreceptor, the activation of which dampens the cell firing rate and limits noradrenaline release.[62] Several animal studies suggest that α_2A -noradrenergic receptors may mediate methylphenidate effects, with administration of α_2 -adrenoceptor antagonists blocking the beneficial effects of methylphenidate.[63,64] A-1291 C>G single nucleotide polymorphism creates an MspI site in the ADRA2A promoter region [65] that appears to be both functional[66] and linked to the inattention domain in studies of ADHD susceptibility.[18-20] Moreover, effects of the C1291G polymorphism on inattentive symptom scores have been documented in two pharmacogenetic studies thus far. The first assessed 106 children and adolescents of varied ADHD subtypes after 1 and 3 months of methylphenidate treatment.[24] The investigators reported that subjects with at least one copy of the less common G allele showed improved methylphenidate response on inattention scores ($F_{1,104} = 8.5$; $p < 0.004$) but not hyperactive-impulsive scores. A subsequent naturalistic study by da Silva et al,[25] the first ADHD pharmacogenetics investigation to enroll an all ADHD-predominantly inattentive type sample, found congruent results: children with the ADRA2A G allele had significantly lower inattentive scores after 1 month of methylphenidate treatment compared with those lacking the G allele.

3.4 Catechol-O-Methyltransferase (COMT)

COMT, found in the synaptic cleft, catabolizes dopamine and noradrenaline. The COMT gene has a functional polymorphism at codon 158 that results in a single amino acid change (methionine [met] for valine [val]). Enzyme activities for the variants are as follows: val/val homozygotes have high, val/met heterozygotes have intermediate, and met/met homozygotes have 4-5 times lower COMT activity.[67] Although some prior studies have identified associations between ADHD and COMT codon 158 polymorphisms,[16,17] a meta-analysis of 13 studies investigating the association between the COMT val158met polymorphism and ADHD did not find a significant association.[68] Nonetheless, the role of COMT in catecholamine catabolism, combined with clear evidence of function for the COMT codon 158 polymorphism, makes it a compelling candidate for ADHD pharmacogenetics studies. Recently, the first study investigating the link between COMT polymorphisms and methylphenidate response was published. This prospective, open-label trial documented a significant interaction between the COMT val/val genotype and good methylphenidate response in terms of hyperactive-impulsive but not inattentive symptoms in a sample of 122 Hungarian children with ADHD.[26]

3.4.1 COMT Amphetamine Studies—One prior study has examined the relationship between COMT polymorphisms and amphetamine response. Mattay et al.[69] documented that working memory efficiency, assessed via functional MRI, was enhanced by amphetamine administration for val/val genotype (high COMT activity) subjects, while amphetamine produced adverse effects under high working memory load conditions for met/met genotype (low activity) subjects.

3.5 Dopamine D₅ Receptor (DRD5)

DRD5 is a G-protein-coupled receptor that stimulates the production of adenylate cyclase. Studies of D₅ null mice suggest that DRD5 may contribute to the activation of dopaminergic pathways relevant to exploratory locomotion, startle and prepulse inhibition.[70] Although the results of individual studies have been inconsistent, three meta-analyses have documented a significant association between ADHD and the 148-base pair allele of a microsatellite marker located 50 to the DRD5 gene.[71-73] Review of the literature indicates a single DRD5 pharmacogenetic study thus far. This study by Tahir et al.[40] in 100 Turkish children did not identify any children with the 148-base pair allele, but did find an association between the 151-base pair allele of the DRD5 50-microsatellite marker and favorable methylphenidate response.

3.6 Noradrenaline (Norepinephrine)Transporter Protein 1 (SLC6A2)

In addition to their effects on the dopamine transporter, stimulant medications block reuptake at noradrenaline transporters.[74] Noradrenaline transporter blockade is also the presumed mechanism of action for the non-stimulant ADHD medication atomoxetine.[75] In addition, although not confirmed in meta-analyses,[76] polymorphisms at several single-nucleotide polymorphisms in SLC6A2 have been associated with ADHD.[21,22] Therefore, noradrenaline transporter polymorphisms are potentially promising candidates to determine variability in ADHD treatment response.

Thus far, one study has evaluated the link between a G1278A polymorphism at exon 9 of SLC6A2 and methylphenidate response in 45 Han Chinese youth with ADHD, and found that individuals homozygous for the A/A genotype had a diminished medication response in terms of hyperactive-impulsive but not inattentive symptoms compared with the G/A or G/G genotypes.[44] However, since the G1278A allele has no known functional activity, the authors noted that the allele might be in linkage disequilibrium with another allele responsible for outcome differences. Recently, an additional SLC6A2 pharmacogenetic study conducted in an adult sample evaluated the association between methylphenidate response and a 4-base pair insertion/deletion polymorphism in the promoter region of SLC6A2.[46] This study, by Kooij et al.,[46] did not identify a significant relationship between the SLC6A2 promoter polymorphism and medication response.

3.6.1 SLC6A2 Amphetamine Studies—The association between eight SLC6A2 polymorphisms and subjective response to dexamphetamine was evaluated in a prospective, double-blind, placebo-controlled study of 99 healthy German adults.[77] This study found that the genotype CC of the 36001A/C single-nucleotide polymorphism and the haplotype GCC from the 28257G/C, 28323C/T and 36001A/C single-nucleotide polymorphisms were associated with higher self-reported positive mood after amphetamine administration. The authors noted that although no functional consequences of the genotype CC or haplotype GCC are presently known, these polymorphisms are located in transcription factor binding sites, and thus may alter SLC6A2 transcription rate and, ultimately, protein levels.[77]

3.7 Synaptosomal-Associated Protein 25 kDa (SNAP25)

A relatively unstudied gene with potential effects on ADHD medication response is SNAP25. SNAP-25 is a neuron-specific vesicle docking protein involved in neurotransmitter exocytosis from storage vesicles into the synaptic space.[78] Several studies have examined the link between ADHD and two single nucleotide polymorphisms (T1069C and T1065G) separated by four base pairs at the 3' end of SNAP25.[79-82] Although study results have not been entirely consistent, pooled analyses for T1065G revealed significant evidence of an association with ADHD.[9] Studies of the mouse mutant strain coloboma, which has a SNAP25 deletion and associated spontaneous hyperactivity,[83] suggest a role for SNAP25 in methylphenidate response. Specifically, hyperactivity in the coloboma mouse was suppressed by administration

of amphetamine but not methylphenidate.[83] This is consistent with presumed differences in the mechanisms of action for these compounds, since amphetamine, but not methylphenidate, compensates for reduced exocytotic catecholamine release by reversing the catecholamine diffusion gradient across the dopamine transporter.

In a study of preschool children with ADHD, McGough et al.[28] found that homozygotes for the T allele of T1065G had moderately improved methylphenidate dose responses, while those homozygous for T at T1069C exhibited poorer methylphenidate responses. In addition, children who were homozygous for the G allele at 1065 were 2-3 times more likely to develop sleep difficulties and irritability than those with at least one copy of the T allele. Those who were homozygous for the C allele at 1069 were 2-4 times more likely to develop tics and other abnormal movements compared with T allele carriers.

3.8 Metabolic Pathways

To date, ADHD pharmacogenetic studies have principally examined the potential effects of genetic variability on drug targets, i.e. transporters and receptors.[23,84] The potential effects of genetic variability on drug metabolism and pharmacokinetics have received relatively little study, although these lines of inquiry frequently provide the basis for pharmacogenetic investigations.[14] This might be due to our relatively poor understanding of methylphenidate metabolic pathways, compared with our more detailed prior study of its mechanism of action.

3.8.1 Methylphenidate Metabolism—It is believed that d,l-methylphenidate undergoes esterification in the bloodstream via the enzymatic activity of carboxylesterase 1 (CES1) to d,l-ritalinic acid and l-ethylphenidate.[85] A recent report described a mutation in the CES1 gene exon 4 at codon 143 leading to a nonconservative amino acid substitution, in addition to a mutation in exon 6 at codon 260, resulting in a premature stop codon. Importantly, both the exon 4 and exon 6 mutations were associated with complete loss of hydrolytic activity toward methylphenidate.[86] Further investigation and replication are required to see if these variants contribute to the pharmacokinetic variability of methylphenidate generally seen in clinical practice. Unfortunately, because these CES1 variants have low allele frequencies (<5% for the exon 4 mutation and <1% for the exon 6 mutation in all races and ethnicities assessed),[86] their future study will likely be complicated by the need for large sample sizes.

3.8.2 Amphetamine Metabolism—In contrast to the renal excretion of the metabolic products of methylphenidate, amphetamine undergoes metabolism via hepatic cytochrome P450 (CYP) isozymes. In mammals, amphetamine is metabolized along two major pathways - the CYP2D6 and CYP3A4 pathways - which are differentially employed by various species.[87]

In the first pathway, hydroxylation of amphetamine via CYP2D6 yields p-hydroxy-amphetamine. Many psychotropic medications are metabolized by CYP2D6, although it is believed to play a minor role for amphetamine.[88] Nonetheless, up to 20% of Caucasians and varying percentages of other racial groups are poor metabolizers due to polymorphisms at CYP2D6, which can have implications for dosing and medication tolerability in individual patients.[89]

In the second pathway, amphetamine undergoes deamination via CYP3A4 to l-phenylpropane-2-one, which is subsequently excreted as inactive benzoic acid. The CYP3A4 amphetamine pathway is dominant in humans. In a study of an extended release preparation of mixed amphetamine salts, mean plasma drug concentrations following acute dosing were 25% higher in African American children.[90] Intriguingly, previous studies have noted ethnic differences in CYP3A4-mediated drug metabolism, with Caucasian subjects demonstrating the highest levels of activity.[91] One allelic variant that is heterozygous in 64% of African

Americans has been associated with decreased metabolic activity.[91] Although a definitive association between polymorphisms at CYP3A4 and racial differences in amphetamine metabolism has not been demonstrated, this may represent a fruitful area of future study, with the potential to advance our understanding of amphetamine pharmacokinetic variability.

3.8.3 Atomoxetine Metabolism—Atomoxetine is metabolized by the CYP2D6 isozyme system. Notably, consideration of the CYP2D6 status of subjects influenced dosage titration algorithms and subsequently derived approved dosage limits during atomoxetine drug development trials. A recent meta-analysis of atomoxetine clinical trials found that poor CYP2D6 metabolizers displayed greater symptom improvement than extensive metabolizers, most likely due to higher plasma drug concentrations, and were more likely to remain in therapy.[92] However, higher rates of appetite decrease and tremor were reported in poor CYP2D6 metabolizers, who also had greater medication-related changes in pulse and blood pressure.

3.9 Genome-Wide Approaches

Candidate gene studies presume some knowledge of the biological system under investigation, and require specific hypotheses regarding effects of the polymorphisms under study. In contrast, genome-wide investigations require no a priori hypotheses related to specific genes, but scan the entire genome to pinpoint areas harboring genes related to outcome. Several genome-wide scans have identified regions related to ADHD susceptibility,[9,93] including fine mapping by one group to SLC6A3.[94]

Genome-wide approaches may also have utility in ADHD pharmacogenetic investigations. One prior study employed quantitative trait analysis in a genome-wide scan assessing for linkage with methylphenidate response.[95] A linkage peak of moderate significance was found on chromosome 7, with additional peaks on chromosomes 3, 5 and 9. Further study, including genome-wide association with high-density, single-nucleotide polymorphism chips, will be necessary to identify the specific genes corresponding to the observed linkage peaks. An additional genome-wide association study recently evaluated response to a methylphenidate transdermal system.[96] In this open-label study of 187 children with ADHD, the strongest association ($p = 3 \times 10^{-6}$) fell short of the threshold for statistical significance in a genome-wide association study. However, intriguing non-significant associations were suggested in the glutamate receptor, metabotropic 7 gene (GRM7) and in two single-nucleotide polymorphisms within SLC6A2. The authors concluded that noradrenergic and possibly glutaminergic genes may be involved in methylphenidate response, although larger, adequately powered samples are necessary to confirm their role in ADHD medication response.

4. Current Research Challenges and Future Directions

While the results of prior ADHD pharmacogenetic studies have been intriguing, many challenges must still be addressed. For example, the majority of ADHD pharmacogenetic studies published to date have examined response to methylphenidate. Study of genetic predictors of response for additional ADHD medication treatment options, including amphetamine preparations, atomoxetine and guanfacine, remains a significant gap in the literature.

Furthermore, the prior methylphenidate pharmacogenetic studies have been hampered by a number of limitations, and have often yielded inconsistent findings. Study design differences may partially account for the heterogeneity of findings. For example, in the case of the dopamine transporter studies, more consistent findings appear to be emerging from placebo-controlled, prospective studies of children with ADHD. However, most prior trials have depended on open-label or retrospective assessment, in which medication doses were not

specified or were considerably lower than those used in community practice for optimal benefit. [23] Since the effects of methylphenidate on ADHD symptoms often follow a linear dose-response curve,[27] these lower doses might bias against finding significant treatment effects.

Current studies are also constrained by the type of outcome measures used, as many studies rely on simple dichotomous outcomes (e.g. responder vs. nonresponder), which have limited power to detect effects compared with analyses of quantitative measures. Correlations between multiple outcome measures in the same subjects are also known to be fairly weak, raising the question as to which outcome measure best defines positive response.[97] In several cases, study results have differed depending on whether parents or teachers are the behavior-rating informants.[28,29]

An additional critical methodological issue is the approach to defining genotypes for analysis. In order to minimize the potential for spurious findings and increased type I errors, investigators must limit their analyses to minimal genotype combinations. For some genes, the risk polymorphisms for ADHD are the less common variants (e.g. the 7-repeat allele of DRD4), while for other genes, such as the dopamine transporter SLC6A3, it is the more common variants that are associated with the disorder. For SLC6A3, the 10/10 and 10/9 genotypes are most common, and earlier studies combined these common genotypes. This practice assumed a dominant effect of either the SLC6A3 9 or 10 allele, but failed to test for a recessive effect of the 9/9 genotype. Alternative grouping of genotypes based on the presence of one or more SLC6A3 9-repeat alleles has led to different results. Future candidate gene studies would benefit from consensus on optimal strategies to define genotype groupings. Genotypes should not be lumped together when evidence of the dominance of one allele is lacking in previous pharmacogenetic studies.

Variation in sample size, composition and environmental exposures may also contribute to differences in ADHD pharmacogenetic study results. Modest sample sizes have limited statistical power to detect mild or moderate genetic effects. Another potential contributor to the observed discrepancies in study findings is that pharmacogenetic effects may vary in different ethnic and racial groups. This suggests that the genetic variants being studied may not be causing the effect observed, but instead may be in linkage disequilibrium with the actual functional genetic variants. In addition, previous investigations may have failed to identify consistent genetic effects due to differences in sample subtype composition, given evidence in some prior studies that certain genetic variants may influence response to medication in terms of hyperactive-impulsive symptoms[26] or inattentive symptoms[24] but not both domains. In addition, although ADHD pharmacogenetic studies to date have not evaluated interactions with additional environmental and toxicant exposures, prior evidence hints that such exposures may be important modifiers of genetic effects on medication response. For example, environmental exposures such as tobacco smoke may influence brain dopamine release by interacting with both catecholamine-related genetic variants[98,99] and methylphenidate.[100] If the suggested three-way interaction between catecholamine genes, methylphenidate and tobacco exposure is in fact at play, we would expect the measured associations between genotype and medication response to vary according to the tobacco exposure level of the different study populations.

Furthermore, failure to evaluate gene-gene interactions, which have received little attention in ADHD pharmacogenetic studies to date,[41] may also be obscuring effects. Moreover, it is increasingly recognized that drug response is the result of a complex matrix of factors, rather than a single factor.[48] As a result, experts have proposed that future pharmacogenetic studies shift their focus from individual genes to pathways encompassing genes for drug-metabolizing enzymes and transporters, as well as genes encoding drug targets and their downstream signals. [101]

To address the limitations of previous studies, guiding principles to promote future ADHD pharmacogenetics research were proposed during the 2006 and 2008 ADHD Molecular Genetics Network (AMGN) meetings.[102,103] These include the recommendation that studies utilize a methodologically rigorous pharmacological intervention, which typically means that investigations should be prospective, randomized and placebo-controlled. The AMGN also proposed that trials employ a full range of dose conditions, since dose-ranging and forced titration designs are more likely to elicit pharmacogenetic effects than flexible dosing. Further suggestions stressed the need for genotyping quality control, ideally including cross-laboratory and cross-method reliability checks. In addition, the AGMN recommended the recruitment of study samples large enough to evaluate effect modification by additional environmental exposures, as well as gene-gene interactions and/or a gene pathways approach. [101] Finally, the group called for assessment of multiple outcomes, including both continuous and categorical outcomes examining both parent and teacher ratings, and proposed that functional outcomes and adverse events should be evaluated as well as symptom ratings.

5. Potential Clinical Applications

Despite great hopes, the potential of candidate gene association studies to yield clinically relevant information regarding ADHD medication response is unclear. Concerns have been raised because the effects of common polymorphisms on drug response have typically been small, not just in ADHD studies but also in pharmacogenetic studies across a wide range of disciplines.[48] Hence, knowledge of small effects due to single polymorphisms may be of dubious clinical utility given the large effect sizes attributed to ADHD stimulant medications in general.[2] Moreover, drug response is increasingly recognized to be the result of a multitude of factors, rather than variations in a single gene. Ultimately, the pharmacogenetic study of individual candidate polymorphisms may not provide the tools for definitive determination of ADHD medication response, but rather may contribute to the development of clinically salient treatment prediction algorithms that incorporate a complex interplay of genetic and environmental factors.

Prediction of adverse effect risk and medication tolerability may be an additional practical clinical application for ADHD pharmacogenetic data. Stimulant medications are the recommended first-line treatments for ADHD.[104] However, open-label follow-up studies of clinical trial subjects taking either methylphenidate or amphetamine have shown that fewer than 60% of previously stabilized patients remained on medication after 12 months of treatment, although those who remained in therapy showed sustained improvements from baseline.[105,106] In one 5-year prospective investigation documenting the discontinuation of ADHD medication, the authors concluded that adverse effects were major factors in the patients' decisions to discontinue treatment by the second study year in over half of participants. [3] In addition, in PATS, development of irritability and increased emotionality were two major reasons subjects discontinued medication therapy.[107] Interestingly, PATS pharmacogenetic analyses revealed genetic predictors of irritability, social withdrawal and abnormal movements.[28] Conceivably, then, awareness of increased adverse effect risk derived from pharmacogenetic data could be used to steer individuals toward tailored treatment regimens that are more likely to be tolerated over time.

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

6. Conclusions

ADHD pharmacogenetics and pharmacogenomics research efforts are expanding worldwide. To date, several promising findings related to prediction of symptom response and adverse effects have been reported, although results have not been entirely consistent. Upcoming investigations should employ more standardized study designs while examining a wider range of stimulant and non-stimulant medications and a variety of outcome measures. Fruitful avenues of future ADHD pharmacogenetic investigation may include study of polymorphisms in drug-metabolizing enzymes, as well as approaches that incorporate gene-gene interactions and effect modification by additional environmental exposures. Furthermore, investigators are increasingly interested in going beyond the study of candidate genes to explore whole-genome approaches. Further research, likely involving multi-site collaborations to obtain larger samples, is clearly necessary before preliminary findings can be applied to contemporary clinical practice. Nonetheless, the promise of ADHD pharmacogenetics is far reaching, and includes the potential to develop individualized medication regimens that improve symptom response, lessen risk of adverse effects and increase long-term tolerability.

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Table 1

Pharmacogenetic studies of methylphenidate (MPH) in children and adolescents with attention-deficit hyperactivity disorder^a

Gene	Study design	Sample size	Outcome	Study location	Reference
Adrenergic α_2A receptor (<i>ADRA2A</i>)	pro, op	106	Improved response on inattention symptoms with G allele	Brazil	[24]
	pro, op	59	Improved response on inattention symptoms with G allele	Brazil	[25]
	pro, op	122	Improved response on hyperactive-impulsive symptoms with homozygous val allele	Hungary	[26]
Catechol-O-methyltransferase (<i>COMT</i>)					
Dopamine transporter (<i>SLC6A3</i>)	pro, db, pc, dose response	47	Different dose-response curves by <i>SLC6A3</i> genotype; decreased response with homozygous 9-repeat	USA (Washington, DC)	[27]
	pro, db, pc	81 (preschoolers)	No effect on primary outcome composite measure based on parent and teacher ratings. Decreased response with homozygous 9-repeat on parent ratings	USA (6 sites)	[28]
	pro, db, pc	159	Decreased response with homozygous 9-repeat on parent ratings	Canada	[29]
	pro, op	30	Decreased response with homozygous 10-repeat	USA (New York)	[30]
	pro, op	50	Decreased response with homozygous 10-repeat	Brazil	[31]
	retro	119	Increased response with number of 10-repeats	Ireland	[32]
	pro, op with SPECT imaging	11	Decreased response with homozygous 10-repeat	Korea	[33]
	retro	168	No effect	UK (Wales)	[34]
	pro, op	82	No effect	Netherlands	[35]
	pro, op	26	No effect of the 10-repeat allele or the 10/3 haplotype	Ireland	[36]
	pro, op	111	No effect	Brazil	[37]
	pro, op	122	No effect	Hungary	[26]
	pro, op	141	Decreased response with homozygous 10-repeat	France	[38]
	retro	156	No effect	USA (Missouri)	[39]
Dopamine D ₂ receptor (<i>DRD2</i>)	pro, op	30	No effect	USA (New York)	[30]

Gene	Study design	Sample size	Outcome	Study location	Reference
Dopamine D ₄ receptor (<i>DRD4</i>)	pro, db, pc	81 (preschoolers)	No effect on symptoms. Increased rate of adverse effects linked to the 7-repeat (social withdrawal) and the 4-repeat (picking)	USA (6 sites)	[28]
	pro, op	30	No effect	USA (New York)	[30]
	pro, op	100	7-repeat transmission more likely in MPH responders than nonresponders	Turkey	[40]
	pro, op	47	Decreased response with 7-repeat allele in combination with serotonin transporter LL homozygosity	Germany	[41]
	pro, op	45	7-repeat associated with need for higher MPH doses	USA (New York)	[42]
	retro	82	Borderline significant (p = 0.09) association between 7-repeat and increased response	Netherlands	[35]
	pro, op	83	Decreased response with 7-repeat allele	Korea	[43]
	pro, op	111	No effect	Brazil	[37]
	pro, op	122	No effect	Hungary	[26]
	retro	159	No effect	USA (Missouri)	[39]
Dopamine D ₅ receptor (<i>DRD5</i>)	pro, op	111	151-base pair allele transmission more likely in MPH responders than nonresponders	Turkey	[40]
Nicotinic acetylcholine α ₄ receptor (<i>CHRNA4</i>)	retro	159	No effect	USA (Missouri)	[39]
Noradrenaline (norepinephrine) transporter protein 1 (<i>SLC6A2</i>)	pro, op	45	Decreased response for homozygous A-allele of the exon 9 polymorphism	China	[44]
Serotonin receptors (<i>HTR1B, HTR2A</i>)	pro, op	111	No effect	Brazil	[37]
Serotonin transporter (<i>SLC6A4</i>)	pro, op	47	Decreased response for LL homozygotes in combination with <i>DRD4</i> 7-repeat allele	Germany	[41]
	pro, op	111	No effect	Brazil	[37]
	retro	159	No effect	USA (Missouri)	[39]
Synaptosomal-associated protein of 25 kDa (<i>SNAP25</i>)	pro, db, pc	81 (preschoolers)	Increased risk of adverse effects for T1065G G homozygotes (sleep difficulties, irritability) and T1069C C homozygotes (tics, abnormal movements)	USA (6 sites)	[28]

db = double-blind; op = open-label; pc = placebo-controlled; pro = prospective; retro = retrospective report; SPECT = single proton emission computerised tomography

^a Studies are listed in chronological order, with prospective, double-blind, placebo-controlled studies (shown in bold type) listed before naturalistic studies for each gene.

Table II
Pharmacogenetic studies of methylphenidate in adults with attention-deficit hyperactivity disorder

Gene	Study design	Sample size	Outcome	Study location	Reference
Dopamine transporter (<i>SLC6A3</i>)	pro, db, pc	66	No effect	USA (Boston, MA)	[45]
	pro, db, pc	42	Decreased response with homozygous 10-repeat	Netherlands	[46]
Dopamine D ₄ receptor (<i>DRD4</i>)	pro, db, pc	42	No effect	Netherlands	[46]
Noradrenaline (norepinephrine) transporter protein 1 (<i>SLC6A2</i>)	pro, db, pc	42	No effect of the SLC6A2 promoter polymorphism	Netherlands	[46]

db = double-blind; pc = placebo-controlled; pro = prospective.