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A Candidate Gene Analysis of Methylphenidate Response in Attention-Deficit/Hyperactivity Disorder

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Abstract

Objective—This study examines the potential role of candidate genes in moderating treatment effects of methylphenidate (MPH) in Attention-Deficit/Hyperactivity Disorder (ADHD).

Method—Eighty two subjects with ADHD aged 6 to 17 participated in a prospective, double-blind, placebo-controlled, multiple-dose, crossover titration trial of immediate release MPH three times daily. Subjects were assessed on a variety of parent and clinician ratings, and a laboratory math test. Data reduction based on principal components analysis identified statistically derived efficacy and side effect outcomes.

Results—ADHD symptom response was predicted by polymorphisms at the serotonin transporter (*SLC6A4*) intron 2 *VNTR* ($p=.01$), with a suggested trend for catechol-O-methyltransferase (*COMT*) ($p=.04$). Gene $\times$ dose interactions were noted on math test outcomes for the dopamine D4 receptor (*DRD4*) promoter ($p=.008$), *DRD4* exon 3 *VNTR* ($p=.006$), and *SLC6A4* promoter insertion/deletion polymorphism (*5HTTLPR*) ($p=.02$). Irritability was predicted by *COMT* ($p=.02$). Vegetative symptoms were predicted by *5HTTLPR* ($p=.003$). No significance effects were noted for the dopamine transporter (*SLC6A3*) or synaptosomal-associated protein 25 (*SNAP25*).

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Conclusions—This report confirms and expands previous studies suggesting that genes moderate ADHD treatment response. ADHD outcomes are not unitary, but reflect both behavioral and learning domains that are likely influenced by different genes. Future research should emphasize candidate gene and genome wide association studies in larger samples, symptom reduction as well as side effects outcomes, and responses over full therapeutic dose ranges to assess differences in both gene and gene $\times$ dose interactive effects.

Keywords

ADHD; pharmacogenetics; candidate genes; methylphenidate

Introduction

There is increasing interest in genetic variability as a potential moderator of medication response in Attention-Deficit/Hyperactivity Disorder (ADHD).¹⁻⁴ Although stimulants are regarded as effective in ADHD management, there is considerable individual variation in both short- and long-term treatment outcomes⁵ and most patients fail to maintain therapy over time.⁶ The potential of ADHD pharmacogenetics lies with the possibility that knowledge of patients' genetic profiles will inform better medication choices and maximize individual treatment outcomes, as well as identify optimal targets for future drug development. ^{1,3,4} Early candidate gene investigations of ADHD susceptibility were directed by an understanding of stimulant activity at the dopamine transporter⁷ and other catecholamine targets.⁸⁻¹⁰ There is increased probability, therefore, that candidate genes associated with disorder risk are likely to predict treatment response.^{1,3}

Numerous investigations have described evidence of genetic influences on methylphenidate treatment outcomes, as summarized in several reviews.¹⁻⁴ Previous studies employed heterogeneous methodologies and yielded conflicting results. Most are retrospective ¹¹⁻¹⁴ or used open-label methylphenidate titrations to “optimal” benefit.¹⁵⁻¹⁸ Randomized, placebo-controlled studies are few,¹⁹⁻²³ as are studies that assessed outcomes at multiple doses.^{21-23,24} Only one explored gene $\times$ dose interactions in addition to gene effects.²¹ Four examined side effects.²²⁻²⁵ Methylphenidate doses ranged from 0.3-2.4 mg/kg/day, typically divided, with all but five studies^{18,20-23} using less than 1.0 mg/kg/day. Outcomes are both categorical and quantitative. Little attention was given to problems from multiple testing or estimating genetic effect sizes.¹ Of prospective reports, almost half had fewer than 50 subjects.^{15,18,20,23,26-28}

The majority of studies examined the variable number of tandem repeat (VNTR) polymorphism located in the 3' untranslated region (UTR) of the dopamine transporter (*SLC6A3*). These suggested decreased^{15,20,25,27,29} or increased¹¹ response with the homozygous 10-repeat; decreased response with the homozygous 9-repeat,^{19,23} or no gene effect.^{12,14,18,21,22,30,31} Several investigated the 48-base pair (bp) VNTR polymorphism in exon 3 of the dopamine D4 receptor (*DRD4*), and demonstrated enhanced response with the homozygous 4-repeat,^{16,18} any 7-repeat allele,¹³ or no effect.^{14,20,21,27,30,31} One described an association between improved response and the homozygous 240-bp polymorphism in the *DRD4* promoter.²¹ A study of the single nucleotide polymorphism (SNP) G1287A at the norepinephrine transporter (*SLC6A2*) suggested decreased response with the homozygous A genotype.²⁸ Two studies of the 44-bp insertion/deletion polymorphism in the promoter region of the serotonin transporter (*SLC6A4*) were negative.^{30,31} Differences in side effects risk have been reported for polymorphisms in synaptosomal-associated protein 25 (*SNAP25*),²¹ *SLC6A3*,²³ and *DRD4*,²¹ while other investigations found no effects.^{24,25} Taken together, these reports and others suggest that

the enormous response variability in ADHD treatment remains unexplained, and initial pharmacogenetic studies, while intriguing, lack consistent replication.

We examined the potential role of candidate genes in moderating ADHD treatment outcomes using a prospective, randomized, blinded, placebo-controlled trial of multiple methylphenidate doses in a relatively large sample. Both efficacy and side effects ratings were obtained, and data reduction techniques were used to derive primary outcome measures. Genotype classifications were defined *a priori* to any analyses to minimize potential problems from multiple comparisons. We considered both gene effects and gene $\times$ dose interactions. Primary hypotheses were that individual candidate genes contribute to variability in symptom changes and side effects profiles during short-term methylphenidate treatment of ADHD.

Method

Participants and Procedures

Male and female subjects aged 6 to 17 years met *DSM-IV* criteria for ADHD, any subtype. Additional inclusion criteria included 1) the absence of a clinically adequate methylphenidate trial comparable to the dose range and treatment duration employed in this study, and 2) normal intelligence ($IQ > 70$) as assessed by the Kaufman Brief Intelligence Test.³² Exclusion criteria were 1) clinical need for other medication with central nervous system effects, 2) current conditions that might require ongoing concomitant medication, or 3) evidence of autism, psychosis, or suicidality. Inclusion and exclusion criteria were determined following clinical evaluation and review of screening data by the study physician.

After receiving a full verbal explanation of study procedures, parents and subjects provided written informed consent/assent approved by the UCLA Institutional Review Board. Psychiatric diagnoses were based on the Schedule for Affective Disorders and Schizophrenia for School Aged Children (KSADS-PL)³³ administered to parent and child, and confirmed by clinical interview. Blood samples were obtained for subsequent genotyping.

Subjects participated in a four- or five-week, double-blind, placebo-controlled, crossover titration trial of immediate-release methylphenidate modeled after other ADHD studies.^{5,34,35} Subjects weighing less than 25 kg received one week each of 5mg, 10mg, 15mg, or placebo three times daily (TID) in random order. Subjects weighing 25kg or more had an additional randomized week of 20mg TID. The first dose in each period was given in the research laboratory and followed after 45 minutes with the Permanent Product Measure of Performance (PERMP) – a 10 minute age-appropriate math test sensitive to stimulant dose effects and scored to provide numbers of problems attempted and correct.³⁶ The ADHD Rating Scale IV (ADHD-RS) measured ADHD symptoms in each previous week, based on the investigator's interview with parent and subject.^{37,38} Parents also rated each prior week's behavior with the Strengths and Weaknesses of ADHD-Symptoms and Normal Behavior (SWAN) scale, an instrument designed to assess population variability in attention, impulsivity, and hyperactivity based on a 7-point scale (-3...0...+3) of *DSM-IV* ADHD related behaviors ranging from far below to far above average.³⁹ Potential side effects on each dose were evaluated via parent completed Side Effects Rating Scales, consisting of 4-point (none, mild, moderate, or severe) ratings on 11 adverse events that commonly occur with stimulants.⁴⁰⁻⁴²

Outcomes

To optimize use of study measures while minimizing risks of multiple testing, we used principal components analyses with varimax rotation to derive composite outcome measures for efficacy and side effects, retaining components with eigenvalues ≥ 1 . Six efficacy variables were initially considered – the ADHD-RS-inattentive subscale, ADHD-RS-hyperactive/impulsive subscale, parent SWAN-inattentive subscale, parent SWAN-hyperactive/impulsive subscale, PERMP-number attempted, and PERMP-number correct. Inattentive and hyperactive subscales of the ADHD-RS and parent SWAN were used in anticipation of their loading on separate components, but they did not. They were replaced respectively by ADHD-RS total and SWAN total scores. A two component solution explained 92% of variance. The first, *ADHD Symptoms* (eigenvalue 2.1, 54% variability explained), comprised equally weighted standardized values from the ADHD-RS and SWAN total scores. The second was comprised of a single item *Math Problems Correct* (eigenvalue 1.5, 38% variability explained). In consideration of potential ethnic heterogeneity, secondary analyses of positive outcomes were conducted limited to white and non-Hispanic white subject subgroups.

Principal components analysis was also employed to suggest logical side effects groupings. Four components explained 61% of variance. These were *Irritability*, comprised of picking, worried/anxious, crabby/irritable, and tearful/sad/depressed (eigenvalue 3.2, 30% variability explained); *Vegetative Symptoms*, comprised of dull/tired/listless, sleep problems, and appetite loss (eigenvalue 1.4, 13% variability explained); *Abnormal Movements*, comprised of motor tics and buccal-lingual movements (eigenvalue 1.1; 10% variability explained), and *Somatic Symptoms*, comprised of headache and stomachache (eigenvalue 1.0; 9% variability explained). Since distributions of side effects ratings within each component were skewed towards “none” or “mild”, we were unable to derive component scores based on weighted rankings of individual items. Instead, consistent with earlier work,²¹ we derived binary outcomes (absent/present) for each component by assigning a value of “0” if ratings for all items within the component were coded “none” or “1” if any item within the component received a rating of “mild”, “moderate”, or “severe.”

Candidate Genes and Genotypes

Selection of candidate genes was guided by previous pharmacogenetic studies, and known roles in catecholamine pathways or as putative stimulant targets. We examined polymorphisms at catechol-O-methyltransferase (*COMT*),^{3,4} the DRD4 promoter region (*DRD4PROM*)²⁸ and exon 3 VNTR (*DRD4VNTR*),^{3,4} *SLC6A2*·*3*·*4* *SLC6A3*·*3*·*4* the *SLC6A4* promoter region insertion/deletion (*5HTTLPR*)^{3,4} and intron 2 VNTR (*SLC6A4VNTR*),⁴³ and *SNAP25* T1065G and T1069C.^{3,4} To minimize multiple testing of non-independent genotypes, allele frequencies were computed and subjects were categorized by a 3-level variable - homozygous (+/+), heterozygous (+/-), or absent (-/-), with reference to the most frequently occurring allele.

PCR Amplification and DNA Sequencing

The *COMT* Val158Met polymorphism was genotyped by PCR, restriction digest, and electrophoresis. PCR amplification of 50ng of genomic DNA was carried out in a volume of 25 μ L reactions containing 0.8mM of dNTP, 0.8 μ M of forward and reverse primers (F- 5'-CTC ATC ACC ATC GAG ATC AA-3'; R- 5'-CCA GGT CTG ACA ACG CCT CA-3'), 1 $\times$ PCR Buffer, 1 $\times$ Q-Solution, 0.6U of HotStarTaq DNA polymerase (Qiagen, California). The DNA was denatured at 95°C for 15 minutes, followed by 35 cycles of 95°C (30 secs), 50°C (45 secs), and 72°C (45 secs), and completed with a 10 min final extension at 72°C. Aliquots of 7.5 μ L of PCR product were digested for 3 hours in 10 μ L reactions consisting of

1 μ g BSA, 1 $\times$ manufacture buffer #4, and 5U NlaIII restriction enzyme (New England Biolab).

The *DRD4PROM* 120/240-bp allele was genotyped using published methods and the following primers: F- 5'-GTT GTC TGT CTT TTC TCA TTG TTT CCA TTG-3'; R 5'-GAA GAA GCA GGC ACC GTG AGC-3'.⁴⁴ The *DRD4VNTR* was genotyped with the following modified primers: F- 5'-CTA CCC TGC CCG CTC ATG-3'; R- 5'-CCG GTG ATC TTG GCA CGC-3' using previously described methods.⁴⁵ The *SLC6A2* G1287A SNP was genotyped following previously published methods.⁴⁶ The *SLC6A3* 480-bp VNTR in the 3' UTR was genotyped using published methods and the following primers: F- 5'-TGT GGT GTA GGG AAC GGC CTG AG-3'; R- 5'-CTT CCT GGA GGT CAC GGC TCA AGG-3'.⁷ The *SLC6A4* promoter region 44-bp insertion/deletion short/long variant (*5HTTLPR*) and intron 2 VNTR were genotyped according to published protocols.⁴³ *SNAP25* T1065G and T1069C were genotyped following published methods and using the following primers: F- 5'-TTC TCC TCC AAA TGC TGT CG-3'; R- 5'-CCA CCG AGG AGA GAA AAT G-3'.⁴⁷

PCR products were electrophoresed in 2% gold agarose (BMA) gels in 1 $\times$ TBE and imaged with ethidium bromide under fluorescent Kodak digital camera. Alleles were determined by comparison with molecular weight standards and control individuals with previously determined genotypes. Samples were double-scored by two technicians independent of clinical information. All assays included positive and negative control samples.

Data Analysis

Analyses used SAS version 9.1.⁴⁸ Descriptive statistics were derived for demographic and genetic characteristics. Genotypes were assessed for Hardy-Weinberg equilibrium (HWE) using SAS PROC ALLELE. Predictors of efficacy were tested using repeated measures ANOVA, fit using SAS PROC MIXED to account for missing data. The fixed effects terms in each model were gene, dose, and gene $\times$ dose interaction. Effect size estimates for efficacy were based on Cohen's f^2 , the ratio of variance explained to unexplained variance for the main and interactive effects.⁴⁹ For side effects, generalized estimating equations based on logistic link functions were conducted using SAS PROC GENMOD to assess genotype contributions on binary outcomes.²¹

Given the pilot nature of this investigation, tests of each candidate gene were considered independently. However, due to our wish to minimize false positive findings and consideration of two primary efficacy measures, α (Type I error rate) was set at 2.5% based on the Bonferroni correction. Since very few previous studies have considered genetic risk for side effects, statistical significance for side effects outcomes remained at 5%. All tests were two tailed. The sample was sufficiently powered to detect small gene and gene $\times$ dose effects at $p < .025$ and medium effects at $p < .0005$ based on Cohen's f^2 .

Results

Subject characteristics appear in Table 1. Candidate genes, allele frequencies, and analyzed genotype groupings appear in Table 2. There were no differences in the frequencies of psychiatric comorbidities across genotypes ($p < .05$). Genotypes met criteria for Hardy Weinberg equilibrium ($p > .01$). Allele frequencies did not differ from established population norms (ALFRED database: <http://alfred.med.yale.edu/alfred/index.asp>).

Active medication was superior to placebo for both *ADHD Symptoms* ($F_{80}=43.01$, $p < .0001$) and *Math Problems Correct* ($F_{81}=50.91$, $p < .0001$). Sex, weight, and age were not significant as covariates for any outcome. For all efficacy models, dose was a positive predictor ($p < .$

01). Gene effects and gene $\times$ dose interactions are summarized in Table 3, with significant outcomes further illustrated in Figures 1 and 2. A significant effect on *ADHD Symptoms* was detected for the *SLCA64VNTR*, with a trend for *COMT*. Gene $\times$ dose interactions were detected for *Math Problems Correct* and the *DRD4PROM*, *DRD4VNTR*, and *5HTTLPR*. There were no significant effects for *SLC6A2*, *SLC6A3*, or *SNAP25*.

When restricted to white subjects ($N=60$), significant gene $\times$ dose interactions remained for *Math Problems Correct* with *DRD4PROM* ($F_{(8,227)}=3.90$, $p=.0002$, $f^2=.14$), *DRD4VNTR* ($F_{(8,227)}=2.91$, $p=.004$, $f^2=.10$), and *5HTTLPR* ($F_{(8,227)}=2.04$, $p=0.04$, $f^2=.07$). When restricted to white/non-Hispanic subjects ($N=42$), a significant gene $\times$ dose interaction remained with the *DRD4PROM* ($F_{(8,148)}=3.93$, $p=.0003$, $f^2=.21$).

The majority of side effects ratings were “none” or “mild”, accounting for an average of 87% of ratings across individual items. *Irritability*, *Vegetative Symptoms*, *Somatic Symptoms*, and *Abnormal Movements* occurred in 75%, 67%, 38%, and 23% of subjects respectively. There were several suggestive predictors of side effects (Table 4). *Irritability* was predicted by *COMT*, with trends noted for *DRD4PROM* and *SNAP25*. Similarly, there were trends for both *COMT* and *DRD4PROM* on *Somatic Symptoms*. *5HTTLPR* predicted *Vegetative Symptoms*. There were no significant predictors of *Abnormal Movements*.

Discussion

This study confirms and expands previous investigations suggesting individual genes moderate response variability in ADHD treatment. Unlike most previous reports, we prospectively examined an expanded set of candidate genes, used blinded ratings of both efficacy and side effects, assessed responses over a range of doses, analyzed statistically derived components as dependent variables rather than arbitrarily choosing outcome measures, and defined genotypes empirically based on allele frequencies without *a priori* consideration of potential effects. While recognizing that sample sizes for certain genotypes are too small to sustain population inferences, the overall study sample is larger than a majority of currently published ADHD pharmacogenetic studies. Apart from any significant findings, study methods described provide a strong basis for future investigations.

For efficacy outcomes, our most striking finding is the observation that differences in response profiles across genotypes, i.e. gene $\times$ dose interactions, are potentially more significant than genotype effects alone. Conflicting results in prior reports might be due in part to failures to titrate medication over a sufficient range to elicit differences in dose-response by genotype. Earlier investigations described both linear⁵⁰ and curvilinear⁵¹ stimulant dose-response profiles. Our results, while requiring replication, suggest that genotype might moderate dose-response outcomes depending on the specific gene and outcome of interest. Assessing response over a full range of doses and consideration of both gene effects and gene $\times$ dose interactions are essential for future ADHD pharmacogenetic studies.

Selection of appropriate outcome measures remains a major issue. Correlations between various ADHD efficacy measures are notably weak,⁵² and there is particular risk for spurious findings in pharmacogenetic studies of outcome variables selected *post hoc*. Our data reduction strategy allowed us to select outcome measures objectively and reduced the risk of false positive findings from multiple exploratory tests. Although our primary results did not meet a level for genome wide significance ($p \leq 1 \times 10^{-8}$), the high prior probability that selected candidates are involved in stimulant medication response and the strength of demonstrated associations support the validity of our findings. Interestingly, our two component solution for efficacy outcomes is consistent with earlier observations describing

two domains of ADHD treatment response, namely learning performance and behavior.⁵¹ Our results support the idea that ADHD outcomes are not unitary, but reflect multiple domains of functioning. Furthermore, our data suggest that different genes differentially influence response on these separate domains.

Our results are largely consistent with previous speculations about the neurobiological significance of these genes. The possibility remains that selected candidates are merely linked with other genes that influence medication response, or are interacting with other unidentified genes to yield observed findings. As such, attempts to provide mechanistic explanations significant effects are speculative.

One prior study of *DRD4VNTR* reported that subjects with the 7-repeat required higher doses to respond on overt ADHD behaviors.¹⁸ The apparent deterioration on *Math Problems Correct* at higher doses in subjects without the homozygous +4/+4 genotype is consistent with older observations that the higher stimulant doses required to control behavior lead to concomitant impairments in learning measures.⁵¹⁻⁵³ For the *SLC6A4VNTR*, findings that individuals with the -12/-12 genotype are less sensitive to symptom improvement are consistent with one report describing increased euphoria from dextro-amphetamine.⁴³ The poorer *Math* response at higher doses in individuals with the +L allele at *5HTTLPR* is consistent with knowledge of the interactions between serotonergic and dopaminergic systems, where serotonin is generally found to constrain dopaminergic signaling.²⁶ Although our results only demonstrated trend effects for *COMT*, several previous reports described a potential role in moderating medication response in both depression and schizophrenia.⁵⁴⁻⁵⁵ Our lack of significant findings with *SLC6A3*, in spite of overall power to detect small effects, reflects the inconsistencies in earlier reports and provides further evidence suggesting that this gene plays less of a role in treatment response than previously proposed.

Examination of genetic moderators of tolerability might ultimately prove more useful than predictions of efficacy.¹⁻³⁻²¹ Side effects findings for the *DRD4PROM* and *SNAP25* are consistent with previous work.²¹ However, relative to investigations of symptom reduction, optimal approaches to assess genetic contributions to side effects are much less developed. For example, the current report describes a brief titration trial and used a low severity threshold to generate sufficient side effect frequencies for reasonable analysis. More clinically relevant information would be derived from a longer term study in larger numbers of patients. Long term investigations of potential genetic moderators of side effects such as growth deceleration, appetite loss, irritability, and tic emergence would, if positive and replicated, have significant impact on clinical practice.

There is an increasing emphasis in ADHD pharmacogenetics on estimating gene effect sizes, $1-19-22$ often based on Cohen's d . While we view the estimation of gene effects as laudable, we are now of the opinion that using Cohen's d for this purpose is a misapplication of that statistic that leads to an overestimation of genetic effects. Cohen's d generally serves to estimate the differences in effect size between two treatments.⁴⁹ However, given the large stimulant treatment effects demonstrated in ADHD, effects of medication dose are likely to outweigh any genetic effects. The proper question is not whether different genotypes should be considered as different treatments, but how much variance in treatment outcome is explained by genetic as opposed to medication effects. Cohen f^2 provides a signal to noise ratio, indicating how much variability is attributable to the model's fixed effects and thus provides a dimensionless measure of the magnitude of gene and gene $\times$ dose signals.⁴⁹ Based on convention, whereby small effects are defined by $f^2 > .02$, moderate effects as $> .15$, and large effects $> .35$, none of the statistically significant effects demonstrated in primary

analyses are likely to represent more than small clinical effects. This is consistent with results from meta-analyses of these same candidate genes for ADHD risk.^{8,10}

Unlike the majority of published ADHD pharmacogenetic studies, we adjusted significance levels for multiple efficacy outcomes based on the Bonferroni correction. We regarded tests of each genotype as independent, but chose to report these within a single study rather than describe each finding separately. Nonetheless, some might argue that a stricter correction should have been applied, i.e. that further corrections should have been made when examining two polymorphisms within a single gene, or that testing two outcomes for each of nine genotypes suggests adjusting for eighteen tests ($p < .003$). Although this pilot investigation was underpowered to detect small effects at this very conservative level of significance, the fact that over 20% of primary outcomes were positive at $p < .025$ is highly suggestive of true findings. Interestingly, our most robust suggested finding was in the restricted sample of white/non-Hispanic subjects which demonstrated a medium sized effect of *DRD4PROM* $\times$ dose on *Math Problems Correct*, at a significance level corrected for 100 tests ($p < .0005$). This highlights the need to conduct future research in more homogeneous samples.

This study has several limitations. Although there is increased probability that candidate genes in the catecholamine system are involved in methylphenidate response, candidate gene investigations have high false positive rates. Our results require replication in independent samples prior to any consideration of clinical relevance. Although the majority of our positive findings remained significant when the sample was restricted to whites only, we were unable, given the size and heterogeneity of the sample, to make further assessments of potential population stratification. We did not correct for comparison of multiple polymorphisms within single genes, but treated these as independent tests. This might prove meaningful as our four positive findings are found within two genes. Future research should consider the possibility of linkage disequilibrium between these variants. While we attempted to reduce problems associated with multiple testing, our decision to define genotypes *a priori* based on allele frequencies negated our ability to assess other previously described genotypes of interest, such as the homozygous 9-repeat allele at *SLC6A3*, or the 7-repeat allele at *DRD4* as primary dependent variables. However, it may prove advantageous to assess the role of these alleles directly after removing minor alleles in future studies of larger samples. Our work did not consider recent findings suggesting that *5HTTLPR* is functionally triallelic, with the +L polymorphism exhibiting a G versus A substitution with nearly equivalent expression as the S allele.⁵⁶ However, whereas this variant was not reported until after we completed genotyping and was not considered in earlier studies involving this deletion/insertion polymorphism, full elucidation of the role of polymorphisms at *5HTTLPR* will require ongoing examination. Finally, we did not explore potential gene $\times$ gene interactions as these would have required additional sets of hypotheses and analyses. These are more suitable for a separate report.

Our investigation assessed short-term outcomes. Large scale studies document that short-term response rates, cited as high as 75-80%, often fall to 55-60% over time. Short-term data are incomplete assessments of treatment response. Long-term outcomes might prove to be much more clinically relevant for investigation. Ultimately, definitive findings in ADHD pharmacogenetics are likely to depend on a combination of candidate gene and genome wide association approaches with extended treatment outcomes in much larger samples.

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Figure 1. Significant gene effects on ADHD symptoms

Subjects lacking any copy of the 12-repeat allele at SLC6A4 showed worsened ADHD symptom ratings with all doses of methylphenidate compared with those subjects having at least one copy of the allele. VNTR = Variable Number Tandem Repeat; TID = Three Times Daily.

Figure 2. Significant gene $\times$ dose effects on Math Problems Correct

Gene $\times$ dose interactions were noted on Math Problems Correct – the total number of correct problems obtained during a 10-minute math test administered 45 minutes after initial dosing in each treatment week. Subjects with any 240 allele at the DRD4 promoter or any L allele at 5HTTLPR showed increased responses at lower doses and a response plateau at higher doses. A positive linear relationship between dose and response was seen in those lacking 240 or L alleles. Subjects not homozygous for the 4-repeat at DRD4 showed increased responses at lower doses and deterioration at higher doses. VNTR = Variable Number Tandem Repeat; TID = Three Times Daily; L = Long Allele.

Table 1
Sample Demographic and Clinical Characteristics

Total N	82	
Male, no. (%)	63 (77)	
Age, yr, mean (SD), range	9.82 (3.08)	6-17
Weight, kg, mean (SD), range	42.14 (19.07)	19-108
Subjects (N) < 25 kg	14	
Subjects (N) ≥ 25 kg	68	
<hr/>		
Race/Ethnicity, no. (%)		
Non-Hispanic White	42 (51)	
Hispanic White	20 (24)	
Black	11 (13)	
Asian	7 (9)	
Other	2 (2)	
<hr/>		
ADHD subtype, no. (%)		
Inattentive	29 (35)	
Hyperactive-impulsive	4 (5)	
Combined	49 (60)	
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Comorbid Conditions, no. (%)		
Agoraphobia	9 (11)	
Conduct disorder	2 (2)	
Generalized anxiety disorder	8 (10)	
Oppositional Defiant disorder	32 (39)	
Separation anxiety disorder	4 (5)	
Social phobia	6 (7)	
Tic disorder	3 (4)	

Table 2

Allele Frequencies and Genotype Classifications

Gene	Description	Allele	Frequency (%)	Genotype	N
COMT (N=82)	Catechol-O-methyltransferase Val158Met polymorphism	Val Met	53 47	+Val/+Val +Val/-Val -Val/-Val	24 39 19
DRD4Prom (N=81)	Dopamine 4 receptor promoter region 240/120 polymorphism	240 120	69 31	+240/+240 +240/-240 -240/-240	39 34 8
DRD4VNTR (N=81)	Dopamine 4 receptor exon 3 variable number tandem repeat (VNTR)	4 7 2, 3, 5, 6	62 27 11	+4/+4 +4/-4 -4/-4	29 42 10
SLC6A2 (N=77)	Norepinephrine transporter G1287A single nucleotide polymorphism (SNP)	G A	77 23	+G/+G +G/-G -G/-G	47 24 6
SLC6A3 (N=79)	Dopamine transporter 3' untranslated region VNTR	10 9 3, 11, 12	78 19 3	+10/+10 +10/-10 -10/-10	48 28 3
5HTTLPR (N=81)	Serotonin transporter promoter region insertion/deletion polymorphism	Long (L) Short (S)	54 46	+L/+L +L/-L -L/-L	22 43 16
SLC6A4VNTR (N=81)	Serotonin transporter intron 2 VNTR	12 10 9	71 28 1	+12/+12 +12/-12 -12/-12	42 31 8
SNAP25-1065 (N=81)	Synaptosomal-associated protein 25 T1065G SNP	T G	68 32	+T/+T +T/-T -T/-T	41 28 12
SNAP25-1069 (N=81)	Synaptosomal-associated protein 25 T1069C SNP	T C	81 20	+T/+T +T/-T -T/-T	56 19 6

VNTR = Variable Number Tandem Repeat; SNP = Single Nucleotide

Table 3

Gene and Gene $\times$ Dose (GXD) Effects

Gene	Effect	Efficacy Outcomes			
		ADHD Total Symptoms		Problems Correct	
	F _{df}	P value	E.S. (f^2)	F _{df}	P value
					E.S. (f^2)
COMT (N=82)	Gene	3.25 _{2,79}	.04	1.75 _{2,79}	.18
	GXD	0.76 _{8,298}	.64	1.01 _{8,300}	.43
DRD4Prom (N=81)	Gene	1.54 _{2,78}	.22	0.53 _{2,78}	.59
	GXD	0.68 _{8,294}	.71	2.64 _{8,296}	.008
DRD4VNTR (N=81)	Gene	0.05 _{2,78}	.95	0.39 _{2,78}	.68
	GXD	1.38 _{8,294}	.20	2.76 _{8,296}	.006
SLC6A2 (N=77)	Gene	0.49 _{2,79}	.61	0.18 _{2,74}	.83
	GXD	1.63 _{8,279}	.12	1.14 _{8,281}	.34
SLC6A3 (N=79)	Gene	0.08 _{2,76}	.92	1.18 _{2,76}	.31
	GXD	1.35 _{8,286}	.22	0.63 _{8,288}	.76
5HTTLPR (N=81)	Gene	1.48 _{2,78}	.23	1.00 _{2,78}	.37
	GXD	0.86 _{8,294}	.55	2.35 _{8,296}	.02
SLC6A4VNTR (N=81)	Gene	4.47 _{2,78}	.01	0.57 _{2,78}	.57
	GXD	1.21 _{8,294}	.29	1.64 _{8,296}	.11
SNAP25-1065 (N=81)	Gene	0.36 _{2,78}	.70	0.53 _{2,78}	.59
	GXD	0.82 _{8,294}	.59	0.93 _{8,296}	.49
SNAP25-1069 (N=81)	Gene	2.22 _{2,78}	.11	0.99 _{2,78}	.38
	GXD	1.18 _{8,294}	.31	0.54 _{8,296}	.82

Significant = $p < .025$; Trend = $.05 < p < .025$; E.S. (f^2) = Effect Size (Cohen's f^2); VNTR = Variable Number Tandem Repeat

Table 4
Genetic Predictors of Side Effects (P value <.15)

Side Effect	Gene	$\chi^2_{df=2}$	P value	Effect
Irritability	<i>COMT</i>	8.09	.02	+Val/+Val increased
	<i>DRD4PROM</i>	4.75	.09	+240/+240 decreased
	<i>SNAP25-1069</i>	4.70	.10	+T/+T decreased
Somatic Symptoms	<i>COMT</i>	5.45	.07	+Val/+Val decreased
	<i>DRD4PROM</i>	6.02	.05	+240/+240 decreased
Vegetative Symptoms	<i>5HTTLPR</i>	11.38	.003	+L/+L decreased

Significant = $p < .05$.