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Can COMT Val158Met Gene Polymorphism Predict Treatment Outcomes for Methylphenidates in ADHD Patients?

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ABSTRACT

The COMT gene encodes for the Catechol-O-methyltransferase (COMT) enzyme, an enzyme responsible for the breakdown of dopamine and norepinephrine in the prefrontal cortical areas. The most common variation of the COMT gene is the Val158Met polymorphism (rs4680) which leads to a valine (Val) to methionine (Met) substitution at codon 158. It is plausible that variations in this gene may predict treatment outcomes to stimulants like methylphenidates used in the treatment of ADHD. The purpose of this study is to statistically evaluate this association to further the clinical implementation of personalized medicine. Quantitative data was collected from clinical trials where patients were genotyped for the COMT gene and were evaluated for treatment response to methylphenidates on a quantifiable scale. Correlational analysis (n=1094) showed a statistically significant association (p=0.003) between this genotype and treatment outcomes. The Odd's ratio calculated from the binary outcomes (n=638 patients) depicted that the Val/Val carriers were 1.86 times more likely to respond positively to methylphenidate treatment compared to the Met allele carriers. Our analysis shows that variations in COMT gene can reliably predict treatment outcomes to Methylphenidates in ADHD patients. However, this association is based on the data extracted from 9 different clinical studies (n= 1094 patients). These studies had different sample sizes, ethnicities, and measurement scales which may have contributed to the heterogeneity in the overall sample data set, thereby diluting the power of the association. Nevertheless, this analysis adds to the body of pharmacogenomic evidence increasing the clinical utility of precision medicine.

Introduction and Background

Attention-deficit/hyperactivity disorder (ADHD) is a neurological disorder that has become increasingly prevalent worldwide. It is characterized by difficulties with inattentiveness, hyperactivity, or impulsivity. This disorder is commonly diagnosed in children, and in up to 70% of childhood cases, symptoms that lead to impairment in functioning persist into adulthood.[1] CDC states that, "the estimated number of children ever diagnosed with ADHD, according to a national 2016 parent survey, is 6.1 million (9.4%)."[2]

Medications approved by the Food and Drug Administration (FDA) for the treatment of ADHD comprise of stimulants (amphetamines and methylphenidate) and nonstimulants (atomoxetine and extended-release clonidine and guanfacine). However, stimulants particularly methylphenidates have generally been recommended as first-line pharmacologic treatment.[3]

The Need for Precision Medicine

Current pharmacological options alleviate symptoms in some, but not all the affected patients, leaving clinicians to implement the conventional trial-and-error approach to treatment. This trial-and-error approach leads to poorer outcomes for patients, in terms of adverse side effects and potential disease progression whilst the effective treatment is delayed and causes patient dissatisfaction.[4]

Many drugs that are currently available are "one size fits all," but they don't work the same way for everyone. It can be difficult to predict who will benefit from a medication, who will not respond at all, and who will experience negative side effects. This is where the need to explore precision medicine becomes important. Genetics may account for much of the variability in the patients' responses to drug therapies. Equipped with the knowledge of the patients' genetic results, clinicians may predict treatment outcomes to certain medications thereby reducing medication failures and lowering the risk of side effects. Improving treatment outcomes would lead to a better cost-benefit ratio.

What are Genes and Genetic Polymorphisms?

National Institutes of Health (NIH) states that, "a gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Every person has two copies (or alleles) of each gene, one inherited from each parent. If the two alleles are the same, the individual is homozygous for that gene. If the alleles are different, the individual is heterozygous."[5] Each pair of alleles represents the *genotype* of a specific gene.

Genetic polymorphism is a term used to describe certain mutations in the genotype, such as single nucleotide polymorphisms. Single nucleotide polymorphisms, frequently called SNPs, are the most common type of genetic variation among people. A SNP is a variation at a single position in a DNA sequence among individuals. The DNA sequence is formed from a chain of four nucleotide bases: A, C, G, and T.[6] If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP.[6] SNPs may lead to variations in the amino acid sequence.

Figure 1: Single Nucleotide Polymorphism (Taken from Fruehwirth et al. 2015) [7]
Patient 1 is heterozygous (i.e. has one copy of A and one copy of G allele) whereas patients 2 and 3 are homozygous (2 copies of the same allele) for the G and A alleles respectively.

What is Pharmacogenomics?

Pharmacogenomics is the study of how these genetic variations (polymorphisms) affect a person's response to drugs. The study of pharmacogenomics shows how genes can affect the way a drug reacts in the body. A pharmacogenomic study analyzes the patient's DNA and can detect variations in the patient's genes that may impact how they metabolize or respond to certain medications. This study normally looks at genes from two categories[8]:

i) Pharmacokinetic genes: These genes are involved in how the body breaks down or metabolizes a particular medication. Variation in these genes can predict the serum levels of the drug in the body.

ii) Pharmacodynamic genes: These genes are involved in how the medication interacts with the body, particularly the receptors or transporters. Variation in these genes can predict likelihood of response and/or risk of side effects with certain medications.

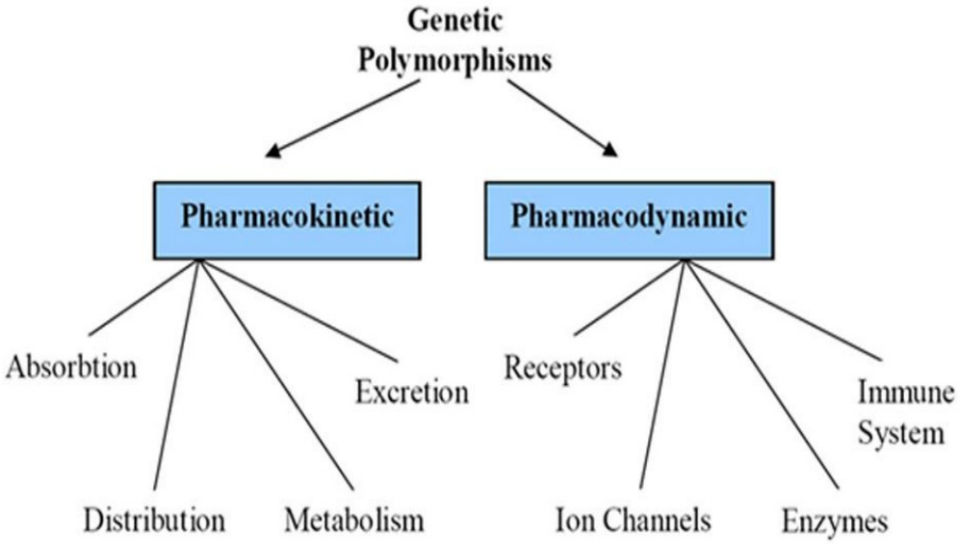


Figure 2: Types of Genetic Polymorphisms (This figure is taken from Adams et al. 2008)[8]

Variations in either or both of categories of genes may change treatment outcomes to medications.

Pathophysiology of ADHD and treatment options

While the exact cause of ADHD is unknown, some researchers have hypothesized the cause to be the dysregulation of dopamine and norepinephrine. This is because dopamine and noradrenaline play important roles in high-level executive functions often reported to be impaired in attention-deficit/hyperactivity disorder (ADHD).[9] According to the American Family Physician[10], the first line treatment for children 6 and above are methylphenidates and amphetamines.

SORT: KEY RECOMMENDATIONS FOR PRACTICE

CLINICAL RECOMMENDATION	EVIDENCE RATING	REFERENCES
Children four years and older and adolescents with poor attention, distractibility, hyperactivity, impulsiveness, poor academic performance, or behavioral problems at home or at school should be evaluated for ADHD.	C	8–10, 16
Behavioral therapy should be the primary treatment for ADHD in children younger than six years, and it may be helpful at older ages.	B	4, 8–10, 30, 31
Treatment of ADHD in children six years and older should start with medication.	B	8–10
Psychostimulants (e.g., methylphenidate [Ritalin], dextroamphetamine) are the most effective therapy for core ADHD symptoms and have generally acceptable adverse effect profiles.	B	9

ADHD = attention-deficit/hyperactivity disorder.

A = consistent, good-quality patient-oriented evidence; B = inconsistent or limited-quality patient-oriented evidence; C = consensus, disease-oriented evidence, usual practice, expert opinion, or case series. For information about the SORT evidence rating system, go to https://www.aafp.org/afpsort.

Figure 3: Current Guidelines for ADHD treatment (This figure is taken from Felt et al. 2014)[10]

According to the above guidelines, most treatment plans including the use of pharmacotherapy like methylphenidates have received an evidence rating of being inconsistent and having limited quality patient-oriented evidence, which is why the need for precise medicine becomes important to improve treatment outcomes.

Mechanism of action of Stimulants

Stimulants like methylphenidate and amphetamine enhance the actions of dopamine and norepinephrine in several brain regions. These medications exert their effects by blocking the transporters that are responsible for the reuptake of dopamine and norepinephrine or by increasing the release of these neurotransmitters, thus leading to an increase in their synaptic availability.[11]

What is the COMT gene?

Sun et al 2013 states that, "Attention-deficit/hyperactivity disorder (ADHD) is a common and highly heritable childhood-onset psychiatric disorder with significant genetic contribution. Considerable evidence has implicated involvement of dopaminergic system and the prefrontal cortex (PFC) in the pathomechanism of ADHD. The catechol-O-methyltransferase (COMT) gene is of particular interest for ADHD as its crucial role in the degradation of dopamine in the PFC."[12]

In short, the COMT gene is a pharmacodynamic gene that codes for Catechol-O-methyltransferase, which is an enzyme that breaks down catecholamines like dopamine (DA) and norepinephrine in the PFC. Stimulants like methylphenidates work by increasing these neurotransmitter levels in the brain. Hence, this gene may have clinical utility in precision medicine, therefore it should be explored as a pharmacogenomic marker for ADHD.

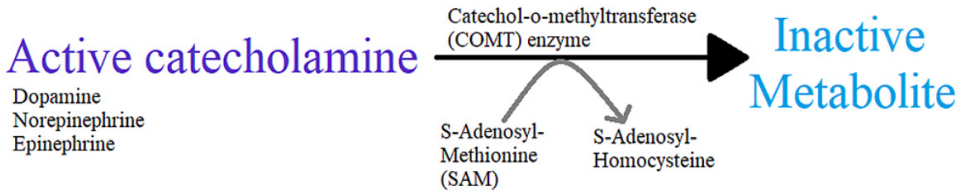


Figure 4: Role of COMT (This figure is taken from Srivastava et al. 2021)[13]

Within the COMT gene, the most widely studied polymorphism is the Val158Met polymorphism (rs4680) which results in an amino acid change from a valine (Val or G) to a methionine (Met or A) at codon 158.[14] This variation is associated with altered enzymatic activity and neurotransmitter levels in the prefrontal cortex. The Met allele is associated with decreased activity in breaking down dopamine and norepinephrine (DA/NE), leading to higher baseline levels of these neurotransmitters[15] and conversely as stated by Chen et al., "Val is a predominant factor that determines higher COMT activity in the prefrontal cortex, which presumably leads to lower synaptic dopamine levels."[16]

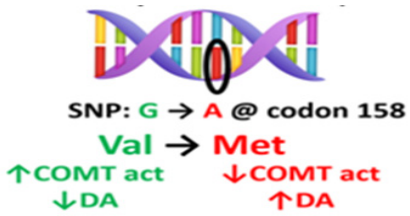


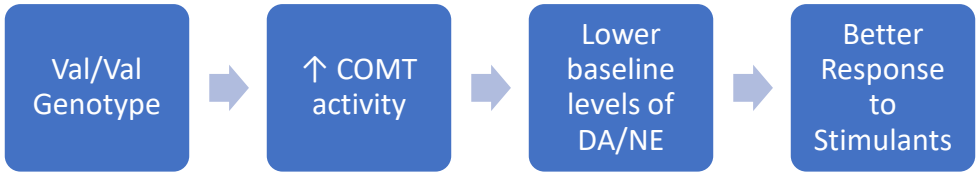
Figure 5: COMT Val158Met Polymorphism (This figure is taken from Smith C 2020)[17]

This differential COMT activity among individuals leads us to the question: ***Can COMT Val158Met polymorphism predict treatment outcomes for stimulants (particularly methylphenidates) in patients with ADHD?***

Research Hypothesis

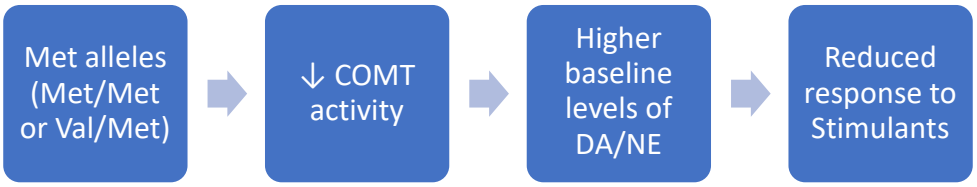
Hypothesis for predicting Methylphenidate treatment outcomes in relation to COMT genotype:

i) The Val/Val genotype [high activity] leads to increased COMT enzyme activity and patients would be expected to have lower baseline DA/NE (due to faster metabolism by COMT). Hence, it can be hypothesized that patients who have the Val/Val genotype may respond better to stimulants like methylphenidates as they work by increasing the dopamine levels.



ii) The Met allele is associated with decreased activity in breaking down dopamine and norepinephrine (DA/NE), leading to higher baseline levels of these neurotransmitters. Since ADHD stimulants work to increase the levels of DA/NE at the synapse, it can be hypothesized that response to these ADHD

medications may be decreased in individuals who already have higher baseline levels of these neurotransmitters.



Methodology

The insights for data extraction and statistical analysis were taken from the published article by Hain et al. 2021[18] that evaluated methylphenidate outcomes in relation to an adrenergic alpha 2A gene called ADRA2A.

Literature Search

Due to the restricted information on patient's genetic results, databases containing genotypes and response rates could not be accessed, therefore raw data was extracted from clinical studies. Systematic guidelines were followed to identify studies where data could be extracted to evaluate methylphenidate outcomes in individuals with the Val158Met variant of the COMT gene. Publications were identified on PubMed database (https://www.ncbi.nlm.nih.gov/pubmed/) using the following Boolean search string: "(Methylphenidate) AND (COMT) AND (ADHD)." Studies that were published up to December 2021 were included. This search gave 26 publications.

The reason for not including amphetamines in our current study is that most studies evaluating amphetamines in relation to COMT genotype were conducted in healthy adults[19-23] and not in ADHD patients. Including the studies that evaluated treatment outcomes only in healthy patients would not meet our inclusion criteria. Additionally, although the primary pharmacologic effect of both amphetamine and methylphenidate is to increase central dopamine and norepinephrine activity, the mechanism of action of amphetamine is complex. Methylphenidates work by blocking the norepinephrine and dopamine transporters, thereby increasing the levels of norepinephrine and dopamine in the brain. The difference with amphetamines is that besides blocking the dopamine transporters, it also promotes dopamine release from synaptic vesicles.[24]

Hence, due to the lack of studies evaluating amphetamines in ADHD patients and the difference in the mechanism of action of these 2 stimulants, we focused our research only on methylphenidate treatment outcomes to minimize confounder effect that may distort the results of the study.

Study Characteristics

After the initial search was completed, each article abstract was screened to assess whether it should be included in this data analysis. Included studies compared methylphenidate outcomes (symptom improvement, response, or adverse events) in patients with ADHD receiving methylphenidate (MPH) treatment across COMT genotypes. Studies that were focusing on the diagnostic utility of COMT rather than pharmacogenomic utility were excluded. Comorbid conditions like Autism and sleep disorders were also excluded. Studies that were not conducted in human subjects and/or were not written in English were also excluded. Studies were included only if the patient population assessed had a diagnosis of ADHD. With these criterions in place, only 10 studies were identified for data extraction to evaluate an association between treatment outcomes of methylphenidates and COMT genotype.

Data extraction

A correlational analysis was performed to assess the association between COMT genotypes and treatment response to methylphenidates. Although, the individual studies reported their own statistical analysis (odds ratio, p value) for their data, the objective of this study was to derive the association by extracting the raw data from the individual studies and then finding a correlation between treatment outcomes and COMT Val158Met genotype.

Raw data was extracted from the publications listed in Table 1. Treatment outcomes were analyzed in terms of number of responders or absolute reduction in ADHD scores in relation to the patient's COMT genotype. Therefore, both dichotomous and continuous outcomes were included.

When collecting raw data, in addition to the number of responders versus non-responders, patient genotype, ethnicity, duration of treatment, patient age, number of patients assessed, validated scales used to access outcomes, and the measure of response was also recorded. Most studies assessed outcomes with the ADHD-RS (ADHD Rating Scale) and the CGI-S (CGI–Severity) scale. Although there are subtle differences in the measure of response recruited by the different studies, the criteria employed for defining responders are all widely accepted in the field of psychiatry and hence, the data is deemed fit to be included in this correlational analysis.

Statistical analysis

There are 2 types of outcomes in which response to methylphenidates are reported. One is called the binary data outcome (dichotomous data) in which the study lists the number of responders versus number of non-responders in relation to COMT genotype. The other is called the continuous data outcome where score reduction or change in mean scores are reported in relation to COMT genotype. To pool the raw data from studies to calculate the odd's ratio and design a forest plot, similar types of outcomes need to be in the cohort. A forest plot is a graphical display of estimated results from several different data sets assessing the same association (hypothesis).

Out of the 10 studies, 5 studies reported MPH response with COMT genotype as binary outcomes[25-29], 3 studies reported response as continuous outcomes[30-32] and 1 only reported on the p value[33]. One study did not report on the COMT genotype and hence, was excluded from our analysis[34].

Since the majority of studies had provided raw data in binary outcomes, data from those 5 studies (Park[27], Kereszturi[25], Cheon[26], Contini[28] and Unal[29]; see Table 2) were extracted which included the total number of responders versus number of non-responders with their genotype. Individual Odds ratio, confidence interval, standard error, z value and p value were calculated in Excel by plugging in formulas:

Odds ratio: $\frac{A\times D}{B\times C}$

Log OR: $\ln\left(\frac{A\times D}{B\times C}\right)$

Standard error of Log OR (SE): $\sqrt{\left(\frac{1}{A}+\frac{1}{B}+\frac{1}{C}+\frac{1}{D}\right)}$

Z score: $\frac{\ln\left(\frac{A\times D}{B\times C}\right)}{\sqrt{\left(\frac{1}{A}+\frac{1}{B}+\frac{1}{C}+\frac{1}{D}\right)}}$

p value: $= 2 \times (1 - Norm.S.Dist\ (\ |z|, True))$

Where A is number of responders to MPH who had Val/Val genotype, B is the number of non-responders to MPH who had Val/Val genotype, C is number of responders to MPH who were Met allele carriers (Met/Met or Val/Met genotype) and D is the number of non-responders to MPH who were Met allele carriers (Met/Met or Val/Met genotype).

This research analysis only analyzed the recessive model of the genomic variant which means that the correlational analysis only compared treatment outcomes with Val/Val versus Met alleles (Val/Met and Met/Met) carriers. This is because most of the raw data from the studies was provided in this format.

Analysis in Revman®

5 Studies (Park, Kereszturi, Cheon, Contini and Unal) were added in the *studies and reference* section of Revman®. In the data and analysis section of the Revman®, we added a comparison called pooled analysis and then added the outcome as the number of responders in each group (Val/Val versus Met Allele carriers). A dichotomous response data type was selected as we only used the raw data that were in binary outcomes. A forest plot was generated used random effects.

Statistical Analysis using Stouffer's Z test

Only 5 studies had reported data in binary outcomes, hence our analysis would not be deemed complete without analyzing the other studies that reported data in continuous outcomes. To include data in binary and continuous outcomes together, a Stouffer's z test was preformed to find an association. This statistical analysis allows us to compare both types of outcomes (continuous and binary).

Descriptive Analysis

Ten studies examined the effect of Val158Met polymorphism on the response to methylphenidate. Three studies showed a positive association (better with Val), one showed an opposite association (better with Met) and six found no association.

Three studies that demonstrated better outcomes with the Val allele. Cheon et al. 2008 assessed the response of 124 stimulant-naïve Korean children to MPH and measured outcomes through ADHD Rating Scale-IV scores from parents and teachers after 8 weeks of treatment. Compared to Met carriers, this group found that Val/Val children had significantly better teacher-rated scores ($p = 0.035$) but not parent-rated scores ($p = 0.60$).[26] Kereszturi et al. 2008 assessed the response of 122 Hungarian children to MPH over a 6 month period. The Val allele was associated with good response to MPH ($p = 0.009$) on the total ADHD-RS scale and Val/Val subjects were more frequent in the responder group ($p = 0.037$).[25] Park et al. 2014 included a sample of 120 stimulant-naïve Korean children treated for 8 weeks. Val/Val was associated with response to MPH on the hyperactivity/impulsivity subscale of ADHD-RS ($p = 0.044$).[27]

Only 1 study showed better outcomes with the Met allele carriers. Salatino-Oliviera et al. 2011 evaluated 126 European-Brazilian male children with ADHD. Using the parent-rated SNAP-IV oppositional subscale, this group found that Met carriers had a better response trajectory to MPH than Val/Val subjects. However, at the end of the 3-month assessment period, Met carriers and Val/Val children had similar SNAP-IV oppositional scores.[31]

Six studies showed a non-significant association between COMT genotype and response to methylphenidates. Sengupta et al. 2008 enrolled 212 Canadian children with ADHD. The study was not able to find a significant interaction between COMT genotype and treatment with MPHs ($p = 0.20$).[32] McGough et al. 2009 assessed Val158Met polymorphism in 82 children treated with MPHs. In this sample, COMT genotype was not associated with MPH treatment efficacy ($p = 0.64$ on ADHD-RS).[33] Contini et al. 2012 also did not find COMT genotype was not associated with response to MPHs (on either SNAP-IV or CGI-S scales) in a sample of 164 Brazilians of European descent ($p = 0.26$).[28] Yatsuga et al. 2014 assessed 50 Japanese male children and found that the Val158Met polymorphism had no significant effect on either methylphenidate response (ADHD-RS; $p = 0.56$) or risk of adverse events ($p = 0.27$).[30] Unal et al. 2016 genotyped 108 Turkish children for the rs4680 polymorphism to look for any association with response to MPH after 4-6 weeks of therapy. This group examined ADHD symptoms and severity (on CPRS/CTRS for parents/teachers and CGI-S) and did not find COMT genotype to be associated with response to MPHs.[29] Pagerols et al. 2017 found that COMT genotype did not affect treatment outcomes (on the CGI-I scale) or risk of adverse events in a sample of 107 Spanish children. Additionally, COMT genotypic frequencies for patient cohort were not provided in the study. Hence, the data from this study could not be used for the analysis.[34]

A detailed description of these ten studies is also depicted in Table 1. The highlighted studies are the ones where the authors have provided results in binary outcomes i.e. the studies have listed the number of responders vs non-responders to MPH with respect to their COMT genotypes. Raw data was extracted from these 5 studies (total number of patients= 638) in terms of responders' vs non-responders which is summarized in table 2.

Table 1: Summary Findings from the 10 Clinical Studies

Reference	Ethnicity	Duration	N	Age	Scales Assessed	Measure of Response (defining Responders)	Genotype Results for COMT
Pagerols et al. 2017	Caucasian (Spanish)	8 weeks	107	5-16 yrs	CGI-S	≥ 2 points improvement in the CGI-S	Not given
Park et al. 2014	Korean	8 weeks	120	6-15 yrs	ADHD-RS (parents), CGI-S (clinician)	≥ 50% ADHD-RS reduction and CGI-I scores of 1 or 2	Given (Binary outcomes)
Yatsuga et al. 2014	Japanese	12 weeks	50	6-16 yrs	ADHD-RS	Δ ADHD-RS	Given (continuous outcomes)
Kereszturi et al. 2008	Caucasian (Hungarian)	24 weeks	122	mean age: 9.6±2.6	ADHD-RS (parent rated), CGI-S (clinician rated)	≥ 25% ADHD-RS reduction and CGI-I scores of 2 or less in last 2 months	Given (Binary outcomes)
Cheon et al. 2008	Korean	8 weeks	124	6-12 yrs	ADHD-RS (parent and clinician rated)	≥ 50% ADHD-RS reduction	Given (Binary outcomes)
Sengupta et al. 2008	Canadian (Montreal)	7 days	212	6-12 yrs	RASS	Δ RASS	Given (continuous outcomes)
Contini et al. 2012	Native Brazilian of European descent	30 days	164	18 yrs or older	SNAP-IV, CGI-S	≥ 30% SNAP IV reduction and CGI-I scores of 2 or less	Given (Binary outcomes)
Salatino-Oliviera et al. 2011	European Brazilian	1 and 3 months	112	4-17 yrs	oppositional subscale of the SNAP-IV (parent-rated)	Δ oppositional subscale of the SNAP-IV	Given (continuous outcomes)
McGough et al. 2009	Mixed Ethnicity	4-5 weeks	82	6-17 yrs	ADHD-RS and SWAN total scores	Δ ADHD-RS and SWAN total scores	Only p value given (no raw data)
Unal et al. 2016	Turkish	4-6 weeks	108	6-18 yrs	CPRS, CTRS, CGI-S, GAS, CPT and TMT A and B	≥ 2 points improvement in the CGI-S and ≥60 on total GAS score and ≥ 50% improvement on any subscales of CPRS/CTRS or improvement in one of the neuropsychological tests applied at follow-up	Given (Binary outcomes)

Scales defined: ADHD-RS: ADHD Rating Scale; CGI-S: CGI–Severity; CPRS: Conners' Parent Rating Scale; CTRS: Conners' Teacher Rating Scale; RASS: The Restricted Academic Situation Scale; GAS: Global Assessment of Functioning Scale; SNAP-IV: Swanson, Nolan, and Pelham Version-IV Scale; TMT: Trail Making Test.

Statistical Analysis and Results

Analysis of data reported in binary outcomes

In table 2, the number of responders versus non responders with reference to the patients' genotypes are segregated.

Table 2: Raw Data extraction from 5 Studies that have Binary outcomes:

	Val/Val			Met alleles		
Studies	# of Responders	# of Non- Responders	Total	# of Responders	# of Non- Responders	Total
Cheon et al. 2008	40	28	68	24	32	56
Contini et al. 2012	40	7	47	96	21	117
Kereszturi et al. 2008	34	5	39	56	27	83
Park et al. 2014	46	24	70	26	24	50
Unal et al. 2016	22	7	29	50	29	79

Table 3: Percent Responders vs non-responders:

	Val/Val (in percent)		Met alleles (in percent)	
Studies	Responders	Non- Responders	Responders	Non- Responders
Cheon et al. 2008	58.8	41.2	42.9	57.1
Contini et al. 2012	85.1	14.9	82.1	17.9
Kereszturi et al. 2008	87.2	12.8	67.5	32.5
Park et al. 2014	65.7	34.3	52	48
Unal et al. 2016	75.9	24.1	63.3	36.7

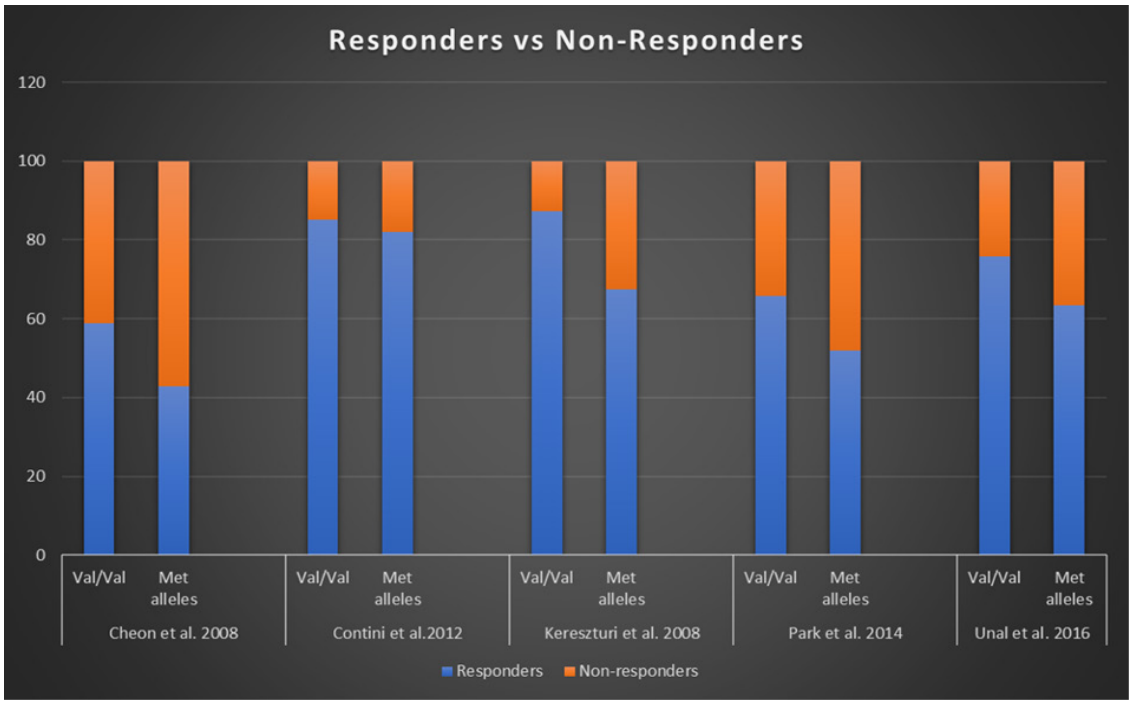


Figure 6: Bar Chart comparing responders and non-responders for Val/Val and Met Allele carriers

Table 3 and Figure 6 compare the raw data listed in Table 2 in terms of percent. Without applying statistics, numerically the number of responders is more in Val/Val carriers compared to Met allele carries. However, statistical analysis needs to be done for the results to be conclusive.

Table 4: Statistical analysis of binary outcomes

Studies	Val/Val Responders	Val/Val Total carriers	Met allele Responders	Met allele Total carriers	Odds Ratio (OR)	95% Confidence Interval (CI)	p value	Result
Cheon et al. 2008	40	68	24	56	1.9	(0.93, 3.90)	0.08	Trends to favor Val/Val
Contini et al. 2012	40	47	96	117	1.25	(0.49,3.17)	0.64	No association
Kereszturi et al. 2008	34	39	56	83	3.28	(1.15,9.32)	0.03	Favors Val/Val
Park et al. 2014	46	70	26	50	1.77	(0.84,3.72)	0.13	No association
Unal et al. 2016	22	29	50	79	1.82	(0.69, 4.79)	0.22	No association

Odd's ratio, 95% confidence interval and p value were calculated using Microsoft Excel; Odds Ratio (OR) is a measure of association between the COMT genotype and MPH treatment outcome; p value is a measure of for probability and

measures how likely it is that any observed difference between groups is due to chance.[35] The lower the p-value, the greater the statistical significance of the effect of COMT genotype on treatment outcomes to MPHs.

The statistical analysis of individual studies resulted in mixed results with 3 studies showing no statistically significant association. However, the level of significance, $p<0.05$, can be negatively impacted by small sample size[36] and none of these studies have evaluated treatment outcomes in large cohorts. Hence, a pooled analysis of all these five studies will enhance the statistical power of small and inconclusive studies and improve our ability to evaluate an association. Revman® software was used to evaluate the pooled analysis.

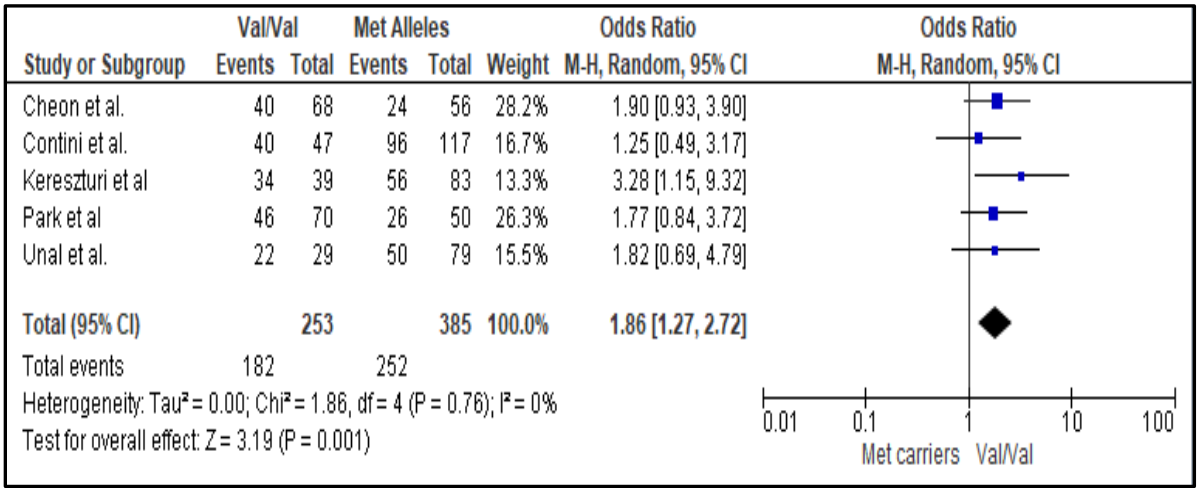


Figure 7: Forest Plot analyzing the pooled data from Binary outcomes (Responders vs non-Responders)

Here, *Events* are number of responders to MPH and *Total* is the total number of patients (responders + non-responders) with that genotype.

The results of the Forest plot showed a statistically significant association (p=0.001) of Val/Val carriers in predicting superior treatment outcomes to MPHs. The odds ratio of the pooled analysis is 1.86 depicting that Val/Val carriers are 1.86 times more likely to respond to MPHs compared to the Met allele carriers in a cohort of 638 patients.

Analysis of data reported as continuous outcomes

Since the above pooled analysis only included data from 5 studies, our results could be biased due to the exclusion of the other 4 studies that reported data in continuous outcomes. Hence, we investigated the continuous data outcomes reported in the other 4 studies (SenGupta[32], Salatino-Oliviera[31], McGough[33] and Yatsuga[30]). Pagerols et al.[34] did not report on the raw data or the p values and hence had to be excluded. Only 1 data set (Yatsuga et al.) out of the four studies provided mean improvement in ADHD scores along with standard deviations. The other three studies reported the p value for association of response to MPHs with COMT genotype, however, did not provide either the mean reduction scores or standard deviation (both of which are needed to calculate the p value in continuous outcomes). Hence, we could only calculate the p value for Yatsuga's study.

	Val/Val			Met Allele carriers				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
Yatsuga	2.1	1	20	2.3	0.9	30	100.0%	-0.20 [-0.74, 0.34]
Total (95% CI)			**20**			**30**	**100.0%**	**-0.20 [-0.74, 0.34]**

Heterogeneity: Not applicable
Test for overall effect: Z = 0.72 (P = 0.47)

Figure 8: p value calculated from continuous outcomes from 1 data set (Mean: mean improvement in ADHD scores)

Results of the Combined Analysis

We employed Stouffer's z method to evaluate if there is an association between the treatment outcomes with MPH and COMT genotype in ADHD patients. Stouffer's z method combines multiple weighted Z-scores that are calculated by using the p-value for association, sample size and estimated direction of effect from each independent data set.[37] With this statistical analysis, we can pool both the dimensional and categorical outcomes together which include the dichotomous and continuous data (i.e. number of responders, changes in ADHD score, symptom improvement).
Table 5 lists all the p values for the 9 studies. For the first 5 studies (n; total=638 patients), the p values were calculated from the dichotomous raw data using Excel (see table 4). For the remaining four studies with continuous outcomes (n; total= 456 patients), the p value was calculated for one data set (Yatsuga et al., n=50) but for the remaining 3 data sets, p values were taken from the studies due to the lack of raw data provided to calculate them.
The p value is converted into the z score using Excel (see formula in statistical analysis section). Adding all of the individual Z-scores and then dividing it by the square root of the total number of studies (k=9) gives the Stouffer's z score which can be converted to the pooled p value using Excel.

Table 5: Pooled analysis of 9 studies using Stouffer's z score method:

Studies	Sample size (# of patients studied)	P value (response outcomes: Val/Val vs Met alleles)	Z Score	Stouffer's z $Z_{Stouffer} = \frac{\sum_{i=1}^{k} Z_i}{\sqrt{k}}$	P Value
Cheon et al. 2008	124	0.08 (calculated)	1.75	2.94	0.003
Contini et al. 2012	164	0.64 (calculated)	0.47		
Kereszturi et al. 2008	122	0.03 (calculated)	2.17		
Park et al. 2014	120	0.13 (calculated)	1.51		
Unal et al. 2016	108	0.22 (calculated)	1.23		
Sengupta et al 2008	212	0.2 (taken from the study)	1.28		
Salatino-Oliviera et al 2011	112	0.019 (taken from the study) (oppo-site effect)	-2.35		
McGough et al. 2009	82	0.04 (taken from the study)	2.05		
Yatsuga et al. 2014	50	0.47 (calculated)	0.72		

TOTAL	1094				

The pooled analysis of the 9 studies (n=1094 ADHD patients) showed a statistically significant association (p=0.003) of Val/Val carriers in predicting superior treatment outcomes to MPH proving our research hypothesis to be true.

Limitations

We could not find a database with COMT genotype and MPH treatment outcomes, hence we had to extract the data for several studies. These studies had different designs, study durations and measured treatment outcomes using different assessment scales. Certain pharmacogenetic variants are more or less common in different ethnic groups. Although all the scales used are validated, the results may have some heterogeneity because of the difference in the scales employed to assess outcomes. While the criteria employed for defining responders are all widely accepted in the field of psychiatry, there are subtle differences in how different researchers defined responders versus non-responders. Psychiatric conditions are multifactorial and several non-genetic clinical factors as well as environmental factors contribute to the treatment outcomes. However, in these data sets it was difficult to assess the impact of those factors.

Conclusion

Current treatments in psychiatric conditions have sizable response variability. There is a need to develop treatment strategies to optimize pharmacological outcomes. Our research analysis showed that COMT is a reliable marker to predict treatment outcomes with methylphenidates. The knowledge of this pharmacogenomic marker can enhance appropriate medication selection in ADHD patients, thereby providing a more personalized treatment plan. In addition to improved clinical outcomes, pharmacogenomic-guided ADHD medication selection may also reduce healthcare resource usage by reducing the time needed to observe benefit from a treatment. Although this correlational analysis has some limitations, it adds to the body of evidence in the field of precision medicine. More studies with larger cohorts are needed to better validate this gene-drug association. Often multiple genes can contribute to the overall disease risk or medication failures. Hence, other genes that may play a role in modulating the neuroplasticity or the neurotransmitter levels in the brain should also be assessed as potential pharmacogenomic markers in the field of ADHD. Precision medicine is widely applicable to all psychological illness, future research can be done observing the correlation with genes and different mental illnesses like depression, anxiety, or schizophrenia.

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