Special Report

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Pharmacogenomics

Systems pharmacogenomics – gene, disease, drug and placebo interactions: a case study in COMT

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Disease, drugs and the placebos used as comparators are inextricably linked in the methodology of the double-blind, randomized controlled trial. Nonetheless, pharmacogenomics, the study of how individuals respond to drugs based on genetic substrate, focuses primarily on the link between genes and drugs, while the link between genes and disease is often overlooked and the link between genes and placebos is largely ignored. Herein, we use the example of the enzyme catechol-O-methyltransferase to examine the hypothesis that genes can function as pharmacogenomic hubs across system-wide regulatory processes that, if perturbed in randomized controlled trials, can have primary and combinatorial effects on drug and placebo responses.

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We begin by discussing how genetic variation in catechol-O-methyltransferase (COMT) influences a broad spectrum of diseases from psychiatric and neurological disorders, to cardiometabolic disease and cancer. We then examine how some drugs and supplements used to prevent and treat these conditions are influenced by COMT in these four disease domains. Next, we discuss how COMT genetic variation influences the placebo response. We continue by discussing the possibility that genetic interactions may lead to different responses in the drug and placebo arms of randomized controlled trials (RCTs), and thereby distort or confound the outcomes. We argue that including the disease and placebo axes with the gene–drug axis in pharmacogenomics has the potential to advance drug development and clinical care.

Catechol-O-methyltransferase in form & function

COMT is a Phase II enzyme (EC2.1.1.6) which, in the presence of magnesium ions, transfers a methyl group from S-adenosylmethionine (SAM) to a hydroxyl group on the catechol ring of endogenous and xenobiotic catechol substrates (Figure 1) [1]. During COMT-catalyzed O-methylation, SAM is converted to a competitive inhibitor, S-adenosylhomocysteine (SAH), resulting in a negative feedback regulatory loop. The endogenous substrates of COMT include the catecholamine neurotransmitters and the hormones dopamine, norepinephrine, and epinephrine (Table 1) [2]. In the absence of methylation, these catecholamines can accumulate and generate semiquinone and quinone free radicals, which promote DNA and lipid damage [3]. Hence, COMT is an important detoxifier of reactive molecules and can protect cells from oxidative stress known to influence neurodegenerative and cardiometabolic disease and cancer (Figure 1).

Catechol estrogens, the product of estradiol hydroxylation by cytochrome P450 Phase I enzymes, represent another class of COMT substrates. Like catecholamines, catechol estrogens undergo oxidation to produce reactive semiquinones and quinones (Table 1) [4]. Beyond the potential for free radical DNA and lipid damage, catechol estrogens have estrogenic properties and are able to promote proliferation of hormone-sensitive tumors [5]. Methylation by COMT, therefore, not only limits the carcinogenicity of catechol estrogens, but also produces

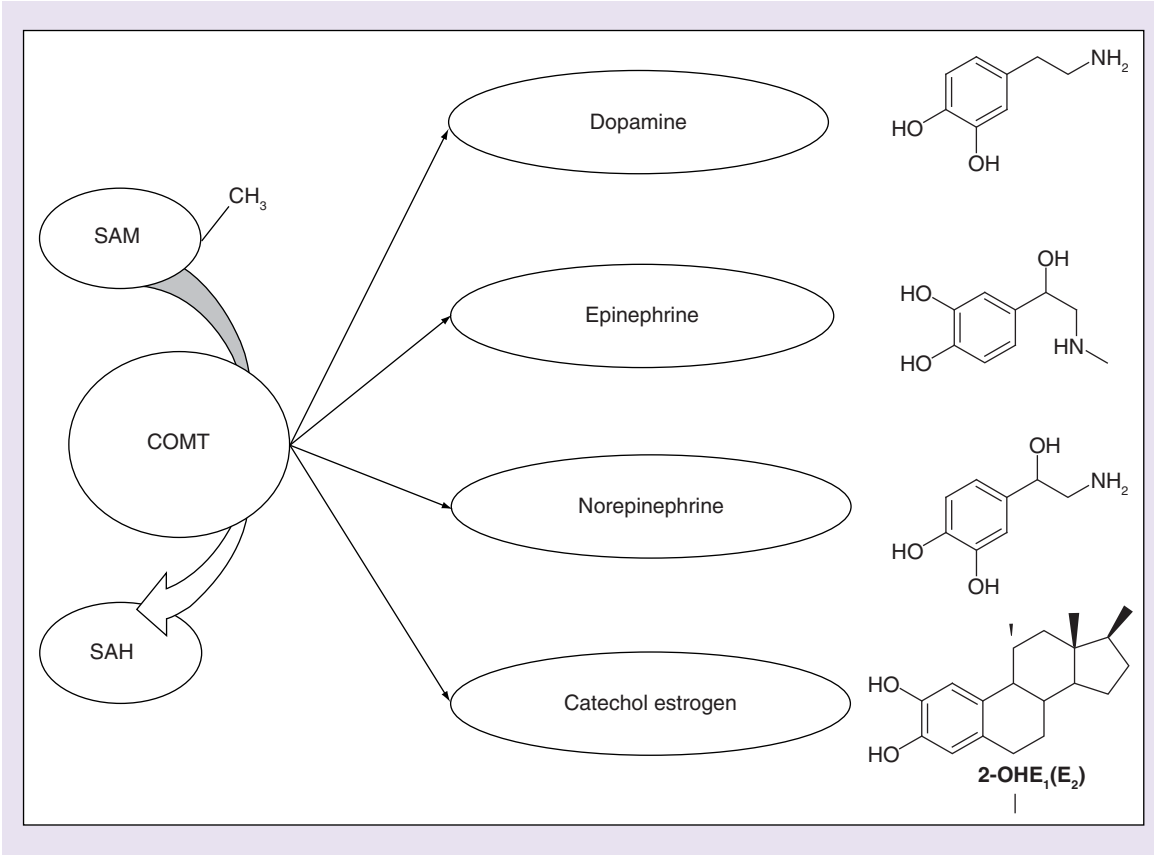

Figure 1. Catechol-O-methyltransferase enzymatic functions. COMT is a Phase II enzyme that, in the presence of magnesium ions, transfers a methyl group (CH_3) from SAM to the hydroxyl group of catechol-containing COMT substrates. SAM is thus converted to SAH, a competitive inhibitor of COMT. Endogenous substrates of COMT include the catecholamines, dopamine, epinephrine, and norepinephrine and the catechol-containing metabolic product of estrogen, catechol estrogen.
COMT: Catechol-O-methyltransferase; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine.

Table 1. Catechol-O-methyltransferase endogenous catechol substrates, their receptors and function.

Endogenous substrates	Receptor	Biological function
Dopamine	Dopamine family of receptors (DRD1–5)	Neurotransmission of reward and incentive salience, motor control. Dopamine inhibits norepinephrine release, acts as a vasodilator, and can increase sodium excretion and urine output. In the pancreas, dopamine reduces insulin production and reduces gastrointestinal motility in the digestive system; in the immune system, it reduces the activity of lymphocytes. Dopamine is converted to norepinephrine by DBH
Norepinephrine	α and β-adrenergic receptors (ADRα and β)	Norepinephrine is a key neurotransmitter in the sympathetic nervous system and is important in the fight-or-flight response. It acts by increasing heart rate, blood pressure and glucose levels, while also increasing blood availability in skeletal muscle and reducing it in the GI tract. Norepinephrine is converted to epinephrine by PNMT
Epinephrine	α and β-adrenergic receptors (ADRα and β)	Like norepinephrine, epinephrine is important in the fight-or-flight response, increasing heart and respiratory rate, muscle contraction, vasomotor tone, blood glucose and lipolysis. Epinephrine is derived from norepinephrine by PNMT
Catechol estrogen	Membrane (mER) and nuclear estrogen receptors (ERα and ERβ)	Estrogen is converted to catechol estrogens by cytochrome P450 enzymes. Catechol estrogens have procarcinogenic, proproliferative, and anti-apoptotic activities. COMT converts catechol estrogens to methoxyestradiols that are protective against carcinogenesis

COMT: Catechol-O-methyltransferase; DBH: dopamine β-hydroxylase; GI: Gastro-intestinal; PNMT: Phenylethanolamine N-methyltransferase

2- and 4-methoxy estradiol, which have antiproliferative, pro-apoptotic, anticarcinogenic, and anti-inflammatory properties [4]. These methoxy-derivatives also appear to exhibit beneficial cardiometabolic effects [6].

The *COMT* gene

The *COMT* gene is located on chromosome 22q11.2 and contains six exons that encode membrane and soluble forms of the enzyme. *COMT* is ubiquitously expressed with the highest levels in the adrenal gland, liver, lung, ovary, urinary bladder, and placenta [7]. Whereas the soluble form is dominant in most tissues, the membrane form is dominant in the brain. Sexual dimorphism in *COMT* expression has been attributed to its regulation by estrogen and its role in estrogen metabolism [8,9]. *COMT* expression also varies with age, increasing in the liver tenfold from infancy to adulthood and then decreasing with age [10]. A three megabase deletion in chromosome 22q11.2, which includes the *COMT* gene, results in DiGeorge/velocardiofacial syndrome [11]. The manifestations of this syndrome, including higher rates of schizophrenia, and susceptibility to cardiovascular disease and cancer, cross many organ systems, and are thought to arise in part because of loss of *COMT* and its role in catecholamine metabolism and detoxification of reactive oxygen species.

The most widely studied *COMT* polymorphism, rs4680 (val158/108met), encodes a G (valine) to A (methionine) transition in exon 4 at codon 158 in the membrane, and 108 in the soluble form [12]. This polymorphism results in a three- to fourfold reduction in thermostability and enzymatic activity, and a commensurate increase in circulating catecholamines in individuals homozygous for the methionine (met/met) versus valine (val/val) form of the enzyme [13]. Rs4680 is a commonly occurring variant, with minor allele frequencies that vary by population ancestry but allow for powerful genetic analysis even in small studies. For example, the frequencies of the val-allele among samples of people of European, African, and Asian ancestry are 0.48, 0.69, and 0.62, respectively [14]. Although most studies focus on rs4680 owing to its functional consequences, the linked synonymous polymorphism rs4818 has also been shown to have clinical phenotypes [15,16], and *COMT* haplotypes have been studied in schizophrenia [17] and pain [15,16].

COMT & disease

COMT effects on executive function & neuropsychiatric symptoms

COMT accounts for most of the dopamine clearance in the prefrontal cortex, where monoamine oxidases and dopamine transporters are poorly expressed [18]. Hence, higher order cognitive functions and behavioral endophenotypes modulated in the prefrontal cortex are more directly influenced by variations in the levels of COMT activity than other regions of the brain. The prefrontal cortex is responsible for a set of cognitive processes that mediate executive function. Although these processes, which include cognitive flexibility, working memory, and attention, have been associated with *COMT*, the magnitude and direction of the effects differ by health status and disease type. For example, among healthy volunteers, meta-analysis of *COMT* association with cognitive flexibility revealed that the met/met genotype was associated with better performance on the Wisconsin Card Sorting Test, compared with those with the val/val genotype (Table 2) [19]. However, this difference was not detected among patients with schizophrenia. Interestingly, during manic episodes, when dopamine levels are thought to be increased in the prefrontal cortex, val/val patients with bipolar disorder performed better on the Wisconsin Card Sorting Test [20]. In general, val/val adults do not perform as well as met/met adults on tests of memory and attention [21,22]. Like humans, transgenic mice humanized with the met allele (met/met) had a 30% reduction in COMT activity and performed better on tests of spatial working memory compared with transgenic val/val mice [23]. Treatment of these val/val mice with the COMT inhibitor tolcapone restored the deficit in spatial working memory.

Given the link between executive function and aggressive behavior, it is not surprising that *COMT* has been shown to influence aggression, as well. In nonhuman primates, val/val macaques of higher rank exhibited higher rates of aggression, but rates of aggression decreased with rank among met/met and val/met animals [25]. Notably, there was no main effect of *COMT* on aggression. *COMT* gene expression was significantly higher in patients with Alzheimer disease (AD) who also display aggressive behavior, and this effect was more pronounced in the later stages of the disease [55].

Executive function is influenced by stress [56], and low-COMT activity seems to predispose individuals across the age spectrum to be more susceptible to stress. In met/met infants, repeated exposure to stress induced higher levels of cortisol and emotionality [26]. Similarly, the met-allele was associated with greater sensitivity to stress-inducing events in childhood and adolescence, again with higher cortisol responses to stress [57,58]. Met/met adults were also found to be more susceptible to stress mindset interventions [27], and practiced greater harm avoidance [29]. In contrast, the val-allele was associated with positive emotionality, extraversion [28], novelty seeking [59], a greater ability to handle stress, and reduced scores evaluating sadness [32]. These differential stress-related phenotypes have also been observed in mice, where the *COMT* gene is highly conserved (almost 80% homology to the human gene),

Table 2. *COMT* rs4680† (val158met) association with disease in selected studies.

Condition	Findings	Ref.
Executive function		
Cognitive flexibility	The met/met genotype was associated with better performance on the WCST, compared with val/val genotype. However, this difference was not detected among patients with schizophrenia. During manic episodes, when dopamine levels are thought to be increased in the prefrontal cortex, val/val patients with BPD performed better on the WCST	[19,20]
Memory and attention	The met-allele was associated with enhanced working memory and better performance on attention-related tasks. Aging also influences cognition and among a biracial cohort of elders, *COMT* was associated with rate of cognitive decline, with the val-allele having a protective effect	[21,24]
Aggression	Although there was no main effect of *COMT* on aggression, in nonhuman primates, val/val macaques of higher rank exhibited higher rates of aggression. Conversely, rates of aggression decreased with rank among met/met and val/met animals	[25]
Stress response	Infants carrying the met allele had significantly higher negative emotionality and a greater cortisol response with repeated exposure to stress. The met-allele was associated with sensitivity to stressful events in childhood and adolescence and higher cortisol response to stress. Met-allele homozygotes were more susceptible to stress mindset interventions	[26–27]
Harm avoidance and novelty seeking	The val-allele was associated with more effective processing of aversive emotional stimuli, extraversion, novelty seeking. The met-allele was associated with harm avoidance which has an inverse relationship with novelty seeking	[21,28,29]
Psychiatric disease		
Attention-deficit hyperactivity disorder	In a recent comprehensive meta-analysis of rs4680 genetic association studies across 15 psychiatric disorders and 363 datasets, the val-allele was more prevalent among ADHD cases versus controls	[30]
Substance-use disorder	In the same meta-analysis as above, the val-allele was more prevalent among SUD cases versus controls	[30]
Panic disorder	Among individuals of Asian ancestry, in the same meta-analysis as above, the val-allele was more prevalent among panic disorder cases versus controls	[30]
Bipolar disorder	BPD tended to be associated with the met-allele among individuals of Asian ancestry	[30]
Obsessive–compulsive disorder	OCD tended to be associated with the met-allele among males, in the same meta-analysis as above	[30]
Suicide	Although there is inconsistency across the many studies looking at *COMT* and suicide, a recent meta-analysis of 17 studies (3282 cases and 3774 controls) found the val-allele was a risk factor in males but protective in females	[31]
Major depressive disorder	The val/val genotype was significantly associated with reduced sadness scores and reduced disposition to depression. Although studies on *COMT* association with MDD are mixed, a recent large meta-analysis found no association overall or in gender subgroups	[32,33]
Neurological disease		
Delirium	Perturbations in executive function can result in delirium which affects mainly attention, and dementia which affects mainly memory. Although COMT does not appear to directly influence delirium, it modified the known association between C-reactive protein (CRP), an acute phase reactant, and postoperative delirium	[34,35]
Functional pain disorders	A recent meta-analyses of *COMT* and functional pain disorders found that the low-activity met-allele was associated with fibromyalgia. An earlier meta-analysis also found that the met-allele was associated with fibromyalgia and widespread chronic pain, but not migraine headache or chronic musculoskeletal pain conditions	[36,37]
Chronic fatigue syndrome	Chronic fatigue syndrome patients were more likely to be homozygous for the met-allele compared with val-allele carriers	[38,39]
Pain	*COMT* low-activity haplotypes were associated with greater sensitivity to nociceptive, but lower sensitivity to neuropathic pain. Conversely, high-activity haplotypes were associated with lower sensitivity to nociceptive but greater sensitivity to neuropathic pain. In breast cancer survivors, pain sensitivity and fatigue was greater among met-allele homozygotes	[40,41]
Parkinson disease	COMT inhibitor drugs are used as adjunctive treatment for patients with Parkinson disease. Researchers have failed to find a clear association between *COMT* and Parkinson disease, but several studies have demonstrated *COMT* genotype modulation of prefrontal cortex dysfunction early in the course of Parkinson disease. Specifically, increasing numbers of met-alleles was associated with diminished performance on tasks that tested prefrontal cortex functions	[42–44]
Alzheimer disease	Similarly, although metabolism of dopamine and other catecholamines can influence Alzheimer disease, results of *COMT* association with the disease have yielded directionally inconsistent findings. Still, recent meta-analyses reported a *COMT* association with AD among Asians but not Caucasians	[45]
Autoimmune disease	COMT's role in limiting the damaging effects of oxidative and emotional stress make its potential role in the etiology of auto-immune diseases plausible. Studies in psoriasis, narcolepsy and systemic lupus erythematosus suggest a link between *COMT* and autoimmune diseases	[46–47]
Cardiometabolic disease		
Cardiometabolic disease risk factors	The *COMT* rs4680 high-activity val-allele, which is associated with lower levels of catecholamines, is consistently associated with lower cardiometabolic risk factors, including triglycerides, systolic blood pressure and hemoglobin A1c	[48–49]
Diabetes	The *COMT* val-allele was modestly protective from risk of Type II diabetes	[[50–51]
Cardiovascular disease	The val-allele was protective from risk of cardiovascular disease in several studies	[48,52,53]

†The *COMT* rs4680 val-allele encodes a form of the enzyme which is 3–4× more active than the met-allele form.
ADHD: Attention-deficit hyperactivity disorder; BPD: Bipolar disorder; COMT: Catechol-O-methyltransferase; OCD: Obsessive-compulsive disorder; MDD: Major depressive disorder; RCT: Randomized controlled trial; SUD: Substance-use disorder; WCST: Wisconsin Card Sorting Test.

Table 2. *COMT* rs4680† (val158met) association with disease in selected studies (cont.).

Condition	Findings	Ref.
Pre-eclampsia	The met-allele and a low-*COMT* activity haplotype were associated with recurrent pre-eclampsia. In murine models of pre-eclampsia, pre-eclampsia-like phenotypes in COMT-deficient pregnant mice were corrected with 2-methoxyestradiol treatment	[6]
Cancer		
Invasive cancers	Although the met-allele is frequently associated with increased risk of cancers, there are as many studies with null or opposite effects. Consistent with this hypothesis, we recently demonstrated that *COMT* was associated with rates of invasive cancer in two large RCTs of vitamin E for cancer prevention	[4,54]

† The *COMT* rs4680 val-allele encodes a form of the enzyme which is 3–4× more active than the met-allele form.
ADHD: Attention-deficit hyperactivity disorder; BPD: Bipolar disorder; COMT: Catechol-O-methyltransferase; OCD: Obsessive-compulsive disorder; MDD: Major depressive disorder; RCT: Randomized controlled trial; SUD: Substance-use disorder; WCST: Wisconsin Card Sorting Test.

and manipulation of the *COMT* gene in murine models has yielded similar cognitive, behavioral, and emotional phenotypes as those observed in humans [60]. For example, *COMT* knockout mice had better working memory compared with wild-type and transgenic mice expressing high levels of *COMT*. Furthermore, anxiety-like behaviors were also reduced in wild-type mice and transgenic mice expressing high levels of *COMT*, whereas knockout mice exhibited increased anxiety in stress tests. Sexual dimorphism in *COMT* effects has also been observed in mice. Male transgenic val/val mice displayed lower prepulse inhibition (a reflex behavior induced by presentation of a weak stimulus to prepare for a subsequent stronger stimulus) compared with their met/met counterparts. This effect was reversed among female mice.

COMT & psychiatric disease

COMT rs4680 is one of a set of polymorphisms related to catecholaminergic neurotransmission that have been extensively studied in psychiatry and human neurosciences. However, *COMT* effects on executive function do not translate to consistent associations with psychiatric or neurological disease. This inconsistency could be due to the greater complexity of these conditions, as well as the variability in study size, population structure, sexual dimorphism, history of stress, and trauma, pharmacogenomic effects and the influence of other genes [30]. Still, with increasing numbers of studies and several recent meta-analyses, some significant trends are beginning to emerge. In a recent comprehensive meta-analysis of rs4680 genetic association studies across 15 psychiatric disorders and 363 datasets, statistically significant associations were reported in attention-deficit hyperactivity disorder (ADHD) and substance abuse disorder (SUD), where the high-activity val-allele was more prevalent among cases versus controls [30]. The link between *COMT* val-allele and increased alcohol consumption was further corroborated in neuroimaging studies demonstrating significant differential opioid release following alcohol consumption by *COMT* genotype in regions of the brain associated with reward (Figure 2) [61]. These results suggested that val/val individuals might gain greater hedonistic benefit from alcohol use.

In subpopulation analyses, panic disorder was significantly associated with the val-allele among individuals of Caucasian ancestry, and bipolar disorder was significantly associated with the met-allele among individuals of Asian ancestry. Obsessive–compulsive disorder was also associated with the met-allele among males [30]. In a separate meta-analysis of 17 studies on *COMT* and suicide, the val-allele was a risk factor in males but protective in females [31]. Finally, although the val/val genotype was significantly associated with reduced sadness scores and reduced disposition to depression [32], overall *COMT* association studies with major depressive disorder (MDD) are mixed. A recent large meta-analysis of 21 case–control studies found no association overall, or in subgroups stratified by sex [33].

Neurological conditions

Delirium

Perturbations in executive function can result in delirium, which affects mainly attention, and dementia, which affects mainly memory. Although *COMT* does not appear to influence delirium directly [34], it modified the known association between C-reactive protein (CRP), an acute phase reactant, and postoperative delirium [35]. Aging also influences cognition and among a biracial cohort of elders, *COMT* was associated with rate of cognitive decline with the val-allele having a protective effect [24].

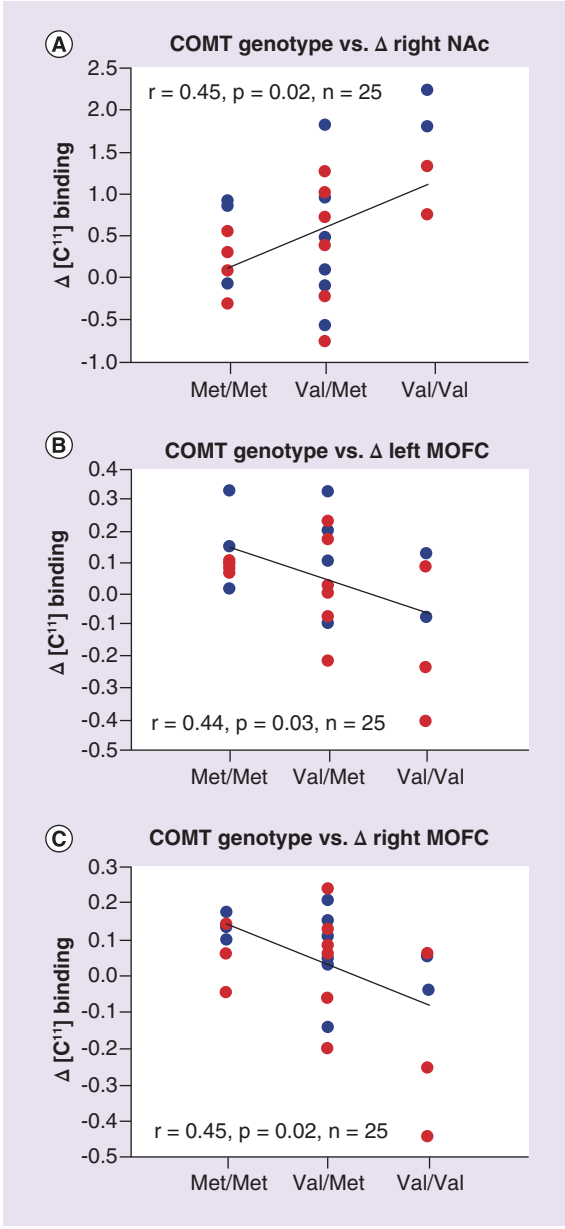

Figure 2. Relationship of the *COMT rs4680* genotype to endogenous opioid release following alcohol consumption. Endorphin release is significantly greater in the right NAc (A: t = 3.25, p = 0.009) and significantly lower in the left mOFC (B: t = 2.34, p = 0.041) and right mOFC (C: t = 2.60, p = 0.027) of individuals with the val/val genotype. Blue circles indicate control subjects, red circles indicate heavy social drinkers.
Reproduced with permission from [61] under the terms of the Creative Commons Attribution License (CC BY) https://creativecommons.org/licenses/by/4.0/
COMT: Catechol-O-methyltransferase; mOFC: Medial orbito-frontal cortex; NAc: Nucleus accumbens.

Pain & fatigue

COMT influences pain perception through catecholamines, which have differential effects based on the regional specificities of α and β adrenergic receptors [40]. One of the first studies on *COMT* and pain perception, published in 2003, found that individuals homozygous for the rs4680 low-activity met-allele were more sensitive to pain induced by hypertonic saline infusion into the masseter muscle than those with the high-activity val-allele [62]. Since that time, our understanding of *COMT* effects on pain sensitivity has broadened to include effects of haplotypes in nociceptive and neuropathic pain. Generally, individuals with low-activity haplotypes tended to be more sensitive to nociceptive pain but less sensitive to neuropathic pain [40]. Conversely, individuals with high-activity haplotypes tended to report lower sensitivity to nociceptive pain but greater sensitivity to neuropathic pain. A recent meta-analyses of functional pain disorders found that the met-allele was associated with fibromyalgia and widespread chronic pain [36]. An earlier meta-analysis also found that the met-allele was associated with fibromyalgia, but not migraine headache or chronic musculoskeletal pain [37]. Consistent with the hypothesis that chronic fatigue syndrome (CFS) results from an overactive autonomic nervous system, CFS patients were more likely to be homozygous for the met-allele compared with val-allele carriers [38,39].

Pain sensitivity and fatigue symptoms co-occur in cancer and have been associated with *COMT*. In several studies, postsurgical cancer patients who were met-allele homozygotes had a lower pain threshold and higher levels of fatigue [41]. Consistent with these profiles, in wild-type mice, sustained delivery of the COMT inhibitor OR486 increased sensitivity to mechanically induced pain [63]. *COMT* knockout mice have enhanced sensitivity to thermal pain, and transgenic mice that overexpress *COMT* are more resistant to thermal pain [60].

Parkinson disease, AD & other diseases

Despite the link between dopamine induced oxidative stress and neuronal damage in the substantia nigra, researchers have failed to establish a clear link between genetic variation in *COMT* and the risk of Parkinson disease [64]. However, several studies have demonstrated *COMT* genotype modulation of prefrontal cortex dysfunction early in the course of Parkinson disease. Specifically, increasing numbers of met-alleles was associated with diminished performance on tasks that tested prefrontal cortex functions [43,44,65]. Furthermore, this reduced function was more pronounced among patients on dopaminergic treatment [66], leading to the hypothesis that impaired function results from an 'overload' of prefrontal cortex dopamine, which is worsened by levodopa.

Similarly, although metabolism of dopamine and other catecholamines can influence AD, results of *COMT* association with the disease have yielded directionally inconsistent findings. Still, recent meta-analyses reported a *COMT* association with AD among Asians but not among Caucasians [45].

COMT's role in limiting the damaging effects of oxidative and emotional stress also make its potential role in the etiology of auto-immune diseases plausible [46]. Studies in psoriasis [67], narcolepsy [68], and systemic lupus erythematosus [47,69] support this hypothesis, but more work needs to be done in this area.

COMT & cardiometabolic disease

Catecholamines influence cardiometabolic functioning through manifold effects on insulin secretion, glucose metabolism, natriuresis, vascular tone, and heart rate. Given COMT's role in catecholamine metabolism, it is not surprising that the *COMT* rs4680 high-activity val-allele, which is associated with lower levels of catecholamines, is consistently associated with lower cardiometabolic risk factors, including triglycerides, systolic blood pressure [48,70,71], and hemoglobin A1c [50].

In terms of disease, a consistent protective association of the val-allele was also observed with Type II diabetes [50,72] with only one exception, men with obesity [51]. COMT deficiency in mouse models resulted in glucose intolerance which was rescued by 2-methoxyestradiol (2-ME) [49]. The *COMT* association extends to cardiovascular disease, specifically to the risk of myocardial infarction and stroke where the rate of incident events is lower among val-allele than met-allele homozygotes [52,53,73]. Interestingly, among women diagnosed with depression, the val-allele was associated with increased risk of cardiovascular disease [74].

Epidemiological and animal studies have demonstrated COMTs role in pre-eclampsia, a condition in pregnancy characterized by high blood pressure and proteinuria [6]. The finding that the met-allele and a low-COMT activity haplotype are associated with recurrent pre-eclampsia was supported by murine models in which pre-eclampsia-like phenotypes in COMT-deficient pregnant mice were corrected with 2-methoxyestradiol treatment, the product of COMT catechol estrogen metabolism [6].

COMT & cancer

Multiple pathways link COMT to cancer. Catecholamines have varied effects on tumor angiogenesis, a process that is necessary for tumor growth and progression [75]. Whereas epinephrine and norepinephrine promote tumor angiogenesis by upregulating the synthesis of proangiogenic factors IL-6 and VEGF via β-adrenergic signaling, dopamine inhibits the formation of new blood vessels by suppressing vascular permeability factor/VEGF receptor-A (VPF/VEGFR-2) [76]. *In vitro* and animal studies support COMT's role in transforming and eliminating the carcinogenic threat of catechol estrogens and other catecholamines which are prone to breaking down into free radicals [77]. High *COMT* expression was associated with survival in colorectal and pancreatic cancer patients [78] and overexpression of the *COMT* gene in colorectal and pancreatic cancer cell lines resulted in reduced proliferation and invasive ability [78,79]. Furthermore, catecholamines are produced in response to stress, and these angiogenic and pro-oxidative stress links provide a potential mechanism through which undue stress might support carcinogenesis [80].

Despite several positive findings on *COMT* and cancer, its association with the risk of hormonal and other cancers remains equivocal. Although the met-allele is frequently associated with increased risk of cancers, there are as many studies with null or opposite effects. There are several potential factors that contribute to this inconsistency,

including heterogeneity in race/ethnicity, sex, age, disease, and potential gene–drug interactions (discussed below). For a recent and detailed discussion of the variability in the *COMT*-cancer relationship, we refer the reader to a recent comprehensive review by Sak [4].

COMT, drugs & supplements

The presence of a catechol moiety makes many drugs, supplements, and their metabolites substrates for COMT. Additionally, drugs that target pathways involved in catecholamine anabolism, signaling or metabolism can also interact with *COMT*. Hence, given the multiple overlapping functions between COMT and pathways targeted by drugs, *COMT* gene–drug interaction effects are prevalent in randomized controlled trial (RCTs) of disease domains influenced by *COMT* (Table 3).

Drugs used in treating psychiatric disorders

Based on its role in neurotransmitter signaling, executive function, and its association with psychiatric endophenotypes and disease, *COMT* has been explored as a candidate gene to explain the variability in treatment response in several neuropsychiatric disorders. Methylphenidate increases catecholamine signaling in the prefrontal cortex, has the potential to improve executive function in patients with ADHD. Not surprisingly, with the higher frequency of the val/val genotype among ADHD patients, the greatest benefit of methylphenidate therapy for ADHD was observed among val/val patients [81].

COMT effects in substance abuse treatment, specifically with regard to nicotine dependence, and alcohol use have been extensively studied. The results, however, have been inconsistent. Hence, early studies that suggested *COMT* may be a predictor of bupropion [83] or nicotine replacement therapy response in the treatment of tobacco dependence, have not been borne out [82].

In MDD, although numerous studies reported significant *COMT* rs4680 associations with antidepressant treatment response (i.e., milnacipran [84], fluoxetine [107], paroxetine [108], fluvoxamine [109], mirtazapine [110], venlafaxine [111], and duloxetine [112]), many of these studies were small and did not include a placebo control. Given the relationship between *COMT* and psychiatric disease and placebo response (discussed below), the absence of a placebo control in these studies, makes it almost impossible to ascribe these effects solely to gene–drug interactions.

COMT effect modification was also observed for venlafaxine for generalized anxiety disorder [86] and several antipsychotic medications used to treat schizophrenia [88,113]. Interestingly, a subset of these drugs form metabolites that are substrates of COMT, for example, paroxetine-catechol and duloxetine-catechol, suggesting other possible mechanisms for the *COMT*–drug interactions reported in the literature [114].

Drugs used to treat neurological disease

Interest in COMT as a druggable target was motivated by the need to inhibit peripheral COMT in patients with Parkinson disease who were treated with the dopamine precursor levodopa. Levodopa is the gold standard treatment for patients with Parkinson disease because unlike dopamine, it is able to cross the blood–brain barrier. Ascorbic acid (vitamin C), a weak competitive inhibitor of COMT [115], was one of the early molecules evaluated, albeit unsuccessfully, as a potential adjunctive therapy to preserve levodopa in the periphery [92]. Although the weak COMT-vitamin C interaction has been largely ignored, in the recent genome-wide association study of human blood metabolites, vitamin C metabolites were found to be strikingly significantly associated with *COMT* [93].

Once the crystal structure of COMT was solved, rational design was used to create nitrocatechol inhibitors, such as entacapone and tolcapone, which contain a catechol with a nitro group at position 4 on the benzene ring [94,116]. These drugs are potent reversible inhibitors of COMT, and although their primary clinical application is as an adjunct to levodopa in Parkinson disease, RCTs have explored their effects in attention [117], cognitive deficits in schizophrenia [118], and based on tolcapone's apoptotic effects, as a chemotherapeutic in neuroblastoma [95,119].

Consistent with the differential pain sensitivity [110], rs4680 and *COMT* haplotypes were associated with opioid requirement for postoperative [96,120] and cancer [97] pain. CFS is thought to be a result of an overactive sympathetic nervous system and in a randomized controlled clinical trial (RCT) of the antihypertensive clonidine, designed to reduce catecholamine tone in CFS, individuals homozygous for the val-allele had poorer outcomes when randomized to clonidine compared with placebo, whereas neither a drug nor placebo effect was observed in the met-allele homozygotes [89]. Finally, val/val individuals in two studies were impaired on cognitive tests when randomized to tetrahydrocannabinol compared with placebo [90,91].

Table 3. Selected drugs that modify catechol-O-methyltransferase effects.

Condition	Drug/treatment	Findings	Ref.
Psychiatric disorders			
Attention deficit hyperactivity disorder	Methylphenidate is a norepinephrine–dopamine reuptake inhibitor	A recent review of *COMT*-stimulant pharmacogenetic effects suggested the strongest benefit of methylphenidate for ADHD was observed among val/val patients	[81]
Substance-use disorder	Nicotine replacement therapy and bupropion a norepinephrine–dopamine reuptake inhibitor	In a large RCT in smokers of European ancestry, compared with placebo, met/met homozygotes were more likely to abstain from smoking than val-allele carriers. In a trial of bupropion, *COMT* haplotypes predicted the response to drug versus placebo	[82,83]
Major depressive disorder	Antidepressants milnacipran, fluoxetine, paroxetine, fluvoxamine, mirtazapine, venlafaxine, duloxetine and electroconvulsive therapy	Many studies report significant *COMT* rs4680 associations with antidepressant treatment response, for example, milnacipran, fluoxetine, paroxetine, fluvoxamine, mirtazapine, venlafaxine and duloxetine. Notably, many of these studies did not include a placebo control. An RCT of duloxetine for MDD did not find a genome-wide association between *COMT* in placebo or drug treatment arms. The val-allele was also found to be influence treatment response to electroconvulsive therapy	[84–85]
Generalized Anxiety disorder	Venlafaxine, a serotonin-norepinephrine reuptake inhibitor	A nominally significant association with response to venlafaxine was reported for the secondary treatment outcome	[86]
Bipolar disorder	Lamotrigine (an anticonvulsant) plus folic acid (vitamin B9)	There was a negative impact of randomized lamotrigine plus folic acid on met-allele carrier	[87]
Schizophrenia	Antipsychotic medications including olanzapine, clozapine and risperidone	In a recent meta-analysis (N = 10 studies), response to antipsychotic treatment was significantly associated with the *COMT* met/met genotype	[88]
Chronic fatigue syndrome	Clonidine, an α-2 adrenergic agonist	In an RCT of clonidine for treating chronic fatigue syndrome, *COMT* modified effects of clonidine in the primary outcome, steps taken per day	[89]
Working memory	Tetrahydrocannabinol (THC) a cannabinoid receptor agonist	Val/Val individuals in two studies were impaired on cognitive tests when randomized to THC compared with placebo	[90,91]
Neurological disease and conditions			
Parkinson disease	Vitamin C (ascorbic acid) is an anti-oxidant	Vitamin C was one of the first drugs evaluated as a possible adjunct therapy to levodopa because of its COMT inhibitory effects. More recently, in the human blood metabolite GWAS, O-methylascorbate a metabolite of vitamin C was significantly associated with rs4680 (p = 5e-178)	[92,93]
	Nitrocatechols for example, entacapone and tolcapone are selective and reversible inhibitors of COMT [94]	Entacapone acts peripherally and is only slightly able to penetrate the blood brain barrier. Tolcapone can penetrate the blood brain barrier and an RCT is underway in patients with relapsed or refractory neuroblastoma	[45,95]
Pain	Morphine and fentanyl are exogenous opioids	*COMT* genetic variation has been associated with variability in opioid requirement for postoperative and cancer pain	[96,97]
Cardiometabolic disease			
Bradycardia	Isoproterenol is an analog of epinephrine and a β adrenoreceptor agonist	An epinephrine analog is used to treat bradycardia	[98]
Diabetes	Insulin detemir	In an RCT of recombinant insulin, reduction of HbA1c was significantly better among met-allele carriers compared with val-allele homozygotes	[99]
Pre-eclampsia	Hydralazine and verapamil	Hydralazine, a widely accepted drug for the treatment of pre-eclampsia, was shown to inhibit placental COMT activity. Verapamil was explored as a potential drug to treat pre-eclampsia. COMT activity was suppressed in term placental explants after 6-hour exposure to verapamil or hydralazine	[100]
Hypertension	Calcium-channel blockers, angiotensin-receptor blockers and diuretics	The val-allele was associated with lower systolic blood pressure in participants treated with Calcium-channel blockers. This effect was even stronger among elderly individuals. Similar effects were seen among participants treated with angiotensin-receptor blockers and there was a significant association with another COMT SNP rs737865 and diuretics	[101]
Cardiovascular disease prevention	Aspirin, an antiplatelet, analgesic and anti-inflammatory drug and vitamin E an anti-oxidant	Significant COMT-by-aspirin and COMT-by-vitamin E effect modification were reported in a randomized trial of cardiovascular disease prevention	[52]
Cancer prevention			
Prostate cancer prevention	Finasteride, inhibits dihydrotestosterone production	In the PCPT RCT, the val-allele was associated with increased risk of prostate cancer among patients randomized to finasteride	[102]
Prevention of all invasive cancers	Vitamin E (α-tocopherol) is an anti-oxidant	In two large RCTs, COMT-by-vitamin E effect modification was reported in cancer prevention such that met-allele homozygotes benefited from vitamin E therapy but val-allele homozygotes did not	[52,103]

ADHD: Attention-deficit hyperactivity disorder; MDD: Major depressive disorder; RCT: Randomized controlled trial.

Table 3. Selected drugs that modify catechol-O-methyltransferase effects (cont.).

Condition	Drug/treatment	Findings	Ref.
Prevention of chronic disease	Quercetin and fisetin are flavonoids used as supplements in chronic disease prevention	These phytochemicals are found in onions, apples and red wine. They have antioxidant and anti-inflammatory effects and inhibit COMT function. They are sold as supplements to prevent cancer and other chronic diseases	
Cancer prevention	Green tea catechins include catechin, epicatechin and epigallocatechin-3-O-gallate [104]	In a population-based case–control study, risk of breast cancer was significantly lower among tea drinkers who were met-allele carriers. The combination of quercetin and green tea was found to enhance inhibition of prostate cancer xenograft tumor growth. Higher energy expenditure and fat oxidation were observed among met/met participants randomized to placebo in an RCT of the cardiometabolic effects of green tea. Differential effects were also observed in the green tea treatment arm	[105–106]

ADHD: Attention-deficit hyperactivity disorder; MDD: Major depressive disorder; RCT: Randomized controlled trial.

Drugs used to treat cardiometabolic disease

Several drugs used to treat various conditions related to cardiometabolic disease physically or pharmacogenomically interact with COMT. For example, isoproterenol is an analog of epinephrine, and, as such, a substrate for COMT. In pharmacogenetic studies of hypertensive medications, the val-allele of rs4680 was associated with lower systolic blood pressure in participants treated with calcium-channel blockers, and this effect was even stronger among elderly individuals [101]. Similar effects were seen among participants treated with angiotensin-receptor blockers; a significant association with another *COMT* SNP, rs737865, and diuretics were also reported. Hydralazine, a widely used drug for the treatment of high blood pressure in pregnancy and pre-eclampsia, was shown to inhibit placental COMT activity [100]. Verapamil, a drug that was also explored as a potential therapy for pre-eclampsia, also suppressed COMT activity in term placental explants [100]. The antiplatelet agent aspirin was found to interact significantly with *COMT* in the Women's Health Study, a large RCT of aspirin for cardiovascular disease prevention, such that met-allele homozygotes had a lower rate of cardiovascular disease when randomized to aspirin treatment compared with placebo [52]. The inverse relationship was observed for val-allele homozygotes who had higher rates of cardiovascular disease with randomized aspirin treatment compared with placebo. Strikingly, a similar directional and statistically significant *COMT* gene–drug effect was observed with vitamin E [52].

Drugs & supplements used to prevent cancer & other chronic diseases

Despite considerable historic controversy over the risks and benefits of vitamin C, there is renewed interest in its potential as an anticancer agent [121]. Vitamin C has several links to COMT. As discussed above, it was one of the first drugs tested as a potential COMT inhibitor to protect levodopa from COMT metabolism. In the human blood metabolite genome-wide association study, O-methylascorbate and other metabolites of vitamin C were strikingly associated with *COMT* rs4680 ($p = 5e{-}178$) [93]. Vitamin C is a water-soluble anti-oxidant that scavenges oxygen radicals in the aqueous phase and interacts with the lipid-soluble antioxidant vitamin E, regenerating it once it has scavenged oxygen radicals [122]. Effects of vitamin E were also found to be modified by genetic variation in *COMT* in two large RCTs of vitamin E for cancer prevention, The Women's Health Study (Figure 3) and the α-Tocopherol, β-Carotene Cancer Prevention Study [123]. Met/met homozygotes randomized to vitamin E had lower rates of all invasive cancers compared with placebo, while the opposite effect was observed for val/val homozygotes. Vitamin E had no effect among heterozygous participants in either treatment arm. Additionally, *COMT* effect modification of vitamin E as well as aspirin was observed in cardiovascular disease prevention in the WHS [52]. Interestingly, the original findings of the overall vitamin E and aspirin effects on cancer and cardiovascular disease in these RCTs were null [124–126]. Hence, accounting for *COMT* effects in *post hoc* analyses of these RCTs allowed for reinterpretation of the benefits and harms of this anti-oxidant supplement. In the Prostate Cancer Prevention Trial, an RCT of finasteride treatment for prostate cancer prevention, the *COMT* high-activity val-allele was associated with greater prostate cancer risk among men with high levels of estrone and cholesterol and lower levels of serum estradiol [102]. In the Prostate Cancer Prevention Trial, the val-allele was also associated with increased risk of prostate cancer among patients randomized to finasteride, a drug that inhibits dihydrotestosterone production. These findings suggest *COMT*'s effects on drug treatment response may also be influenced by its role in hormone metabolism.

Catechol-containing catechins found in teas are strong inhibitors of COMT activity in the liver [104]. In a population-based case–control study, the risk of breast cancer was significantly lower among tea drinkers who were met-allele carriers [105]. The flavonoids quercetin and fisetin can also modify COMT function by both competing

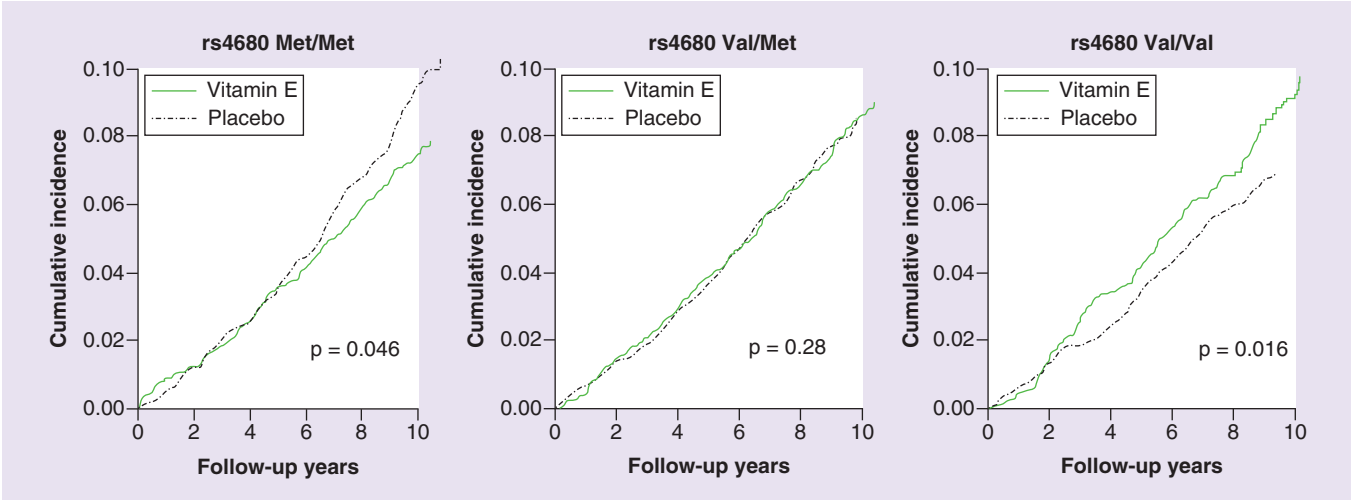

Figure 3. Kaplan–Meier curves demonstrating differential effects of vitamin E versus placebo by *COMT* rs4680 genotype strata over the 10 years of the Women's Health Study, a randomized placebo controlled clinical trial. (A) met/met women; **(B)** val/met women; **(C)** val/val women.

with endogenous catechols and by the production of S-adenosylhomocysteine, a competitive COMT inhibitor generated with SAM demethylation (Figure 1) [104,127]. The combination of quercetin and green tea was found to enhance inhibition of prostate cancer xenograft tumor growth in one study [128]. In another study of vitamin C and quercetin as micronutrients consumed in the form of blueberry/apple juice, vitamin E levels increased but so did the level of adduct formation in met/met lymphocytes examined *ex vivo* [129]. In a recent comprehensive review of *COMT* and cancer, Sak also proposed that use of flavonoids through supplements or diet could further interact with *COMT* masking its effects on cancer [4]. Although these supplements are marketed as preventive for cancer and cardiovascular disease, there is evidence in animals that they can increase tumors and metastases [104].

COMT & placebo (inert treatments)

Before we begin our discussion of *COMT* and the placebo response, it is helpful to distinguish between 'placebo response' and 'placebo effect'. A placebo response is defined as an improvement in target outcome detected in the placebo arm of RCTs. Placebo responses include spontaneous remission, normal fluctuations, and regression to the mean [130]. In preventive RCTs, they may reflect an inherent risk of disease. A placebo response can at times also include effects related to participation in the therapeutic encounter, characterized by verbal and nonverbal cues embedded in rituals, symbols, and behaviors [131–133]. These latter effects are placebo effects, and they rely on complex cognitive and neural processing mechanisms, including involvement with catecholaminergic and opioid signaling and activation of specific, quantifiable, and relevant regions of the brain, including the prefrontal cortex [134].

While great strides have been made in establishing the veracity and neurobiological basis of the placebo effect, the field is challenged with many methodological hurdles [135]. In order to separate spontaneous improvement from genuine placebo effects in pharmaceutical and procedural RCTs, an additional no-treatment control (NTC) arm is required to capture and control for regression to the mean, and the natural history of the disease, in studies of the placebo response. Given that these distinctions are not viewed to be directly relevant to drug discovery, an NTC arm is rarely included in RCTs; this limits discussion of placebo effects based on routine RCTs. In the last decade, however, there have been a significant number of RCTs that deliberately included NTC arms demonstrating that there is, indeed, a genuine, statistically meaningful and clinically relevant placebo effect embedded in the placebo arms of RCTs for multiple diseases [136–139]. Placebo research conducted in a laboratory setting (often on healthy volunteers), is more likely to utilize a NTC arm to measure accurately the placebo effect, but the results are often not generalizable to RCTs or the clinical setting. A further limitation of gaining information for placebo (and drug) effects in typical routine RCTs, is that at times placebo effects can be different in the drug and placebo arm of an RCT; that is, the drug and placebo responses are not directionally additive [130,140–143]. Genetic interactions in disease, drug and placebo effects may play a significant role in this phenomenon. Given the overlap between

Table 4. *COMT* and placebo response.

Condition	Study design, N	Outcome	Results	Ref.
Psychiatric studies				
Working memory	RCT of tolcapone in healthy normal volunteers N = 68	N-back	In the placebo group, met-allele homozygotes outperformed val-allele homozygotes on tests of memory and were more risk averse. Tolcapone reversed these effects	[150]
MDD	RCT in MDD patients, N = 132	HAM-D17 and MADRS	Met-alleles showed a statistical trend toward a lower magnitude of placebo response. But in a recent GWAS of a placebo controlled RCT of duloxetine for MDD, *COMT* association did not reach genome-wide significance	[144,151]
Pain and neurological studies				
Pain	Experimental pain in healthy normal volunteers. Repeated measures, N = 296	VAS	The *COMT* met allele plus *OPRM1* rs1799971 Asn/Asn associated with placebo analgesic response	[152]
Pain	Experimental Pain in healthy normal volunteers. N = 223	VAS and FOP	Met allele was associated with increased fear of pain but not placebo analgesia	[148]
Pain	Experimental Pain in healthy normal volunteers, N = 48	fMRI measures	Met alleles were linearly associated with stronger experimental placebo analgesia	[153]
IBS	RCT in IBS patients with NTC, N = 104	IBSSS	Met/met had greater placebo response in placebo arm augmented with positive clinical engagement	[149]
Cardiometabolic disease studies				
CVD	RCT in healthy normal women, N = 104	CVD events	Val allele associated with lower rates of CVD in placebo treatment arm	[52]
Energy expenditure and fat oxidation	Green tea (capsules) versus placebo in randomized cross-over study among healthy normals, N = 14	Energy expenditure and fat oxidation	Differential *COMT* genetic green tea effects were observed with higher energy expenditure and fat oxidation among met/met participants with placebo compared with val/val participants	[106]
Other conditions				
Inflammatory disease	Meta-analysis of RCTs in asthma and other inflammatory disease	Quality of Life (QOL)	No effect was observed for *COMT*	[154]
Nocebo effects in immunosuppression	RCT of immunosuppression	General side effects	The val-allele was found to be positively associated the perception and reporting of side effects in a study of placebo in immunosuppression	[155]

Asn/Asn: Asparagine polymorphism in OPRM1; CVD: Cardiovascular disease; FOP: Fear of pain; HAM-D17: Hamilton Depression Rating Scale score; IBS: Irritable bowel syndrome; IBSSS: IBS symptom severity score; MADRS: Montgomery-Asberg and Depression Rating Scales; MDD: Major depressive disorder; met: Methionine; NTC: No-treatment control; RCT: Randomized clinical trial; rs-fMRI: Resting-state functional MRI; val: Valine; VAS: Visual analog pain scale.

catecholaminergic and opioid signaling and placebo response pathways, *COMT* is a strong candidate among a growing number of 'placebome' genes/proteins implicated in modifying the placebo response [144–147]. It is, therefore, important to account for main and interaction effects of genes like *COMT* in both the drug and placebo treatment arms of RCTs.

As with drug response, the directionality of *COMT* associations with outcomes in placebo control arms of RCTs are variable (Table 4). The most consistent associations of *COMT* with placebo responses are in pain-related studies. In these studies, the *COMT* rs4680 low-activity met-allele alone or with opioid receptor polymorphisms was associated with placebo analgesia and with placebo-induced fear of pain [148]. Similarly, in an RCT of placebo response in irritable bowel syndrome (IBS), a functional pain syndrome, the strongest effects were observed among met-allele homozygotes in response to placebo treatment augmented with a purposefully positive clinical interaction, suggesting that variability in placebo response may also be influenced by the nature of the placebo treatment (Figure 4) [149].

There are several studies in which significant *COMT* association with outcomes in the placebo arm is directionally reversed in the drug treatment arm. For example, in an RCT of tolcapone and working memory and behavioral effects, the higher response observed among met/met participants in the placebo arm was reduced with tolcapone treatment and vice versa (Figure 5). In an RCT of CFS, where higher levels of catecholamines were associated with disease [53,74], the positive response in the placebo arm among val-allele homozygotes was abrogated with randomized clonidine treatment [89]. The potential for differential responses in the placebo and drug treatment arm, underscore the importance of including and analysing genetic effects in both active and control arms in clinical trials. This problem is evident in several single-arm trials of MDD, see Table 3. Still there is considerable variability

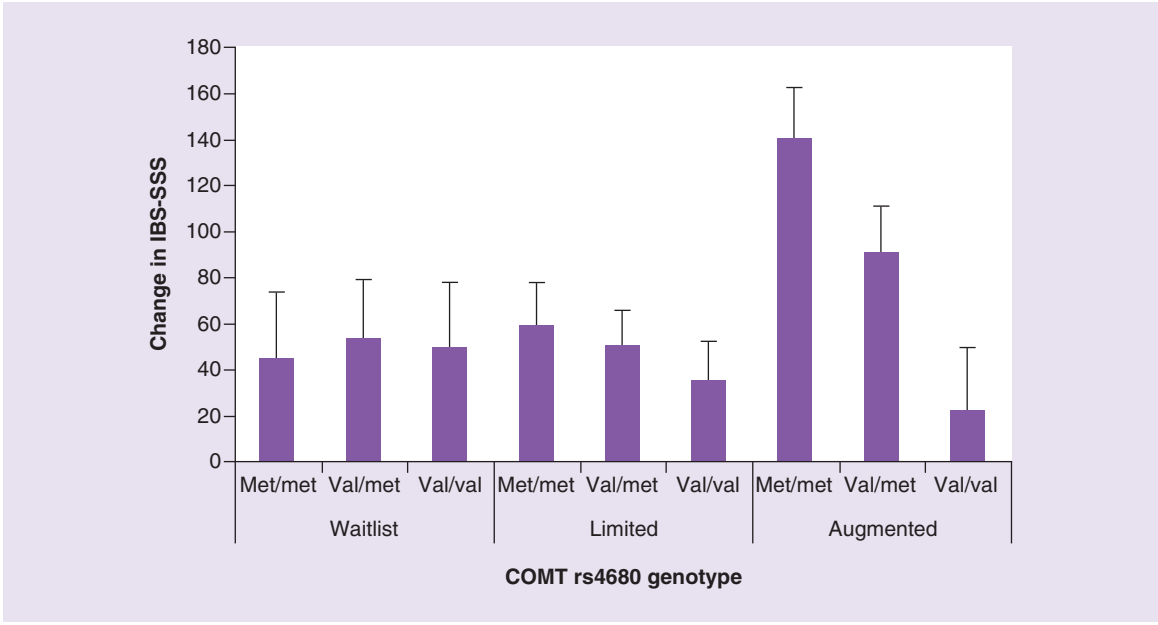

Figure 4. Interaction effect of catechol-O-methyltransferase genotype and treatment arm on change in IBS-SSS. The interaction between COMT genotype (number of met alleles) and treatment arm was statistically significant ($\beta = 0.17$; $p = 0.035$). Regression model included the following parameters: COMT genotype (number of met alleles), treatment arm, baseline IBS-SSS and their interaction (COMT genotype x treatment arm). Error bars indicate the standard error of the mean. $n = 104$.
IBS-SSS: Irritable Bowel syndrome – Symptom Severity Scale.

in trials that did include placebo controls. For example, whereas in one trial there was a trend for higher placebo response with the val-allele [151], in a genome-wide association study from an RCT for duloxetine versus placebo in MDD no genome-wide significant effects of *COMT* were reported in either arm [144].

These differential placebo and drug treatment arm effects are not limited to subjective outcomes. Higher energy expenditure and fat oxidation were observed among met/met participants randomized to placebo in an RCT of the cardiometabolic effects of green tea [106]. Differential *COMT* effects were also observed in the green tea treatment arm. In a large RCT of cardiovascular disease prevention, the high-activity val-allele was associated with better outcomes with randomized placebo treatment, but increased risk of cardiovascular disease with randomization to aspirin or vitamin E [52]. Although a meta-analysis of RCTs in inflammatory disease found no association of *COMT* with placebo response [154], the val-allele was found to be positively associated the perception and reporting of adverse effects in a study of placebo in immunosuppression [155].

One hypothesis for the differential drug and placebo responses in some of these trials is that the drug is perturbing the placebo response/placebo effect. That drugs can disrupt the placebo response is not a novel concept. Early studies on placebo effects (that included NTCs) in molar tooth extractions demonstrated that naloxone could block a robust placebo effect [156,157]. A series of interesting studies followed which used drugs that targeted neurotransmission [158,159], and one even earlier study which included aspirin [160], provided evidence of different placebo effect pathways. Although the findings from these studies laid the groundwork for our understanding of the biochemical pathways induced by placebo treatment and led to the assertion that there are multiple placebo response pathways, little is known about the influence of genetic variation in *COMT* and other genes on these effects.

Although *COMT* association with outcomes among individuals treated with placebo seem to vary by health status of the patient (healthy normal versus patient), condition (pain, IBS, anxiety), and study design (RCT versus laboratory experiment), there are enough statistically significant findings to warrant consideration and further investigation. A deeper understanding of potential, different placebo effects in the placebo arm of RCTs may help

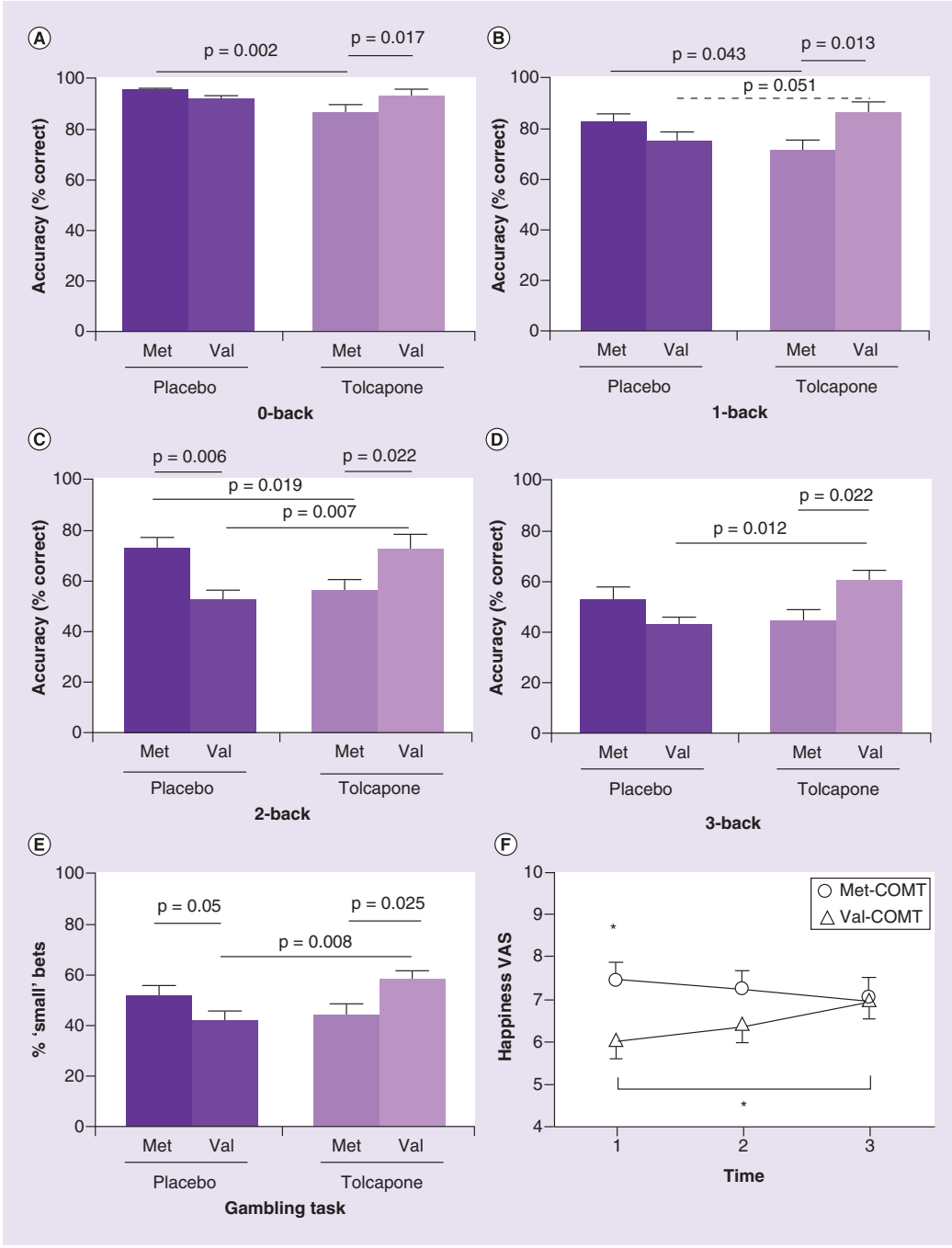

Figure 5. Interactive behavioral effects of catechol-O-methyltransferase genotype and catechol-O-methyltransferase inhibition. (A) 0-back: main effect of drug ($F[1,56] = 4.14$; $p = 0.047$) and genotype × drug interaction ($F[1,56] = 6.75$; $p = 0.012$). Met-COMT subjects given tolcapone perform worse than those given placebo. **(B)** 1-back: genotype × drug interaction ($F[1,56] = 9.26$; $p = 0.006$). Tolcapone impairs performance in Met-COMT subjects and, as a trend, improves it in Val-COMT subjects, compared with their respective placebo groups. **(C)** 2-back: genotype × drug interaction ($F[1,56] = 13.62$; $p = 0.001$). Met-COMT subjects perform better than Val-COMT subjects on placebo. Tolcapone reverses this difference, impairing Met-COMT subjects and enhancing Val-COMT subjects, compared with their respective placebo groups. **(D)** 3-back. Genotype × drug interaction ($F[1,56] = 8.03$; $p = 0.006$). Val-COMT subjects given tolcapone perform better than those given placebo. **(E)** Gambling task, showing percentage of times when 5 not 25 was gambled. Genotype × drug interaction ($F[1,61] = 7.91$; $p = 0.007$). On placebo, Met-COMT subjects are more likely than Val-COMT subjects to make a small rather than a large bet. Tolcapone reverses this difference, making Val-COMT subjects significantly more risk averse, compared with those given placebo. **(F)** Happiness VAS ratings for tolcapone-treated subjects. Data are adjusted means with standard errors. Time 1: immediately before drug administration. Time 2: T1 + 90 min. 3: T1 + 210 min.
*$p = 0.026$.
VAS: visual analogue scale.

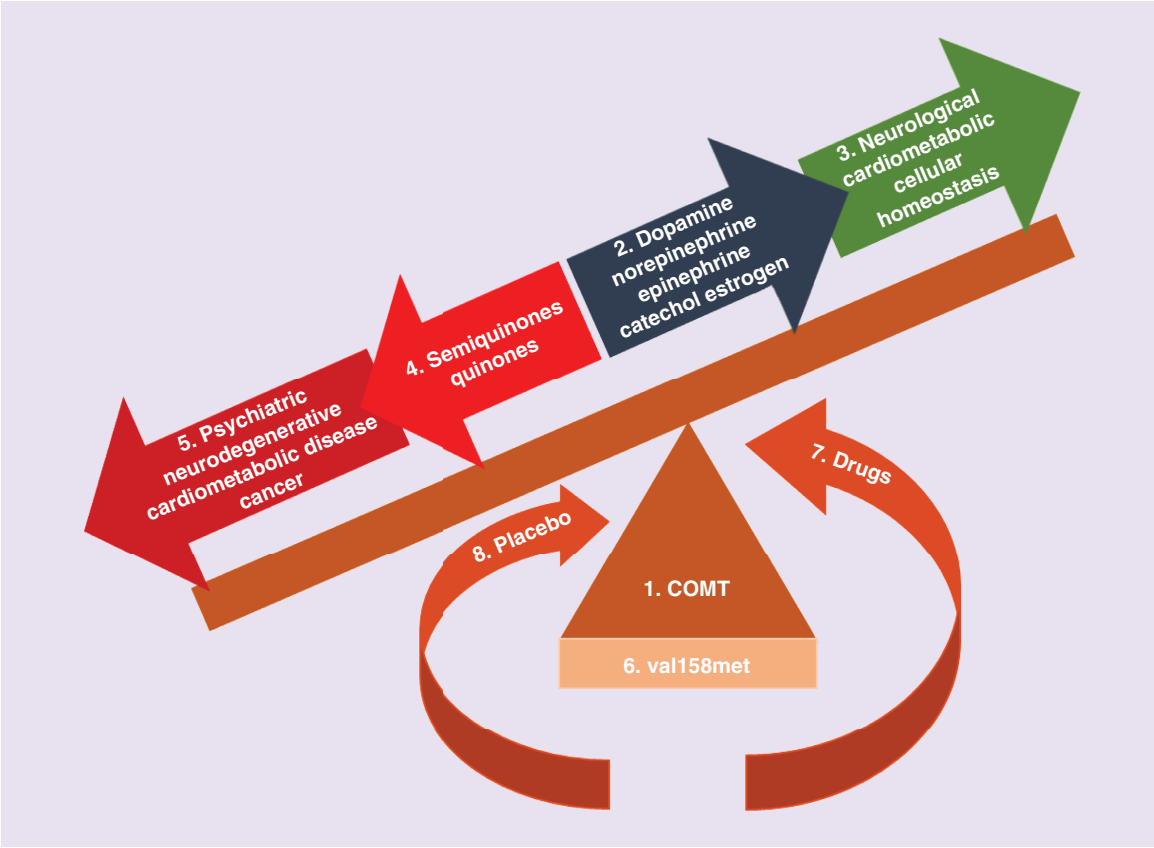

Figure 6. The COMT dynamic disease-placebo-pharmacogenomic hub. 1. COMT metabolizes catecholamine substrates. **2.** COMT substrates include the catecholamine neurotransmitters and hormones – dopamine, norepinephrine, epinephrine and catechol estrogen. **3.** COMT substrates are important in maintaining neurological, cardiometabolic and cellular homeostasis. **4.** When COMT enzymatic activity is perturbed, the catecholamines form chemically reactive semiquinones and quinones that can damage DNA and lipids. **5.** Perturbation in the *COMT* gene is associated with psychiatric disorders, neurodegenerative disease, cardiometabolic diseases and cancer. **6.** *COMT* can be perturbed by genetic variation at loci like rs4680 (val158met). **7.** Drugs can also modify COMT activity. **8.** Genetic variation in *COMT* can also modify placebo effects/response.

elucidate unexpected absences or variability in detecting drug–placebo differences that is not uncommon to find in drug development.

COMT is a member of the placebome, a network of proteins implicated in the placebo response [161]. Mapping the placebome to the larger network of human protein–protein interactions, the interactome, indicated that the placebome was also enriched for proteins implicated in disease and targeted by a wide cross-section of drugs, for example, analgesics, antidepressive agents, antioxidants, and adrenergic agonists [146]. Hence, there are likely to be several other genes/proteins involved in the drug–disease–placebo axis, and the understanding of how genetics can perturb potential drug–placebo interactions will require much additional research.

Conclusion

Genetic variation in *COMT* can have main and combinatorial effects on disease, drug response, and placebo effects/response, and thereby influence outcomes in RCTs. Hence, *COMT* is an example of a pharmacogenomic hub that has not previously been synthesized into a systems regulatory model as a means to integrate the evidence (Figure 6). This is a first attempt to examine this hypothesis using COMT as a model. *COMT* effects, as reviewed here, are many and varied in direction and magnitude, and may sometimes (or frequently) be masked by the many interacting and overlapping pathways within which it functions.

This potential for genetic variation to modify disease as well as drug and placebo effects/response differentially poses a challenge to the RCT, a cornerstone of drug development. The RCT was designed to determine the efficacy

of novel pharmaceutical treatments above any placebo response. However, the current approach to analyzing efficacy does not account for differences in disease progression or susceptibility and treatment or placebo response as a function of genetic variation. This limitation, in part, stems from the fact that we do not have many strong candidate genes known to perturb clinical outcomes predictably. Further, for the handful of candidate genes we do have, the mechanisms and sources of variability in treatment response are not fully understood. Given the potential for system-wide effects, we propose that pharmacogenomics broaden its scope to include investigation of genes like *COMT* that might not only influence drug response, but disease and placebo responses, as well.

No doubt, physicians increasingly bombarded by questions derived from personal genotyping are frustrated by how clinical utility of pharmacogenomics has lagged behind the sharp rise in online accessibility to genotyping data. Although, this review raises several interesting and potential points of intervention, given the considerable variability in outcomes, it is still too soon for clinical recommendations based on *COMT* genotype. Still, with the recent increase in big clinical data and developments in systems biology, network medicine, and machine learning/artificial intelligence, the promise of precision medicine to improve medicine has been revitalized and we anticipate, that considerable progress can now be made to actualize this area of research.

Our goal here has been to provide the reader with a sense of the network of influence a potential pharmacogenomic mediator like *COMT* can have. To simplify our exploration of this network, we focused on just one locus in *COMT*. With almost 5000 entries in PubMed, this review is by no means an exhaustive exploration of the associations between *COMT* and disease, drugs, or placebos. It is a reasonable attempt at a comprehensive review. Furthermore, there are likely many more genes like *COMT* that affect disease, as well as drug and placebo responses, differently. Owing to this complexity, systems pharmacogenomics approaches [162] will be needed to harness the potential to perturb these networks for clinical benefit and to optimize our ability to discern the efficacy and safety of drugs being tested.

Executive summary

Catechol-O-methyltransferase in form & function

- COMT endogenous substrates include catechol estrogen a key metabolite in estrogen metabolism and the catecholamines dopamine, epinephrine and norepinephrine.
- In the presence of magnesium ions, COMT transfers a methyl group from S-adenosylmethionine (SAM) to a hydroxyl group on the catechol ring of endogenous and xenobiotic catechol substrates. SAM is then converted to S-adenosylhomocysteine (SAH) a competitive inhibitor of COMT.
- The most widely studied polymorphism in COMT is rs4680 (val158met). It encodes a valine to methionine change that results in a 3-4-fold reduction in COMT enzymatic activity.

COMT & disease

- COMT influences diseases in at least four domains – psychiatric, neurological, and cardiometabolic diseases and cancer.
- COMT effects in psychiatric (ADD and bipolar disorder) and neurological (pain) diseases likely result from perturbations in dopamine and norepinephrine signalling in the brain.
- COMT effects in cardiometabolic (preeclampsia) and cancer likely result from perturbations in catechol estrogen and epinephrine.

COMT, drugs & supplements

- Genetic variation in COMT can modify thye effects of both active and pharmacologically inactive (placebo) treatments.
- COMT is modified by drugs and supplements containing a catechol ring. COMT effects can also be modified by drugs and supplements that modify the action or availability of COMT enymatic substrates.
- Genetic variation in COMT may differentially influence placebo and drug treatment responses and thus mask drug efficacy in clinical trials.

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