Carboxylesterase 1 and Precision Pharmacotherapy: Pharmacogenetics and Non-Genetic Regulators

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List of Abbreviations:
ADHD  attention deficit hyperactivity disorder
API    active pharmaceutical ingredient
AT I   angiotensin i
AT II  angiotensin ii
AUC    area under the curve
BCS    biopharmaceutics classification system
CES1   carboxylesterase 1
CES2   carboxylesterase 2
CI     confidence interval
Cmax   maximum plasma concentration
CNVs   copy number variations
CPIC   Clinical Pharmacogenetics Implementation Consortium
CYPs   cytochrome p450
DL-EPH dl-ethylphenidate
DMEs   drug-metabolizing enzymes
FDA    Food and Drug Administration
GWAS  genome-wide association study
INH    isoniazid
LD     loading dose
LOF    loss-of-function
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<th>Abbreviation</th>
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<tr>
<td>MAF</td>
<td>minor allele frequency</td>
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<td>MD</td>
<td>maintenance dose</td>
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<td>OR</td>
<td>odds ratio</td>
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<td>PCI</td>
<td>percutaneous coronary intervention</td>
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<td>pharmacodynamics</td>
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<td>PNPA</td>
<td>p-nitrophenyl acetate</td>
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<td>PTM</td>
<td>post-transcriptional modification</td>
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<td>SNPs</td>
<td>single-nucleotide polymorphisms</td>
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<td>T2DM</td>
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Abstract

Carboxylesterase 1 (CES1) is the most abundant drug-metabolizing enzyme in human livers, comprising approximately 1% of the entire liver proteome. CES1 is responsible for 80-95% of total hydrolytic activity in the liver and plays a crucial role in the metabolism of a wide range of drugs (especially ester-prodrugs), pesticides, environmental pollutants, and endogenous compounds. Expression and activity of CES1 vary markedly among individuals, which is a major contributing factor to interindividual variability in the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs metabolized by CES1. Both genetic and non-genetic factors contribute to CES1 variability. Here, we discuss genetic polymorphisms, including single-nucleotide polymorphisms (SNPs) and copy number variants, and non-genetic contributors, such as developmental status, genders, and drug-drug interactions, that could influence CES1 functionality and the PK and PD of CES1 substrates. Currently, the loss-of-function SNP G143E (rs71647871) is the only clinically significant CES1 variant identified to date, and alcohol is the only potent CES1 inhibitor that could alter the therapeutic outcomes of CES1 substrate medications. However, G143E and alcohol can only explain a small portion of the interindividual variability in the CES1 function. A better understanding of the regulation of CES1 expression and activity and identification of biomarkers for CES1 function in vivo could lead to the development of a precision pharmacotherapy strategy to improve the efficacy and safety of many CES1 substrate drugs.
Significance Statement

Carboxylesterase 1 (CES1) is a major phase I drug-metabolizing enzyme responsible for 80-95% of total hydrolytic activity in human livers. CES1 plays a crucial role in metabolizing a wide range of drugs, pesticides, environmental pollutants, and endogenous compounds. The clinical relevance of CES1 has been well demonstrated in various clinical trials with methylphenidate, oseltamivir, and clopidogrel. Here, we discuss genetic polymorphisms and non-genetic contributors that influence CES1 functionality and the PK and PD of drugs metabolized by CES1.
1. Introduction

Carboxylesterase 1 (CES1) is a phase I drug-metabolizing enzyme (DME) responsible for 80-95% of total hydrolytic activity in the liver (Imai et al., 2006); it metabolizes a wide range of drugs, pesticides, environmental pollutants, and endogenous compounds, including lipid esters (Table 1). CES1-mediated metabolism can lead to the biotransformation of a pharmacologically active drug into its inactive metabolite, as exemplified by methylphenidate hydrolysis in the liver. CES1 also plays an important role in activating prodrugs since most ester-containing prodrugs are exclusively dependent on CES1 for their activation. The clinical relevance of CES1 has been well demonstrated in various clinical trials with oseltamivir, methylphenidate, and clopidogrel (Zhu et al., 2008; Tarkiainen et al., 2012; Lewis et al., 2013; Jiang et al., 2016). Recent studies have also revealed that CES1 acts as a cholesteryl ester hydrolase in lipid metabolism in human macrophages and hepatocytes, and suggest CES1 as a potential drug target for the treatment of metabolic diseases, such as diabetes and atherosclerosis (Dolinsky et al., 2004; Zhao et al., 2007; Ghosh et al., 2010; Ross et al., 2010; Lian et al., 2018b).

2. Importance of CES1 in drug metabolism

CES1 plays an important role in metabolizing many clinically significant medications, especially the ester-prodrugs (Table 1). A prodrug refers to an inactive drug molecule that needs to be enzymatically biotransformed in vivo to its active metabolite to produce its intended pharmacological effect (Rautio et al., 2008). Prodrug design offers an attractive method to overcome the issue of low bioavailability for Biopharmaceutics Classifications System (BCS) class III drug molecules. Drug
molecules can be categorized into four BCS classes based on permeability and solubility, and a BCS class III substance is a hydrophilic compound with low permeability and high solubility (Shah and Amidon, 2014). In particular, hydrophilic compounds with –OH or –COOH functional groups usually have difficulty being absorbed into the body, and drug developers often mask these functional groups using an ester-prodrug design. The prodrug market has been growing: 20% of drugs approved in 2015 were prodrugs, compared to ~6% of all currently approved drugs (Rautio et al., 2017).

Two major assumptions behind the ester-prodrug design are that prodrugs are rapidly activated via unspecific esterases in the body, and that the interindividual variability in activating a prodrug is clinically insignificant. These incorrect assumptions may have stemmed from the fact that many hydrolytic enzymes exist in the body, such as CES1, CES2, acetylcholinesterase, butyrylcholinesterase, paraoxonases, and arylesterase. However, these hydrolases differ in their tissue-specific expression, cellular localization, and most importantly, substrate selectivity (Fukami and Yokoi, 2012). In humans, CES1 is highly abundant in the liver and expressed to a less extent in the lung and brain; CES1 expression is considered negligible in the human intestine, kidney, and plasma. CES1 is substrate-selective towards carboxyl esters with a large ethyl group and a small alcohol group. In comparison, CES2, another major carboxylesterase in humans, is highly expressed in the intestine, kidney, and liver, and is more efficient at metabolizing compounds with a small ethyl group and a large alcohol group (Jewell et al., 2007). Numerous in vivo and in vitro studies have demonstrated the specificity of CES1, and many CES1 substrates cannot be metabolized by other esterases (Table 3).
CES1 expression and activity vary significantly among individuals (Wang et al., 2016a); this variability could result in treatment failure and unexpected adverse effects of CES1 substrate drugs. A better understanding of the genetic and non-genetic factors contributing to CES1 variability will improve the design and clinical use of many drugs that are metabolized (deactivated/activated) by CES1.

3. Pharmacogenetics of drug-metabolizing enzymes

Traditionally, fixed-dose regimens have been used for most medications. However, different individuals taking the same dose of medication do not necessarily achieve the same drug exposure and hence drug response. More individualized, patient-centered dosing regimens have been developed based on a patient’s characteristics, such as renal clearance, liver function, body weight, and surface area (DiPiro, 2017). In addition, genetic polymorphisms of DMEs have been found to play an important role in the response to pharmacotherapy, and pharmacogenomics has been increasingly utilized in the clinic to improve the efficacy and safety of drug treatment. DMEs serve to primarily detoxify digested xenobiotics through four general mechanisms: hydrolysis (e.g., carboxylesterase), reduction (e.g., carbonyl reductase), oxidation (e.g., cytochrome P450), and conjugation (e.g., UDP-glucuronosyltransferase) (Foti and Dalvie, 2016). The expression and activity of DMEs vary significantly among individuals, and studying pharmacogenomics of DMEs is one means of better understanding interindividual variability in the PK and PD of a drug. For example, the active metabolite of irinotecan, SN-38, is primarily metabolized by the enzyme UDP glucuronosyltransferase family 1 member A1 (UGT1A1 enzyme) (Ando et al., 2000). If a patient carries the common UGT1A1*28 polymorphism, the decrease it causes in
UGT1A1 enzymatic activity would impede the metabolism of SN-38, leading towards the accrual of toxic concentrations. Accordingly, the Food and Drug Administration (FDA) recommended that patients with UGT1A1*28/*28 start irinotecan at a lower dose (Innocenti et al., 2004). However, given that both genetic and environmental factors contribute to DME function, we should also pay close attention to non-genetic contributors when studying the variability of DMEs.

4. CES1 pharmacogenetics

Although CES1 plays a critical role in the metabolism of many clinically important medications, CES1 pharmacogenetics is understudied relative to other major DMEs (e.g., CYP450s). CES1 is encoded by the CES1 gene, consisting of 14 exons located on chromosome 16q13-q22.1. CES1 VAR is a variation of the CES1 gene that differs in exon 1 DNA sequences and has an average minor allele frequency (MAF) of 17%. Although one study claimed that CES1 VAR mRNA was undetectable (Fukami et al., 2008), an in vitro human liver study showed that the protein expressions of CES1 and CES1 VAR were not statistically different (Wang et al., 2016a). CES1P1 is a pseudogene due to a premature stop codon in exon 4 and lies tail-to-tail with CES1 (Figure 1) (Wang et al., 2016a). Interestingly, a CES1P1 variant named CES1P1 VAR is a functional coding gene with a DNA sequence identical to CES1 VAR. However, the transcription efficiency of CES1P1 VAR is only 2% of that of CES1, due to the transcription factor Sp1, and the enhancer-binding protein C/EBPα preferring to bind to the CES1 promoter over the CES1P1 VAR promoter (Hosokawa et al., 2008; Yoshimura et al., 2008). Due to the existence of the CES1 VAR and CES1P1 VAR variants, four CES1/CES1P1 haplotypes can be formed (Figure 1). In addition to these structural
variations, there are over 7000 CES1 SNPs registered in the NCBI SNP database, and
approximately 300 of them have MAFs over 1%. These common CES1 variants (MAF >
1%) are distributed in various regions of the gene, including 13 in 5'-UTR and 3'-UTRs,
14 in exons, and 308 in introns. Of the exonic SNPs, 12 are non-synonymous SNPs and
two are synonymous SNPs. In the following section, we discuss the clinical findings and
mechanistic bases of functional CES1 variants identified to date.

4.1 Pharmacogenetics of the first loss-of-function CES1 variant G143E
(rs71647871)

In SNP notation, G143E indicates an amino acid change from glycine to
 glutamic acid at amino acid position 143. G143E is also termed 428G>A, indicating that
the nucleotide guanine is changed to adenine at position 428 of the CES1 mRNA
(DiPiro, 2017). The MAF of G143E is 3.7%, 4.3%, and 2%, in White, Hispanic, and
African American populations, respectively, while the SNP is extremely rare in Asian
populations (Zhu et al., 2008; Suzaki et al., 2013a).

G143E is a non-conservative amino acid substitution located near the active-site
triad residues of CES1 (serine 221, glutamic acid 354, and histidine 468). Serine
hydrolases shares similar catalytic mechanism involving (1) nucleophilic attack from
oxygen in the serine residue on a substrate ester bond, (2) formation of a tetrahedral
intermediate where the deprotonated oxygen is stabilized via an oxyanion hole, (3)
formation of an acyl-enzyme intermediate, and (4) water-catalyzed hydrolysis (Satoh
and Hosokawa, 2006). For CES1 to maintain its enzymatic function, the catalytic triad
and oxyanion hole need to be conserved (Zhu et al., 2008; Arena de Souza et al., 2015).
The change from glycine (hydrophobic residue) to glutamic acid (electrostatic residue) at
codon 143 disrupts the hydrophobicity needed for the oxyanion hole (Gly 141-131), resulting in a complete loss of function of CES1. The G143E is only CES1 SNP that has been subjected to in vitro kinetics studies, in which the variant exhibited null catalytic activity on all tested CES1 substrates except for oseltamivir (Table 2). The $V_{\text{max}}$ of G143E on oseltamivir hydrolysis was 37 nmol/min/mg with catalytic efficiency of 17.2 μl/min/mg protein — this was approximately 16% of wild type CES1 catalytic efficiency (Zhu and Markowitz, 2009).

4.1.1 Discovery of G143E and its impacts on methylphenidate PK and PD

G143E is the first loss-of-function (LOF) variant known for CES1 and was originally discovered in a methylphenidate (Ritalin®) PK study in healthy volunteers. Methylphenidate is a central nervous system stimulant, the most commonly prescribed medication for ADHD treatment and has high abuse potential when used with alcohol (Neos Therapeutics, 2017). Its drug product comes as a racemic mixture of d- and l-methylphenidate hydrochloride; d-methylphenidate is approximately 10 times more pharmacologically potent than l-methylphenidate (Heal and Pierce, 2006).

Methylphenidate is metabolized by de-esterification via CES1 to ritalinic acid, an inactive metabolite which accounts for approximately 80% of the recovered dose in human urine (Figure 2) (Laizure et al., 2013; Neos Therapeutics, 2017). In 2007, a prospective single-dose (0.3 mg/kg) PK study was conducted in twenty healthy volunteers to examine the DDI between methylphenidate and alcohol (Patrick et al., 2007). During this study, the researchers unexpectedly found a participant that showed significantly elevated pharmacokinetic parameters (e.g., AUC, $C_{\text{max}}$) of methylphenidate. Specifically, dl-methylphenidate $C_{\text{max}}$ was seven times higher and l-methylphenidate
C_{\text{max}} was 100-fold higher in this poor metabolizer compared to the rest participants. Later analysis found that this poor metabolizer carried the G143E polymorphism in CES1 and the D260fs polymorphism in CES1P1 (Zhu et al., 2008). This study also concluded that while CES1 metabolism is substantially stereoselective towards L-methylphenidate, d-methylphenidate metabolism is also significantly impacted by CES1 dysfunction.

Following the discovery of the G143E variant, a retrospective study was conducted to examine the methylphenidate response in Hungarian ADHD patients with G143E (n=7) and non-carrier patients (n=115). Even though the CES1 genotype could not explain the entire interindividual variability between responders (n=90) and non-responders (n=32), the study demonstrated an association between G143E polymorphism and methylphenidate dose reduction: five responders who had the G143E polymorphism required lower doses of methylphenidate for symptom reduction (0.410 vs 0.572 mg/kg, P=0.022) (Nemoda et al., 2009). In 2017, a healthy volunteer study confirmed the significance of G143E in the PK of methylphenidate. In this open-label, prospective clinical trial (n=22), study participants carrying the G143E SNP (n=6) had approximately 152.4% higher median AUC of d-methylphenidate (53.3 ng×ml^{-1}×h^{-1}) than the non-carrier group (21.4 ng×ml^{-1}×h^{-1}) (P<0.0001) (Stage et al., 2017a).

The above studies suggest that G143E carriers may be at high risk of being exposed to a toxic methylphenidate concentration. This result is clinically impactful because methylphenidate is considered as the first-line pharmacotherapy for ADHD, with approximately 40 million prescriptions dispensed every year (Schubert et al., 2010). This result could potentially explain why many patients have an unsatisfactory response
to the treatment. Further clinical studies in ADHD patients with larger sample sizes are needed to fully understand the effect of CES1 variants on the efficacy and toxicity of methylphenidate, and how methylphenidate doses should be adjusted based on a patient’s CES1 genotypes.

4.1.2 G143E and clopidogrel (Plavix®)

Clopidogrel is a P2Y12 inhibitor and has several clinical indications, including myocardial infarction prophylaxis, cerebrovascular accident prophylaxis, and peripheral arterial occlusive disease prophylaxis. Clopidogrel is usually considered as the first-line antiplatelet agent due to its proven efficacy and cost-effectiveness (Wiviott et al., 2007; Wallentin et al., 2009; Roe et al., 2012). Clopidogrel is a non-ester-prodrug that needs to be activated by two oxidation reactions via several CYPs (Figure 3). CYP2C19 pharmacogenetics and its impact on clopidogrel activation have been extensively studied. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline and the FDA both recommend intermediate and poor metabolizers of CYP2C19 to use an alternative antiplatelet agent, such as ticagrelor or prasugrel (Scott et al., 2013). Clopidogrel, its intermediate, and active metabolites are all CES1 substrates and metabolized by CES1 to inactive hydrolytic metabolites (Figure 3). Approximately, 85% of clopidogrel is hydrolyzed by CES1, and only 15% clopidogrel enters the CYPs-mediated activation pathway (Zhu et al., 2013). Thus, patients with CES1 dysfunction would have a higher concentration of clopidogrel active metabolite compared to normal CES1 metabolizers when taking the same dose. However, the impact of CES1 on the PK and PD of clopidogrel is less studied than the impacts of CYPs.

Two clinical trials support that CES1 G143E carriers have significantly higher plasma concentrations of clopidogrel active metabolite compared to non-carriers. A
retrospective sub-analysis was performed on participants of the Pharmacogenomics of Antiplatelet Intervention (PAPI) Study (n=506) and on clopidogrel-treated patients at Sinai Hospital (n=350) to examine the effect of CES1 G143E on clopidogrel metabolism. Study participants received a 300mg loading dose of clopidogrel followed by a 75mg maintenance dose for six days, and platelet aggregation was measured as a PD marker. A 50% higher active metabolite concentration was observed in G143E carriers (n=7, 30.3 ng/ml) compared to non-carriers (n=499, 19.0 ng/mL) (P=0.001). In addition, the inhibition of adenosine diphosphate (ADP)-induced platelet aggregation was 24% higher in G143E carriers (reduced to 71% from baseline) relative to non-carriers (reduced to 57% from baseline) (P=0.003) (Lewis et al., 2013; Bozzi et al., 2016; Jiang et al., 2016). Another prospective, single-dose, healthy volunteer (n=22) clinical study was conducted by Tarkiainen et al. to determine the effect of CES1 G143E on clopidogrel metabolism. The authors found that the AUC$_{0-\infty}$ ratio of clopidogrel carboxylic acid (inactive metabolite (1) in Figure 3) to clopidogrel was 53% less in G143E carriers (n=10) than non-carriers (n=12) (P=0.009). The G143E carriers also exhibited significantly higher plasma concentrations of the parent compound clopidogrel (P = 0.004) and its active metabolite (P = 0.009) compared to non-carriers. In agreement with the PK findings, the average inhibition of P2Y12-mediated platelet aggregation in the carriers was 19 percentage points higher than in non-carriers (P = 0.036) (Zhu et al., 2013; Tarkiainen et al., 2015a). The findings of the above two studies are especially important for patients on triple antithrombotic therapy with a high bleeding risk (Mehta et al., 2001; Steinhubl et al., 2002; Shmyr et al., 2017). Clopidogrel dose adjustment may be necessary to prevent potential toxicity (i.e., bleeding) in patients with CES1 dysfunction.
4.1.3 G143E and angiotensin-converting enzyme inhibitors (ACEIs)

Angiotensin-converting enzyme inhibitors (ACEIs) are generally considered to be the first-line therapy for heart failure and hypertension, and approximately 150 million ACEI prescriptions are filled in the US annually (Mahmoudpour et al., 2015). Currently, eight out of ten FDA approved ACEIs are ester-containing prodrugs, and all ACEI prodrugs need to be activated by CES1 in order to exert their intended therapeutic effects (Chaturvedi, 2004; Yancy et al., 2017). The activation is essential for the pharmacological effects as the active metabolites are 10-1,000 times more potent than their prodrug forms (Foye et al., 2013). Therefore, patients with CES1 dysfunction would have a lower concentration of the ACEI active metabolite relative to normal CES1 metabolizers (Figure 4).

A prospective, single-dose pharmacokinetic clinical study was conducted in healthy volunteers to examine the effect of the G143E variant on the activation of the ACEI prodrugs enalapril and quinapril. The AUC\(_{0-\infty}\) of the enalapril active metabolite enalaprilat was found to be 20% lower in the G143E carriers (n=10) than in non-carriers (n=12) (P=0.049) (Tarkiainen et al., 2015b). This finding is consistent with an in vitro study that showed that enalapril activation was impaired in liver samples carrying the G143E variant (Wang et al., 2016a). However, the AUC\(_{0-\infty}\) of the quinapril and its active metabolite (quinaprilat) were not significantly different between carriers and non-carriers (P=0.114). Further investigations are warranted to fully understand the effect of CES1 variants on the PK and PD of ACEI prodrugs.

4.1.4 G143E and oseltamivir (Tamiflu®)
Oseltamivir is an antiviral drug that has an FDA indication for influenza types A and B infections. Even though oseltamivir is rarely effective due to its specific administration requirement (i.e., this medication should be taken within 2 days of onset of symptoms in order to reduce flu duration by approximately one day), oseltamivir remains one of the most prescribed drug products due to flu epidemics (Singh et al., 2003; Dahlgren et al., 2018). As an ester-prodrug, oseltamivir needs to be activated by CES1 into its active metabolite, oseltamivir carboxylate (Shi et al., 2006). An in vitro study based on cell lines stably transfected with CES1 variants suggested the G143E SNP markedly impaired CES1 activity in oseltamivir activation (Zhu and Markowitz, 2009).

To examine the effect of G143E on oseltamivir PK and activation, a prospective, single-dose pharmacokinetic clinical study was conducted in healthy volunteers consisting of nine G143E heterozygotes, one G143E homozygote, and 12 non-carriers. The $AUC_0-\infty$ ratio of oseltamivir carboxylate (active metabolite) to oseltamivir (parent molecule) was 23% lower in G143E heterozygotes compared to non-carriers ($P = 0.006$). The one G143E homozygous individual had an $AUC_0-\infty$ of oseltamivir that was approximately 360% greater than the non-carriers, indicating that loss of CES1 activity could profoundly impair oseltamivir activation (Tarkiainen et al., 2012).

### 4.1.5 G143E and dabigatran and sacubitril

Dabigatran and sacubitril are both prodrugs that need to be activated by CES1 in the liver (Figure 5). In vitro studies showed that the formation rates of the active metabolites of dabigatran and sacubitril were significantly lower in human livers carrying the G143E variant than in non-carrier samples (Shi et al., 2016c; Shi et al., 2016d).
However, it remains undetermined whether the variant can affect the activation and therapeutic response of these two drugs in patients.

4.2 Pharmacogenetics of other CES1 genetic variants

In addition to G143E, many other CES1 variants have been studied for their effects on the PK and PD of CES1 substrate drugs. However, the results were generally inconclusive, and further studies are needed to determine the clinical significance of these variants.

4.2.1 E220G (rs200707504)

A nonsynonymous variant E220G, commonly referred to as c.662A>G, was suggested to decrease CES1 enzymatic activity in an *in silico* analysis (Oh et al., 2017). In agreement with that prediction, an *in vitro* study on transfected cell lines found E220G markedly decreased CES1 activity and the metabolisms of several CES1 substrates including enalapril, clopidogrel, and sacubitril (Wang et al., 2017a). Notably, E220G has a MAF of 0.55% in East Asians but is rare in other populations. To determine the clinical impact of E220G on the PK of a CES1 substrate, a single-dose oseltamivir (75mg) PK study was conducted in 20 healthy Korean volunteers. In this study, the variant was observed to have a marginal effect on the PK of oseltamivir and its active metabolite (oseltamivir carboxylate); however, the differences were statistically insignificant. In the E220G carriers (n=8), the AUC_{0-48h} of oseltamivir was increased by 10% (P = 0.334), and the AUC_{0-48h} of oseltamivir carboxylate was decreased by 5% (P = 0.513) relative to the non-carriers (n=12) (Oh et al., 2017).

4.2.2 S75N (rs2307240)
S75N is one of the most common CES1 nonsynonymous SNP with MAFs ranging from 2% to 7% in different populations. A retrospective pharmacodynamics analysis was conducted to examine the effect of CES1 S75N on the outcome of clopidogrel therapy in patients with the coronary syndrome (n=851). The result showed that CES1 S75N carriers (n=372) had higher incidence of cerebrovascular events (P < 0.001), acute myocardial infarction (P < 0.001), and unstable angina (P < 0.001) compared to non-carriers. The study also found that the S75N polymorphism was more frequent in acute coronary syndrome patients (MAF 22%) than the general population (MAF 5%). The authors concluded that there was a significant association between the S75N polymorphism and the outcome of clopidogrel therapy (Xiao et al., 2017). However, this result conflicts with another study that found the S75N variant to be not associated with the outcomes of ADHD patients treated with methylphenidate (Johnson et al., 2013). Furthermore, an in vitro study showed the S75N variant did not significantly alter the expression and activity of CES1 in transfected cells and human livers (Wang et al., 2017a).

4.2.3 -816A>C (rs3785161)

The -816A>C polymorphism is located in the promoter region of CES1P1 VAR and has been suggested as a potential up-regulator of CES1P1 VAR expression (Yoshimura et al., 2008). A prospective clinical study was conducted to examine the impact of -816A>C on the outcome of the ACEI prodrug (imidapril) therapy in hypertensive patients (n=105). The study found that after eight weeks of imidapril therapy, -816A>C homozygotes and heterozygotes (n=47) had greater systolic blood pressure reduction (24.1 mmHg) compared to non-carriers (17.6 mmHg) (P = 0.0184),
indicating increased CES1 functionality in the carriers. The follow up in vitro study claimed that the -816A>C SNP may have enhanced transcription of the CES1P1 VAR gene (Geshi et al., 2005). The -816A>C SNP was also evaluated for its impact on the outcomes of dual antiplatelet therapy (i.e., aspirin and clopidogrel) in patients with coronary heart diseases (n=162). The -816A>C carriers (n=75) had decreased vasodilator stimulated phosphoprotein-platelet reactivity index (VASP-PRI) (P=0.014), indicating increased CES1 function in the carriers (Xie et al., 2014).

However, conflicting findings were reported by other studies. A study involving the outcome of clopidogrel treatment in patients undergoing percutaneous coronary intervention, -816A>C carriers showed a lower ADP-induced maximum platelet aggregation (21.5%, n=125) compared to non-carriers (31.7%, n=124) (P=0.001), indicating decreased CES1 function (Zou et al., 2014). Zhu et al. also performed a retrospective pharmacogenetic analysis of the INternational VErapamil SR Trandolapril (INVEST) study (n=486) and did not find an association between -816A>C and the blood pressure-lowering effect of trandolapril. The follow up in vitro study also showed -816A>C genotype was not significantly associated with CES1 protein expression and trandolapril activation in human liver samples (n=100) (Zhu et al., 2016). Other researchers also noted that the CES1P1 VAR gene, which contains -816A>C, is considered functionally insignificant due to its low transcription efficiency (Tanimoto et al., 2007; Hosokawa et al., 2008).

4.2.4 -75G>T (rs3815583)

The -75G>T SNP is located in the promoter region of CES1 and was suspected to alter CES1 expression in the liver; however, the findings are conflicted. A study was
performed to determine the association between the variant and appetite reduction (a side effect of methylphenidate) in children with ADHD (n=213). Appetite reduction was measured by the Barkley Stimulant Side Effect Rating Scale, and methylphenidate dose was titrated up for three months as tolerable. The carrier group (n=129) had worse appetite reduction compared to non-carriers (n=76) (41% vs 77%, P=0.01), indicating that the variant was associated with decreased CES1 function (Bruxel et al., 2013). A study in patients treated with irinotecan, however, showed a contrary finding, suggesting that the -75G>T variant confers greater CES1 function (Sai et al., 2010). CES1 is involved in the conversion of the prodrug irinotecan to its active metabolite, SN-38, and then further metabolized by UGT1As to inactive SN-38G. Following irinotecan treatment, patients who carried the T allele of this variant had higher plasma (SN-38 + SN-38G)/irinotecan AUC ratios relative to non-carriers (P=0.027) following irinotecan treatment (Sai et al., 2010).

Other CES1 substrates, isoniazid, and ACEI prodrugs were also studied in the context of -75G>T, however, no significant relationships were found between the variant and the medication responses. In one such study, the variant was evaluated for its effect on the outcomes of ACEI prodrugs in congestive heart failure patients (n=200) that underwent ACEI prodrug dose titrations. The study reported -75G>T did not significantly impact plasma ATII/ATI ratios, furthermore, the -75G>T variant was not significantly associated with fatal outcomes (i.e. cardiovascular death and all-cause death) (Nelveg-Kristensen et al., 2016). The study with isoniazid had similar results showing no significant association between the variant and isoniazid-induced hepatotoxicity (n=170) (Yamada et al., 2010).

4.2.5 1168-33C>A (rs2244613)
Dabigatran (Pradaxa®) is a prodrug that needs to be activated by both CES1 and CES2 to exert its anticoagulant effect (Figure 5). Paré and associates conducted a genome-wide association study of dabigatran in participants (n=2944) of the Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY) clinical trial. The researchers concluded the CES1 intronic variant 1168-33C>A (rs2244613) is associated with lower trough concentrations of the active metabolite (15% decrease per allele; 95% CI 10-19%) and a lower risk of any bleeding (odds ratio, 0.67; 95% CI 0.55-0.82) compared to non-carriers (Pare et al., 2013). However, an in vitro study did not find the variant to be associated with CES1 protein expression and dabigatran metabolism in human livers (Shi et al., 2016a). A prospective study also examined the impact of 1168-33C>A in ADHD patients treated with methylphenidate, and found the variant to be associated with the occurrence of sadness, a side effect of short-acting methylphenidate. However, researchers concluded this might be due to linkage disequilibrium with two SNPs of the noradrenaline transporter gene (Johnson et al., 2013).

4.2.6 Copy number variation (i.e. CES1P1/CES1P1 VAR)

Many researchers have studied the impact of CNVs on CES1 functionality; however, the results are conflicted. Stage et al. found that participants with four functional copies of CES1 (n=5) had an increased AUC of d-methylphenidate relative to the control group with two functional copies of CES1 (n=17) (61% increase, P = 0.011); participants with three copies of CES1 (n=2) had 45% increased AUC compared to the control group (P = 0.028) (Stage et al., 2017a). Stage et al. conducted a similar study with enalapril (n=43), however, they could not find a statistically significant correlation...
between CNV and enalapril PK (Stage et al., 2017b). When Sai et al. examined the effect of CNV on the irinotecan exposure, they found patients with multiple CES1 copies (i.e., three or four) to have 1.24 fold higher irinotecan AUC relative to patients with two copies of CES1 (P = 0.0134) (Sai et al., 2010). Many researchers, however, did not find the relationship between CNVs and CES1 function. Suzaki et al. evaluated the relationship between CNVs of CES1 and oseltamivir PK parameters but did not find any correlation (Suzaki et al., 2013b). Nelveg-Kristensen et al. studied the relationship between CNV and ACEI prodrugs, and again, no association was found (Nelveg-Kristensen et al., 2016). Moreover, an in vitro study showed CES1 protein expression levels to be comparable among human livers with different copy numbers of functional CES1 gene (Wang et al., 2016a).

4.2.7 Other CES1 SNPs

In addition to the polymorphisms discussed above, sporadic reports have stated several CES1 SNPs to be associated with the outcomes of CES1 substrate medications. For example, the SNP 1315 + 2025A>C (rs8192950) was associated with a decreased risk of ischemic events in patients (n=64) having symptomatic extracranial or intracranial stenosis and receiving dual antiplatelet therapy with clopidogrel for a minimum of five days (Zhao et al., 2016). Another retrospective sub-analysis of a capecitabine clinical study identified associations of 1168-41C>T (rs2244614), 690 + 129del (rs3217164), 95346T>C (rs7187684), -1232A>G (rs1186118) with severe early-onset of capecitabine-induced toxicity (Hamzic et al., 2017). None of these findings has been validated independently.
A rare LOF variant, D260fs (c.70DelT), was reported in a clinical study (Zhu et al., 2008). D260fs causes a deletion in exon 6, resulting in a frameshift and premature truncation. Moreover, an in vitro study with CES1 variants transfected cell lines examined the SNPs proximate to the CES1 active site, and identified four LOF nonsynonymous SNPs: G142E, G147C, Y170D, and R171C. However, these variants appear to be clinically insignificant due to their low MAFs (< 0.4%) (Wang et al., 2017b).

The above-mentioned CES1 SNPs and their impacts on the PK and PD of CES1 substrate medications are summarized in Table 3.

5. Non-genetic factors affecting CES1 expression and activity

5.1 Developmental expression of CES1

The developmental expression patterns of CES1 in human and mouse livers were similar, and many in vitro studies have suggested that hepatic CES1 protein expression increases with age (Zhu et al., 2009a; Hines et al., 2016; Boberg et al., 2017). An in vitro study with human liver samples (n=104) demonstrated the adult group (≥ 18 years of age) to have had higher CES1 expression than children (0 days–10 years); meanwhile child group had higher CES1 expression than fetuses (82–224 gestation days). A follow-up study with liver microsomes showed that, in parallel with expression level, CES1 activity on hydrolyzing its substrate oseltamivir was also positively correlated with age (Yang et al., 2009). The same group did a similar in vitro human liver study with a slightly different age bracket, in which the liver samples were divided into five age groups: 1–31 days old (group 1), 35–70 days old (group 2), 89–119 days old (group 3), 123–198 days old (group 4), and over 18 years old (group 5). Neonates (group 1) had 10% of the CES1 expression and hydrolysis levels compared to
the adult group (group 5); pediatric groups (Group 2-4) had approximately 50% of the CES1 expression and hydrolysis levels compared to an adult (Shi et al., 2011). Lastly, a similar in vitro study quantified CES1 protein levels in human liver samples of various ages (n=165). CES1 expression levels were 4.76 pmol/mg from birth to three weeks (n=36); 15.8 pmol/mg for those aged three weeks to six years (n=90); and 16.6 pmol/mg for ages six years to 18 years (n=36). The study team concluded that the median CES1 expression level is directly correlated with age (P < 0.001) (Hines et al., 2016). Overall, CES1 expression and activity levels are lower in neonates and pediatric patients; further studies are warranted to investigate the potential effect of CES1 maturation on the treatment outcome of CES1 substrate medications in patients in the early stages of development.

5.2 Sex difference of CES1 expression

Both in vitro and clinical studies have suggested that CES1 expression is higher in females than in males (Patrick et al., 2007; Zhu et al., 2009a; Shi et al., 2016d). A PK study on healthy volunteers revealed that males had significantly higher exposure to d-methylphenidate than females (Patrick et al., 2007). Nonetheless, females experienced a more pronounced stimulant effect, despite their lower exposure. Shi et al. observed significantly higher CES1 activity in female human liver samples (n = 56) compared to male samples (n = 46). A follow-up in vitro study with dabigatran suggested CES1 activity was higher in females than males (Shi et al., 2016d). However, such difference was not observed in another in vitro study using human liver samples (n=32) and mouse liver samples (n=9) (Zhu et al., 2009a). Further study is needed to examine the impact of sex on the CES1 expression level and the PK and PD of CES1 substrates.
5.3 Drug-drug interactions

5.3.1 CES1 inhibitor - alcohol

To date, ethanol is the only known CES1 inhibitor that has been confirmed in multiple \textit{in vivo} and \textit{in vitro} studies. The impact of ethanol on the metabolism of the CES1 substrate, methylphenidate, was tested in healthy volunteers (n=14) (Zhu et al., 2017). D-methylphenidate comes as a single active ingredient (Focalin®) or in combination with l-methylphenidate (racemic mixture, Ritalin®). D-methylphenidate is approximately ten times more pharmacologically potent than l-methylphenidate, while l-methylphenidate is a more efficient CES1 substrate (Figure 2). This clinical study used a pulsatile dosing regimen with methylphenidate (dl-methylphenidate 40 mg or d-methylphenidate 20 mg) and ethanol (0.6 g/kg, four hours after methylphenidate dose) to eliminate any potential confounding effect of ethanol on methylphenidate absorption, as the methylphenidate drug products (i.e., Ritalin® and Focalin®) might undergo faster gastric dissolution in the stomach if administered with alcohol. When alcohol and d-methylphenidate (Focalin®) were co-administered, the \(C_{\text{max}}\) of d-methylphenidate was elevated by 27\% (\(P = 0.001\)) and the \(\text{AUC}_{4\to8}\) was elevated by 20\% (\(P < 0.01\)); when alcohol and dl-methylphenidate (Ritalin®) were co-administered, the \(C_{\text{max}}\) of d-methylphenidate was elevated by 35\% (\(P < 0.01\)) and the \(\text{AUC}_{4\to8}\) was elevated by 25\% (\(P < 0.05\)) (Zhu et al., 2017). These results are consistent with the previous clinical trial by Patrick et al. In that study, when alcohol and d-methylphenidate (Focalin®) were co-administered, the d-methylphenidate AUC was increased by 14\%; when alcohol and dl-methylphenidate (Ritalin®) were co-administered, the d-methylphenidate AUC was increased by 21\% (Patrick et al., 2013). Patrick and colleagues also showed that the co-administration of alcohol 30 min before
or 30 min after methylphenidate had a similar impact on methylphenidate exposure (Patrick et al., 2007). Both authors concluded that alcohol is a strong inhibitor of CES1, and the impact of CES1 inhibition is greater for dl-methylphenidate (Ritalin®) than for d-methylphenidate (Focalin®). Additionally, the DDI between methylphenidate and ethanol produced the transesterification metabolites d-ethylphenidate and l-ethylphenidate, and the plasma concentrations of l-ethylphenidate were much higher than d-ethylphenidate due to l-ethylphenidate being a more efficient CES1 substrate (Zhu et al., 2011; Zhu et al., 2017). Other in vivo studies with mice demonstrated similar results (Griffin et al., 2010; Bell et al., 2011b; Griffin et al., 2013).

The impact of alcohol on the CES1 function was also examined in the context of a different CES1 substrate, oseltamivir. A prospective health volunteer PK study (n=18) examined the interaction between oseltamivir 150mg (a recommended daily dose for the treatment of influenza) and alcohol. Alcohol increased the oseltamivir AUC_{0-6h} by 27% (P=0.011) and decreased the AUC_{0-6h} ratio of the active metabolite oseltamivir carboxylate to the parent compound oseltamivir by 34% (p<0.001) (Parker et al., 2015). However, co-administration of alcohol did not significantly affect the AUC_{0-24h} of oseltamivir carboxylate. These results are consistent with in silico analysis of the DDI between alcohol and oseltamivir (Hu et al., 2014).

5.3.2 Other CES1 inhibitors: cannabis, protease inhibitors, aripiprazole, isradipine, tacrolimus, valproate

Besides alcohol, many drug products on the market have been suggested to be potent inhibitors of CES1 mainly by in vitro investigations (Table 4). A further clinical
study with a validated CES1 substrate is needed to determine the clinical significance of these CES1 inhibitors.

An *in vitro* study with CES1 transfected cells suggested that cannabis (i.e., THC, CBD, and CBN) can act as a potential CES1 inhibitor. The inhibition constant (Ki) values for THC, CBD and CBN were 0.541, 0.974, and 0.263 µM (0.170, 0.306, and 0.0817 µg/ml), respectively (Qian et al., 2019). This result could be clinically impactful as the use of cannabis is expected to increase in the next few years (Hasin, 2018).

Several protease inhibitors (i.e. nelfinavir, amprenavir, atazanavir, ritonavir, and saquinavir) were identified as CES1 inhibitors by an *in silico* analysis and later confirmed by an *in vitro* incubation study. Among those, nelfinavir had a significantly higher inhibitory effect than the other agents. The relative CES1 activity towards PNPA (a CES1 substrate) was 5.2%, 74.2%, 51.7%, 76.9%, and 67.8% of the control after incubation with nelfinavir, ritonavir, amprenavir, saquinavir, and atazanavir, respectively (Rhoades et al., 2012).

An *in vitro* study suggested aripiprazole, perphenazine, thioridazine, and fluoxetine to be potent inhibitors of CES1, and a complementary animal study (n=10) with FVB mice demonstrated that co-administration of aripiprazole and methylphenidate (CES1 substrate) significantly increased the plasma concentrations of dl-methylphenidate (P < 0.01) (Zhu et al., 2010).

Moreover, a total of 27 cardiovascular, antiplatelet, anticoagulant and immunosuppressant drugs have been tested for CES1 inhibition using human liver microsomes and recombinant CES1. The results suggested isradipine (a dihydropyridine calcium antagonist, DHP) and tacrolimus (an immunosuppressive agent)
to be potent CES1 inhibitors. CES1 activity towards PNPA was decreased to 17.6% with isradipine, and 28.4% with tacrolimus (Thomsen et al., 2014).

An \textit{In vitro} study suggested valproate could inhibit CES1 function and affect rufinamide metabolism in both microsomes and cytosol. This result could be clinically significant as the two antiepileptic medications are often prescribed together when monotherapy is ineffective (Williams et al., 2011).

A combined ensemble docking and machine learning approach was utilized to identify potential CES1 inhibitors from 1114 FDA-approved drugs. Among the identified inhibitor candidates, four drugs including diltiazem, benztropine, iloprost, and treprostinil were found to inhibit CES1 activity \textit{in vitro} with IC$_{50}$ values ranging from 13.9 µM to 391.6 µM (Briand et al., 2019).

Lastly, an \textit{in vitro} study suggested that some naturally occurring oxysterols and fatty acids might significantly inhibit CES1 activity with IC$_{50}$ values within the micromolar range (Crow et al., 2010). These compounds could potentially affect CES1-mediated detoxification and drug metabolism \textit{in vivo}.

5.3.3 CES1 inducers

Overall, CES1 inducers are understudied relative to its inhibitors. Evidence suggests that various nuclear receptors might be involved in the regulation of CES1 expression (Staudinger et al., 2010). For example, several agonists of peroxisome proliferator-activated receptors (PPARs) induced the mRNA expressions of several CES1 isoforms in mouse livers (Jones et al., 2013). A moderate increase of CES1 expression was observed in human hepatocytes treated with rifampicin, a prototypical human PXR-activating agent (Shi et al., 2008). An \textit{in vivo} study with mice suggested
that glucose could induce hepatic CES1 expression by stimulating CES1 promoter activity and increasing acetylation of histone 3 and histone 4 in the CES1 chromatin, indicating a potential role of CES1 in glucose homeostasis (Xu et al., 2014). Moreover, phenobarbital induced CES1 expression in mouse livers, and the inducibility was more prominent in neonatal mice relative to adult mice (Xiao et al., 2012). Again, a further clinical investigation is needed to determine the impacts of CES1 inducers on the PK and PD of CES1 substrate medications.

5.3.4 Drug-drug interactions between CES1 substrates

In addition to CES1 inhibitors and inducers, concomitant use of multiple CES1 substrate drugs can theoretically impact the substrate metabolism by competitively inhibiting the CES1. This hypothesis has been tested in several studies. An in vitro study suggested trandolapril and enalapril might increase clopidogrel activation (Kristensen et al., 2014). Consistent with the in vitro study, a follow-up retrospective clinical study reported the concomitant use of ACEI prodrugs and clopidogrel increases the risk of clinically important bleeding in patients with myocardial infarction (n=70,934) (P=0.002). The clinical significance of this finding is, however, debatable as the hazard ratio of clinically significant bleeding for patients on concomitant therapy was 1.10 (95% CI 0.97-1.25) (Kristensen et al., 2014). Another clinical study with the similar design did not report a significant association between the composite cardiovascular outcome and the concomitant use of ACEI prodrugs and clopidogrel in patients with myocardial infarction (n=45,918). The adjusted odds ratio (aOR) for the perindopril was 0.94 (95% CI 0.76-1.16), and for ramipril was 0.97 (95% CI 0.80-1.18), relative to lisinopril, an ACEI not metabolized by CES1 (Cressman et al., 2015).
6. Disease states related to CES1

A prospective clinical study was conducted in monozygotic and dizygotic twin subjects (62–83 years) with (n=48) or without (n=247) type 2 diabetes mellitus (T2DM) to examine the association of CES1 with adiposity and metabolic function. CES1 mRNA expression level in adipose tissue was positively associated with body-mass index (P<0.001), fasting glucose level (P=0.002), insulin (P=0.006), and triglycerides (P=0.003) (Friedrichsen et al., 2013). Recent studies have also found that CES1 function was positively correlated with increased liver lipid storage and plasma lipid concentrations, indicating that CES1 might be heavily involved in lipid metabolism and is a potential drug target for the treatment of human metabolic disorders (Kaddurah-Daouk et al., 2018; Lian et al., 2018a; Lian et al., 2018b).

7. Conclusion and future directions

In sum, G143E (rs71647871) is the only clinically significant LOF CES1 variant identified to date, and alcohol is the only potent CES1 inhibitor that significantly affect CES1-mediated drug metabolism both in vivo and in vitro. However, G143E (MAF 2–4%, carrier frequency 4-8%) and alcohol-induced DDI can only be able to explain a small portion of the interindividual variability in the CES1 function. Previous in vitro studies have demonstrated marked variability of CES1 activity and expression in human liver samples not carrying G143E (Shi et al., 2016b; Wang et al., 2016a). In fact, analysis of the correlation between CES1 expression and activity revealed that the majority of interindividual variability in the CES1 function is due to variation in CES1 protein expression (Wang et al., 2016a).
Unfortunately, the mechanism by which CES1 protein expression is regulated remains largely unexplored. Notably, most of the existing gene expression regulation studies were based upon the measurement of mRNA expression levels. However, increasing evidence suggests that mRNA expression correlates poorly with protein expression for many genes including CES1 and most DMEs, which could result in false identification of gene expression regulators (Ohtsuki et al., 2012). Recent advances in LC-MS/MS-based proteomics have allowed for accurate CES1 protein quantification. The application of CES1 proteomics in a large set of clinical samples (e.g. human livers) is expected to uncover important factors influencing CES1 expression, such as genetic polymorphisms, disease conditions, inducers, and post-transcriptional modification (Wang et al., 2016b; He et al., 2019); the findings from such research will lead to the development of an individualized pharmacotherapy approach for improving the efficacy and safety of many medications metabolized by CES1.
Acknowledgements

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Lucy Her, Hao-Jie Zhu

Conflict of interest

The authors state no conflict of interests
Reference


LEGENDS FOR FIGURES

Figure 1. CES1 gene structure and haplotypes. CES1 gene consists of 14 exons located on chromosome 16q13-q22.1, and CES1P1 is a pseudogene, lying tail-to-tail with CES1. CES1, CES1P1 and their variants CES1 VAR and CES1P1 VAR form four major haplotypes. Red represents where stop codon is located at. Transcription efficiency of CES1P1 VAR is approximately 2% of CES1.

Figure 2. D-methylphenidate comes as a single active ingredient (Focalin®) or in combination with L-methylphenidate (racemic mixture) (Ritalin®). D-methylphenidate is approximately 10 times more pharmacologically potent than L-methylphenidate, while L-methylphenidate is a better CES1 substrate. Ethylphenidate can be formed via transesterification with ethanol.

Figure 3. Clopidogrel metabolic pathway. Clopidogrel is a non-ester-prodrug that needs to be activated by two oxidation reactions via CYPs. Clopidogrel and its intermediate and active metabolites are all metabolized (deactivated) by CES1.

Figure 4. ACE inhibitors (enalapril and Trandolapril) metabolism. Enalapril and trandolapril are ester-prodrugs that need to be activated by CES1.

Figure 5. Dabigatran metabolic pathway. Dabigatran is a prodrug that activated by both CES1 and CES2.
**Table 1. List of CES1 Substrates.** *Prodrugs that need CES1 activation*

<table>
<thead>
<tr>
<th>ACE Inhibitors</th>
<th>CNS Agents</th>
<th>Antihyperlipidemia Agents</th>
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<tbody>
<tr>
<td>Enalapril*</td>
<td>Methylphenidate</td>
<td>Clofibrate</td>
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<tr>
<td>Imidapril*</td>
<td>Cocaine</td>
<td>Fenofibrate</td>
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<tr>
<td>Benzapril*</td>
<td>Heroin</td>
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<tr>
<td>Quinapril*</td>
<td>Mepridine</td>
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<tr>
<td>Ramipril*</td>
<td>Flumazenil</td>
<td></td>
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<tr>
<td>Trandolapril*</td>
<td>Rufinamide</td>
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<tr>
<th>Antiviral Agents</th>
<th>Anticancer Agents</th>
<th>Chemical Warfare Agents</th>
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<tbody>
<tr>
<td>Oseltamivir*</td>
<td>Capecitabine*</td>
<td>Sarin</td>
</tr>
<tr>
<td>Sofosbuvir*</td>
<td>Irinotecan*</td>
<td>Soman</td>
</tr>
<tr>
<td>Tenofovir alafenamide*</td>
<td>Telotristat etiprate*</td>
<td>Tabun</td>
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<tr>
<th>Endogenous Compounds</th>
<th>Antiplatelets/Anticoagulants</th>
<th>Pesticides</th>
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<tr>
<td>Cholesterol</td>
<td>Clopidogrel</td>
<td>trans-permethrin</td>
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<tr>
<td>Fatty acid ethyl esters</td>
<td>Dabigatran*</td>
<td>Para-nitrophenyl valerate</td>
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<tr>
<th>ARNi</th>
<th>Immunosuppressive Agents</th>
<th>Others</th>
</tr>
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<tbody>
<tr>
<td>Sacubitril*</td>
<td>Mycophenolate mofetil*</td>
<td>Dimethyl fumarate*</td>
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<td></td>
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<td>Oxybutynin</td>
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Table 2. *In vitro* kinetics of wild type CES1 in human liver S9 fractions (HLS9).

<table>
<thead>
<tr>
<th>CES1 Substrates</th>
<th>HLS9</th>
<th></th>
<th></th>
<th>Reference</th>
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<tr>
<td></td>
<td>Vmax (pmol/min/mg protein)</td>
<td>Km (μM)</td>
<td>Catalytic Efficiency (Vmax/Km, μl/min/mg protein)</td>
<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>3558.6</td>
<td>62.7</td>
<td>56.8</td>
<td>(Zhu et al., 2013)</td>
</tr>
<tr>
<td>2-oxoclopidogrel</td>
<td>158.1</td>
<td>2.4</td>
<td>65.9</td>
<td></td>
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<tr>
<td>(clopidogrel intermediate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Enalapril</td>
<td>67.5</td>
<td>60.1</td>
<td>1.1</td>
<td>(Wang et al., 2016a)</td>
</tr>
<tr>
<td>Ramipril</td>
<td>18100</td>
<td>690.4</td>
<td>26.2</td>
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<tr>
<td>Perindopril</td>
<td>18100</td>
<td>1767</td>
<td>23.3</td>
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<td>Moexipril</td>
<td>4400</td>
<td>1457</td>
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<td>Fosinopril</td>
<td>1400</td>
<td>471.3</td>
<td>3.0</td>
<td></td>
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<tr>
<td>l-methylphenidate</td>
<td>1701</td>
<td>775.7</td>
<td>2.2</td>
<td>(Zhu et al., 2008)</td>
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<tr>
<td>d-methylphenidate</td>
<td>177.2</td>
<td>663.5</td>
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<td>Oseltamivir</td>
<td>145000</td>
<td>1380</td>
<td>105.1</td>
<td>(Zhu and Markowitz, 2009)</td>
</tr>
<tr>
<td>Trandolapril</td>
<td>103600</td>
<td>639.9</td>
<td>161.9</td>
<td>(Zhu et al., 2009b)</td>
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<tr>
<td>Dabigatran</td>
<td>1174</td>
<td>33.5</td>
<td>35.0</td>
<td>(Laizure et al., 2014)</td>
</tr>
</tbody>
</table>
Table 3. CES1 SNPs and their impacts on the PK and PD of CES1 substrate medications

<table>
<thead>
<tr>
<th>AA/Nucleotide Change (dbSNP ID)</th>
<th>Citation</th>
<th>Treatment</th>
<th>Population</th>
<th>Design/Outcome</th>
<th>Result</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>G143E (rs71647871)</td>
<td>Patrick et al., 2007; Zhu et al., 2008b</td>
<td>Methylphenidate SINGLE dose of 0.3mg/kg</td>
<td>n=20 (with 1 carrier)</td>
<td>Prospective study</td>
<td>The study unexpectedly found one volunteer with elevated PK parameters of methylphenidate and alcohol</td>
<td>The later analysis found this volunteer had G143E and D260fs SNPs, which resulted in elevated plasma concentration of methylphenidate</td>
</tr>
<tr>
<td></td>
<td>Nemoda et al., 2009</td>
<td>Methylphenidate Dose adjusted based on symptom reduction × 1 month</td>
<td>n=122 (with 7 carriers)</td>
<td>Retrospective study</td>
<td>G143E carriers needed lower doses of methylphenidate for symptom reduction compared to non-carriers (0.410 vs 0.572 mg/kg, ( p=0.022 ))</td>
<td>G143E impaired methylphenidate metabolism in vivo</td>
</tr>
<tr>
<td></td>
<td>Stage et al., 2017a</td>
<td>Methylphenidate Single dose 10mg</td>
<td>n=22 (with 6 carriers)</td>
<td>Open labeled, prospective, PK study</td>
<td>G143E carriers showed 152.4% higher active metabolite concentration (53.3ng×ml(^{-1})×h(^{-1})) compared to the non-carrier group (21.4 ng×ml(^{-1})×h(^{-1})) (( P&lt;0.0001 ))</td>
<td>G143E carriers had higher exposure to methylphenidate compared to non-carriers</td>
</tr>
<tr>
<td></td>
<td>Lewis et al., 2013</td>
<td>Clopidogrel (1) PAPI patients received 300mg LD with 75mg MD (2) Patients from Sinai Hospital received either 300 or 600 mg LD (n= 204) with MD, or just</td>
<td>n=506 (with 7 carriers)</td>
<td>Retrospective subanalysis of two clinical studies: (1) Pharmacogenomics of Antiplatelet Intervention (PAPI) Study (2)Clopidogrel-treated patients at Sinai Hospital</td>
<td>(1) A 50% higher active metabolite concentration was observed in G143E carriers (n=7, 30.3 ng/ml) compared to non-carriers (n=499, 19.0 ng/ml) (( P=0.001 ))</td>
<td>G143E carriers had higher plasma concentrations of clopidogrel active metabolites, and consequently had a higher antiplatelet effect</td>
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<tr>
<td>Study</td>
<td>Drug</td>
<td>Dose/Details</td>
<td>n</td>
<td>Participants</td>
<td>Study Design</td>
<td>PD Outcome</td>
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<tr>
<td>Tarkiainen et al., 2015a</td>
<td>Clopidogrel</td>
<td>Single-dose 600 mg</td>
<td>22 (with 10 carriers)</td>
<td>Healthy volunteers</td>
<td>Prospective, PK/PD study</td>
<td>Inhibition of P2Y12-mediated platelet aggregation</td>
</tr>
<tr>
<td>Tarkiainen et al., 2015b</td>
<td>Enalapril, Quinapril</td>
<td>Single Dose 10 mg Enalapril or Quinapril</td>
<td>22 (with 10 carriers)</td>
<td>Healthy volunteers</td>
<td>Prospective PK study</td>
<td>(1) AUC$_0$–$\infty$ of the enalapril active metabolite enalaprilat was 20% lower in the G143E carriers (n=10) compared to non-carriers (n=12) ($P=0.049$) (2) AUCs$_0$–$\infty$ of the quinapril and its active metabolite (i.e., quinaprilat) were not significantly different between the non-carriers and carriers ($P=0.114$)</td>
</tr>
<tr>
<td>Tarkiainen et al., 2012</td>
<td>Oseltamivir</td>
<td>Single Dose 75mg</td>
<td>22 (with 9 G143E heterozygotes, 1 G143E homozygote)</td>
<td>Healthy volunteers</td>
<td>Prospective PK Study</td>
<td>(1) The AUC$_0$–$\infty$ ratio of oseltamivir carboxylate (active metabolite) to oseltamivir (parent molecule) was 23% lower in G143E heterozygotes compared to non-carriers ($P = 0.006$) (2) The one G143E homozygous individual had an AUC$_0$–$\infty$ of oseltamivir that was</td>
</tr>
<tr>
<td>SNP</td>
<td>Study Details</td>
<td>Treatment/Study Design</td>
<td>Results</td>
<td>Comments</td>
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<td>E220G (rs200707504)</td>
<td>Shi et al., 2016c</td>
<td>Dabigatran n=102 human liver samples</td>
<td>The activation rates of DABE, M1 and M2 in G143E carriers were 53% (P = 0.018), 43% (P = 0.004), and 37% (P = 0.001) of normal carriers (after normalized by CES1 expression). G143E carriers had a lower dabigatran activation rate, potentially resulting in a lower dabigatran active metabolite concentration in the carriers.</td>
<td>G143E carriers had a lower dabigatran activation rate, potentially resulting in a lower dabigatran active metabolite concentration in the carriers.</td>
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<tr>
<td>G143E</td>
<td>Shi et al., 2016d</td>
<td>Sacubitril n= 53 (with 5 carrier) human liver samples</td>
<td>The activation rates of sacubitril were lower in the carriers compared to the non-carriers (4.2 vs. 7.2 nmol/mg protein/min, P = 0.025). G143E carriers had a lower sacubitril activation rate, potentially resulting in a lower sacubitril active metabolite plasma concentration in the carriers.</td>
<td>G143E carriers had a lower sacubitril activation rate, potentially resulting in a lower sacubitril active metabolite plasma concentration in the carriers.</td>
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<tr>
<td>E220G (rs200707504)</td>
<td>Oh et al., 2017</td>
<td>Oseltamivir 75mg single dose n=20 (with 8 carriers)</td>
<td>AUC_{0-48h} of oseltamivir was increased by 10% (P = 0.334) and AUC_{0-48h} of oseltamivir carboxylate was decreased by 5% (P = 0.513) in carriers. E220G appears to have no significant impact on oseltamivir activation in humans.</td>
<td>E220G appears to have no significant impact on oseltamivir activation in humans.</td>
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<tr>
<td>S75N (rs2307240)</td>
<td>Xiao et al., 2017</td>
<td>Clopidogrel 75mg x 1 year n=851 (with 372 carriers)</td>
<td>CES1 S75N carriers (n=372) had more cerebrovascular events (P&lt;0.001), acute myocardial infarction (P&lt;0.001), and unstable angina (P&lt;0.001) compared to non-carriers. S75N appears to increase the function of CES1, resulting in the decreased efficacy of clopidogrel.</td>
<td>S75N appears to increase the function of CES1, resulting in the decreased efficacy of clopidogrel.</td>
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<td>S75N (rs2307240)</td>
<td>Johnson et al., 2013</td>
<td>Methylphenidate Weight based dosing x 6 weeks n=44 (with 2 carriers)</td>
<td>No significant differences in methylphenidate side effect were found between carriers and non-carriers (p = 1). S75N does not appear to affect the function of CES1.</td>
<td>S75N does not appear to affect the function of CES1.</td>
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<tr>
<td>Study</td>
<td>Treatment</td>
<td>No. of Participants</td>
<td>Study Design</td>
<td>Findings</td>
<td>Summary</td>
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<td>Wang et al., 2017</td>
<td>Enalapril</td>
<td>n= 36 (with 3 carriers)</td>
<td><em>In vitro</em> study with human liver samples</td>
<td>No statistical difference in enalapril activation rate or CES1 protein expression level between carriers and no carriers.</td>
<td>S75N does not appear to affect the function of CES1.</td>
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<tr>
<td>Geshi et al., 2005</td>
<td>Imidapril</td>
<td>n=105 (with 47 carriers)</td>
<td><strong>Prospective clinical study</strong></td>
<td>Greater systolic blood pressure reduction (24.1 mmHg) was observed compared to non-carriers (17.6 mmHg) after 8 weeks of imidapril therapy (P = 0.0184).</td>
<td>-816A&gt;C appears to up-regulate the CES1P1 VAR expression.</td>
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<td>Xie et al., 2014</td>
<td>Clopidogrel</td>
<td>n=162 (with 75 carriers)</td>
<td><strong>Retrospective PD analysis</strong></td>
<td>The carriers had decreased Vasodilator Stimulated Phosphoprotein-Platelet Reactivity Index (VASP-PRI) (45.93 vs 53.18%) (P=0.014).</td>
<td>-816A&gt;C appears to up-regulate the CES1P1 VAR expression.</td>
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<td>Zou et al., 2014</td>
<td>Clopidogrel</td>
<td>n=249 (with 108 heterozygous carrier, 17 homozygous carrier)</td>
<td><strong>Retrospective PD analysis</strong></td>
<td>A lower ADP-induced maximum platelet aggregation (21.5%, n=125) was observed compared to non-carriers (31.7%, n=124) (P=0.001).</td>
<td>-816A&gt;C appears to down-regulate the CES1P1 VAR expression.</td>
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<td>Zhu et al., 2016</td>
<td>Trandolapril</td>
<td>(1) n=486 (with 109 heterozygous carriers, 10 homozygous carriers) (2) n=100 (<em>in vitro</em> study) (26)</td>
<td><strong>Retrospective analysis of the INternational VErapamil SR Trandolapril Study</strong></td>
<td>(1) No association between the -816A&gt;C and the blood pressure lowering effect of trandolapril (2) Not associated with CES1 protein expression and trandolapril activation in human liver samples</td>
<td>-816A&gt;C does not appear to be associated with overall CES1 function.</td>
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<tr>
<td>SNPs</td>
<td>Study Details</td>
<td>Outcome Measures</td>
<td>Observations</td>
<td>Conclusion</td>
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<td>-75G&gt;T (rs3815583)</td>
<td>Bruxel et al., 2013</td>
<td>Blood Pressure</td>
<td>The carriers had worse appetite reduction compared to non-carriers (41% vs 77%, P=0.01)</td>
<td>-75G&gt;T appears to be associated with decreased CES1 function</td>
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<td>Methylphenidate</td>
<td>Dose titrated up x 3 months as tolerable</td>
<td>Retrospective PD analysis</td>
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<td>n=205 (with 129 carriers)</td>
<td>Outcome: appetite reduction - the side effect of methylphenidate</td>
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<td>-75G&gt;T (rs3815583)</td>
<td>Sai et al., 2010</td>
<td>Appetite reduction</td>
<td>The carriers had higher plasma (SN-38 + SN-38G)/irinotecan AUC ratios relative to non-carriers (P=0.027)</td>
<td>-75G&gt;T appears to be associated with higher CES1 function</td>
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<td>Irinotecan 100 mg m(^{-2}) weekly or 150 mg m(^{-2}) biweekly</td>
<td>n=177 177 Japanese cancer patients</td>
<td>Retrospective PK analysis</td>
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<tr>
<td>-75G&gt;T (rs3815583)</td>
<td>Nelveg-Kristensen et al., 2016</td>
<td>Appetite reduction</td>
<td>The -75G&gt;T genotypes did not significantly impact the plasma ATII/ATI ratios in the study subjects or fatal outcomes (i.e. cardiovascular death and all-cause death)</td>
<td>-75G&gt;T was not associated with CES1 function</td>
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<td>ACEI</td>
<td>n=200 Congestive heart failure patients</td>
<td>Retrospective PD analysis</td>
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<td>1168-33C&gt;A (rs2244613)</td>
<td>Pare et al., 2013</td>
<td>Trough concentrations of dabigatran, bleeding risk</td>
<td>The carriers had lower trough concentrations of the active metabolite (15% decrease per allele; P=1.2×10(^{-8})) and a lower risk of any bleeding (odds ratio, 0.67; P=7×10(^{-5})) compared to non-carriers</td>
<td>1168-33C&gt;A appears to be associated with lower CES1 function</td>
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<td>Dabigatran 110 or 150 mg twice daily</td>
<td>n=2944 (with 587 carriers) Patients with atrial fibrillation (within 6 months) and additional risk factors for stroke</td>
<td>Retrospective GWAS of Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY) clinical trial</td>
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<td>Outcome: Trough concentrations of dabigatran, bleeding risk</td>
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<td>1168-33C&gt;A (rs2244613)</td>
<td>Shi et al., 2016a</td>
<td>In vitro study with human liver samples</td>
<td>No association between 1168-33C&gt;A and dabigatran activation</td>
<td>1168-33C&gt;A appears to be not associated with CES1 function</td>
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<td>Dabigatran</td>
<td>n=102 (with 29 heterozygous carriers and 5 homozygous carriers)</td>
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</table>
Table 4. Drug-Drug Interaction Summary

<table>
<thead>
<tr>
<th>CES1 inhibitors</th>
<th>CES1 Substrates</th>
<th>Interaction Summary</th>
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<tbody>
<tr>
<td><strong>Alcohol</strong></td>
<td>Methylphenidate</td>
<td>Many <em>in vitro</em> and <em>in vivo</em> studies confirmed alcohol inhibits CES1 and mediates biotransformation of methylphenidate to ethylphenidate; methylphenidate plasma concentrations were increased when patients took methylphenidate with alcohol (Griffin et al., 2010; Bell et al., 2011a; Bell et al., 2011b; Zhu et al., 2011; Griffin et al., 2013; Patrick et al., 2013; Parker et al., 2015; Zhu et al., 2017).</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td>Oseltamivir</td>
<td>When alcohol was administered with oseltamivir in humans, the AUC of oseltamivir increased by 37% (Hu et al., 2014).</td>
</tr>
<tr>
<td><strong>Cannabis</strong></td>
<td>Oseltamivir</td>
<td><em>In vitro</em> study with CES1-transfected cells suggested THC, CBD and CBN to be the potent CES1 inhibitors. The inhibition constant (Ki) values for THC, CBD and CBN were 0.541, 0.974, and 0.263 µM (0.170, 0.306, and 0.0817 µg/ml) (Qian et al., 2019).</td>
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<tr>
<td><strong>Protease Inhibitors</strong></td>
<td>Methylphenidate, <em>p</em>-nitrophenyl acetate (PNPA) and <em>p</em>-nitrophenol (PNP)</td>
<td><em>In vitro</em> study showed that protease inhibitors (i.e., nelfinavir, amprenavir, atazanavir, ritonavir, and saquinavir) inhibited the catalytic activity of CES1 (<em>p</em> &lt;0.01). Among protease inhibitors, nelfinavir had a significantly higher inhibitory effect compared to other agents (Rhoades et al., 2012).</td>
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<tr>
<td><strong>Aripiprazole</strong></td>
<td>Methylphenidate, <em>p</em>-nitrophenyl acetate (PNPA)</td>
<td><em>In vitro</em> study suggested aripiprazole, perphenazine, thioridazine, and fluoxetine to be potent inhibitors of CES1. Among the medications tested, aripiprazole was the most potent inhibitor of CES1, and an <em>in vivo</em> study with FVB mouse confirmed this result (Zhu et al., 2010).</td>
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<td><strong>Isradipine /Tacrolimus</strong></td>
<td>PNPA, Trandolapril</td>
<td><em>In vitro</em> study with human liver microsomes suggested isradipine (dihydropyridine calcium antagonist, DHP) and tacrolimus</td>
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<td>Immunosuppressive agent</td>
<td>CES1 inhibitors (Thomsen et al., 2014).</td>
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<td>Valproate</td>
<td><strong>In vitro</strong> study suggested valproate could inhibit CES1 function and affect rufinamide metabolism (Williams et al., 2011).</td>
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<td>ACEI</td>
<td>Clopidogrel</td>
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<td>ACEIs and clopidogrel are often administered together as both of them are cardiovascular medications; both ACEIs and clopidogrel are suggested to be inhibitors of CES1. A clinical study with myocardial infarction patients (n=70,934) demonstrated concomitant use of ACEIs increased the rate of clinically significant bleeding compared to the clopidogrel monotherapy (P=0.002) (Kristensen et al., 2014). Another clinical study with myocardial infarction patients (n=45,918) with clopidogrel showed that concomitant use of clopidogrel and ACEI (perindopril and ramipril) was not associated with the re-infarction, heart failure or death (Cressman et al., 2015).</td>
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Figure 2

DL-Ethylphenidate

CES1 mediated-Trans-esterification (Minor)
CH₃CH₂OH (Alcohol)

D-Methylphenidate (Focalin®)

L-Methylphenidate (Better CES1 substrate)

Alcohol

CES1 (Major)

DL-Ritalinic Acid (inactive metabolite)
Figure 3

Clopidogrel (Prodrug) → Intermediate Metabolite → Active Metabolite → Inactive Metabolite (1) → Inactive Metabolite (2) → Inactive Metabolite (3)

CYP5 → CES1 → CES1 → CES1
Figure 4

Enalapril \[\overset{\text{CES1}}\rightarrow\] Enalaprilat

Trandolapril \[\overset{\text{CES1}}\rightarrow\] Trandolaprilat
Figure 5

Dabigatran Etxilate

CES2

M2 Metabolite

CES1

Dabigatran