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Association of Oseltamivir Activation with Gender and Carboxylesterase 1 Genetic Polymorphisms

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Abstract: Oseltamivir, an inactive anti-influenza virus prodrug, is activated (hydrolyzed) *in vivo* by carboxylesterase 1 (CES1) to its active metabolite oseltamivir carboxylate. CES1 functions are significantly associated with certain *CES1* genetic variants and some non-genetic factors. The purpose of this study was to investigate the effect of gender and several *CES1* genetic polymorphisms on oseltamivir activation using a large set of individual human liver samples. CES1-mediated oseltamivir hydrolysis and CES1 genotypes, including the G143E (rs71647871), rs2244613, rs8192935, the -816A>C (rs3785161) and the CES1P1/CES1P1VAR, were determined in 104 individual human livers. The results showed that hepatic CES1 protein expression in females was 17.3% higher than that in males ($P = 0.039$) while oseltamivir activation rate in the livers from female donors was 27.8% higher than that from males ($P = 0.076$). As for *CES1* genetic polymorphisms, neither CES1 protein expression nor CES1 activity on oseltamivir activation was significantly associated with the rs2244613, rs8192935, -816A>C or CES1P1/CES1P1VAR genotypes. However, oseltamivir hydrolysis in the livers with the genotype 143G/E was approximately 40% of that with the 143G/G genotype (0.7 ± 0.2 *versus* 1.8 ± 1.1 nmole/mg protein/min, $P = 0.005$). In summary, the results suggest that hepatic oseltamivir activation appears to be more efficient in females than that in males, and the activation can be impaired by functional *CES1* variants, such as the G143E. However, clinical implication of CES1 gender differences and pharmacogenetics in oseltamivir pharmacotherapy warrants further investigations.

Oseltamivir (Tamiflu®), an important antiviral medication on the World Health Organization's Essential Drugs List, is widely used for the prevention and treatment of influenza A and influenza B infection [1, 2]. However, significant inter-individual variability in oseltamivir pharmacokinetics and antiviral effects has been demonstrated in several clinical studies [2-5]. Oseltamivir was designed as an ethyl ester prodrug to improve its bioavailability, and it has to be converted to its active metabolite, oseltamivir

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carboxylate, to exert its intended antiviral effect *in vivo*. Oseltamivir activation is primarily catalyzed by carboxylesterase 1 (CES1) in human liver (supplemental fig. 1) [6, 7]. CES1, the predominant hepatic hydrolase encoded by the *CES1* gene in human beings, is responsible for the metabolism of many ester- and amide-containing medications, such as oseltamivir, clopidogrel, dabigatran, methylphenidate and many angiotensin-converting enzyme inhibitors [8]. Previous studies have provided evidence to suggest that genetic polymorphisms of *CES1* could significantly affect the pharmacokinetics of, and/or response to CES1 substrate drugs [9-15].

The first clinically significant *CES1* non-synonymous single nucleotide polymorphism (SNP) G143E (rs71647871) was discovered in a clinical pharmacokinetic study of methylphenidate [15]. This variant was found to be significantly associated with altered pharmacokinetics and/or pharmacodynamics of several drugs metabolized by CES1 [10, 12, 13, 15]. We have reported that the activation of oseltamivir was markedly impaired by the G143E variant using the Flp-In™ 293 cells stably transfected with the wild type (WT) CES1 and the G143E variant [16]. Consistent with our *in vitro* findings, a healthy volunteer pharmacokinetic study showed that the area under the plasma concentration-time curve (AUC) of oseltamivir was 18% greater in the G143E carriers relative to the non-carriers [13]. However, whether the G143E variant may affect oseltamivir activation in the livers and to what extent is unclear.

A recent genome-wide association study (GWAS) identified two *CES1* intronic variants, rs2244613 and rs8192935, associated with lower plasma concentrations of dabigatran, the active metabolite converted from the prodrug dabigatran etexilate by CES1 and CES2 [17, 18], in the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) study participants [19]. The rs2244613 was also associated with lower risk of any bleeding. However, whether the two variants are associated with altered CES1 expression/activity remains unknown.

The *CES1P1* gene is a non-functional pseudogene located in proximity of the *CES1* gene. DNA sequences of *CES1P1* are highly similar to those of *CES1*; however, *CES1P1* does not encode any functional protein due to a premature stop codon in the

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exon 3 [20]. It is interesting that a common *CES1P1* variation termed as *CES1P1VAR* is functional, with the protein product identical to that expressed from the *CES1* gene. Thus, it has been speculated that the CES1P1/CES1P1VAR genotypes may affect total CES1 expression levels and contribute to inter-individual variability in CES1-mediated drug metabolism.

The SNP -816 A>C (rs3785161) is a common variant located in the promoter region of the *CES1P1* gene, and it was reported to be associated with therapeutic responses to the CES1 substrate drugs imidapril and clopidogrel, although the results from the two clopidogrel studies were contradictory [14, 21, 22].

Besides genetic variations, non-genetic factors, such as gender and age can also affect the expression and/or activity of many drug-metabolizing enzymes in human beings [23, 24]. It has been reported that CES1 expression and activity were very low in neonates and gradually increased with age through childhood [25-28]. As for gender effect, it has been speculated that sex-related differences in CES1 activity exist based on the results from several clinical pharmacokinetic studies of CES1 substrate drugs [29-32].

In the present study, a total of 104 individual normal human liver samples were utilized to investigate the effect of gender and the selected *CES1* genetic variants on CES1 expression and oseltamivir activation. Due to that only two out of the 104 individuals were under the age of 20 years, the impact of developmental status on hepatic CES1 function was not studied in the present investigation.

Materials and Methods

Materials

Oseltamivir was obtained from Toronto Research Chemicals (Toronto, Canada). Oseltamivir carboxylate was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Ritalinic acid, LC–MS grade methanol, acetonitrile and formic acid were all purchased from Sigma–Aldrich (St. Louis, MO, USA). The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) solution (1M) was purchased from Life Technologies (Carlsbad, CA). Taq DNA polymerase with standard Taq buffer and

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deoxynucleotide (dNTP) solution mix were obtained from New England Biolabs (Ipswich, MA, USA). All other chemicals and reagents were of the analytical grade, and they were commercially available.

A total of 104 individual normal human liver samples were obtained from the Cooperative Human Tissue Network (Columbus, OH) and the XenoTech LLC (Kansas City, KS, USA). The demographic information were unknown for two samples, and the rest samples consisted of 46 males, 56 females with ages ranging from 1 to 83 years (56.5 ± 16.6 years). The donors included 94 Caucasians, 5 African-Americans, 1 Hispanics and 2 classified as 'others'.

Genotyping

Genomic DNA was extracted from human liver samples using the PureLink® Genomic DNA Mini Kit (Life Technologies, CA, USA) in accordance with the manufacturer's instructions. To determine the G143E, rs2244613, rs8192935, and -816A>C genotypes, PCR was performed to amplify the DNA fragments containing the variants. The PCR conditions and primers' sequences are listed in the supplemental table 1. The PCR products were purified using the PureLink® PCR Purification Kit (Life Technologies, CA, USA) and analysed with agarose (2%) gel electrophoresis before being subjected to Sanger sequencing. The CES1P1/CES1P1VAR genotypes were determined using the restriction fragment length polymorphism (RFLP) method described in our previous publication [33].

Oseltamivir hydrolysis in human liver S9 fractions

An *in vitro* incubation study was conducted to determine oseltamivir hydrolysis in 104 normal human liver S9 fraction (HLS9) samples. HLS9 was prepared according to the method detailed in a previous publication [34]. The incubation was carried out in 1.5 ml Eppendorf tubes at a total volume of 80 µl. Oseltamivir and individual HLS9 were prepared in PBS buffer containing 20 mM HEPES. The hydrolytic reaction was initiated by mixing 40 µl of oseltamivir solution with an equal volume of individual HLS9. The final concentration of oseltamivir was 100 µM. Our preliminary study indicated that the formation of oseltamivir carboxylate was linear with a series of HLS9 protein

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concentrations (0.05– 0.2 mg/ml) and incubation times (5–15 min.). In the present study, hydrolytic reactions were performed with the final HLS9 protein concentration of 0.1 mg/ml and an incubation period of 10 min. at 37℃. After incubation, the reactions were terminated by adding 320 µl of methanol containing the internal standard ritalinic acid (5 µM). The mixture was vortexed for 3 min. and centrifuged at 17,000 x *g* for 10 min. to precipitate proteins. The LC-MS/MS assay described below was used to analyse the supernatants for the concentrations of the active metabolite oseltamivir carboxylate.

LC-MS/MS method for oseltamivir carboxylate quantification

The LC–MS/MS analysis was performed on a Shimadzu HPLC system (Shimadzu, Tokyo, Japan) coupled with an Applied Biosystems API 4000 triple quadruple mass spectrometer (Foster City, CA, USA). The analytes were separated on a Shimadzu VP-ODS column (5um, 150×2.0mm, Shimadzu, Japan). Oseltamivir carboxylate quantification was performed based on a previously described assay with some modifications [35]. The mobile phase consisted of water containing 0.05% formic acid (v/v) (phase A) and methanol containing 0.05% formic acid (v/v) (phase B), and it was delivered at a flow rate of 0.2 ml/min. A gradient elution was applied to the separation, with the time program set as follows: phase B increased from 10% to 80% during the period of 0 to 8 min, then returned to 10% at 9 min., and maintained the same level until the end of elution at 12.5 min. MS was operated in positive ion mode using turbo electrospray ionization. The following parameters were adopted for the MS analysis: curtain gas= 20 psi; ion source gas 1= 40 psi; ion source gas 2= 70 psi; ion spray voltage= 5500 V; source temperature= 500 ℃; entrance potential= 10 V; dwell time= 50 ms; collision cell exit potential= 15 V for ritalinic acid and 11 V for oseltamivir carboxylate; declustering potential= 40 V for oseltamivir carboxylate and 50 V for ritalinic acid; and collision energy= 25 eV for oseltamivir carboxylate and 35 eV for ritalinic acid. The following transitions were monitored in a Multiple Reaction Monitoring (MRM) mode: oseltamivir carboxylate, m/z 285.3 > 138.0 and ritalinic acid, m/z 220.5 > 84.6. Quantifications were performed based on the peak area ratios of oseltamivir carboxylate to ritalinic acid (internal standard). The regression coefficients of calibration curves were found to be greater than 0.99. Three quality controls of oseltamivir

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carboxylate (5, 25, and 250 nM) representing low, medium and high concentrations were included in every batch of analysis. Inter-day and intra-day relative standard deviations were found to be less than 10%.

Absolute quantification of CES1 protein expression in the liver

Absolute CES1 protein expression levels in individual human liver samples were quantified using the stable isotope labeling by amino acids in cell culture (SILAC)-based LC-MS/MS assay recently established in our laboratory [36].

Data analysis

The data are presented as mean ± standard deviation (SD). Mann–Whitney test was utilized to analyse the differences of CES1 expression and activity between genders and among different genotype groups (GraphPad Prism software version 6.0; GraphPad Software, San Diego, CA, USA). A *P*-value less than 0.05 was considered statistically significant.

Results

Effect of gender on CES1 expression and oseltamivir activation in human livers

As shown in fig. 1, oseltamivir activation and CES1 expression varied markedly in the human liver samples. CES1 protein expression in female livers was 17.3% higher than that in male's (10.7 ± 4.4 *versus* 9.1 ± 4.1 ng/µg protein, $P = 0.039$). Consistent with the CES1 expression, oseltamivir activation rate was 27.8% higher in females than males (2.0 ± 1.2 *versus* 1.5 ± 0.9 nmole/min/mg protein), though the difference did not reach a statistically significant level ($P = 0.076$).

The -816A>C, rs2244613, rs8192935 and CES1P1/CES1P1VAR genotypes had no effect on oseltamivir activation and CES1 protein expression in human livers

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Several selected genetic polymorphisms of CES1 were genotyped in the 104 human liver samples. The -816A>C, rs2244613, rs8192935 and CES1P1/CES1P1VAR genotypes had no significant effect on the hydrolysis rates of oseltamivir in the liver samples. In addition, CES1 expressions did not differ significantly between these genotypes (fig. 2).

Oseltamivir activation was impaired in human livers carrying the 143E allele

Five G143E heterozygotes were identified from the 104 human liver samples, giving a minor allele frequency (MAF) of 2.4%, which is consistent with the previous report [15]. As shown in fig. 2, CES1 activity on oseltamivir activation in the G143E carriers was approximately 40% of the non-carriers (0.7 ± 0.2 *versus* 1.8 ± 1.1 nmole/mg protein/min, $P = 0.005$) while CES1 expression levels were comparable between the two genotypes.

Correlation of CES1 expression and activity on oseltamivir activation

CES1 activity on oseltamivir activation correlated significantly with CES1 expression in the individual human livers (fig. 3). The *R*-squared value was 0.34 for the 104 samples overall, indicating that the variation of CES1 protein expression can explain approximately one-third of variability in oseltamivir activation (fig. 3A). The *R*-squared value increased to 0.38 when the G143E carriers were removed from the dataset (fig. 3B). Thus, the G143E variant appears to account for about 4% of the variability in oseltamivir activation in the present study.

Discussion

Significant inter-individual variability in oseltamivir pharmacokinetics and in antiviral effects has been consistently reported in clinical studies [2-5]. Varied CES1 function can be an important contributor to the variability in response to oseltamivir therapy, since CES1 is the only enzyme responsible for the activation of this prodrug. The studies from our as well as other laboratories have demonstrated that CES1 expression and activity vary significantly in human livers, and genetic polymorphisms and non-genetic factors

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play an important role in regulating CES1 function [6, 25, 27, 29-32, 37-40]. In the present study, an *in vitro* human liver study was carried out to investigate the effect of gender and several genetic variants, including the G143E, rs2244613, rs8192935, -816A>C and CES1P1/CES1P1VAR, on oseltamivir activation.

Sex-related differences in the pharmacokinetics of several CES1 substrates, including lovastatin, simvastatin, methylphenidate and oseltamivir, have been observed in clinical studies [30-32, 41]. For example, Patrick *et al.* reported that the AUC of d-methylphenidate was significantly higher in males than female after the administration of a single dose of dl-methylphenidate (0.3 mg/kg) [32]. Consistent with this finding, an oseltamivir pharmacokinetic study conducted by Wattanagoon and colleagues showed a 28% increase of oseltamivir AUC in males than females after a male outlier was excluded from data analysis [30]. The data indicate that women may exhibit greater CES1 activity than men. In the present study, we demonstrated that the CES1 protein expression in female livers was significantly higher than males (10.7 ± 4.4 *versus* 9.1 ± 4.1 ng/µg protein, $P = 0.039$). Consistent with the CES1 expression, oseltamivir activation rate in female livers was 27.8% higher than in male livers (2.0 ± 1.2 *versus* 1.5 ± 0.9 nmole/min/mg protein), though the difference did not reach a statistical significance level ($P = 0.076$). It is noted that a previous study also observed an increase of CES1 activity towards oseltamivir activation in female liver samples compared to male liver samples in both paediatric and adult individuals, though the differences were not statistically significant [28].

The *CES1* variant G143E (rs71647871) is a non-synonymous mutation resulting from a substitution of G to A in exon 4, which leads to the change of glycine to glutamic acid at the coding position of 143. This variant was originally discovered in an individual who participated in a pharmacokinetic study of methylphenidate [15]. The MAFs of G143E were 3.7%, 4.3%, 2.0% and 0% in White, Black, Hispanic and Asian populations, respectively [15]. *In vitro* functional studies based on *CES1* transfected cell lines have shown that catalytic activity of the G143E was substantially impaired in the metabolism of CES1 substrate drugs, including oseltamivir, methylphenidate, clopidogrel, sacubitril and many angiotensin-converting enzyme (ACE) inhibitors [15, 16, 34, 42, 43].

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Furthermore, clinical significance of this variant has been demonstrated in several clinical studies. For instance, G143E carriers required significantly lower doses of methylphenidate for ADHD symptom reduction [44] and had significantly increased plasma levels of clopidogrel active metabolite and greater clopidogrel responses compared to non-carriers [11, 12].

The catalytic efficiency (V_{max}/K_m) of the G143E variant expressed in stably transfected HEK293 cells on oseltamivir activation was less than 20% of the WT CES1 in an *in vitro* study published previously by our group [16]. Consistent with the *in vitro* findings, a clinical study showed that G143E heterozygotes exhibited 18% greater AUC of oseltamivir and 23% smaller AUC ratios of oseltamivir carboxylate to oseltamivir compared to individuals with the 143GG genotype [13]. In the present study, we genotyped 104 normal individual human livers and identified five G143E heterozygotes. The average CES1 activity on oseltamivir activity in the heterozygotes was approximately 40% of that in non-carriers. This study provided a definitive evidence that the G143E is a loss-of-function variant for the activation of oseltamivir in the liver. It is noted that marked inter-individual variability in oseltamivir activation present in the human livers without the G143E variant and the correlations between CES1 expression and activity suggest that the G143E can only explain approximately 4% variability in the activation of oseltamivir. Thus, in addition to the G143E, many other factors, such as other unknown genetic variants and non-genetic factors, may have contributed to the variation.

The CES1 variants rs2244613 and rs8192935 were recently identified to be associated with lower dabigatran plasma concentrations in the RE-LY GWAS study participants [19]. The SNP rs2244613 was also associated with lower risk of any bleeding. Both SNPs are located in the intronic region of the *CES1* gene. Given that CES1 and CES2 are the enzymes responsible for the activation of the prodrug dabigatran etexilate [17, 18], the GWAS data indicate that the individuals carrying the two SNPs might have lower CES1 activity relative to the non-carriers. However, in the present study, we were not able to find any significant association of the two SNPs with CES1 expression or

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activity on oseltamivir activation in human livers. Thus, further investigations are needed to determine the function of the two variants.

The non-functional pseudogene *CES1P1* has over 90% sequence identity to the CES1 gene, but it does not encode any functional proteins due to a premature stop codon in its exon 3. However, the common *CES1P1* variation CES1P1VAR, which results from several SNPs being in complete linkage disequilibrium in exon 1, expresses protein product identical to that of *CES1*. Therefore, it has been suspected that the CES1P1/CES1P1VAR genotypes may affect CES1 expression levels and consequently alter pharmacokinetics and pharmacodynamics of drugs metabolized by CES1. However, our human liver study suggests that neither CES1 expression nor activity on oseltamivir activation is associated with the CES1P1/CES1P1VAR genotypes. A previous study has shown that the transcription efficiency of *CES1P1VAR* was only about 2% of the *CES1* gene [20], indicating that the contribution of *CES1P1VAR* to CES1 protein expression is negligible. Thus, the CES1P1/CES1P1VAR genotypes are not expected to have significant effect on therapeutic responses to drugs metabolized by CES1.

The SNP -816A>C (rs3785161) is located in the promoter region of the *CES1P1* gene, and it was reported to be associated with greater response to imidapril, a prodrug activated by CES1 to reduce blood pressure in hypertensive patients, suggesting that carriers of this variant may exhibit greater CES1 expression [14]. In addition, the -816A>C was found to be significantly associated with antiplatelet activity of the CES1 substrate drug clopidogrel in two independent clinical studies [21, 22]. However, the effects of the SNP determined from the two studies were contradictory. The present human liver study demonstrated that the -816A>C variant did not significantly affect CES1 expression and activity on oseltamivir activation. These results are in agreement with the fact that the -816A>C variant is located in the *CES1P1* gene locus and the *CES1P1* and *CES1P1VAR* do not significantly contribute to hepatic CES1 expression.

In summary, the study suggests that gender differences in hepatic CES1 expression may exist, although its clinical implications require further investigations. Furthermore,

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we have demonstrated that the variant G143E can significantly impair oseltamivir activation in human livers while the common variants -816A>C, rs2244613, rs8192935 and CES1P1VAR had no effect on oseltamivir activation and CES1 expression. Thus, CES1 genetic variants have the potential to serve as a valid biomarker for the prediction of oseltamivir activation and the optimization of oseltamivir pharmacotherapy. It should be noted that, although over 2000 *CES1* variants have been identified, only very few of them have been studied for their effect on CES1 expression and activity; therefore, a comprehensive *CES1* functional pharmacogenetic study is urgently needed. In addition, a prospective clinical investigation is imperative to establish *CES1* pharmacogenetics as a valuable approach to improve therapeutic outcomes of oseltamivir treatment.

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References

1. Jefferson T, Jones M, Doshi P, Spencer EA, Onakpoya I, Heneghan CJ. Oseltamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. BMJ. 2014;348:g2545.
2. Dobson J, Whitley RJ, Pocock S, Monto AS. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. Lancet. 2015;385:1729-37.
3. Eyler RF, Heung M, Pleva M, Sowinski KM, Park PK, Napolitano LM, et al. Pharmacokinetics of oseltamivir and oseltamivir carboxylate in critically ill patients receiving continuous venovenous hemodialysis and/or extracorporeal membrane oxygenation. Pharmacotherapy. 2012;32:1061-9.

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4. Robson R, Buttimore A, Lynn K, Brewster M, Ward P. The pharmacokinetics and tolerability of oseltamivir suspension in patients on haemodialysis and continuous ambulatory peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21:2556-62.
5. Treanor JJ, Hayden FG, Vrooman PS, Barbarash R, Bettis R, Riff D, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. Jama. 2000;283:1016-24.
6. Shi D, Yang J, Yang D, LeCluyse EL, Black C, You L, et al. Anti-influenza prodrug oseltamivir is activated by carboxylesterase human carboxylesterase 1, and the activation is inhibited by antiplatelet agent clopidogrel. J Pharmacol Exp Ther. 2006;319:1477-84.
7. Ward P, Small I, Smith J, Suter P, Dutkowski R. Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. J Antimicrob Chemother. 2005;55 Suppl 1:i5-i21.
8. Imai T, Taketani M, Shii M, Hosokawa M, Chiba K. Substrate specificity of carboxylesterase isozymes and their contribution to hydrolase activity in human liver and small intestine. Drug Metab Dispos. 2006;34:1734-41.
9. Rasmussen HB, Bjerre D, Linnet K, Jurgens G, Dalhoff K, Stefansson H, et al. Individualization of treatments with drugs metabolized by CES1: combining genetics and metabolomics. Pharmacogenomics. 2015:1-17.
10. Tarkiainen EK, Tornio A, Holmberg MT, Launiainen T, Neuvonen PJ, Backman JT, et al. Effect of carboxylesterase 1 c.428G > A single nucleotide variation on the pharmacokinetics of quinapril and enalapril. Br J Clin Pharmacol. 2015;80:1131-8.
11. Tarkiainen EK, Holmberg MT, Tornio A, Neuvonen M, Neuvonen PJ, Backman JT, et al. Carboxylesterase 1 c.428G>A single nucleotide variation increases the antiplatelet effects of clopidogrel by reducing its hydrolysis in humans. Clin Pharmacol Ther. 2015;97:650-8.
12. Lewis JP, Horenstein RB, Ryan K, O'Connell JR, Gibson Q, Mitchell BD, et al. The functional G143E variant of carboxylesterase 1 is associated with increased

Author Manuscript

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clopidogrel active metabolite levels and greater clopidogrel response. Pharmacogenet Genomics. 2013;23:1-8.
13. Tarkiainen EK, Backman JT, Neuvonen M, Neuvonen PJ, Schwab M, Niemi M. Carboxylesterase 1 polymorphism impairs oseltamivir bioactivation in humans. Clin Pharmacol Ther. 2012;92:68-71.
14. Geshi E, Kimura T, Yoshimura M, Suzuki H, Koba S, Sakai T, et al. A single nucleotide polymorphism in the carboxylesterase gene is associated with the responsiveness to imidapril medication and the promoter activity. Hypertens Res. 2005;28:719-25.
15. Zhu HJ, Patrick KS, Yuan HJ, Wang JS, Donovan JL, DeVane CL, et al. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: clinical significance and molecular basis. Am J Hum Genet. 2008;82:1241-8.
16. Zhu HJ, Markowitz JS. Activation of the antiviral prodrug oseltamivir is impaired by two newly identified carboxylesterase 1 variants. Drug Metab Dispos. 2009;37:264-7.
17. Laizure SC, Parker RB, Herring VL, Hu ZY. Identification of carboxylesterase-dependent dabigatran etexilate hydrolysis. Drug metabolism and disposition: the biological fate of chemicals. 2014;42:201-6.
18. Ishiguro N, Kishimoto W, Volz A, Ludwig-Schwellinger E, Ebner T, Schaefer O. Impact of endogenous esterase activity on in vitro p-glycoprotein profiling of dabigatran etexilate in Caco-2 monolayers. Drug metabolism and disposition: the biological fate of chemicals. 2014;42:250-6.
19. Pare G, Eriksson N, Lehr T, Connolly S, Eikelboom J, Ezekowitz MD, et al. Genetic determinants of dabigatran plasma levels and their relation to bleeding. Circulation. 2013;127:1404-12.
20. Fukami T, Nakajima M, Maruichi T, Takahashi S, Takamiya M, Aoki Y, et al. Structure and characterization of human carboxylesterase 1A1, 1A2, and 1A3 genes. Pharmacogenet Genomics. 2008;18:911-20.
21. Zou JJ, Chen SL, Fan HW, Tan J, He BS, Xie HG. CES1A -816C as a genetic marker to predict greater platelet clopidogrel response in patients with percutaneous coronary intervention. J Cardiovasc Pharmacol. 2014;63:178-83.

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22. Xie C, Ding X, Gao J, Wang H, Hang Y, Zhang H, et al. The effects of CES1A2 A(-816)C and CYP2C19 loss-of-function polymorphisms on clopidogrel response variability among Chinese patients with coronary heart disease. Pharmacogenetics and genomics. 2014;24:204-10.
23. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. N Engl J Med. 2003;349:1157-67.
24. Scandlyn MJ, Stuart EC, Rosengren RJ. Sex-specific differences in CYP450 isoforms in humans. Expert Opin Drug Metab Toxicol. 2008;4:413-24.
25. Zhu HJ, Appel DI, Jiang Y, Markowitz JS. Age- and sex-related expression and activity of carboxylesterase 1 and 2 in mouse and human liver. Drug Metab Dispos. 2009;37:1819-25.
26. Hines RN, Simpson PM, McCarver DG. Age-Dependent Human Hepatic Carboxylesterase 1 (CES1) and Carboxylesterase 2 (CES2) Postnatal Ontogeny. Drug metabolism and disposition: the biological fate of chemicals. 2016.
27. Yang D, Pearce RE, Wang X, Gaedigk R, Wan YJ, Yan B. Human carboxylesterases HCE1 and HCE2: ontogenic expression, inter-individual variability and differential hydrolysis of oseltamivir, aspirin, deltamethrin and permethrin. Biochem Pharmacol. 2009;77:238-47.
28. Shi D, Yang D, Prinssen EP, Davies BE, Yan B. Surge in expression of carboxylesterase 1 during the post-neonatal stage enables a rapid gain of the capacity to activate the anti-influenza prodrug oseltamivir. J Infect Dis. 2011;203:937-42.
29. Stangier J. Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate. Clinical pharmacokinetics. 2008;47:285-95.
30. Wattanagoon Y, Stepniewska K, Lindegardh N, Pukrittayakamee S, Silachamroon U, Piyaphanee W, et al. Pharmacokinetics of high-dose oseltamivir in healthy volunteers. Antimicrob Agents Chemother. 2009;53:945-52.
31. Vree TB, Dammers E, Ulc I, Horkovics-Kovats S, Ryska M, Merkx I. Differences between lovastatin and simvastatin hydrolysis in healthy male and female volunteers:gut hydrolysis of lovastatin is twice that of simvastatin. ScientificWorldJournal. 2003;3:1332-43.

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32. Patrick KS, Straughn AB, Minhinnett RR, Yeatts SD, Herrin AE, DeVane CL, et al. Influence of ethanol and gender on methylphenidate pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther. 2007;81:346-53.
33. Wang X, Wang G, Shi J, Aa J, Comas R, Liang Y, et al. CES1 genetic variation affects the activation of angiotensin-converting enzyme inhibitors. The pharmacogenomics journal. 2015.
34. Wang X, Wang G, Shi J, Aa J, Comas R, Liang Y, et al. CES1 genetic variation affects the activation of angiotensin-converting enzyme inhibitors. Pharmacogenomics J. 2015:DOI: 10.1038/tpj.2015.42 tpj201542 [pii].
35. Chang Q, Chow MS, Zuo Z. Studies on the influence of esterase inhibitor to the pharmacokinetic profiles of oseltamivir and oseltamivir carboxylate in rats using an improved LC/MS/MS method. Biomedical chromatography : BMC. 2009;23:852-7.
36. Wang X, Liang Y, Liu L, Shi J, Zhu HJ. Targeted absolute quantitative proteomics with SILAC internal standards and unlabeled full-length protein calibrators (TAQSI). Rapid communications in mass spectrometry : RCM. 2016;30:553-61.
37. Hosokawa M, Endo T, Fujisawa M, Hara S, Iwata N, Sato Y, et al. Interindividual variation in carboxylesterase levels in human liver microsomes. Drug Metab Dispos. 1995;23:1022-7.
38. Yoshimura M, Kimura T, Ishii M, Ishii K, Matsuura T, Geshi E, et al. Functional polymorphisms in carboxylesterase1A2 (CES1A2) gene involves specific protein 1 (Sp1) binding sites. Biochem Biophys Res Commun. 2008;369:939-42.
39. Hagihara K, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, et al. A possible mechanism for the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. Drug Metab Dispos. 2009;37:2145-52.
40. Ross MK, Borazjani A, Wang R, Crow JA, Xie S. Examination of the carboxylesterase phenotype in human liver. Arch Biochem Biophys. 2012;522:44-56.
41. Laizure SC, Herring V, Hu Z, Witbrodt K, Parker RB. The role of human carboxylesterases in drug metabolism: have we overlooked their importance? Pharmacotherapy. 2013;33:210-22.

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42. Zhu HJ, Appel DI, Johnson JA, Chavin KD, Markowitz JS. Role of carboxylesterase 1 and impact of natural genetic variants on the hydrolysis of trandolapril. Biochem Pharmacol. 2009;77:1266-72.
43. Zhu HJ, Wang X, Gawronski BE, Brinda BJ, Angiolillo DJ, Markowitz JS. Carboxylesterase 1 as a determinant of clopidogrel metabolism and activation. J Pharmacol Exp Ther. 2013;344:665-72.
44. Nemoda Z, Angyal N, Tarnok Z, Gadoros J, Sasvari-Szekely M. Carboxylesterase 1 gene polymorphism and methylphenidate response in ADHD. Neuropharmacology. 2009;57:731-3.

Figure legends

Figure 1. Sex differences in oseltamivir activation and CES1 expression in human livers. Horizontal bars show mean values in each group. Mann–Whitney test was utilized to test the differences between genders.

Figure 2. Effects of *CES1* genetic polymorphisms on oseltamivir hydrolysis (A) and CES1 expression (B) in 104 human livers. The samples were categorized based on different genotypes. Horizontal bars show mean values in each group. Mann–Whitney test was utilized to test the differences of CES1 expression and activity among different genotypes. A *P*-value less than 0.05 was considered statistically significant.

Figure 3. Correlation analysis of the hydrolytic rates of oseltamivir with CES1 expression levels in 104 human liver S9 fraction (HLS9) samples. Data are means from two independent experiments. A: all samples (n=104); B: G143E non-carriers only (n=99).

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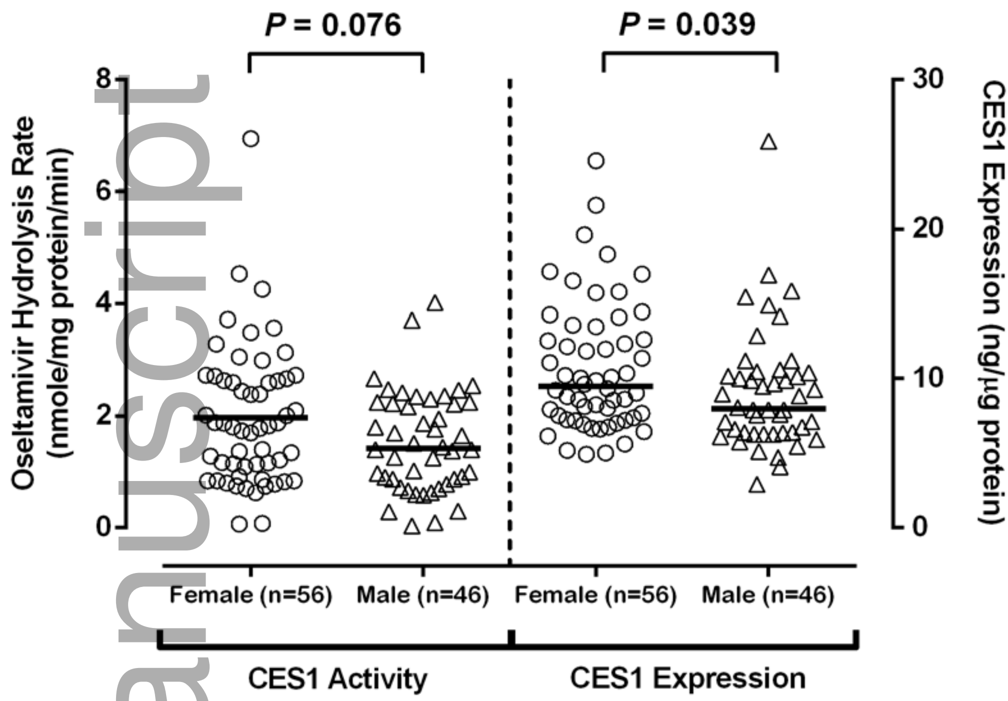
Figure 1



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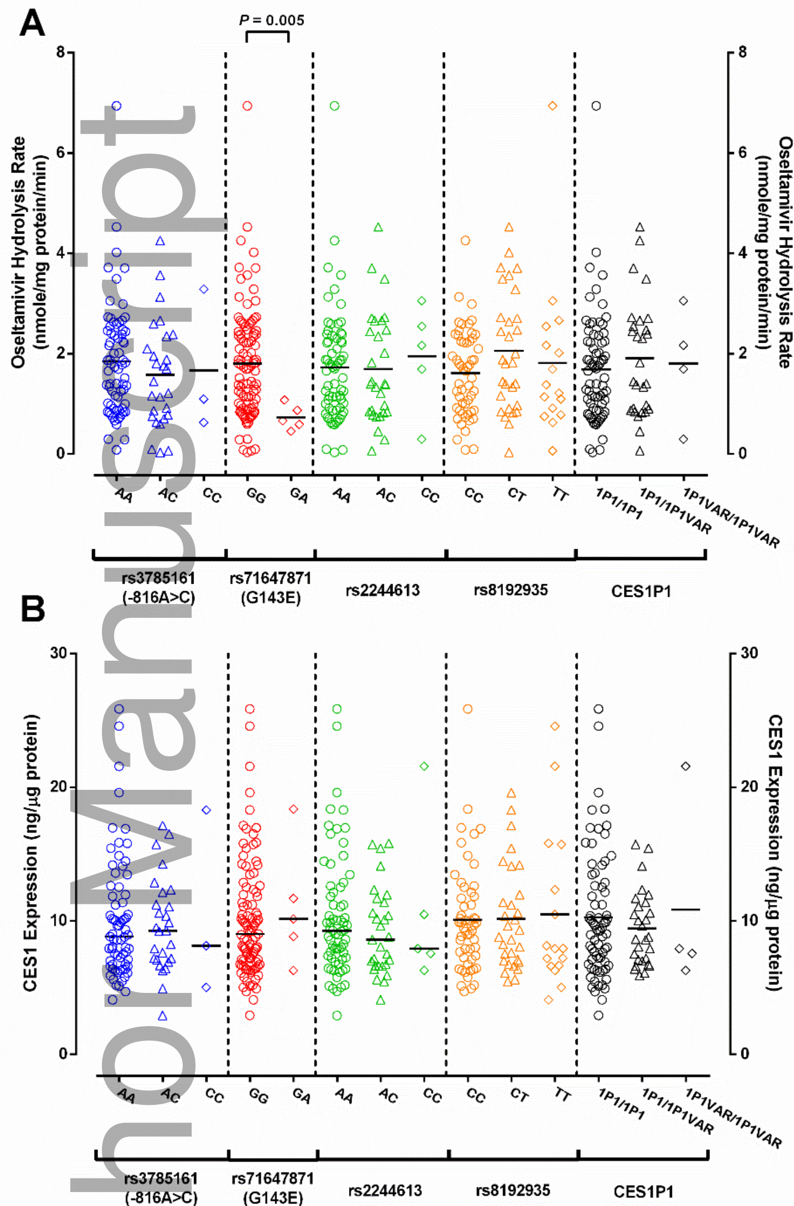

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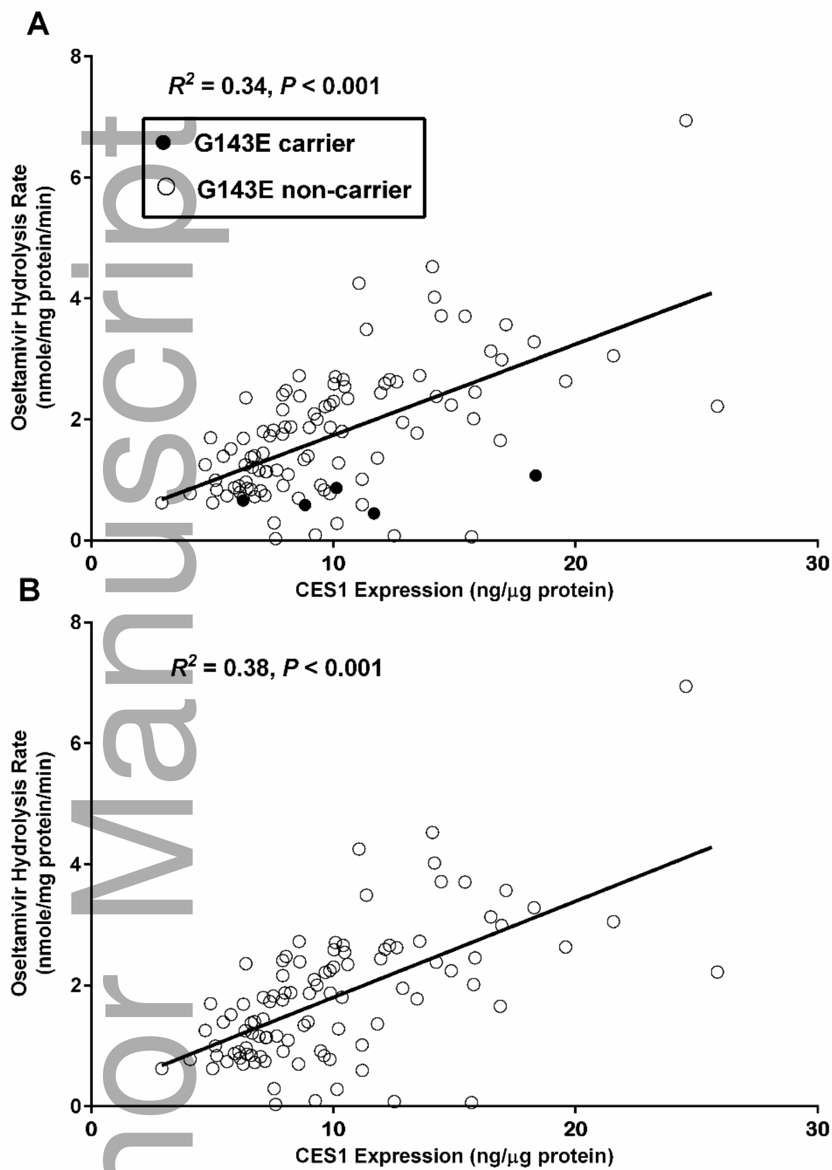

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