Supplemental Materials

**Title:**
A comprehensive functional assessment of carboxylesterase 1 nonsynonymous polymorphisms

**Authors:**
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**Journal Title:** Drug Metabolism and Disposition
## Supplementary materials for “Comprehensive functional assessment of CES1 nonsynonymous polymorphisms”

**Table S1. Sequences of the primers used in this study**

<table>
<thead>
<tr>
<th>Site Directed Mutagenesis</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tr>
<td>L40Ter</td>
<td>5'-TGTGCAATCTTCTCAGTGAGCAATCTCCCCA-3'</td>
<td>5'-TCCTTGGAATTTGTCAGCGAGGATTTGCACA-3'</td>
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<tr>
<td>S75N</td>
<td>5'-TGGCGATTCTCTACAAAGTTCCATGGTCTGCAGGCA-3'</td>
<td>5'-GCCTGCAACATGGAACCTTTGTTGAGAATTCACA-3'</td>
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<tr>
<td>G142E</td>
<td>5'-CACCACGGCCTCTCTCCGGATGCA-3'</td>
<td>5'-TGATCCAGGAGGGCTGATGGTG-3'</td>
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<td>G147C</td>
<td>5'-GATGAGGAGGATGCTGAGCAACATCAGGCC-3'</td>
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<td>A158V</td>
<td>5'-CGTTTTCATGGGCAACAAGGGCAACAGCCA-3'</td>
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<td>T167S</td>
<td>5'-CAGGCAGATATTGAATGCTCACCACACCAGCT-3'</td>
<td>5'-AACGCTGATGTTGAGGACTCAATTGACCTG-3'</td>
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<td>Q169P</td>
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<td>Y170D</td>
<td>5'-GCCAGGGACATTTGAAATTGGCACCACACAC-3'</td>
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<table>
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<tr>
<th>Nested PCR</th>
<th>Outer P</th>
<th>Inner P</th>
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<tr>
<td>cDNA P-1</td>
<td>5'-TGTGCGACTTCCAGGCCTTCATGAC-3'</td>
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### Table S2. Thermocycling conditions for PCR

<table>
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<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td></td>
<td>Temperature</td>
<td>time</td>
<td>Temperature</td>
</tr>
<tr>
<td>Initial Denaturation</td>
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<td>30s</td>
<td>95 °C</td>
</tr>
<tr>
<td>30 Cycles</td>
<td>95 °C</td>
<td>30s</td>
<td>95 °C</td>
</tr>
<tr>
<td></td>
<td>60°C</td>
<td>45s</td>
<td>72 °C</td>
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<tr>
<td></td>
<td>68 °C</td>
<td>2min10s</td>
<td>68 °C</td>
</tr>
<tr>
<td>Final Extension</td>
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<td>5min</td>
<td>68 °C</td>
</tr>
<tr>
<td>Hold</td>
<td>4 °C</td>
<td>∞</td>
<td>4 °C</td>
</tr>
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</table>

A: the PCR conditions for the amplification of outer region of CES1 cDNA (nested PCR-1<sup>st</sup> outer);
B: the PCR conditions for the amplification of inner region of CES1 cDNA (nested PCR-2<sup>nd</sup> inner)
C: the real time PCR conditions for CES1 mRNA quantification
**Table S3.** The gradient conditions of HPLC-MS/MS analysis

<table>
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<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
<th>Time (min)</th>
<th>A (%)</th>
<th>C (%)</th>
<th>Time (min)</th>
<th>A (%)</th>
<th>C (%)</th>
<th>Time (min)</th>
<th>D (%)</th>
<th>E (%)</th>
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**Mobile Phase:**
- A: water containing 2 mM ammonium acetate and 0.2% formic acid
- B: methanol containing 2 mM ammonium acetate and 0.2% formic acid
- C: acetonitril with 0.2% formic acid
- D: water containing 0.1% formic acid
- E: acetonitril with 0.1% formic acid
**Figure S1:** Correlation between CES1 mRNA and protein expressions in different CES1 variants and WT CES1 transfected cell lines. The CES1 nsSNPs exhibiting significant impact on protein but not mRNA expressions are labeled. Data are the means from three independent experiments.
Figure S2. Correlation between CES1 protein, mRNA expressions and CES1 activities on enalapril, clopidogrel and sacubitril hydrolysis. (A) enalapril vs protein; (B) enalapril vs mRNA; (C) clopidogrel vs protein; (D) clopidogrel vs mRNA; (E) sacubitril vs protein; (F) sacubitril vs mRNA. CES1 nsSNPs showing significant impact on CES1 activity on a CES1 substrate are labeled. Data are the means from three independent experiments.