Supplemental Data

Two *CES1* Gene Mutations Lead to Dysfunctional Carboxylesterase 1 Activity in Man: Clinical Significance and Molecular Basis

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Figure S1. The Major Metabolic Pathway of Racemic MPH in Humans

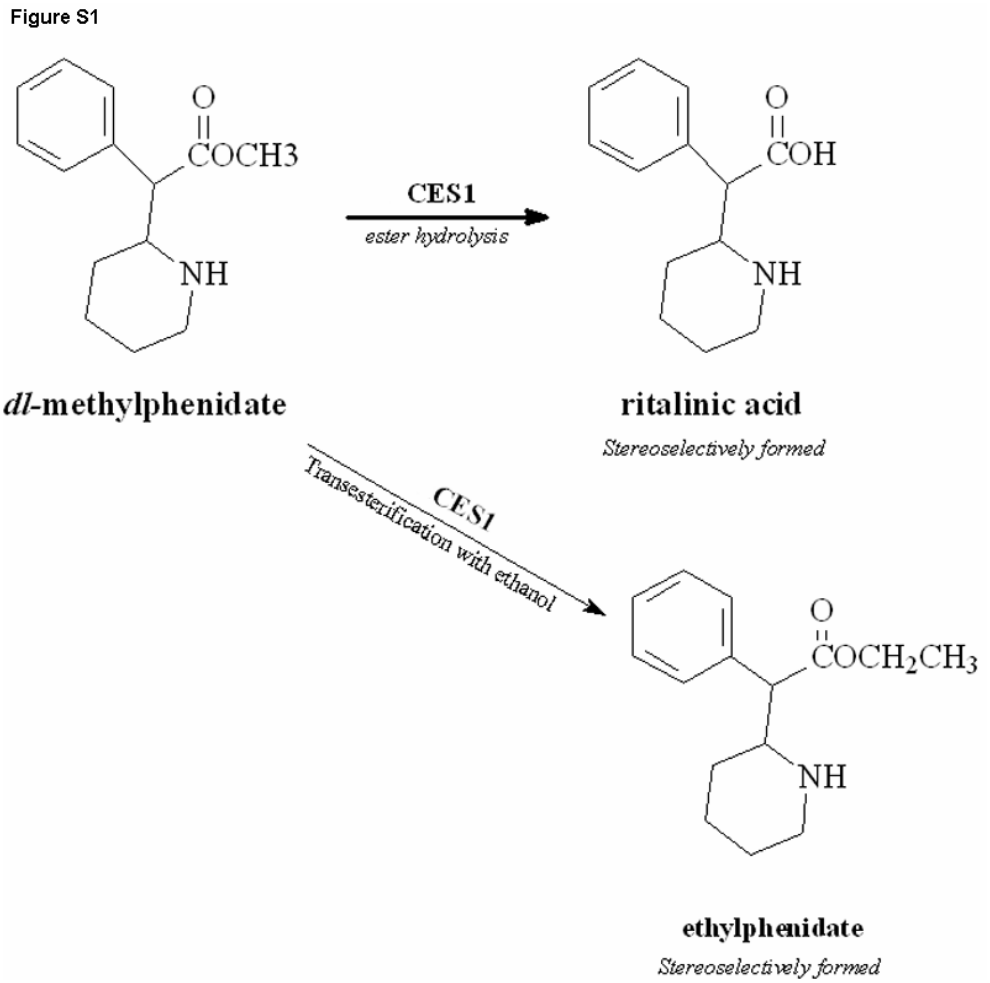
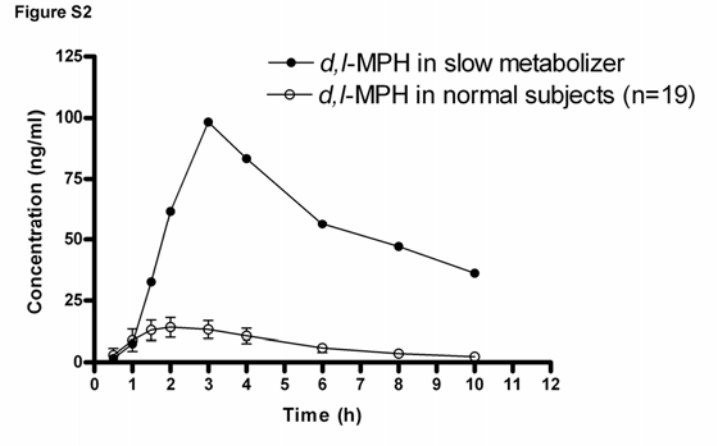


Figure S2.



The plasma concentration versus time curve of total (*d*-MPH and *l*-MPH) concentrations of MPH in an apparent aberrant metabolizer compared to a similar plot of mean isomer concentrations from 19 study peers following a single 0.3 mg/kg dose of racemic MPH.

Figure S3. Alignment of the Predicted Protein Sequences of WT CES1 and the Two Mutations Identified in the Aberrant Metabolizer

Figure S3

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                                                                      50
wildtype      MWLRAFILATLSASAAWGHPSSPPVVDTVHGKVLGKFVSLEGFAQPVAIF
p.Gly143Glu MWLRAFILATLSASAAWGHPSSPPVVDTVHGKVLGKFVSLEGFAQPVAIF
p.Asp260fs  MWLRAFILATLSASAAWGHPSSPPVVDTVHGKVLGKFVSLEGFAQPVAIF

                                                                     100
wildtype      LGIPFAKPPLGPLRFTPPQPAEPWSFVKNATSYPPMCTQDPKAGQLLSEL
p.Gly143Glu LGIPFAKPPLGPLRFTPPQPAEPWSFVKNATSYPPMCTQDPKAGQLLSEL
p.Asp260fs  LGIPFAKPPLGPLRFTPPQPAEPWSFVKNATSYPPMCTQDPKAGQLLSEL

                                                        øøø          150
wildtype      FTNRKENIPLKLSEDCLYLNIYTPADLTKKNRLPVMVWIHGGGLMVGAAS
p.Gly143Glu FTNRKENIPLKLSEDCLYLNIYTPADLTKKNRLPVMVWIHGGELMVGAAS
p.Asp260fs  FTNRKENIPLKLSEDCLYLNIYTPADLTKKNRLPVMVWIHGGGLMVGAAS

                                                                     200
wildtype      TYDGLALAAHENVVVVTIQYRLGIWGFFSTGDEHSRGNWGHLDQVAALRW
p.Gly143Glu TYDGLALAAHENVVVVTIQYRLGIWGFFSTGDEHSRGNWGHLDQVAALRW
p.Asp260fs  TYDGLALAAHENVVVVTIQYRLGIWGFFSTGDEHSRGNWGHLDQVAALRW

                                                                     250
wildtype      VQDNIASFGGNPGSVTIFGESAGGESVSVLVLSPLAKNLFHRAISESGVA
p.Gly143Glu VQDNIASFGGNPGSVTIFGESAGGESVSVLVLSPLAKNLFHRAISESGVA
p.Asp260fs  VQDNIASFGGNPGSVTIFGESAGGESVSVLVLSPLAKNLFHRAISESGVA

                                                                     300
wildtype      LTSVLVKKGDVKPLAEQIAITAGCKTTTSAVMVHCLRQKTEEELLETTLK
p.Gly143Glu LTSVLVKKGDVKPLAEQIAITAGCKTTTSAVMVHCLRQKTEEELLETTLK
p.Asp260fs  LTSVLVKKGESSPWLSKLLSLLGAKPPPLLSWFTACDRRRKRSSWRRH*

                                                                     350
wildtype      MKFLSLDLQGDPRESQPLLGTVIDGMLLLKTPEELQAERNFHTVPYMVGI
p.Gly143Glu MKFLSLDLQGDPRESQPLLGTVIDGMLLLKTPEELQAERNFHTVPYMVGI

                                                                     400
wildtype      NKQEFGWLIPMQLMSYPLSEGQLDQKTAMSLLWKSYPLVCIAKELIPEAT
p.Gly143Glu NKQEFGWLIPMQLMSYPLSEGQLDQKTAMSLLWKSYPLVCIAKELIPEAT

                                                                     450
wildtype      EKYLGGTDDTVKKKDLFLDLIADVMFGVPSVIVARNHRDAGAPTYMYEFQ
p.Gly143Glu EKYLGGTDDTVKKKDLFLDLIADVMFGVPSVIVARNHRDAGAPTYMYEFQ

                                                                     500
wildtype      YRPSFSSDMKPKTVIGDHGDELFSVFGAPFLKEGASEEEIRLSKMVMKFW
p.Gly143Glu YRPSFSSDMKPKTVIGDHGDELFSVFGAPFLKEGASEEEIRLSKMVMKFW

                                                                     550
wildtype      ANFARNGNPNGEGLPHWPEYNQKEGYLQIGANTQAAQKLKDKEVAFWTNL
p.Gly143Glu ANFARNGNPNGEGLPHWPEYNQKEGYLQIGANTQAAQKLKDKEVAFWTNL

                              567
wildtype      FAKKAVEKPPQTEHIEL*
p.Gly143Glu FAKKAVEKPPQTEHIEL*
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p.Gly143Glu is the Gly143Glu substitution and is denoted by the boxed amino acid. p.Asp260fs is the Asp260Glu frameshift mutation and the altered amino acid sequence is underlined. The amino acids of the catalytic triad are bolded, the residues of the oxyanion hole are indicated by the symbol 'ø', and * designates a stop codon.

Table S1. Pharmacokinetic Parameters in the Slow Metabolizer versus the 19 Study Peers

Pharmacokinetic Parameter	***Mean (±SD)***	***Outlier***	***ESD Statistic***	**p *Value***
AUC (ng/ml · hr)	78.6 (35.7)	208.7	3.6	< 0.01
C_{max} (ng/ml)	13.8 (3.2)	36.7	7.3	< 0.01
$t_{1/2}$ (hr)	3.0 (0.7)	5.4	3.3	< 0.01

Table S2. Minor Allele Frequencies of *CES1* SNP p.Gly143Glu

	Caucasian	***Black***	***Hispanic***	***Asian***	***Total***
GA	n = 34 (7.5%)	n = 10 (8.5%)	n = 12 (4.0%)	n = 0 (0.0%)	n = 56 (6.0%)
GG	n = 421 (92.5%)	n = 107 (91.5%)	n = 287 (96.0%)	n = 54 (100.0%)	n = 869 (94.0%)
MAF	C = 3.7%	C = 4.3%	C = 2.0%	C = 0%	C = 3.0%
Totals	455	117	299	54	925

* The 95% confidence interval of average minor allele frequencies (MAF) from four tested populations is -0.57% ~5.57%