Supplemental Information For

Impact of cytochrome P450 variation on meperidine *N*-demethylation to the neurotoxic metabolite normeperidine

Supplemental Figure 1. LC-MS/MS analysis of meperidinic acid from chemical synthesis and meperidine metabolism in pooled human liver microsomes.

Supplemental Figure 2. Kinetic analysis of meperidinic acid formation in human liver microsomal incubations with meperidine.

Supplemental Figure 3. *S*-Mephenytoin 4´-hydroxylation by individual *CYP2C19*-genotyped human liver microsomes.

Supplemental Figure 4. Correlation of meperidine *N*-demethylation to CYP2B6, CYP2C19, and CYP3A activities in human liver microsomes from BioreclamationIVT.

Chemical synthesis and analysis of meperidinic acid formation.

Additional experiments were performed to investigate the generation of meperidinic acid from hydrolysis of meperidine. First, we sought to chemically synthesize meperidinic acid from base-catalyzed hydrolysis of meperidine. For these studies, meperidine, over a range of concentrations (1-1000 μM), was incubated with 0.1 M potassium hydroxide for 10 minutes at room temperature (pH ~13). Following the incubation period, samples were adjusted to approximately pH 3 by addition of formic acid (10 μl of formic acid was added to a reaction volume of 200 μl). Samples were mixed with a vortex device. Then, 400 μl of ice-cold methanol containing normeperidine-d_4 (0.4 μM, internal standard) was added, and samples were transferred to LC-MS vials for analysis.

An LC-MS/MS method was developed to detect meperidinic acid (*m/z* 220) using electrospray ionization (ESI+) in positive ion mode. LC-MS and MS/MS analyses were carried out on a Shimadzu LCMS 8030 triple quadrupole mass spectrometer system interfaced with two HPLC LC-20AD XR pumps and a SIL-20AC XR autosampler, as described in "*Materials and Methods*". The LC gradient program was the same as that used for LC-MS/MS analysis of meperidine and normeperidine (See "*Materials and Methods*"). Samples from incubation of meperidine with potassium hydroxide were subjected to full scan Q3 MS analysis (range *m/z* 100-300) and MS/MS product ion scan analysis for the predicted precursor ion of meperidinic acid, *m/z* 220, using collision-induced dissociation (collision energy -25 V). LC-MS/MS analysis of *m/z* 220 by ESI+ in positive ion mode generated a fragmentation pattern indicative of meperidinic acid. The major product ions of *m/z* 220 (meperidinic acid) were *m/z* 174, corresponding

to loss of the carboxylic acid moiety, and *m/z* 70, which is a product ion common to meperidine (Wang et al., 2011). The product ion spectrum of *m/z* 220 (meperidinic acid) is shown in **Supplemental Figure S1A**.

Based on this fragmentation pattern, multiple reaction monitoring (MRM) of the precursor-to-product ion transition *m/z* 220 → 174 was used for selective detection of meperidinic acid (**Supplemental Figure S1B**). Next, samples from kinetic assays with pooled human liver microsomes incubated with meperidine (1-1000 μM) were analyzed by LC-MS/MS utilizing MRM to detect the formation of meperidinic acid from meperidine.

Meperidinic acid was detected by LC-MS/MS from microsomal incubations with meperidine utilizing the MRM method described above (**Supplemental Figure S1C**). Meperidinic acid was formed in a concentration-dependent manner (**Supplemental Figure 2**). We sought to measure meperidinic acid formation by HPLC-UV detection and LC-MS/MS analysis. However, the actual levels of meperidinic acid could not be quantified in the absence of an authentic chemical standard of known concentration. Relative levels of meperidinic acid were estimated by the LC-MS/MS MRM peak area ratio of meperidinic acid (*m/z* 220 → 174) to internal standard, meperdine-d_4 (*m/z* 252 → 224). Metabolite formation data were fit to the Michaelis-Menten equation by non-linear regression analysis using GraphPad Prism 7 (GraphPad Software In., San Diego, CA, USA) to estimate the relative kinetic parameters (V_{max} and apparent K_m) of meperidine hydrolysis to meperidinic acid.

Supplemental Figure 1. LC-MS/MS analysis of meperidinic acid from chemical synthesis and from meperidine metabolism in pooled human liver microsomes. (A) Representative product ion spectrum from MS/MS analysis of meperidinic acid (*m/z* 220) synthesized from incubation of meperidine (1000 μM) with 0.1 M potassium hydroxide. LC-MS/MS analysis was carried out by electrospray ionization in positive ion mode (ESI+). (B) Representative LC-MRM chromatogram of meperidine (*m/z* 248 → 220) and meperidinic acid (*m/z* 220 → 174) generated from chemical synthesis with potassium hydroxide. (C) Representative LC-MRM chromatogram of internal standard meperidine-d_4 (*m/z* 252 → 224) and meperidinic acid (*m/z* 220 → 174) generated from incubation of meperidine (1000 μM) with pooled human liver microsomes.

Supplemental Figure 2. Kinetic analysis of meperidinic acid formation in human liver microsomal incubations with meperidine. Meperidine (1-1000 μM) was incubated with pooled human liver microsomes (0.1 mg protein/ml) for 10 minutes in the presence of NADPH-regenerating system. Meperidinic acid formation was detected by LC-MS/MS analysis and is expressed as peak area ratio of meperidinic acid (*m/z* 220 → 174) to internal standard, meperdine-d_4 (*m/z* 252 → 224). Metabolite formation data were fit to the Michaelis-Menten equation by non-linear regression analysis using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA) to estimate the relative kinetic parameters (V_{max} and apparent K_m) of meperidine hydrolysis to meperidinic acid. Values for V_{max} and apparent K_m are the mean ± standard error from a single experiment performed in triplicate.

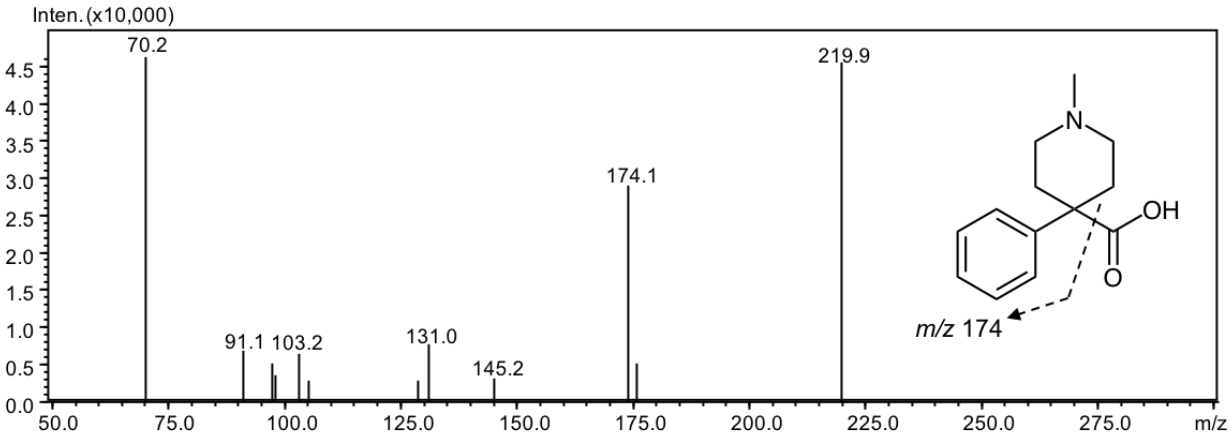
Supplemental Figure 3. *S*-Mephenytoin 4´-hydroxylation by individual *CYP2C19*-genotyped human liver microsomes. *S*-Mephenytoin (60 μM) was incubated with *CYP2C19*-genotyped human liver microsomes (0.2 mg protein/ml) for 40 minutes to measure CYP2C19 activity, as measured by 4´-hydroxymephenytoin formation. Formation of 4´-hydroxymephenytoin was quantified by LC-MS/MS using a standard curve with known concentrations of 4´-hydroxymephenytoin. Genotype groups included the following: *CYP2C19*1/*1* (n = 6), *CYP2C19*1/*2* (n = 2), *CYP2C19*2/*2* (n = 4), *CYP2C19*1/*17* (n = 3), and *CYP2C19*17/*17* (n = 3). Bars represent the average metabolite formation for each *CYP2C19* genotype. Each point represents the average metabolite formation for each donor determined from a single experiment performed in triplicate. Results were graphed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA).

Supplemental Figure 4. Correlation of meperidine *N*-demethylation to CYP2B6, CYP2C19, and CYP3A activities in human liver microsomes from BioreclamationIVT. Meperidine (50 μM) was incubated with CYP2C19-genotyped human liver microsomes (0.1 mg protein/ml) from 11 individual donors for 10 min. Rates of probe substrate marker reactions (bupropion hydroxylation for CYP2B6 activity, *S*-mephenytoin 4´-hydroxylation for CYP2C19 activity, and testosterone 6*β*-hydroxylation for CYP3A activity) were provided by the supplier, BioreclamationIVT. Meperidine *N*-demethylation was correlated with (A) bupropion hydroxylation, (B) *S*-mephenytoin 4´-hydroxylation, and (C) testosterone 6*β*-hydroxylation. Each point represents the average normeperidine formation by a single-donor liver microsomal sample determined from

three experiments, performed in triplicate each (n = 11 donors). Linear regression analysis was performed by GraphPad Prism 7 Software to determine r^2 values, and Pearson *r* correlation analysis was performed to determine p-values.

Supplemental Figure 1.

A.



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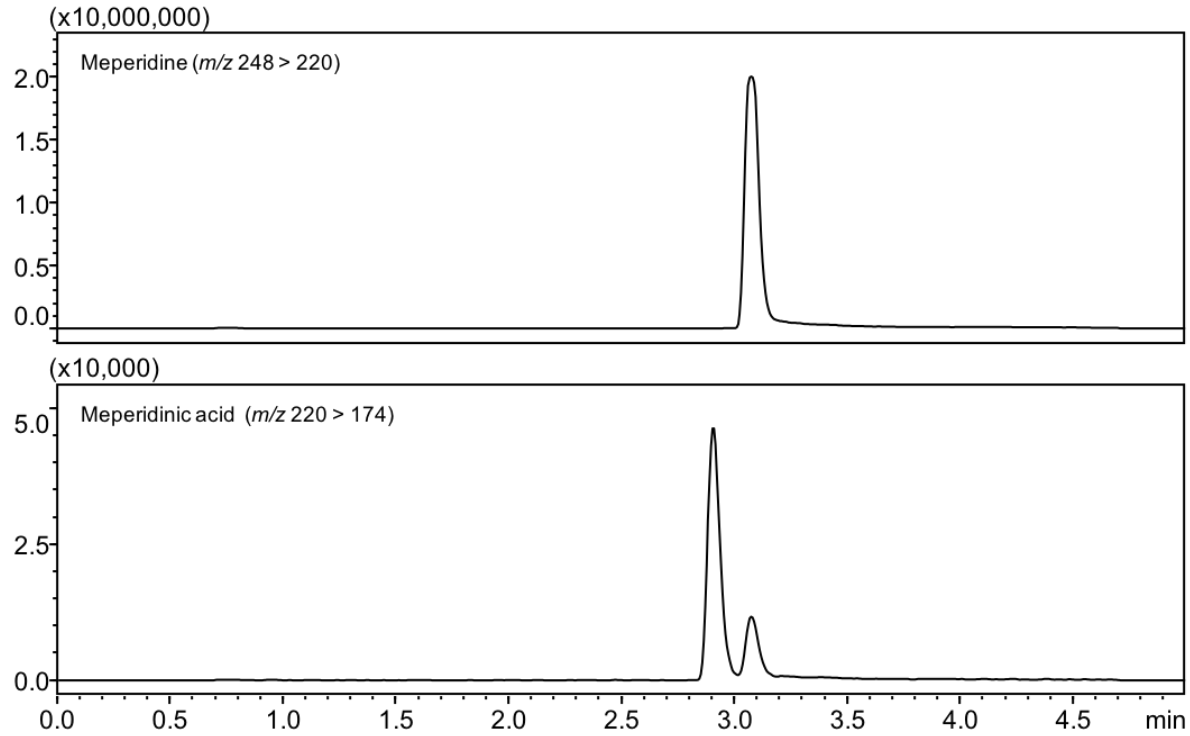

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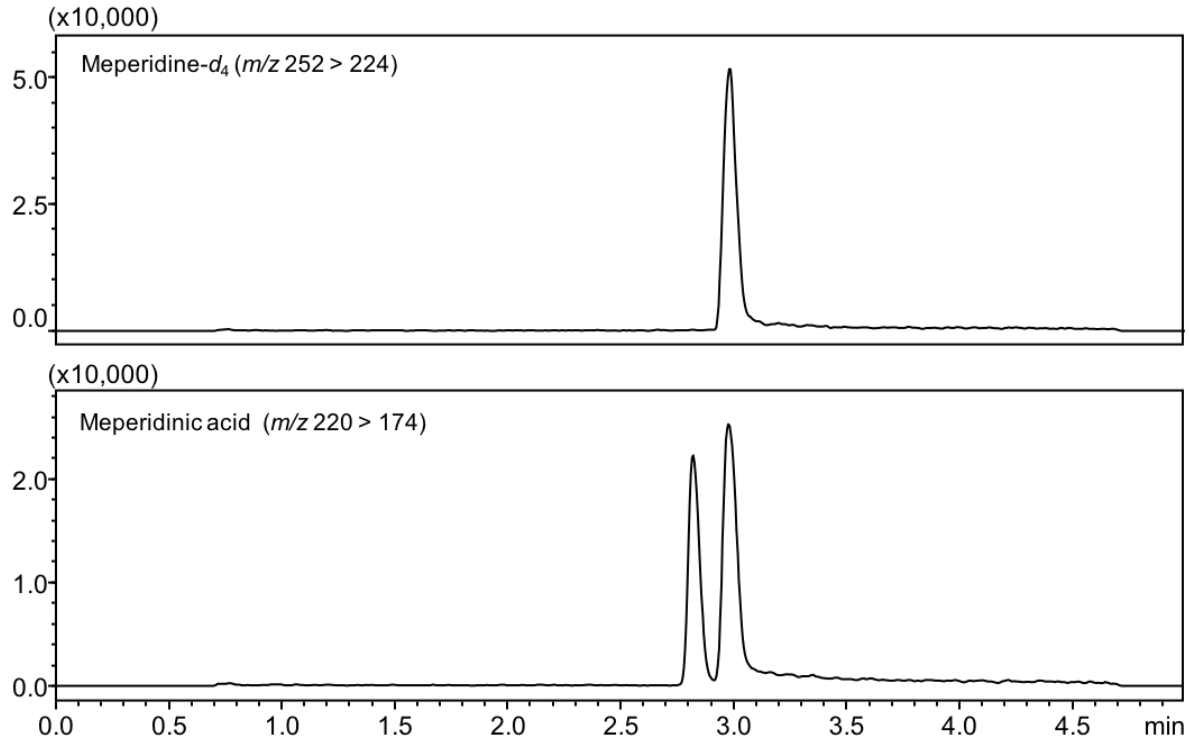
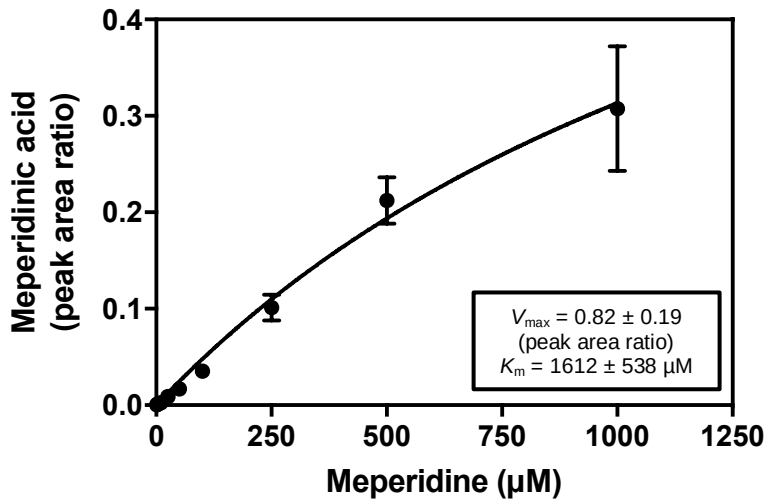
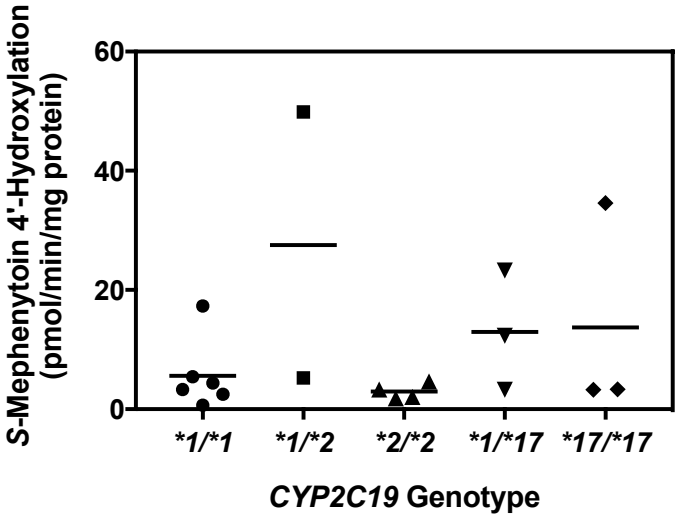

(x10,000)
Meperidine-d_4 (*m/z* 252 > 224)
5.0
2.5
0.0
(x10,000)
Meperidinic acid (*m/z* 220 > 174)
2.0
1.0
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
min

Supplemental Figure 2.

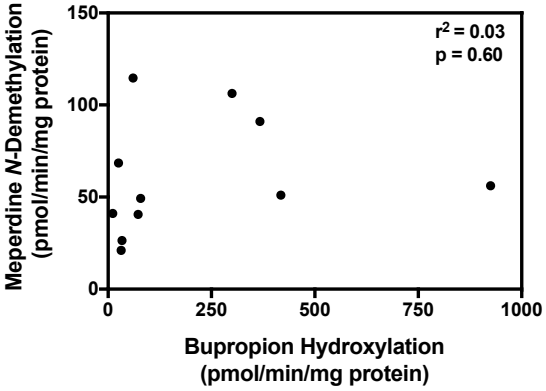


Supplemental Figure 3.



Supplemental Figure 4.

A.



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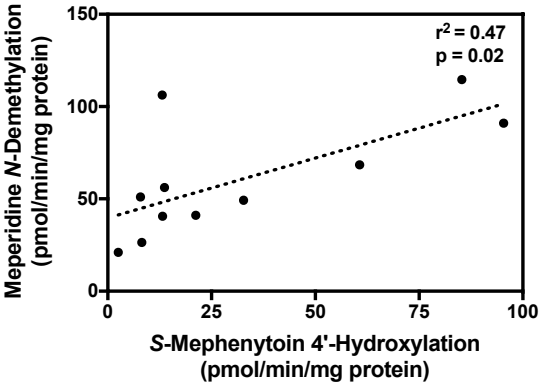

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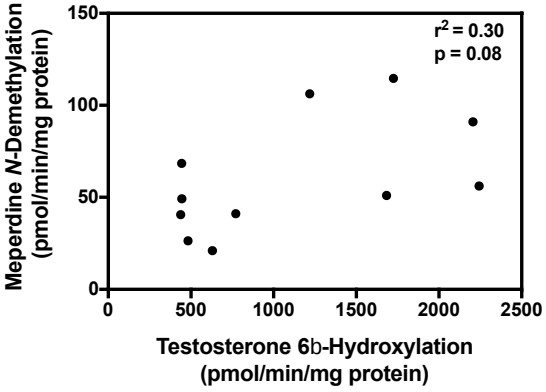

References:

Wang X, Xiang Z, Cai X, Wu H, Wang X, Li J, and Zhang M (2011) Determination of pethidine in human plasma by LC-MS/MS. *Biomed Chromatogr* **25:**833-837.