Differential expression of cytochrome P450 enzymes from the CYP2C subfamily in human brain

Authors: Iris M Booth Depaz, Francesca Toselli, Peter A Wilce and Elizabeth M J Gillam

School of Biomedical Sciences (IMBD), and

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia, 4072 (FT, PAW, EMJG).

Present address (FT): Department of Pharmacology and Toxicology, University of Toronto, 1 King's College Circle, Toronto, ON, M5S 1A8, Canada.

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: CYP2C expression in the human brain

Address correspondence to:

Professor Elizabeth M.J. Gillam, School of Chemistry and Molecular Biosciences,

The University of Queensland, Brisbane, Australia, 4072.

Telephone: +61 7 3365 1410**, Email:** e.gillam@uq.edu.au

Number of text pages: 16

Number of tables: 0

Number of figures: 4

Number of references: 19

Number of words in *Abstract*: 144

Number of words in *Introduction*: 213

Number of words in *Results and Discussion*: 866

Non-standard abbreviations: P110 000, pellet from centrifugation at 110 000 x g; P450, Cytochrome P450, heme-thiolate protein P450.

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Cytochrome P450 enzymes from the CYP2C subfamily play a prominent role in the metabolic clearance of many drugs. CYP2C enzymes have also been implicated in the metabolism of arachidonic acid to vasoactive epoxyeicosatrienoic acids. CYP2C8, CYP2C9, and CYP2C19 are expressed in the adult liver at significant levels; however the expression of CYP2C enzymes in extrahepatic tissues such as the brain is less well characterized. Form-specific antibodies to CYP2C9 and CYP2C19 were prepared by affinity purification of antibodies raised to unique peptides. CYP2C9 and CYP2C19 were located in microsomal fractions of all five human brain regions examined, namely frontal cortex, hippocampus, basal ganglia, amygdala and cerebellum. Both CYP2C9 and CYP2C19 were detected predominantly within neuronal soma but with expression extending down axons and dendrites in certain regions. Finally, a comparison of cortex samples from alcoholics and age-matched controls suggested CYP2C9 expression was increased in alcoholics.

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Cytochrome P450 enzymes play a dominant role in the metabolic clearance of drugs and other environmental chemicals. However it is increasingly apparent that P450s are expressed differentially in extrahepatic tissues in a region-specific fashion that may influence the tissue-specific clearance of drugs. In particular, P450s are expressed in the brain in a highly cell-specific fashion. Localized metabolism of drugs in the brain may have significant implications for the efficacy and side-effect profile of neuroactive medicines. Given the role of CYP2C forms in the metabolism of a number of drugs affecting the central nervous system (Guengerich, 2005), it is of significant interest to characterize their expression in the brain. Moreover CYP2C enzymes in animals have been proposed to contribute to the regulation of cerebral blood flow via generation of epoxyeicosatrienoic acid metabolites from arachidonic acid (Alkayed et al., 1996; Iliff et al., 2007). While a few studies have been undertaken to detect CYP2C transcript expression in human brain (McFadyen et al., 1998; Klose et al., 1999; Dauchy et al., 2008; Dutheil et al., 2009), the detection of CYP2C proteins has been limited by the paucity of human brain tissue and form-specific antibodies. The aim of this study was to characterize the expression of CYP2C9 and CYP2C19 proteins in discrete regions of the human brain.

Materials and Methods

Polyvinylidene fluoride (PVDF) and BioTrace™ NT nitrocellulose membranes were obtained from Pall Corporation (East Hills, New York). AlexaFluor 680- and AlexaFluor 488-labeled goat anti-rabbit IgG antibodies were purchased from Invitrogen (Carlsbad California) and IRDye 800-labeled donkey anti-mouse IgG antibody was obtained from Rockland Immunochemicals (Gilbertsville, PA). Mouse anti-human-α-tubulin monoclonal primary

antibody was purchased from Sigma (St. Louis, MO). Fluorescence mounting medium (DAKO, Glostrup, Denmark) was used to maintain fluorophore stability.

All work described here was done under protocols approved by the University of Queensland Molecular Biosciences Animal Ethics Committee and the University of Queensland Human Ethics Committee. CYP2C9- and CYP2C19-specific antibodies were raised in rabbit, against two unique His-tagged 13-amino acid sequences for each P450 in regions predicted to be immunogenic: L**M**KMEKEK**H**NQ**P**C-HHHHHH and GGNFKKS**K**YFC-HHHHHH (CYP2C9) and L**I**KMEKEK**Q**NQ**Q**C-HHHHHH and GGNFKKS**N**YFC-HHHHHH (CYP2C19) respectively (bold type indicates residues that differ from the corresponding peptide in other CYP2C forms). The peptides were conjugated to keyhole limpet hemocyanin then used to immunize two 12-week old New Zealand white rabbits (150 μg initially using Freund's complete adjuvant with three subsequent boosters of 75 μg each fortnightly thereafter in Freund's incomplete adjuvant). A third antibody preparation was raised against recombinant CYP2C18 protein, expressed in *E. coli* with a C-terminal hexa-His tag, and purified by Ni^{2+} chelate chromatography as described previously (Cuttle et al., 2000; Shukla et al., 2005). Purified CYP2C18 was used to immunize another pair of rabbits as above but with 100 μg initially followed by three subsequent boosters of 50 μg each fortnightly thereafter. Prior to injection of antigen, 20 mL of pre-immune serum was collected from each animal to check background immunogenicity. Animals were exsanguinated 11 weeks after initial immunization, whole blood was collected, IgG fractions were prepared, and antibodies were affinity-purified as described previously (Booth Depaz et al., 2013) using bacterial membrane preparations containing recombinant CYP2C enzymes. Isolated antibodies raised against CYP2C9, CYP2C19 or CYP2C18 were tested for specificity to all four recombinant human CYP2C proteins (CYP2C9, CYP2C19, CYP2C8 and CYP2C18)

expressed in bacterial membrane fractions (Supplementary table 1) (Cuttle et al., 2000; Shukla et al., 2005).

Frozen human brain samples were obtained from the New South Wales Tissue Resource Centre (NSW TRC). Five brain regions (frontal cortex (Brodmann area 9 – BA9), anterior hippocampus, basal ganglia, amygdala and cerebellum) from three different alcohol- and illicit drug-free patient samples (a total of 15 tissue samples) were provided as dissected sections from the right hemisphere. The histological appearance was reported as normal at both the macroscopic and microscopic level. Patient histories are given in Supplementary table 2. Samples utilized to compare the expression of P450s in the alcoholic and non-alcoholic brain were obtained from 12 different male subjects matched for age (Supplementary table 3). Brain microsomes were prepared as described previously (Booth Depaz et al., 2013) from ~500 mg frozen brain tissue.

Either 10 µg (CYP2C18 antibody) or 20 µg protein (CYP2C9 and CYP2C19 antibodies) were subjected to immunoblotting as described previously (Booth Depaz et al., 2013). β-Actin immunoreactivity detected with a mouse anti-β-Actin monoclonal primary antibody (used at a dilution of 1:20,000, Sigma, St. Louis, MO) was utilized as a loading control. Immunoreactive protein was detected with an Odyssey near-infrared imaging system (LI-COR, Lincoln, NE) using AlexaFluor 680-labeled goat anti-rabbit IgG (1:5,000; Invitrogen) and IRDye 800-labeled donkey anti-mouse IgG (1:20,000; Rockland Immunochemicals, Gilbertsville, PA) secondary antibodies. For quantitation of the alcoholic frontal cortex immunoblots, individual sample optical densities (OD) were corrected against β-actin immunoreactivity (OD of target protein/OD β-actin) and the corrected measurements were used for statistical analysis. Data were subjected to *t*-test analysis with a confidence interval (CI) of 95 %. Fluorescent immunohistochemistry of paraffin-embedded sections was done as

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

described previously using an AlexaFluor 488-labeled goat anti-rabbit IgG secondary antibody (Booth Depaz et al., 2013).

Results and Discussion

The affinity purified CYP2C9 and CYP2C19 peptide antibodies specifically detected their respective target proteins; no cross reactivity against the other known human CYP2C P450s was observed in immunoblot analyses under the conditions used (Supplementary figure 1). By contrast, the antibody raised against recombinant CYP2C18 protein detected all four CYP2C forms tested in this study and is referred to hereafter as the “all-2C” antibody (Supplementary figure 1). CYP2C9 and CYP2C19 were both expressed in the microsomal (110 000 x g pellet) fractions of the cortex, hippocampus, amygdala, basal ganglia and cerebellum of the human brain (Figure 1A and 1B). The “all-2C” antibody detected CYP2C in microsomal fractions of all five brain regions as well as liver microsomes (Figure 1C). CYP2C9 and CYP2C19 were expressed predominantly in the somatic region of neuronal cells, with expression frequently extending to the axonal hillock (Figures 2 and 3), and further down axons and dendrites in some brain regions. Both CYP2C9 and CYP2C19 were detected from layer 2 through to layer 6 of the frontal cortex, although CYP2C9 was detected to a lesser extent in layer 2 (Figure 2A). CYP2C9 and 2C19 were both located in the hippocampus, where expression of both P450s appeared to be highest in the CA1 and CA3 regions (Figure 2B). CYP2C9 and CYP2C19 expression was predominantly localized in the soma of neurons in the amygdala, but both somatic and axonal expression was observed in the basal ganglia. Both forms were localized to the pyramidal cells and granular cell layer of the cerebellar cortex (Figure 3).

To the best of our knowledge this is the first study to characterize the expression of individual CYP2C forms at the protein level in the human brain; however several groups

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

have reported CYP2C mRNA in brain. McFadyen et al. (1998) detected CYP2C mRNA in frontal and temporal cortex, basal ganglia, midbrain and cerebellum but not pons or medulla, by PCR using primers designed to amplify all CYP2C forms, subsequently identifying the product as CYP2C8. Klose et al. (1999) found both CYP2C8 and CYP2C18 but not CYP2C9 or CYP2C19 mRNA in whole brain. Dauchy et al. (2008, 2009) also found CYP2C8 in cortex and microvessels, and both CYP2C8 and CYP2C18 in a cerebral microvascular endothelial cell line. Dutheil et al. (2009) found sufficient CYP2C8 expression to quantify in total human brain, whereas CYP2C9 was detected but not quantifiable, and neither CYP2C18 nor CYP2C19 was detected. In addition, CYP2C9 was observed in several different types of brain tumor (Knupfer et al., 1999). Finally, data from vast transcriptomic studies reported in the Genecard database (http://www.genecards.org/, accessed in November 2014) suggest all CYP2C transcripts might be expressed, to some extent, in several human brain regions; however specificity and tissue source for those data are unclear, thus may not be directly comparable to those obtained in our study and those cited above.

There is evidence also that specific CYP2C forms are expressed in rodent brain including cortex and hippocampus (Alkayed et al.; Luo et al., 1998; Riedl et al., 2000; Iliff et al., 2007). CYP2C11 expressed in astrocytes and perivascular neurons was proposed to be an arachidonic acid epoxygenase involved in the regulation of cerebral blood flow in rats (Alkayed et al.; Iliff et al., 2007). Human CYP2C family enzymes may play a similar role in regulating cerebral blood flow (Gervasini et al., 2004); CYP2C8 and CYP2C9 were shown to have arachidonic acid epoxygenase activity (Rifkind et al., 1995) and CYP2C9 has been suggested to regulate blood flow in skeletal muscle during exercise (Hillig et al., 2003).

The fact that CYP2C9 and CYP2C19 protein were clearly detected in specific cell types across several brain regions in the current study contrasts with the limited detection of mRNA for these two forms in previous studies. While the affinity purified antibodies

appeared specific for the cognate antigens when tested on immunoblots, we cannot exclude the possibility that the antibodies are detecting an altered CYP2C antigen in the immunohistochemical analyses. However a BLAST search failed to find any proteins other than CYP2C enzymes with the relevant linear epitopes, so any non-CYP2C proteins detected would represent conformational mimics, an unlikely, but not impossible, prospect. Alternatively, the P450 proteins, or at least peptide antigens derived therefrom, may be more resistant to degradation during the postmortem delay compared to mRNA. Significantly, both CYP2C18 and CYP2C19 were expressed in brain at the mRNA level in mice transgenic for these two genes (Lofgren et al., 2008). Moreover, in liver, P450 protein expression correlates with mRNA transcript levels for only some P450s (Ohtsuki et al., 2012).

CYP2C9 expression was higher in the microsomal fractions of the frontal cortex of brains from individuals with a history of alcohol abuse than in age- and sex-matched controls (Figure 4A; $P < 0.05$, 95 % CI). By contrast, no difference was observed in the expression of CYP2C19 in the microsomal fractions between brains from alcoholics and controls (Figure 4B). CYP2C9 expression does not appear to be regulated by alcohol exposure in liver (Guengerich, 2005); however other reports have shown that the regulation of other P450s differs both quantitatively and qualitatively between liver and brain (Hesse et al., 2004), so this statistically significant association, albeit of small effect size, merits further exploration.

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgments

We are grateful to the donors and their next of kin and the neuropathologists from the NSW TRC for providing the tissue samples used in this study. Thanks are extended to Prof. P. Beaune (Univ. Rene Descartes) and Dr. F.J. Gonzalez (NIH) for donation of the CYP2C18 and CYP2C8 cDNAs and to Dr. Peter Dodd for helpful comments.

Authorship contributions

Participated in research design: Booth Depaz, Wilce, Gillam

Conducted experiments: Booth Depaz, Toselli

Contributed new reagents or analytic tools: Wilce

Performed data analysis: Booth Depaz, Toselli, Gillam

Wrote or contributed to the writing of the paper: Booth Depaz, Toselli, Gillam

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

References

Alkayed NJ, Birks EK, Hudetz AG, Roman RJ, Henderson L, and Harder DR (1996) Inhibition of brain P-450 arachidonic acid epoxygenase decreases baseline cerebral blood flow. *Am J Physiol-Heart Circul Physiol* **271:**H1541-H1546.

Booth Depaz IM, Toselli F, Wilce PA, and Gillam EMJ (2013) Differential expression of cytochrome P450 enzymes from the CYP3A subfamily in the drug free and alcoholic human brain. *Drug Metab Dispos* **41:**1187-1194.

Cuttle L, Munns AJ, Hogg NA, Scott JR, Hooper WD, Dickinson RG, and Gillam EMJ (2000) Phenytoin metabolism by human cytochrome P450: Involvement of P450 3A and 2C forms in secondary metabolism and drug-protein adduct formation. *Drug Metab Dispos* **28:**945-950.

Dauchy S, Dutheil F, Weaver RJ, Chassoux F, Daumas-Duport C, Couraud PO, Scherrmann JM, De Waziers I, and Decleves X (2008) ABC transporters, cytochromes P450 and their main transcription factors: expression at the human blood-brain barrier. *J Neurochem* **107:**1518-1528.

Dutheil F, Dauchy S, Diry M, Sazdovitch V, Cloarec O, Mellottee L, Bieche I, Ingelman-Sundberg M, Flinois JP, de Waziers I, Beaune P, Decleves X, Duyckaerts C, and Loriot MA (2009) Xenobiotic-metabolizing enzymes and transporters in the normal human brain: Regional and cellular mapping as a basis for putative roles in cerebral function. *Drug Metab Dispos* **37:**1528-1538.

Gervasini G, Carrillo JA, and Benitez J (2004) Potential role of cerebral cytochrome P450 in clinical pharmacokinetics - Modulation by endogenous compounds. *Clin Pharmacokinet* **43:**693-706.

Guengerich FP (2005) Human cytochrome P450 enzymes, in: *Cytochrome P450* (Ortiz de Montellano PR ed), pp 377-530, Kluwer Academic/Plenum Publishing, New York.

Hesse LM, He P, Krishnaswamy S, Hao Q, Hogan K, von Moltke LL, Greenblatt DJ, and Court MH (2004) Pharmacogenetic determinants of interindividual variability in bupropion hydroxylation by cytochrome P450 2B6 in human liver microsomes. *Pharmacogenetics* **14:**225-238.

Hillig T, Krustrup P, Fleming I, Osada T, Saltin B, and Hellsten Y (2003) Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J Physiol-London* **546:**307-314.

Iliff JJ, Close LN, Selden NR, and Alkayed NJ (2007) A novel role for P450 eicosanoids in the neurogenic control of cerebral blood flow in the rat. *Exp Physiol* **92:**653-658.

Klose TS, Blaisdell JA, and Goldstein JA (1999) Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. *J Biochem Mol Toxicol* **13:**289-295.

Knupfer H, Knupfer MM, Hotfilder M, and Preiss R (1999) P450-expression in brain tumors. *Oncol Res* **11:**523-528.

Lofgren S, Baldwin RM, Hiratsuka M, Lindqvist A, Carlberg A, Sim SC, Schulke M, Snait M, Edenro A, Fransson-Steen R, Terelius Y, and Ingelman-Sundberg M (2008) Generation of mice transgenic for human CYP2C18 and CYP2C19: Characterization of the sexually dimorphic gene and enzyme expression. *Drug Metab Dispos* **36:**955-962.

Luo G, Zeldin DC, Blaisdell JA, Hodgson E, and Goldstein JA (1998) Cloning and expression of murine CYP2Cs and their ability to metabolize arachidonic acid. *Arch Biochem Biophys* **357:**45-57.

McFadyen MCE, Melvin WT, and Murray GI (1998) Regional distribution of individual forms of cytochrome P450 mRNA in normal adult human brain. *Biochem Pharmacol* **55:**825-830.

Ohtsuki S, Schaefer O, Kawakami H, Inoue T, Liehner S, Saito A, Ishiguro N, Kishimoto W, Ludwig-Schwellinger E, Ebner T, and Terasaki T (2012) Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of individual human liver: Comparison with mRNA levels and activities. *Drug Metab Dispos* **40:**83-92.

Riedl AG, Watts PM, Douek DC, Edwards RJ, Boobis AR, Rose S, and Jenner P (2000) Expression and distribution of CYP2C enzymes in rat basal ganglia. *Synapse* **38:**392-402.

Rifkind AB, Lee C, Chang TKH, and Waxman DJ (1995) Arachidonic acid metabolism by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Arch Biochem Biophys* **320:**380-389.

Shukla A, Gillam EM, Mitchell DJ, and Bernhardt PV (2005) Direct electrochemistry of enzymes from the cytochrome P450 2C family. *Electrochem Commun* **7:**437-442.

Footnote

The NSW TRC and Australian Brain Donor Program are supported by the University of Sydney, the Australian National Health and Medical Research Council (NHMRC), the Schizophrenia Research Institute, the National Institute on Alcohol Abuse and Alcoholism, and the NSW Department of Health. Financial support for the NSW TRC was provided by the NIAAA [grant NIH AA12404] and the NHMRC [grant #401551]. This work was supported by the NHMRC [Project grant #210215].

DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1. CYP2C protein expression in human brain microsomes. Affinity-purified polyclonal antibodies raised against CYP2C9, CYP2C19 and recombinant CYP2C18 ("All CYP2C") were used to assess CYP2C9 (A), CYP2C19 (B) and total CYP2C expression (C) in cortex, hippocampus, basal ganglia, amygdala and cerebellum. Twenty micrograms (20 µg) of protein from the 3 samples described in Table 2 were pooled and loaded in each lane for CYP2C9 and CYP2C19 blots. For the blot incubated with the all-2C-antibody, 10 µg of protein were loaded from one representative brain sample. CTX, cortex; HP, hippocampus; BG, basal ganglia; AM, amygdala; CB, cerebellum; HL, human liver microsomes; 2C9, recombinant CYP2C9; 2C19, recombinant CYP2C19; 2C8, recombinant CYP2C8; 2C18, recombinant CYP2C18; M, protein marker; Lµs, human liver P110 000 (microsomal) fraction; LP10, human liver P10 000 fraction. Recombinant proteins used as standards in panels A and B contained truncated N-terminal peptide sequences compared to the full length, native proteins in liver and brain fractions. Recombinant CYP2C9 and CYP2C18 in panel C were expressed from full length constructs (Cuttle et al., 2000; Shukla et al., 2005).

Figure 2. Immunohistochemical detection of CYP2C9 and CYP2C19 expression in the human frontal cortex (A) and hippocampus (B). Negative controls incubated with preimmune sera for CYP2C9 and CYP2C19 were performed with layers 5/6 (A) and CA4 (B) respectively and were representative of other layers. Scale bar = 50 µm. (A) CYP2C9 and CYP2C19 proteins were detected in layer 2 through to layer 6 of the frontal cortex, predominantly in the soma of neuronal cells. Expression of CYP2C9 was also observed in neuronal axons and dendrites, predominantly in layers 5 and 6. (B) CYP2C9 and CYP2C19 were both located throughout the hippocampus, however expression appeared to be highest in the CA1 region in both cases. In addition, CYP2C9 was also highly expressed in the CA3 region.

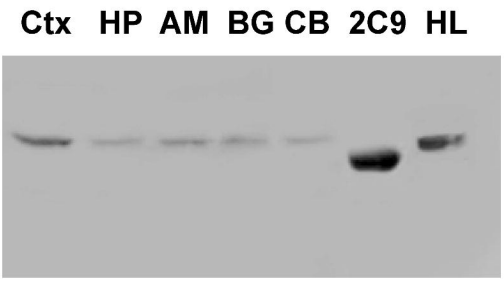
DMD Fast Forward. Published on December 11, 2014 as DOI: 10.1124/dmd.114.061242
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3. Immunohistochemical detection of CYP2C9 and CYP2C19 expression in the human amygdala, basal ganglia and cerebellum. Negative controls incubated with preimmune sera for CYP2C9 and CYP2C19 were performed with the molecular layer of the cerebellum but were representative of other areas. Scale bar = 50 μm. Both P450s were expressed in the amygdala, basal ganglia and cerebellum. In the amygdala, expression was predominantly localized in the soma of neurons, whilst both somatic and axonal expression of both CYP2C9 and CYP2C19 was observed in the basal ganglia. Expression of both CYP2C9 and CYP2C19 was localized to the pyramidal cells and granular cell layer of the cerebellar cortex.

Figure 4. Immunoblot of P110 000 (microsomal) fractions of the frontal cortex of six individual control and six individual alcoholic case-matched human brain samples using antibodies detecting CYP2C9 (A) and CYP2C19 (B). An equivalent amount (20 μg) of total protein was loaded in each lane. C: Individual sample densities were corrected against alpha-tubulin and the corrected data were subjected to a *t*-test with a confidence interval (CI) of 95 %. Data are expressed as mean ± SEM. * CYP2C9 expression in samples from alcoholics was significantly elevated over expression in controls ($P < 0.05$, 95 % CI, $n = 6$). No significant differences were seen in CYP2C19 expression between samples from alcoholics and controls.

Figure 1

A CYP2C9



B CYP2C19

C All CYP2C

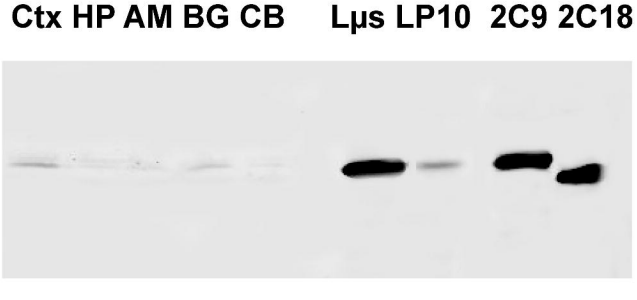


Figure 2

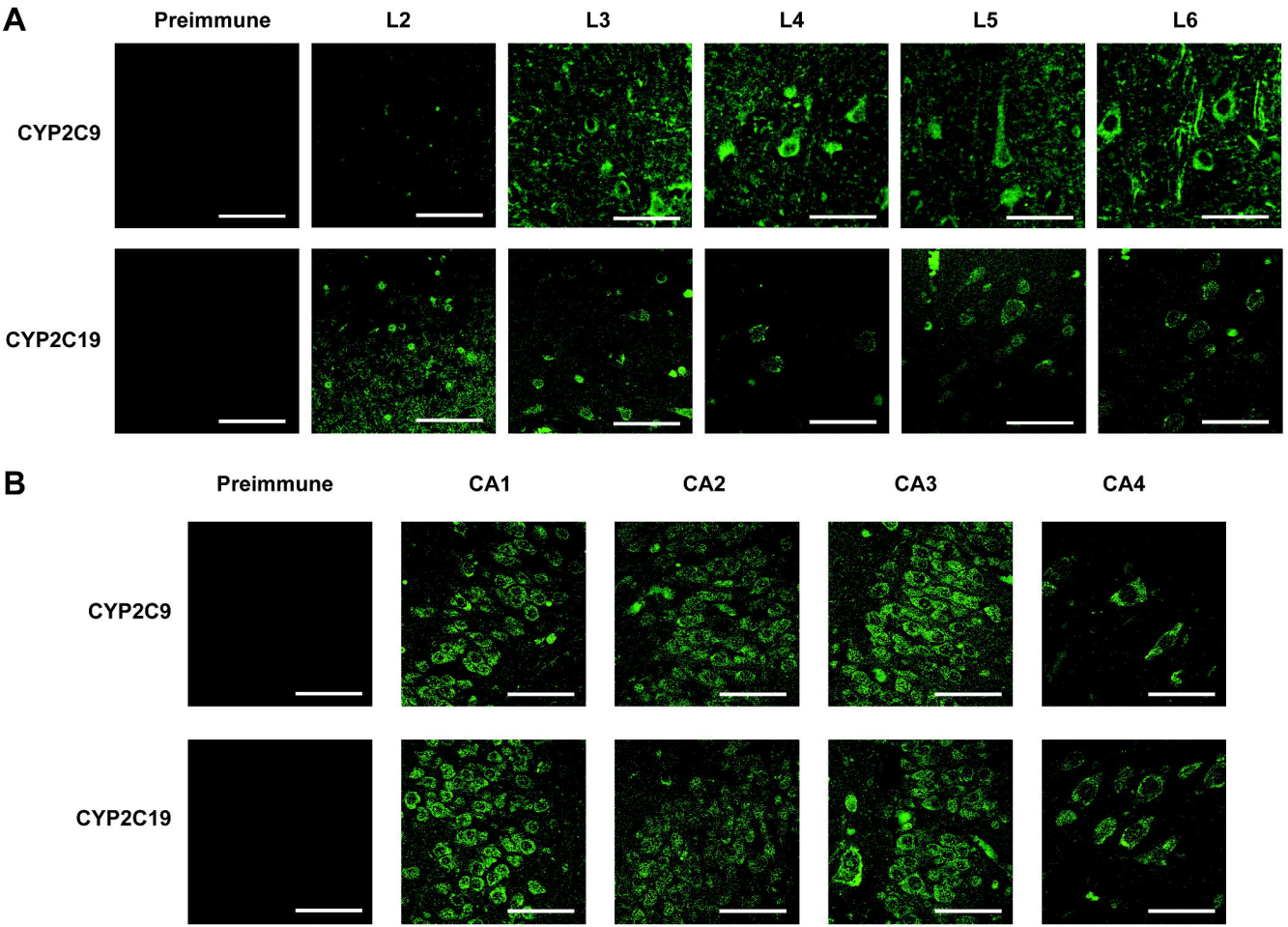

A
Preimmune
L2
L3
L4
L5
L6
CYP2C9
CYP2C19
B
Preimmune
CA1
CA2
CA3
CA4
CYP2C9
CYP2C19

Figure 3

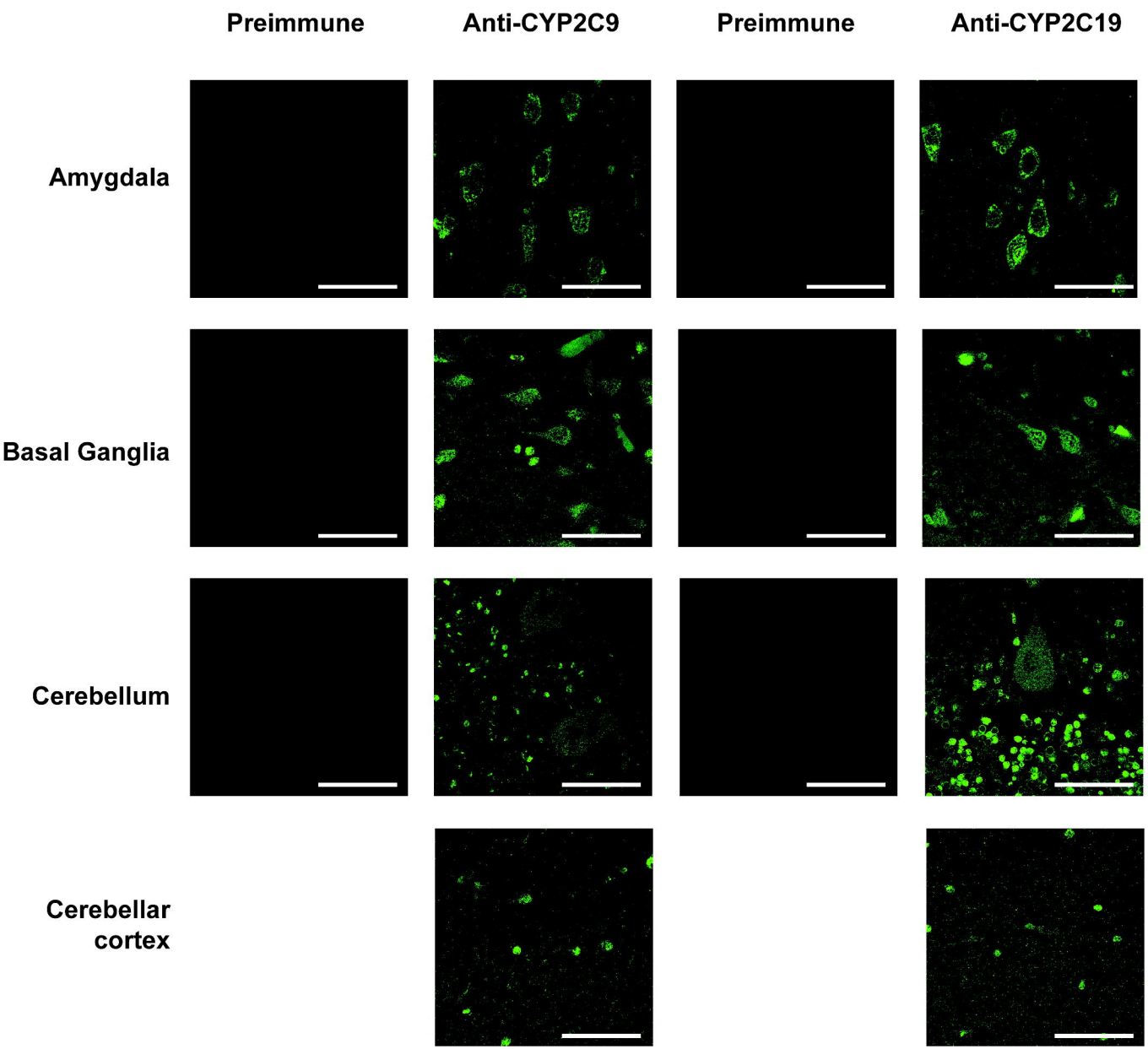
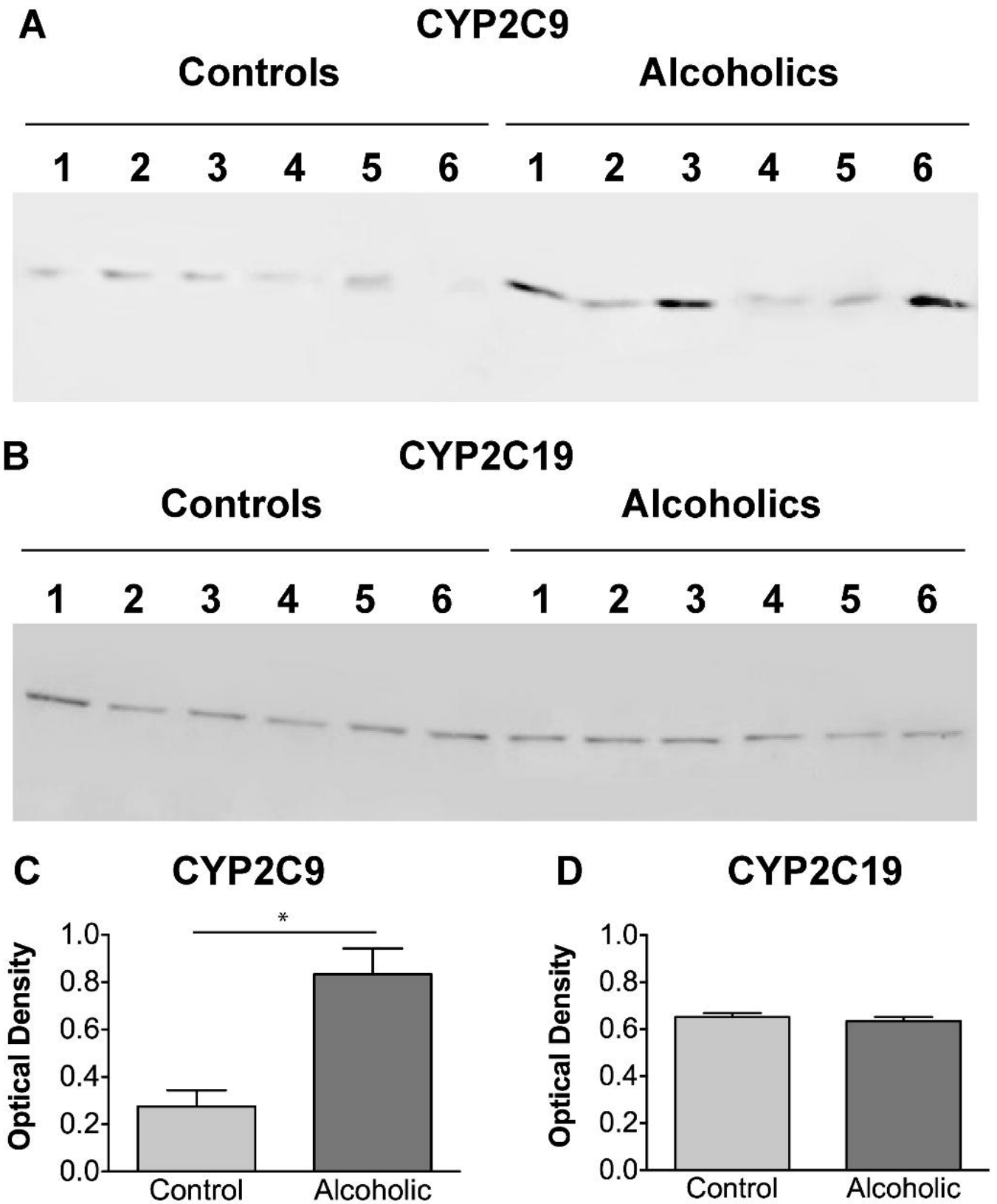

Preimmune
Anti-CYP2C9
Preimmune
Anti-CYP2C19
Amygdala
Basal Ganglia
Cerebellum
Cerebellar cortex

Figure 4


A
CYP2C9
Controls
Alcoholics
1 2 3 4 5 6 1 2 3 4 5 6
B
CYP2C19
Controls
Alcoholics
1 2 3 4 5 6 1 2 3 4 5 6
C
CYP2C9
Optical Density
1.0
0.8
0.6
0.4
0.2
0.0
*
Control
Alcoholic
D
CYP2C19
Optical Density
1.0
0.8
0.6
0.4
0.2
0.0
Control
Alcoholic

Differential expression of cytochrome P450 enzymes from the CYP2C subfamily in human brain

Authors: Iris M Booth Depaz, Francesca Toselli, Peter A Wilce and Elizabeth M J Gillam

School of Biomedical Sciences (IMBD), and

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia, 4072 (FT, PAW, EMJG).

Present address (FT): Department of Pharmacology and Toxicology, University of Toronto, 1 King's College Circle, Toronto, ON, M5S 1A8, Canada.

Address correspondence to:

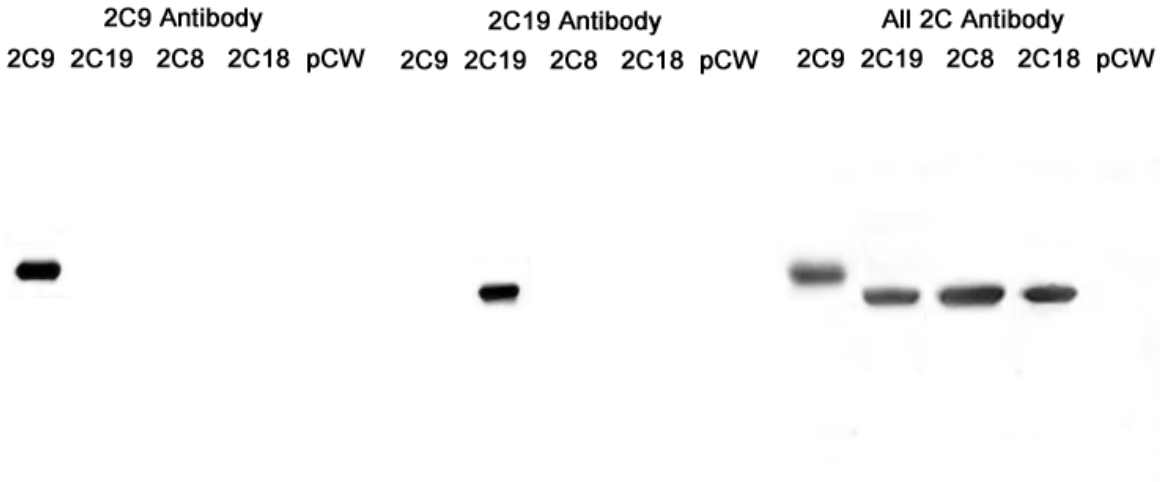
Professor Elizabeth M.J. Gillam, School of Chemistry and Molecular Biosciences,

The University of Queensland, Brisbane, Australia, 4072.

Telephone: +61 7 3365 1410**, Email:** e.gillam@uq.edu.au

Supplementary Information

Supplementary figure 1. Immunoblots to test specificity of antibodies raised against peptides targeted against CYP2C9 (A), CYP2C19 (B) or recombinant CYP2C18 (C) expressed in bacterial membrane fractions. Bacterial membranes containing recombinant CYP2C forms were subjected to SDS PAGE and immonoblotting with antibodies raised against CYP2C9 (A) and CYP2C19 (B) specific peptides and purified recombinant CYP2C18 protein (C). Lanes 1-4, bacterial membranes containing recombinant CYP2C9, CYP2C19, CYP2C8 and CYP2C18 . Lane 5, bacterial membranes from cells transformed with the pCW vector alone.



Supplementary table 1. N-terminal amino acid sequences of recombinant CYP2C forms used for assessing the specificity of antibodies used in the current study

CYP2C9																									
Native:	M	D	S	L	V	V	L	V	L	C	L	S	C	L	L	L	L	S	L	W	R	Q	S	S	G	R	G
Truncated:	M	A																			R	Q	S	S	G	R	G
Expressed:	M	A	L	L	L	A	V	F	L	G	L	S	C	L	L	L	L	S	L	W	R	Q	S	S	G	R	G
CYP2C19																									
Native:	M	D	P	F	V	V	L	V	L	C	L	S	C	L	L	L	L	S	I	W	R	Q	S	S	G	R	G
Truncated:	**M**	**A**																			**R**	**Q**	**S**	**S**	**G**	**R**	**G**
Expressed:	**M**	**A**	**L**	**L**	**L**	**A**	**V**	**F**	**L**	**G**	**L**	**S**	**C**	**L**	**L**	**L**	**L**	**S**	**I**	**W**	**R**	**Q**	**S**	**S**	**G**	**R**	**G**
CYP2C8																									
Native:	M	E	P	F	V	V	L	V	L	C	L	S	F	M	L	L	F	S	L	W	R	Q	S	C	R	R	R
Expressed:	**M**	**A**	**L**	**L**	**L**	**A**	**V**	**F**	**L**	**G**	**L**	**S**	**C**	**L**	**L**	**L**	**F**	**S**	**L**	**W**	**R**	**Q**	**S**	**C**	**R**	**R**	**R**
CYP2C18:																									
Native:	M	D	P	A	V	A	L	V	L	C	L	S	C	L	F	L	L	S	L	W	R	Q	S	S	G	R	G
Expressed:	**M**	**A**	**L**	**L**	**L**	**A**	**V**	**F**	**L**	**G**	**L**	**S**	**C**	**L**	**L**	**L**	**L**	**S**	**L**	**W**	**R**	**Q**	**S**	**S**	**G**	**R**	**G**

Supplementary table 2. Patient history details for control brain samples used for studies of the regio-selective expression of CYP2C proteins.

Sample Code	S1	S2	S3
Age (years)	39	46	42
Gender	Male	Male	Male
Post mortem delay (h)	43	40	20
Cause of Death	Carbon monoxide poisoning	Myocardial Infarction	Mallory-Weiss tear of gastro-oesophageal junction

Supplementary table 3. Clinical details and characteristics pertaining to alcoholic and control samples.

Sample Code	Gender	Age	Post Mortem Delay (h)	Cause of death
C1	Male	50	17	Cardiac coronary artery atheroma
C2	Male	48	19	Coronary thrombosis
C3	Male	51	15	Pulmonary embolism
C4	Male	42	18	Suicide by hanging. Asphyxia
C5	Male	45	16	Dilated cardiomyopathy
C6	Male	40	20	Ischaemic heart disease
A1	Male	51	18	Alcohol toxicity
A2	Male	42	21	Ischaemic heart disease
A3	Male	45	20	Consequences of cirrhosis
A4	Male	40	21	Acute alcohol poisoning
A5	Male	48	22	Bleeding oesophageal varices
A6	Male	50	19	Cardiomyopathy