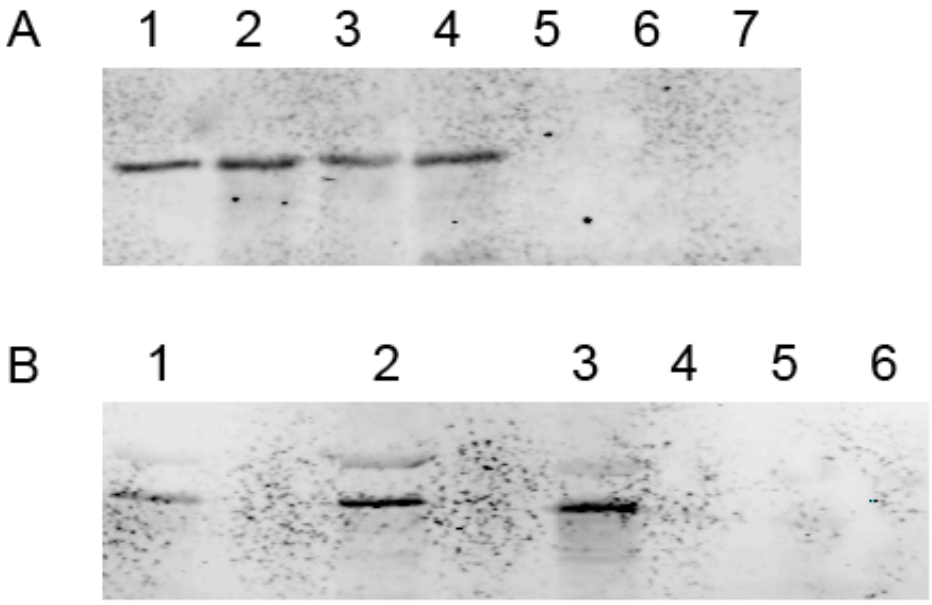
Differential expression of human cytochrome P450 enzymes from the CYP3A subfamily in the brains of alcoholics and drug free controls

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

Drug Metabolism and Disposition

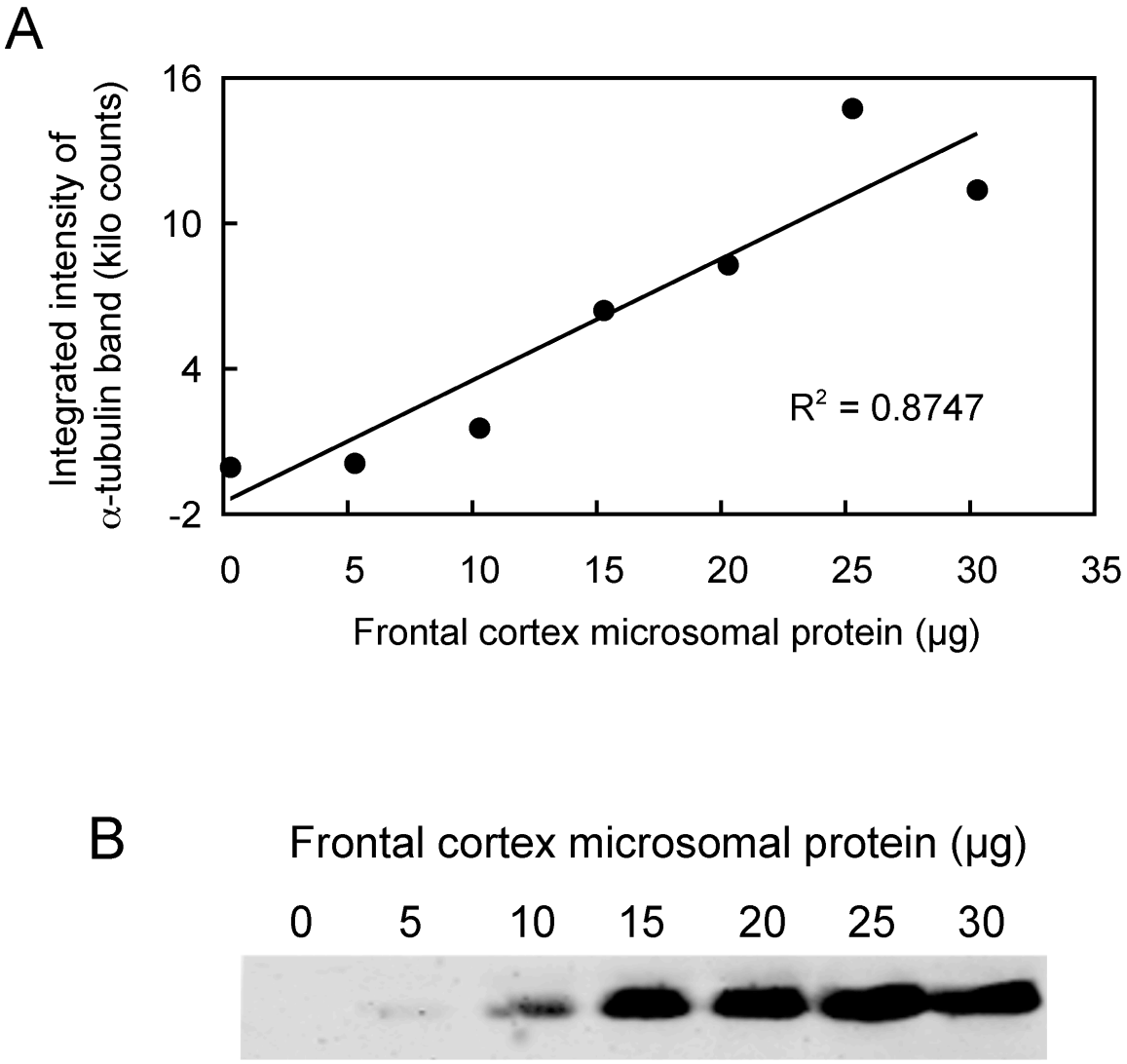
Supplementary information



Supplementary Figure 1. Cross-reactivity of affinity-purified anti-CYP3A4 (A) and anti-CYP3A5 (B) antibodies towards recombinant CYP3A4, CYP3A5, CYP3A7 and CYP3A43 expressed in bacterial membrane fractions.

Bacterial membranes containing recombinant CYP3A forms were subjected to SDS-PAGE and immnoblotting with antibodies raised against purified recombinant CYP3A4 (A) and 3A5 (B). A. Lane 1, purified CYP3A4; lanes 2-5, bacterial membranes containing recombinant CYP3A4, CYP3A5, CYP3A7 and CYP3A43; lane 6, bacterial membranes containing recombinant human NADPH-P450 reductase; lane 7, bacterial membranes from cells transformed with the pCW vector alone. B. Lanes 1-4, bacterial membranes containing recombinant CYP3A4, CYP3A5, CYP3A7 and CYP3A43; lane 5, bacterial membranes containing recombinant human NADPH-P450 reductase; lane 6, membranes from bacteria

transformed with the pCW vector alone. The amount of sample loaded was 0.5 pmol of P450 enzyme, 1.9 pmoles of recombinant reductase and 4.8 μg of total protein for pCW. The cross-reactivity of the anti-CYP3A4 antibody was expressed as the ratio of the band intensity for each form over that for CYP3A4 and was 0.51 and 0.93 for CYP3A5 and 3A7 respectively. The cross-reactivity of the antibody raised against recombinant CYP3A5 for each other form was expressed as the ratio of the blot intensity for each form over that for CYP3A5 and was 0.51 and 0.81 for CYP3A4 and 3A7 respectively.



Supplementary Figure 2 . Linearity of α-tubulin signal in microsomes from human frontal cortex. (A) Detection of α-tubulin signal in increasing amounts of frontal cortex microsomal protein in the range of 0 to 30 µg. Integrated intensity was calculated using the LiCor Odyssey software and expressed in arbitrary units of kilo counts. The line of best fit was obtained by linear regression of the integrated intensity values and shows an R^2 value of 0.8747. (B) Immunoblot showing the linearity of α-tubulin detection under the conditions used.