Gene expression profiling of cytochromes P450, ABC transporters and their principal transcription factors in the amygdala and prefrontal cortex of alcoholics, smokers and drug-free controls by qRT-PCR

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Abstract
1. Ethanol consumption and smoking alter the expression of certain drug-metabolizing enzymes and transporters, potentially influencing the tissue-specific effects of xenobiotics.
2. Amygdala (AMG) and prefrontal cortex (PFC) are brain regions that modulate the effects of alcohol and smoking, yet little is known about the expression of cytochrome P450 enzymes (P450s) and ATP-binding cassette (ABC) transporters in these tissues.
3. Here, we describe the first study on the expression of 19 P450s, their redox partners, three ABC transporters and four related transcription factors in the AMG and PFC of smokers and alcoholics by quantitative RT-PCR.
4. CYP1A1, CYP1B1, CYP2B6, CYP2C8, CYP2C18, CYP2D6, CYP2J2, CYP2S1, CYP2U1, CYP4X1, CYP46, adrenodoxin and NADPH-P450 reductase, ABCB1, ABCG2, ABCA1, and transcription factors aryl hydrocarbon receptor AhR and proliferator-activated receptor α were quantified in both areas. CYP2A6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, adrenodoxin reductase and the nuclear receptors pregnane X receptor and constitutive androstane receptor were detected but below the limit of quantification. CYP1A2 and CYP2W1 were not detected.
5. Adrenodoxin expression was elevated in all case groups over controls, and smokers showed a trend toward higher CYP1A1 and CYP1B1 expression.
6. Our study shows that most xenobiotic-metabolizing P450s and associated redox partners, transporters and transcription factors are expressed in human AMG and PFC.

Keywords
ABC transporters, amygdala, cortex, cytochrome P450, smoking

Introduction
Cytochrome P450 enzymes (P450s) are involved in the metabolism of many drugs that act in the central nervous system including addictive drugs such as nicotine and alcohol (Miksys & Tyndale, 2013). P450s appear to be expressed in a region- and cell-specific manner in the human and rodent brain (Meyer et al., 2007), suggesting that they may catalyze the biotransformation of neuroactive drugs in situ. Recently, P450s have been shown to be active in vivo in rat brain (Miksys & Tyndale, 2013), supporting this proposition. ATP-binding cassette (ABC) transporters work in tandem with P450s to clear xenobiotics and certain endobiotics from the brain and thereby maintain brain homeostasis. Differences in the expression of P450s or ABC transporters may contribute to inter-individual variability in the therapeutic response to, and side effects of, neuroactive drugs.

In addition to their role as substrates, alcohol and nicotine and/or other components of cigarette smoke induce certain P450 proteins in human brain. Variations in the expression of CYP2B6, CYP2D6, CYP2E1 and CYP3A4 proteins in several regions in the human brain have been attributed to alcohol and/or tobacco use (Booth Depaz et al., 2013; Howard et al., 2003; Miksys et al., 2002). Importantly ABC transporters and P450s appear to be subject to similar transcriptional regulation in many instances (Miller, 2010; Pelkonen et al., 2008), suggesting the possibility of coordinate regulation of these functionally related proteins.

The expression of P450s and ABC transporters remains poorly characterized in regions of the brain involved in the reinforcing effects of addictive substances such as the amygdala (AMG) (Booth Depaz et al., 2013; Stark et al., 2008). Moreover, even in regions such as the prefrontal cortex...
(PFC) which have received more attention, the effects of factors such as smoking and alcohol use on P450 and ABC transporter transcript expression are not clear. The aim of this study was to determine whether a subset of drug-metabolizing P450s and their associated proteins were expressed in the AMG and PFC at the mRNA level and determine the degree of variation in individuals classified by alcohol consumption and smoking status.

Materials and methods

Materials

QIAzol, RNeasy Lipid Tissue Mini Kits and QIAquick PCR purification kits were purchased from Qiagen (Qiagen GmbH, Hilden, Germany). Experion RNA StdSens analysis kits were obtained from Bio-Rad Laboratories (Hercules, CA). Superscript III First-Strand Synthesis SuperMix for qRT-PCR and Escherichia coli RNase H were from Invitrogen (Carlsbad, CA). MicroAmp™ Optical 384-well plates and MicroAmp™ Optical Adhesive Film were supplied by Applied Biosystems (Foster City, CA). Nuclease-free water was purchased from Ambion (Life Technologies Ltd., Paisley, UK). ABsolute™ Blue QPCR SYBR® Green ROX Mix was obtained from ABGene (Epsom, Surrey, UK). Taq DNA polymerase was bought from New England Biolabs Inc. (Beverly, MA).

Tissue collection

Unfixed frozen human autopsy brain tissue was collected by the NSW Tissue Resource Centre (University of Sydney, Australia). Samples were collected by qualified pathologists with full ethical clearance (Central Sydney Area Health Service and Ethics Committee of the University of Sydney) and informed consent from the next of kin. Gray matter from PFC (Brodmann area 9) and basolateral AMG were sampled as described previously (Flatscher-Bader & Wilce, 2006) and stored at −80°C.

Case selection and classification

For each case a complete medical history was available, as well as results from the autopsy, which included diagnostic neuropathological examination. None of the patients used in this study showed any histological, neurological or psychiatric disorders. All alcoholic cases satisfied the criteria of the Diagnostic and Statistical Manual for Mental Disorders (DSM IV) of the American Psychiatric Association. Subjects were classified as smokers when they had a record of heavy smoking at the time of death, and non-smokers when they had never smoked. Twenty-two subjects were selected for this study, and were classified as the following: alcoholic smoker (AS: ≥80 g ethanol and ≥5 cigarettes/day or diagnosed as long-term heavy drinker and heavy smoker), alcoholic non-smoker (ANS: ≥80 g ethanol/day or diagnosed as long-term heavy drinker and non-smoker), non-alcoholic smoker (NAS: ≤30 g of ethanol per day, no history of alcohol abuse; ≥10 cigarettes/day or diagnosed as heavy smoker), and non-alcoholic non-smoker (NANS: ≤10 g of ethanol/day and non-smoker). For some subjects only one brain region was available; a summary of the case histories and tissue samples for each subject is given in Supplementary Table 1.

RNA extraction and first-strand cDNA synthesis

All tissue samples (≤10 mg) were homogenized while still frozen in 1 ml of QIAzol on ice. RNA was isolated using an RNeasy Lipid Tissue Mini Kit according to the manufacturer’s instructions. RNA concentration and purity were estimated as previously described (Dutheil et al., 2009). RNA samples were diluted to a concentration of ≤900 ng/µl where necessary and RNA integrity was assayed on an Experion automated electrophoresis system (Bio-Rad Laboratories) using the Experion RNA StdSens analysis kit, following the manufacturer’s instructions. RNA quality indicator (RQI) values were determined by the Experion 3.20 software (Hercules, CA). Total RNA from each sample (700 ng at a final concentration of 35 ng/µl) was reverse-transcribed to cDNA using Superscript III First-Strand Synthesis SuperMix for qRT-PCR, according to the manufacturer’s instructions. Samples were then treated with 2 U of E. coli RNase H (Invitrogen) for 20 min at 37°C and stored at −20°C until use.

qRT-PCR

Primers for qRT-PCR were designed and validated as detailed in Section 1.1 of the Supplementary Information. All PCR reactions were performed using an Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems). The final reaction mixture contained 1 µl cDNA in nuclease-free water (corresponding to 3.5 ng of reverse-transcribed total RNA), 5 µl of ABsolute™ Blue QPCR SYBR® Green ROX Mix, 0.2 µl of each primer (0.2 µM final concentration) and 3.6 µl nuclease-free water. PCR conditions comprised an initial DNA polymerase activation step at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 65°C for 1 min. For each reaction, amplification cycles were followed by a dissociation stage to obtain a melting curve. All reactions were set up manually and performed in triplicate. All samples amplified with the same primer pair were analyzed on the same plate, to allow direct comparison of expression levels between samples, without any effect of between-run variation.

Analysis of qRT-PCR data

No published validation of reference genes (RGs) in brain tissue from alcoholics and smokers exists currently, so an extensive analysis of five alternative RGs was undertaken (see Section 1.5 of the Supplementary Information). Differences in expression of genes of interest (GOI) between case groups and between different brain regions in the same individual were analyzed by the △ΔCq method (Livak & Schmittgen, 2001). GraphPad Prism 5 (La Jolla, CA) was used for all statistical analyses.

CYP1A1 genotyping. cDNA samples from each individual (n = 22) were analyzed for the CYP1A1 A1384G (462V) variant by allele-specific PCR as described previously using the forward primer from (Zhang et al., 1996) and the reverse primer from (Vibhuti et al., 2010). A plasmid expressing the
I462V variant (pCW/1A1(I462V)/CPR) was used as a positive control. Products amplified using non-allele-specific primers were sequenced at the Australian Genome Research Facility (Brisbane node, University of Queensland).

Results

RNA quality and demographic variables

The average RQI across all samples was 6.6 (Min, 3.7; Max, 7.9; on a scale of 1–10 representing most degraded to most intact, respectively). RQI values were significantly lower in AMG versus PFC samples from the same individual (6.1 ± 1.1 and 7.1 ± 0.7 in AMG and PFC, respectively; p = 0.0019, paired, two-tailed Student’s t-test) (Supplementary Figure 1A). RQI values from all smokers and alcoholics were slightly but not significantly higher compared to the NANS group (6.4 ± 0.6 versus 5.3 ± 1.6 for cases and NANS in AMG; 7.2 ± 0.5 versus 6.7 ± 1.0 for cases and NANS in PFC) (Supplementary Figure 1B). The average patients’ age across all samples was 57 years (range 34–82); mean post-mortem interval (PMI) was 25.3 h (range 8.5–59.4); mean brain pH was 6.6 (range 6.2–6.9), and mean brain weight was 1418 g (range 1140–1635). None of these demographic variables differed significantly between cases and NANS controls.

Validation of RGs for normalization

Candidate reference transcripts were validated using three methods: the software packages geNorm and NormFinder, as well as manually (see the Section 1.5 of the Supplementary Information for more details). Upon analysis of RG expression across all samples, as well as between case groups or brain areas, both algorithms returned a comparable ranking of candidate RGs, with the top three being ALG9, ACTB and GAPDH. However, the stability of reference transcripts as determined by geNorm and NormFinder can sometimes disagree with the apparent inter-sample and inter-group variation as judged by relative quantification (Gebhardt et al., 2010). Therefore, expression data were also inspected manually to determine the inter-individual variability and relative expression between brain areas and case groups. Most transcripts showed a trend toward lower expression in NANS subjects than in cases, significantly lower expression in AMG than in PFC samples from the same individual, and higher inter-individual variability in AMG than in PFC. An exception to this pattern was represented by RPL13A, which showed no statistically significant differences between the two brain areas, the lowest variation between NANS and cases, as well as the lowest inter-individual variability in both brain areas. RNA quality and RG expression were strongly positively correlated, with two-tailed Pearson’s correlation returning p values lower than 0.0001 and r coefficients between 0.89 and 0.92 for most transcripts; again, RPL13A represented an exception, showing the lowest correlation with RQI values (r coefficient of 0.77).

This result is consistent with a previous report that expression of RPL13A is very stable in human brain samples (Gebhardt et al., 2010). As a result, the expression pattern of all other candidate RGs reflected the RQI variation seen between case groups or brain areas, whereas RPL13A underestimated (and PGK1 over compensated for) differences in RNA quality. Accordingly, ALG9 was chosen for normalization of GOI expression and all results shown hereafter reflect normalization with this RG. ALG9 has the added advantage of showing expression levels within the same linear range as that of target transcripts (data not shown). However, generally comparable results were also obtained after normalization with ACTB, GAPDH and the geometric mean of ALG9 and GAPDH (results not shown).

Expression of target genes in AMG and PFC

Of the 25 transcripts examined, CYP1A1, CYP1B1, CYP2B6, CYP2C8, CYP2C18, CYP2D6, CYP2E1, CYP2J2, CYP2S1, CYP2U1, CYP4X1, CYP46, cytochrome P450 reductase (CPR), Adrenodoxin (Adx), ABCA1, ABCB1, ABCG2, aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor α (PPARα) were expressed in both brain regions at levels sufficient for quantification (C_q < 33). CYP2A6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, adrenodoxin reductase (Adr) and the transcription factors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) were also detected in all replicates of the majority of samples, but at low levels (C_q > 33) and with more than twofold differences in expression between technical triplicates of the same sample, and were therefore not quantified. CYP1A2 and CYP2W1 were detected in some technical triplicates of a small number of samples, but always at C_q values greater than 36. Due to their extremely low levels and sporadic detection, CYP1A2 and CYP2W1 were considered not to be detectable.

Correlation with demographic variables

Using a p value of less than 0.01 and r coefficient greater than 0.5 to define a significant correlation (two-tailed Pearson’s correlation), PMI, brain pH and brain weight had no statistically significant influence on the expression of any GOI. CYP2U1, ABCA1, CPR and PPARα levels showed a positive correlation with age, but only in AMG (r values of 0.70, 0.73, 0.76 and 0.71, respectively; p values of 0.0024, 0.0013, 0.0007 and 0.0020, respectively; Supplementary Figure 2), whereas a trend toward significance was observed for a positive correlation of CYP2D6 expression with age in PFC (r value = 0.5482; p value = 0.0151; Supplementary Figure 2).

Relative expression between case groups

Fold changes in expression in AS, ANS and NAS case groups over the controls (NANS) were analyzed separately for each brain area using a one-way ANOVA with Tukey’s post hoc test where appropriate; p < 0.05 was considered statistically significant. The same expression patterns could be identified regardless of the RG used for normalization, but the relative quantities varied. No statistically significant differences were observed between any subject groups except that in AMG, Adx expression was significantly higher in AS (1.4 ± 0.1 fold, p < 0.05), ANS (1.7 ± 0.1 fold, p < 0.01) and NAS (1.4 ± 0.2 fold, p < 0.05) than NANS (Figure 1A and B). In addition, a clear, but not statistically significant, trend toward higher expression of CYP1A1 and CYP1B1 was noted in both brain areas (for CYP1A1) and in AMG (for CYP1B1) of smoker subjects.
Relative expression between brain areas

Six GOIs showed higher expression in AMG than in PFC within the same individual (paired, two-tailed, Student’s t-test), namely CYP2D6 (2.5 ± 0.9-fold higher, \( p = 2.2 \times 10^{-6} \)), CYP2J2 (3.6 ± 2.2-fold higher, \( p = 5.4 \times 10^{-6} \)), CYP2U1 (1.4 ± 0.3, \( p = 7.4 \times 10^{-3} \)), CPR (1.3 ± 0.3, \( p = 0.0022 \)), PPARα (2.5 ± 1.3, \( p = 0.0035 \)) and ABCA1 (4.3 ± 1.3, \( p = 3.2 \times 10^{-5} \)) (Figure 2). Conversely, CYP2E1 and CYP4X1 showed higher expression in PFC (0.7 ± 0.3 and 0.8 ± 0.2-fold changes over PFC, respectively; \( p = 0.0045 \) and \( p = 0.0093 \), respectively; Figure 2).

Correlation with expression of transcription factors

Co-variation of expression of several GOIs with their related transcription factors was examined across all samples \( (n = 35) \), and separately in each brain area \( (n = 16 \) AMG; \( n = 19 \) PFC). Three correlations were found to be significant \( (p < 0.01 \) and \( r \) coefficient ≥ 0.5, two-tailed Pearson’s correlation), all involving PPARα. PPARα expression (Figure 3) correlated positively with that of: ABCA1 \( (r = 0.91, 0.91 \) and 0.85 for all samples, AMG and PFC, respectively, all \( p \) values <0.0001); CYP2J2 \( (r = 0.82, 0.68 \) and 0.73 for all samples, AMG and PFC, respectively, \( p \) values <0.0001, 0.0035 and 0.0004, respectively) and CYP2U1 \( (r = 0.73, 0.83 \) and 0.67 for all samples, AMG and PFC, respectively, \( p \) values <0.0001, <0.0001 and 0.0016, respectively).

**CYP1A1 genotyping**

CYP1A1 expression in AMG from case AS2 and PFC from AS4 was markedly higher (11.2- and 4.5-fold, respectively) than NANS (Figure 1). A PCR designed to detect any and all CYP1A1 variants amplified a band of the expected size in cDNA from all individuals (Supplementary Figure 3A). However, an allele-specific PCR revealed the A1384G (I462V) polymorphic variant in two NANS samples (NANS3 and NANS5) only (Supplementary Figure 3B and C). Sequencing showed that AS4 and AS2 expressed the homozygote wild-type CYP1A1 genotype (Supplementary Figure 3D), whereas the two NANS individuals were heterozygous for the polymorphic variant (Supplementary Figure 3E). The allele frequency for the variant A1384G (I462V) in this population was calculated to be 4.5% \( (n = 22 \) individuals).

**Discussion**

The quantification of mRNA by qRT-PCR is sensitive to differences in RNA quality, and RT and amplification efficiency, necessitating the use of appropriate RGs for
normalization of expression levels (Tunbridge et al., 2011). Optimal RGs must not be co-regulated with the target GOIs but should vary in response to all other confounding experimental variables (including RNA degradation) to a similar extent. ALG9 was determined to be the most suitable RG for comparison of GOI expression between brain samples of alcoholics, smokers and drug-free controls. To our knowledge, this is the first report on the validation of RGs for these conditions.

Of the transcripts investigated here, CYP1A1, CYP1B1, CYP2B6, CYP2C8, CYP2C18, CYP2D6, CYP2E1, CYP2J2, CYP2S1, CYP2U1, CYP4X1, CYP46, CPR, Adx, ABCB1, ABCG2, ABCA1, AhR and PPARα could be quantified in PFC and AMG. Detection of CYP1A1, CYP1B1, CYP2B6, CYP2D6, CYP2E1, CYP2J2, CYP2S1, CYP2U1, CYP4X1, CYP46, CPR, Adx, ABCB1, ABCG2, ABCA1, AhR and PPARα could be quantified in PFC and AMG. Detection of CYP1A1, CYP1B1, CYP2B6,
CYP2C8, CYP2D6, CYP2E1, CYP2J2, CYP2S1, CYP2U1 and PPARα mRNA in PFC agrees with previous studies on human cortex (Dauchy et al., 2008; Dutheil et al., 2009) and a single whole brain sample (Nishimura et al., 2003). All P450s quantified here have also been detected in human cortex at the protein level, except for CYP2C8, CYP2C18 and CYP4X1, which have not been tested (Bhagwat et al., 2000; Dutheil et al., 2009; Howard et al., 2003; Miksys et al., 2002, 2003; Siegle et al., 2001). While CYP2C18 mRNA was not detected previously in human cortex using the same primers (Dauchy et al., 2008), the cortical region studied was not specified, so differential expression within the cortex, as observed for other P450s (Miksys et al., 2002; Stark et al., 2008), may explain this discrepancy. Of the 19 transcripts quantified here, only ABCA1 (Langmann et al., 1999) and CYP4X1 (Stark et al., 2008) had previously been reported in human AMG and none have been documented at the protein level to date. This is the first study to report AdR mRNA expression in the human brain, but CPR, AdR and Adx proteins have been detected previously in the human cortex (Bhagwat et al., 2000; Dutheil et al., 2009), in accordance with results from both brain regions examined here.

In previous studies, CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2W1, CYP3A4 and CYP3A5 transcripts were either barely, or not, detectable in human cortex depending on the C_q threshold used for detection (Dauchy et al., 2008; Dutheil et al., 2009; Nishimura et al., 2003) consistent with the present results. However, of these P450s, CYP2C9, CYP2C19, CYP3A4 and CYP3A5 have been reported at the protein level in human brain, including in frontal cortex and AMG (Booth Depaz et al., 2013, 2015). In addition, CYP2C9 and CYP3A4 protein expression was found to be elevated in the frontal cortex from alcoholic subjects (Booth Depaz et al., 2013, 2015).

It is important to stress that while mRNA detection and quantification is a valuable initial step in gene expression studies, as it is a sensitive approach that allows screening for a relatively high number of transcripts, it is not always predictive of protein expression levels or enzyme activity in human tissues or in vivo (Guo et al., 2008; Tian et al., 2004). This can be explained by technical discrepancies, as techniques for transcript detection have generally better sensitivity and specificity, or biologically, as RNA may have higher transcript instability during the post-mortem delay, as

Figure 3. Correlation of ABCA1, CYP2J2 and CYP2U1 with PPARα expression. Correlation was examined with a two-tailed Pearson’s correlation for -ΔCq values, with all samples considered together, as well as separately for each brain area. Relative expression of GOIs was normalized to ALG9. (A–C) ABCA1 expression showed consistent positive correlation with that of PPARα in both brain areas. (D–F) CYP2J2 expression showed positive correlation with that of PPARα in both AMG and PFC, with normalization to any reference transcript except RPL13A. (G–I) CYP2U1 expression showed positive correlation with that of PPARα in both brain areas.
compared with protein, and it can be subjected to post-transcriptional regulation.

As such, while no effect of alcohol consumption or cigarette smoking was observed here on the expression of any P450 transcript, increased CYP2E1 and CYP2U1 protein expression was observed in the same alcoholic brain samples analyzed here, with CYP2U1 protein also being elevated in smokers (Toselli et al., 2015). This is consistent with a post-transcriptional regulatory mechanism of expression and might occur through substrate-mediated stabilization or protection from proteolytic degradation. This has been suggested previously for CYP2E1 (Eliasson et al., 1990; Roberts et al., 1995), and might be extended to CYP2U1, which metabolises long-chain saturated and unsaturated fatty acids (Chuang et al., 2004), known to be elevated in the steatotic alcoholic liver (as reviewed in Altamirano & Bataller, 2011).

ABCA1 mRNA (Langmann et al., 1999) and ABCB1 and ABCG2 mRNA and protein have been detected previously in the human brain (Dauchy et al., 2008; Dutheil et al., 2009; Warren et al., 2009) in line with results obtained here for the PFC and AMG. Expression of the transcription factors AhR, CAR, PXR and PPARγ in the human cortex (Dauchy et al., 2008; Dutheil et al., 2009; Nishimura & Naito, 2005; Nishimura et al., 2004) was confirmed here and extended to the human AMG. The expression of PPARγ was positively correlated with that of CYP2J2 and CYP2U1 in both regions suggesting coregulation of their expression. PPARγ regulates several other P450s that metabolize fatty acids (FAs) which are also substrates for CYP2J2 and CYP2U1 (Chinetti et al., 2001; Chuang et al., 2004; Wu et al., 1996). Interestingly, CYP2J2-generated arachidonic acid (AA) metabolites have been shown to activate PPARγ mediated transcription in vitro and in vivo (Wray et al., 2009). A common mechanism that increases both CYP2J2 and PPARγ, thereby also regulating the expression of other FA-metabolizing enzymes, could represent a homeostatic response to increased intracellular AA levels. Similarly, the strong correlation between the expression of the cholesterol transporter ABCA1 and its transcription factor PPARγ could reflect a concerted response to increased intracellular cholesterol levels. Further work is needed, however, to test these hypotheses.

A positive correlation of CYP2U1, CPR, ABCA1 and PPARγ mRNA expression with age was observed in AMG, and a trend toward a positive correlation of CYP2D6 expression with age was found in PFC. While this is in contrast with previous findings on PPARγ mRNA and protein expression with age in rat heart and liver (Iemitsu et al., 2002; Ye et al., 2005), and on CPR protein expression in human liver (Gan et al., 2009; George et al., 1995), a positive correlation of CYP2D6 protein expression with age has been reported in human frontal cortex, cerebellum and substantia nigra (Mann et al., 2012). This increase in CYP2D6 expression in the brain of aged individuals might represent a physiological response to prolonged exposure to xenobiotics, and may alter the effect of several neurochemicals, and brain-acting drugs and toxins. Changes in ABCA1 and CYP2U1 expression with age have not been previously documented. However, cholesterol levels increase in the brain of older rats (Calderini et al., 1983; Cutler et al., 2004). Thus, up-regulation of its efflux transporter ABCA1 could represent a mechanism to maintain cholesterol homeostasis. Conversely, levels of AA and docosahexaenoic acid, known substrates of CYP2U1 (Chuang et al., 2004), decrease with age in rat brain, probably due to a decrease in phospholipase activity (Barcelo-Coblijn et al., 2003; Lynch & Voss, 1994). A parallel increase in CYP2U1-mediated hydroxylation of these lipids could also partially explain such findings, although this has not been proven directly.

Statistically significant effects of smoking or alcohol consumption on mRNA expression were only observed in the current study for Adx, which was modestly but significantly induced in the AMG of alcoholic and smoker subjects. Adx is regulated by adrenocorticotropic hormone (ACTH) at both the transcript and protein level (Golos et al., 1987; Hanukoglu et al., 1990). Chronic alcohol consumption and nicotine both increase ACTH and cortisol secretion in animal models (Matta et al., 1987; Rivier, 1996), and increased hypothalamo–pituitary–adrenal axis activity is found in human alcoholics (Eskay et al., 1995), so increased ACTH levels may lead to Adx induction in alcoholics and smokers.

Elevated CYP1A1 and CYP1B1 mRNA expression has been shown in extrahepatic tissues from smokers and in human cell lines treated with cigarette smoke condensate or the cigarette smoke component, benzo[a]pyrene (Buchthal et al., 1995; Chi et al., 2009; Hakkola et al., 1997; Iwanari et al., 2002; Nagaraj et al., 2006; Thum et al., 2006). In the current study, while no statistically significant differences were evident, a trend toward induction was observed for CYP1A1 (in PFC and AMG) and CYP1B1 (in AMG) in samples from smokers, with two AS individuals showing particularly high CYP1A1 expression levels. The A1384G (I462V) mutation at 2454 bp on the CYP1A1 gene sequence, rs1048943, has been associated with increased inducibility (Crofts et al., 1994; Hayashi et al., 1991). Interestingly, the same SNP has been associated with the risk of chronic obstructive pulmonary disease (COPD) (Vibhuti et al., 2010), and AS2, the individual with the highest relative CYP1A1 expression, died from chronic airflow limitation, a symptom of COPD (Supplementary Table 1). However, the A1384G variant was only present in two NANS individuals, not AS2, so no association between the A1384G polymorphism and elevated CYP1A1 mRNA expression was detected here.

Interindividual variation in relative expression levels was much lower for Adx in the AMG than for CYP1A1, CYP1B1 and all other transcripts quantified here, a factor that may have facilitated detection of a statistically significant effect. A priori statistical power analyses require an estimate of the degree of variation in a sample population; however, such information was not available prior to this study due to the lack of previous studies on P450 transcriptional regulation by smoking or alcohol in human brain. Gene expression is uniquely regulated in brain as compared with other tissues, and data from animal models cannot be quantitatively compared with human data, due to their higher homogeneity of genetic background, drug doses and exposure duration and to the lack of post-mortem delay. Using our data as basis for an a priori power analysis, in a population with a relatively
large differences in expression, but also a high variance, such as that for CYP1A1 expression in smokers, a total of 228 samples was computed to be necessary to achieve a power of 0.8 when the highest standard deviation among all groups was used in the calculations (performed with G*Power 3, version 3.1.7 (Düsseldorf, Germany); (Faul et al., 2007)). Unfortunately, not only is a large variance in gene expression inevitable in human cases, but it increases particularly when expression is altered by drug exposure (as may be the case for CYP1A1 and CYP1B1), due to differences in the drug dose/exposure, delay from last exposure to the drug, genetics and other concurrently used drugs, etc. Thus, even if the very large sample size needed had been known before the study, the practical limitations of sourcing sufficient samples from such specific human cases would have precluded achievement of this sample size. Indeed, our study has the largest sample populations for specific case types (ANS, AS, NAS) used to date for the study of modulation of P450 and ABC transporter expression in the human brain (Howard et al., 2003; Miksys et al., 2002, 2003). This study represents a useful pilot study, the results of which will facilitate a priori power analysis to aid in the planning of future studies to examine the effect of alcohol and smoking on the expression in brain of enzymes and transporters responsible for xenobiotic clearance.

Conclusions

In summary, this study provides evidence that the majority of drug-metabolizing P450s and some of their associated regulatory factors and redox partners as well as associated transporters are expressed at the transcript level in human AMG and PFC and may influence the effects of drugs in those regions. It also provides important pilot data that will be useful for the design of future studies to assess the effect of alcohol use and smoking on the expression of these genes in the human brain.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Supplementary material available online
Supplementary Figures 1–7 and Supplementary Tables 1–6