DMD Fast Forward. Published on October 16, 2007 as doi:10.1124/dmd.107.017228

DMD#17228

Title Page

Transcription factors and drug metabolizing enzymes genes expression in lymphocytes from human healthy subjects

Gérard Siest, Elise Jeannesson, Jean-Brice Marteau, Anastasia Samara, Bérangère Marie, Michèle Pfister, Sophie Visvikis-Siest

Equipe Inserm «Génétique Cardiovasculaire» CIC 9501 ; Faculté de Pharmacie ; Université Henri Poincaré - Nancy I, 30 rue Lionnois, 54000 Nancy, France.

Running title Page

Running title: Transcription factors and CYP450 expression in lymphocytes

Corresponding author:

Pr Gérard Siest

Faculté de Pharmacie

Equipe Inserm «Génétique Cardiovasculaire» CIC 9501

30 rue Lionnois

54000 Nancy - France

Tél. 33 (0)3 83682170

Fax. 33 (0)3 83321322

E-mail : gerard.siest@pharma.uhp-nancy.fr

Number of text pages: 32

Number of tables: 2

Number of figures: 5

Number of references: 49

Number of words in Abstract: 217

Number of words in Introduction: 614

Number of words in Discussion: 1579

List of standard abbreviations:

AHR Aryl Hydrocarbon Receptor

ARNT Aryl Hydrocarbon Receptor Nuclear Translocator

BMI Body Mass Index

CAR Constitutive Androstane Receptor

CYP Cytochrome P450

DME Drug Metabolizing Enzyme

EH Epoxyde Hydrolase

FXR Farnesoid X-activated Receptor

GR Glucocorticoid Receptor

GST Glutathion S Transferase

MEF2A Myocyte Enhancer Factor-2

LXR Liver X Receptor

NAT N Acetyl Transferase

PBMC Peripheral Blood Mononuclear Cell

PPAR Peroxisome Proliferator-Activated Receptor

PXR Pregnane X Receptor

ST Sulfo Transferase

TCF7 Transcription Factor 7

TF Transcription factor

VDR Vitamin D Receptor

Abstract

We aimed to measure simultaneously in healthy subjects lymphocytes the expression of drug metabolizing enzymes (DME) and transcription factors (TF) with high importance in cardiovascular physiopathology. RNA was isolated from peripheral blood mononuclear cells (PBMC) of twenty subjects from the Stanislas Cohort. We used a microarray approach to measure sixteen DME and thirteen TF. Cytochromes P450 including CYP2C19, CYP2C9, CYP2J2, CYP2D6, CYP1A1, CYP4F2, CYP4A11, CYP2E1, CYP11B2, CYP2C18 and CYP2A6 were expressed in all subjects. CYP3A4 and CYP3A5 were not expressed. GST were expressed but GSTM1 only in some subjects. PXR, MEF2A, VDR, LXRα, AHR, TCF7, CAR and ARNT were expressed in the majority of the subjects. GR, PPARγ, and LXRβ, were expressed only in some individuals. PPARα mRNA was found in one subject only and FXR was not expressed. In addition, we found significant correlations between the expression of AHR, ARNT and CYP1A1 and between PXR and CYP involved in leukotrienes metabolism (CYP2C, CYP4F2, CYP4A11, CYP2J2 and CYP11B2). We describe here for the first time the presence of the majority of TF and DME in PBMC of healthy subjects without prior induction. The expression of these genes in lymphocytes could be a useful tool for further studying the physiological and pathological variations of DME and TF related to environment, to drug intake and to cardiovascular metabolic cycles.

Introduction

Drug metabolizing enzymes (DME) and Cytochromes P450 (CYP) in particular, are central players in cardiovascular health and disease (Elbekai and El-Kadi, 2006). DME are important in the follow up of cardiovascular drugs since many drugs are metabolised by them. These enzymes are also involved in the metabolism of natural substrates (such as leukotrienes, steroids and bile acids) that are in their turn implicated in several cardiovascular related pathways including inflammation, lipid metabolism and blood pressure regulation. In addition, many environmental factors (tobacco, polycyclic hydrocarbons and dioxins, alcohol and nutrients) are modulating their patterns of expression and expression of several of these genes is under control of transcription factors (TF) such as the Pregnane X Receptor (PXR), the Constitutive Androstane Receptor (CAR), the Glucocorticoid Receptor (GR) or the Aryl Hydrocarbon Receptor (AHR). Finally, some polymorphisms in genes coding for these DME are well known to influence the level of expression with clinical and pharmacological relevances.

Some DME, including glutathione S-transferases (GST*)*, N-acetyltransferases (NAT) and sulfotransferases (ST) are soluble enzymes measurable as phenotypes in the plasma. However, the majority is mainly localized in the endoplasmic reticulum and is rarely excreted or found in the plasma. That is one of the reasons we looked for the expression of DME in an easily accessible type of cells: the peripheral blood mononuclear cells (PBMC). The second reason is the involvement of lymphocytes in cardiovascular events, i. e. through inflammation. Lymphocytes could be a useful target and tool for investigating relationships between DME, inflammation and other metabolic pathways related to cardiovascular physiopathology. In addition, the mechanisms of inflammation and immune defences are regulated by the same transcription factors.

During the last ten years, a large number of CYP has been studied in PBMC of healthy subjects including CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A3, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CYP4B1 and CYP4F. Epoxyde hydrolase (EH), GST, NAT and ST have been also described (Raucy et al., 1997; Baron et al., 1998; Dassi et al., 1998; Spencer et al., 1999; Starkel et al., 1999; Takeda et al., 1999; Boucher et al., 2000; Krovat et al., 2000; Nakamoto et al., 2000; Nguyen et al., 2000; Smart and Daly, 2000; Finnstrom et al., 2001; Hannon-Fletcher et al., 2001; Asghar et al., 2002; Finnstrom et al., 2002; Carcillo et al., 2003; Gashaw et al., 2003; Landi et al., 2003; Lin et al., 2003; Toide et al., 2003; Tuominen et al., 2003; Furukawa et al., 2004; Lampe et al., 2004; Yamamoto et al., 2004; Haas et al., 2005; Landi et al., 2005; Liangpunsakul et al., 2005; Miura et al., 2006). However, study of the expression of both DME and FT are rarely conducted at the same time. The existing work in the field examined preferentially CYP1A1, AHR and the Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) (Smart and Daly, 2000; Landi et al., 2003; Lin et al., 2003; Yamamoto et al., 2004).

The global objectives of this investigation are to propose useful biomarkers easily measurable without any activation on a large scale i.e. during clinical trials. The main objective consisted in investigating patterns of expression of DME together with their related TF in PBMC, a type of cells easily accessible and closely related to inflammation. Before studying these genes in pathological states or in patients undergoing treatment, looking for them in healthy subjects is an obligatory step. Therefore, we studied the simultaneous expression of an important number of DME and TF RNA in lymphocytes (without prior induction) of twenty supposed healthy subjects. Finally, we have been reviewing the papers which described expression of DME and TF in PBMC of healthy subjects and we discussed the biological variation factors found in these papers.

Methods

Population

Twenty supposed healthy individuals, eleven males and nine females, from the Stanislas Cohort were chosen for measurement of PBMC gene expression by an in house micro-array assay (Visvikis-Siest et al., 2007). The investigation conforms to the principles outlined in the Declaration of Helsinki. Individuals were of French origin and exempt of any acute or chronic diseases. Some of them have slight cardiovascular risk (i. e. obesity and hypertension). Specific exclusion criteria were medication; except contraceptives; current smoking and heavy alcohol consumption. Characteristics are shown in Table I.

PBMC collection

The PBMC bank was constituted according to a well validated protocol, with a high recovery in lymphocytes (97%). Briefly, fresh whole blood (10 ml) was collected from 8 a.m. to 10 a.m. by standardized venipuncture in EDTA tubes (Vacutainer^{TM}; Becton Dickinson) during a period of 5 months (November to March). PBMC were isolated by centrifugation on a density gradient of Ficoll (Ficoll-Paque^{TM} PLUS; Amersham). Percentages of lymphocytes, monocytes and polynuclear cells were determined in some samples by microscopic observation after May–Grunwald–Giemsa staining. PBMC were stored at -80°C until RNA extraction. RNA quality and stability were carefully tested (Marteau et al., 2005).

Microarray design

An in-house microarray was designed as previously described (Visvikis-Siest et al., 2007). Briefly, we have selected numerous genes including 16 DME and 13 TF. In addition, we included a non-human RNA (Arabidopsis thaliana) to test unspecificity. Oligonucleotides were selected from MWG database and the chip was manufactured by MWG Biotech AG.

DMD Fast Forward. Published on October 16, 2007 as DOI: 10.1124/dmd.107.017228
This article has not been copyedited and formatted. The final version may differ from this version.

RNA was extracted by an automated isolation procedure (MagNA Pure LC instrument). Concentration and quality were determined by the spectrophotometer NanoDrop ND-1000 (Labtech International). RNA was amplified using the Amino allyl MessageAmp^{TM} II aRNA Amplification Kit (Ambion) and a T7-$(dT)_{24}$ primer. The double-strand cDNA obtained was transcribed in amplified RNA using 5-(3-aminoallyl)-UTP (Ambion). The RNA yield ranged from 1.3 to 8 µg. RNA samples were labeled with fluorochrome Cy3 and a reference RNA (Universal Human Reference RNA, Stratagene) was labelled with fluorochrome Cy5. The labeled RNA sample and the corresponding quantity of labeled reference RNA were pre-hybridized on each slide in 5% bovine serum albumin. Cy3- and Cy5-labeled RNA were co-hybridized to the micro array at 50°C for one night. Slides were scanned using an Axon GenePix 4000B scanner and GenePix version 6 software (Axon Instruments).

Data normalization and analysis

Normalization and analysis were assessed using Genespring v6.1 software (Silicon Genetics, Agilent). The ratio of Cy3 intensity on Cy3 background noise was calculated for each spot. Then, to evaluate the expression or the non expression, we used a Student *t* test ($p<0.01$). If the mean of ratios for a given RNA (2 spots per slide) is not significantly different from the mean of ratios for Arabidopsis thaliana RNA (8 spots per slide), the gene was considered as non-detectable. Spearman's correlation coefficients were calculated to look for potential relationship between gene expression, age and Body Mass Index (BMI). Mann-Whitney test was used to identify significant difference between men and women for the gene expression.

Validation of microarray results (Visvikis-Siest et al., 2007)

This chip was used to study expression of other genes such as genes involved in the two principal biological pathways related to leukocytes: inflammation and cellular adhesion.

DMD Fast Forward. Published on October 16, 2007 as DOI: 10.1124/dmd.107.017228
This article has not been copyedited and formatted. The final version may differ from this version.

Validation of the results obtained with this chip was performed by quantitative real time PCR on 4 genes expressed in a significant number of individuals: ICAM1, TNFα, SELL and IL6.

Results

Expression of DME genes (Fig. 1)

All the subjects expressed CYP2C19, 2C9, 2J2, 2D6, 1A1, 4F2, 4A11, 11B2 and GSTP1. In addition, CYP2E1, 2C18, 2A6 and GSTT1 were expressed in 19, 18, 19 and 17 subjects respectively. Concerning CYP3A4 and CYP3A5, they were not measurable here. GSTM1 was expressed in only 4 individuals.

The variability is important (coefficients of variation shown in Figure 1). We obtained no difference depending on gender, age or BMI. We observed no variation due to the time of the day or the day or month of collection (data not shown).

Expression of TF genes (Fig. 2)

Among thirteen TF, twelve were found in PBMC. PXR, CAR, Liver X Receptor (LXR)-α, AHR, ARNT, Vitamin D Receptor (VDR), Transcription Factor 7 (TCF7) and Myocyte Enhancer Factor-2 (MEF2A) were expressed in the majority of subjects. Peroxisome Proliferator-Activated Receptor (PPAR)-γ, LXRβ and GR were expressed in some subjects, PPARα in one individual and Farnesoid X-activated Receptor (FXR) was not detected.

The variability is also high (coefficients of variation shown in Figure 2). No trends of age, sex and BMI were observed which could have explained part of this variability (data not shown).

Association of TF and DME

We found results of interest as concern CYP involved in inflammation and blood pressure regulation. There is a significant correlation between the expression of PXR and the CYP2C ($p<0.0001$, Fig. 3). The CYP2C were also correlated with LXRα ($p<0.001$) and LXRβ ($p<0.07$ except CYP2C9) and with CAR ($p\leq0.001$) but not with GR (data not shown).

Like the two other CYP involved in leukotrienes metabolism, namely 2C19 and 2C9, CYP4F2 (leukotriene ß4 omega hydroxylase), CYP4A11 (fatty acid omega hydroxylase) and CYP2J2 (epoxygenase active on eicosanoids) were correlated with PXR. In addition, CYP11B2 (aldosterone synthase) was also correlated with PXR ($p<0.0005$, Fig. 4).

Finally, we found also a significant correlation between ARNT and AHR ($p<0.0001$), ARNT and CYP1A1 ($p<0.006$) and a trend between AHR and CYP1A1 ($p=0.022$, Fig. 5).

Validation of microarray results

The quantitative real time PCR results were in agreement with those observed by microarray analysis (Visvikis-Siest et al., 2007).

Discussion

CYP and GST expression in PBMC of healthy subjects

The choice of the DME was based on their importance in cardiovascular drugs pharmacogenetics (CYP2D6, 2C19, 2C9, 3A4, 3A5, 2C8), their environmental interest (CYP2E1, 1A1, 2A6, GST) and their possible involvement in hypertension or vascular regulation *via* endogenous metabolites (CYP2C, 4F2, 2J2, 4A11, 11B2).

We measured simultaneously eleven CYP and three GST mRNA in lymphocytes without any prior induction or cell culture. Most of the genes are expressed in each individual, contrary to what was observed in other studies (Asghar et al., 2002; Haas et al., 2005). GSTM1 is only expressed in four subjects. This is not surprising since the complete gene is only present in 50% of the Caucasians.

We report here for the first time, the expression of CYP2C18, CYP2J2 and CYP4F2 in lymphocytes of healthy subjects. In addition, we also found CYP2A6 in contrast to previous studies (Koskela et al., 1999; Krovat et al., 2000). As for the lack of expression of CYP3A4 and CYP3A5 in our subjects, these findings are not in agreement with other previously reported (Nakamoto et al., 2000; Finnstrom et al., 2001; Gashaw et al., 2003). Krovat *et al*, who were not able to detect CYP3A5 and whose detection of CYP3A4 was near the detection limit of the assay, proposed as an explanation the preferential localisation of CYP3A in B cells which constitute only a small part of total lymphocytes (Krovat et al., 2000).

TF and CYP expression in PBMC of healthy subjects

To our knowledge, this is the first time that such an important number of DME and TF is simultaneously studied. We have found a large panel of TF in PBMC. Only few authors have been interested in measuring TF expression in PBMC of healthy subjects (Table II). In

addition, study of the expression of both CYP and FT are rarely conducted at the same time. AHR pathway is associated with induction of CYP1A1, 1A2 and 1B1 (Gueguen et al., 2006). Four authors have looked for the TF regulating CYP1A1 expression: Lin *et al* (Lin et al., 2003) (AHR), Smart and Daly (Smart and Daly, 2000), Landi *et al* (Landi et al., 2003) (AHR, ARNT) and Yamamoto *et al* (Yamamoto et al., 2004) (AHR, ARNT and AHR repressor). Expression of RAR and RXR has been described in healthy subjects PBMC but only qualitatively (Szabova et al., 2003). No one has looked in healthy lymphocytes for the three other transcriptional activation mechanisms involved in CYP regulation: CAR-RXR, PXR-RXR and PPARα-RXR. It is generally admitted that PXR regulates CYP2B6, 2C8, 2C9, 3A4, 3A7, GST, ST, UGT1A1 and ABCB1, mainly in the liver (Gueguen et al., 2006) while CAR modulates CYP2B6, 2C9, 2C19.

CYP are often regulated simultaneously by two or more mechanisms (Miao et al., 2004) including VDR and GR (Pascussi et al., 2003). The different TF influence mutually their relative expression. CAR and PXR are regulated, at least in part, by the GR and a signal transduction cascade GR-[CAR-PXR]-CYP exist at least for CYP3A4 and CYP2C9 (Dvorak et al., 2003) and maybe CYP2C19 (Chen et al., 2003). In addition, VDR probably regulates CYP3A4, CYP2C9 and CYP2B6 (Drocourt et al., 2002). Only limited information is available on the regulation of CYP3A5 expression but it appears to be inducible via the GR, PXR and CAR, as for CYP3A4 (Daly, 2006) and CYP2C9 (Kirchheiner, 2004). CYP2C8 expression is regulated by CAR, PXR, GR ad HNF4α in the liver (Ferguson et al., 2005). Finally, constitutive hepatic expression of CYP2A6 is governed by interplay between the transcription factors HNF4α, CEBPα, CEBPβ and OCT1 (Pitarque et al., 2005).

It is also possible to describe activation through the antioxidant response element which could cross-talk with the xenobiotic response elements (Miao et al., 2004). CAR could in addition be regulated by activation through phosphorylation which permits its translocation in the

nucleus (Sueyoshi and Negishi, 2001). Finally, regulation could also be mediated through mRNA or protein stabilisation (CYP2E1). These mechanisms are mainly studied in the liver (Sueyoshi and Negishi, 2001; Pascussi et al., 2003; Miao et al., 2004; Handschin and Meyer, 2005).

Choice of the PBMC as a tool for measuring DME and TF

As previously mentioned, a large number of CYP has been described in PBMC of healthy subjects (Table II). Gene's expression in lymphocytes is not always representative of expression in other tissues. However, considering that they are involved in cardiovascular related diseases, mainly through the inflammation pathway, lymphocytes expression could be used to evaluate modification of expression observed with this system. In addition, gene's expression in lymphocytes stay a good biomarker to evaluate CYP and transcription factors phenotypes and thus to monitor for example, exposure to, and risk associated with, xenobiotics. We would like to mention here the work of Wibaut-Berlaimont and collaborators (Wibaut-Berlaimont et al., 2005). Using an Affymetrix chip, they described the significant regulation of 240 genes (among 12650 genes) in PBMC of dyslipidemic patients after atorvastatin treatment. Unfortunately, neither CYP nor TF that we studied here appear among the regulated genes.

PBMC are easily accessible cells. They could be a great tool to investigate biomarkers in large population studies if only a small quantity of blood is taken from the patient (we recommend 5 to 10 ml). Quantities of blood taken are often too high (Table II), the main reason being the need of enough material for measuring protein levels of DME in microsomes after ultracentrifugation (Raucy et al., 1997; Baron et al., 1998; Hannon-Fletcher et al., 2001) or after lymphocytes cultures (Spencer et al., 1999; Smart and Daly, 2000; Landi et al., 2003; Lin et al., 2003).

We consider that the choice of PBMC is a good one, since CYP are being largely represented in lymphocytes (Raucy et al., 1997). In PBMC we found essentially T lymphocytes which are the richest cells in CYP content. In addition, monocytes, even if there are contaminating PBMC have a low CYP content. Our experimental conditions were well defined for PBMC preparation and the lymphocytes purity obtained was very high (97 %) compared to the 70-90 % described in papers cited in Table II. Total white blood cells (WBC) were used without any problem by Finnstrom *et al* (Finnstrom et al., 2001) and buffy coat with all WBC by Furukawa and collaborators (Furukawa et al., 2004). The heterogeneity of the cell population is no longer an argument. It is not more heterogeneous than a liver extract which is also a mixture of more than four different cell types. In comparison with the liver, the concentration of CYP in PBMC is 20 to 2000 times lower creating thus some limitations. Some authors have used cultures and inducers to increase the CYP levels but such a strategy is not applicable to studies in large populations or to new drug trials. We show here that it is possible to measure the majority of them without induction. However, we observed a high variability in expression of these DME. This, and the very low level for CYP3A4, could be attributed to expression in a limited number of cells (Gashaw et al., 2003). We are looking essentially with our PBMC preparation to T lymphocytes with a low content of B (< 5%) and monocytes (< 5%). The difficulties which should be more deeply studied are linked to the low levels of expression and to the non-systematic expression in all subjects.

The work of Whitney *et al*, which also use microarray expression to study inter-individual and temporal variations in healthy subjects, was a report stimulating the interest on studying WBC and lymphocytes for health surveillance. However, they did not investigate CYP expression (Whitney et al., 2003). The majority of the published data on CYP mRNA content in PBMC has been obtained by using the technique of real-time PCR (Table II), except two who used a microarray technology (Nguyen et al., 2000; Lampe et al., 2004).

Biological variations

Determination of reference values and biological variations in healthy individuals is an obligatory step in the development of any candidate biomarker prior to its application in diagnostic or in pharmacogenetic studies. In laboratory medicine, biological variations data which are two times higher than the analytical variations could be retained. A biomarker with high variability is of great interest in health screening and pre-pathological state studies.

The main factors affecting biological variability of clinical chemistry constituents are age, gender, biological rhythms, BMI, alcohol, tobacco, diet, drug intake and genetic variations. We tested the possible contribution of some factors but the biological variation observed could not by explained by them. We should study these factors on a greater set of healthy subjects. Finally, the mRNA profiling approaches by microarray technologies should be confirmed by RT-PCR.

We demonstrate here that the majority of DME and TF are expressed in lymphocytes of twenty healthy subjects. This is of importance not only for pharmacokinetic studies in drug clinical trials but also because it gives the perspective of further studying cardiovascular related pathways such as inflammation, blood pressure regulation and lipid metabolism by using PBMC. These TF are involved in cholesterol (LXR), triglycerides (PPAR), bile acids (VDR, FXR, LXR) steroids (CAR, PXR) and bilirubin (CAR, PXR) (Handschin and Meyer, 2005) metabolisms. TF also interact with many other cardiovascular related pathways including the cytokines ones for PPAR (Jones et al., 2002; Trifilieff et al., 2003). To conclude, a biological system approach could be defined for a better understanding on the relation of each TF with every CYP or other constituent candidate marker measured in PBMC.

Acknowledgments

We are grateful for the excellent technical assistance of Dr Pascal Soularue and his team (PARTNERCHIP CEA/DSV/DRR/SGF Evry, France) and thank Dr Steve Mohr for his scientific involvement. We thank the families of the Stanislas Survey and the staff of the Centre for Preventive Medicine for their involvement.

References

Asghar A, Gorski JC, Haehner-Daniels B and Hall SD (2002) Induction of multidrug resistance-1 and cytochrome P450 mRNAs in human mononuclear cells by rifampin. *Drug Metab Dispos* 30:20-26.

Baron JM, Zwadlo-Klarwasser G, Jugert F, Hamann W, Rubben A, Mukhtar H and Merk HF (1998) Cytochrome P450 1B1: a major P450 isoenzyme in human blood monocytes and macrophage subsets. *Biochem Pharmacol* 56:1105-1110.

Boucher P, Seree E, Vidon C, de Souza AC, Villard PH, Chambon R, Barra Y and Vallon JJ (2000) Dietary lipids affect human ethanol-inducible CYP2E1 gene expression in vivo in mononuclear cells. *Life Sci* 67:1307-1316.

Carcillo JA, Adedoyin A, Burckart GJ, Frye RF, Venkataramanan R, Knoll C, Thummel K, Roskos L, Wilson JW, Sereika S, Romkes M, Bebia Z and Branch RA (2003) Coordinated intrahepatic and extrahepatic regulation of cytochrome p4502D6 in healthy subjects and in patients after liver transplantation. *Clin Pharmacol Ther* 73:456-467.

Chen Y, Ferguson SS, Negishi M and Goldstein JA (2003) Identification of constitutive androstane receptor and glucocorticoid receptor binding sites in the CYP2C19 promoter. *Mol Pharmacol* 64:316-324.

Daly AK (2006) Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet* 45:13-31.

Dassi C, Signorini S, Gerthoux P, Cazzaniga M and Brambilla P (1998) Cytochrome P450 1B1 mRNA measured in blood mononuclear cells by quantitative reverse transcription-PCR. *Clin Chem* 44:2416-2421.

Drocourt L, Ourlin JC, Pascussi JM, Maurel P and Vilarem MJ (2002) Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J Biol Chem* 277:25125-25132.

Dvorak Z, Modriansky M, Pichard-Garcia L, Balaguer P, Vilarem MJ, Ulrichova J, Maurel P and Pascussi JM (2003) Colchicine down-regulates cytochrome P450 2B6, 2C8, 2C9, and 3A4 in human hepatocytes by affecting their glucocorticoid receptor-mediated regulation. *Mol Pharmacol* 64:160-169.

Elbekai RH and El-Kadi AO (2006) Cytochrome P450 enzymes: central players in cardiovascular health and disease. *Pharmacol Ther* 112:564-587.

Ferguson SS, Chen Y, LeCluyse EL, Negishi M and Goldstein JA (2005) Human CYP2C8 is transcriptionally regulated by the nuclear receptors constitutive androstane receptor, pregnane X receptor, glucocorticoid receptor, and hepatic nuclear factor 4alpha. *Mol Pharmacol* 68:747-757.

Finnstrom N, Ask B, Dahl ML, Gadd M and Rane A (2002) Intra-individual variation and sex differences in gene expression of cytochromes P450 in circulating leukocytes. *Pharmacogenomics J* 2:111-116.

Finnstrom N, Thorn M, Loof L and Rane A (2001) Independent patterns of cytochrome P450 gene expression in liver and blood in patients with suspected liver disease. *Eur J Clin Pharmacol* 57:403-409.

Furukawa M, Nishimura M, Ogino D, Chiba R, Ikai I, Ueda N, Naito S, Kuribayashi S, Moustafa MA, Uchida T, Sawada H, Kamataki T, Funae Y and Fukumoto M (2004) Cytochrome p450 gene expression levels in peripheral blood mononuclear cells in comparison with the liver. *Cancer Sci* 95:520-529.

Gashaw I, Kirchheiner J, Goldammer M, Bauer S, Seidemann J, Zoller K, Mrozikiewicz PM, Roots I and Brockmoller J (2003) Cytochrome p450 3A4 messenger ribonucleic acid

induction by rifampin in human peripheral blood mononuclear cells: correlation with alprazolam pharmacokinetics. *Clin Pharmacol Ther* 74:448-457.

Gueguen Y, Mouzat K, Ferrari L, Tissandie E, Lobaccaro JM, Batt AM, Paquet F, Voisin P, Aigueperse J, Gourmelon P and Souidi M (2006) [Cytochromes P450: xenobiotic metabolism, regulation and clinical importance]. *Ann Biol Clin (Paris)* 64:535-548.

Haas CE, Brazeau D, Cloen D, Booker BM, Frerichs V, Zaranek C, Frye RF and Kufel T (2005) Cytochrome P450 mRNA expression in peripheral blood lymphocytes as a predictor of enzyme induction. *Eur J Clin Pharmacol* 61:583-593.

Handschin C and Meyer UA (2005) Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. *Arch Biochem Biophys* 433:387-396.

Hannon-Fletcher MP, O'Kane MJ, Moles KW, Barnett YA and Barnett CR (2001) Lymphocyte cytochrome P450-CYP2E1 expression in human IDDM subjects. *Food Chem Toxicol* 39:125-132.

Jones DC, Manning BM and Daynes RA (2002) A role for the peroxisome proliferator-activated receptor alpha in T-cell physiology and ageing immunobiology. *Proc Nutr Soc* 61:363-369.

Kirchheiner JT, M.; Jabrane, W.; Roots, I.; Brockmöller, J. (2004) The CYP2C9 polymorphism: from enzyme kinetics to clinical dose recommendations. *Personnalized Medicine* 1:63-84.

Koskela S, Hakkola J, Hukkanen J, Pelkonen O, Sorri M, Saranen A, Anttila S, Fernandez-Salguero P, Gonzalez F and Raunio H (1999) Expression of CYP2A genes in human liver and extrahepatic tissues. *Biochem Pharmacol* 57:1407-1413.

Krovat BC, Tracy JH and Omiecinski CJ (2000) Fingerprinting of cytochrome P450 and microsomal epoxide hydrolase gene expression in human blood cells. *Toxicol Sci* 55:352-360.

Lampe JW, Stepaniants SB, Mao M, Radich JP, Dai H, Linsley PS, Friend SH and Potter JD (2004) Signatures of environmental exposures using peripheral leukocyte gene expression: tobacco smoke. *Cancer Epidemiol Biomarkers Prev* 13:445-453.

Landi MT, Bergen AW, Baccarelli A, Patterson DG, Jr., Grassman J, Ter-Minassian M, Mocarelli P, Caporaso N, Masten SA, Pesatori AC, Pittman GS and Bell DA (2005) CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. *Toxicology* 207:191-202.

Landi MT, Bertazzi PA, Baccarelli A, Consonni D, Masten S, Lucier G, Mocarelli P, Needham L, Caporaso N and Grassman J (2003) TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 20 years after the accident. *Carcinogenesis* 24:673-680.

Liangpunsakul S, Kolwankar D, Pinto A, Gorski JC, Hall SD and Chalasani N (2005) Activity of CYP2E1 and CYP3A enzymes in adults with moderate alcohol consumption: a comparison with nonalcoholics. *Hepatology* 41:1144-1150.

Lin P, Hu SW and Chang TH (2003) Correlation between gene expression of aryl hydrocarbon receptor (AhR), hydrocarbon receptor nuclear translocator (Arnt), cytochromes P4501A1 (CYP1A1) and 1B1 (CYP1B1), and inducibility of CYP1A1 and CYP1B1 in human lymphocytes. *Toxicol Sci* 71:20-26.

Marteau JB, Mohr S, Pfister M and Visvikis-Siest S (2005) Collection and storage of human blood cells for mRNA expression profiling: a 15-month stability study. *Clin Chem* 51:1250-1252.

Miao W, Hu L, Kandouz M, Hamilton D and Batist G (2004) A cell-based system to identify and characterize the molecular mechanism of drug-metabolizing enzyme (DME) modulators. *Biochem Pharmacol* 67:1897-1905.

Miura R, Nakamura K, Miura D, Miura A, Hisamatsu K, Kajiya M, Hashimoto K, Nagase S, Morita H, Fukushima Kusano K, Emori T, Ishihara K and Ohe T (2006) Aldosterone synthesis and cytokine production in human peripheral blood mononuclear cells. *J Pharmacol Sci* 102:288-295.

Nakamoto T, Hase I, Imaoka S, Hiroi T, Oda Y, Asada A and Funae Y (2000) Quantitative RT-PCR for CYP3A4 mRNA in human peripheral lymphocytes: induction of CYP3A4 in lymphocytes and in liver by rifampicin. *Pharmacogenetics* 10:571-575.

Nguyen LT, Ramanathan M, Weinstock-Guttman B, Dole K, Miller C, Planter M, Patrick K, Brownscheidle C and Jacobs LD (2000) Detection of cytochrome P450 and other drug-metabolizing enzyme mRNAs in peripheral blood mononuclear cells using DNA arrays. *Drug Metab Dispos* 28:987-993.

Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P and Vilarem MJ (2003) The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim Biophys Acta* 1619:243-253.

Pitarque M, Rodriguez-Antona C, Oscarson M and Ingelman-Sundberg M (2005) Transcriptional regulation of the human CYP2A6 gene. *J Pharmacol Exp Ther* 313:814-822.

Raucy JL, Schultz ED, Wester MR, Arora S, Johnston DE, Omdahl JL and Carpenter SP (1997) Human lymphocyte cytochrome P450 2E1, a putative marker for alcohol-mediated changes in hepatic chlorzoxazone activity. *Drug Metab Dispos* 25:1429-1435.

Smart J and Daly AK (2000) Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics* 10:11-24.

Spencer DL, Masten SA, Lanier KM, Yang X, Grassman JA, Miller CR, Sutter TR, Lucier GW and Walker NJ (1999) Quantitative analysis of constitutive and 2,3,7,8-

tetrachlorodibenzo-p-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* 8:139-146.

Starkel P, Sempoux C, Van Den Berge V, Stevens M, De Saeger C, Desager JP and Horsmans Y (1999) CYP 3A proteins are expressed in human neutrophils and lymphocytes but are not induced by rifampicin. *Life Sci* 64:643-653.

Sueyoshi T and Negishi M (2001) Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu Rev Pharmacol Toxicol* 41:123-143.

Szabova L, Macejova D, Dvorcakova M, Mostbock S, Blazickova S, Zorad S, Walrand S, Cardinault N, Vasson MP, Rock E and Brtko J (2003) Expression of nuclear retinoic acid receptor in peripheral blood mononuclear cells (PBMC) of healthy subjects. *Life Sci* 72:831-836.

Takeda Y, Furukawa K, Inaba S, Miyamori I and Mabuchi H (1999) Genetic analysis of aldosterone synthase in patients with idiopathic hyperaldosteronism. *J Clin Endocrinol Metab* 84:1633-1637.

Toide K, Yamazaki H, Nagashima R, Itoh K, Iwano S, Takahashi Y, Watanabe S and Kamataki T (2003) Aryl hydrocarbon hydroxylase represents CYP1B1, and not CYP1A1, in human freshly isolated white cells: trimodal distribution of Japanese population according to induction of CYP1B1 mRNA by environmental dioxins. *Cancer Epidemiol Biomarkers Prev* 12:219-222.

Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E and Whittaker P (2003) PPAR-alpha and -gamma but not -delta agonists inhibit airway inflammation in a murine model of asthma: in vitro evidence for an NF-kappaB-independent effect. *Br J Pharmacol* 139:163-171.

Tuominen R, Warholm M, Moller L and Rannug A (2003) Constitutive CYP1B1 mRNA expression in human blood mononuclear cells in relation to gender, genotype, and environmental factors. *Environ Res* 93:138-148.

Visvikis-Siest S, Marteau JB, Samara A, Berrahmoune H, Marie B and Pfister M (2007) Peripheral blood mononuclear cells (PBMCs): a possible model for studying cardiovascular biology systems. *Clin Chem Lab Med* In press.

Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA and Brown PO (2003) Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci U S A* 100:1896-1901.

Wibaut-Berlaimont V, Randi AM, Mandryko V, Lunnon MW, Haskard DO and Naoumova RP (2005) Atorvastatin affects leukocyte gene expression in dyslipidemia patients: in vivo regulation of hemostasis, inflammation and apoptosis. *J Thromb Haemost* 3:677-685.

Yamamoto J, Ihara K, Nakayama H, Hikino S, Satoh K, Kubo N, Iida T, Fujii Y and Hara T (2004) Characteristic expression of aryl hydrocarbon receptor repressor gene in human tissues: organ-specific distribution and variable induction patterns in mononuclear cells. *Life Sci* 74:1039-1049.

Footnotes

This work was supported by the Région Lorraine and the Communauté Urbaine du Grand Nancy for their support. This work was also made possible thanks to an INSERM Grant CRB "Collection de Ressources Biologiques" 4CH07H.

Person to receive reprint requests:

Pr Gérard Siest

Faculté de Pharmacie

Equipe Inserm «Génétique Cardiovasculaire» CIC 9501

30 rue Lionnois

54000 Nancy - France

E-mail : gerard.siest@pharma.uhp-nancy.fr

Legends for figures

Figure 1: Lymphocyte drug metabolizing enzymes mRNA levels in 20 healthy subjects (CV Coefficient of variation).

Figure 2: Lymphocyte transcription factors mRNA levels in 20 healthy subjects (CV Coefficient of variation).

Fig. 3. Correlation between PXR and CYP2C RNA expressions in peripheral blood mononuclear cells of healthy subjects (Expression = Cy3 intensity/Cy3 background noise).

Fig. 4: Correlation of CYP involved in blood pressure regulation and PXR expressions in peripheral blood mononuclear cells of healthy subjects (Expression = Cy3 intensity/Cy3 background noise).

Fig. 5: Correlation between ARNT, AHR and CYP1A1 RNA expressions in peripheral blood mononuclear cells of healthy subjects (Expression = Cy3 intensity/Cy3 background noise).

Tables

Table 1. Characteristics of the 20 selected individuals from the Stanislas Cohort.

sex	age (yrs)	BMI (kg/m²)	WBC (10*9/L)	cholesterol (mmol/L)	HDL (mmol/L)	TG (mmol/L)	glucose (mmol/L)	GGT (u/L)	GTP (u/L)	CRP (mg/L)	alcohol consumption
male	43	24,40	4,5	5,58	1,49	0,85	4,96	17	25	0,16	o
male	50	25,82	4,9	5,42	1,85	0,86	5,21	22	17	0,55	++
female	48	23,30	6,5	4,50	1,96	0,60	4,96	12	15	0,18	o
female	50	27,62	8,1	5,89	1,77	0,71	4,90	18	15	0,72	o
female*	51	31,66	5,5	5,70	1,14	1,03	5,60	25	54	8,53	o
male	52	34,83	5,9	6,50	1,70	2,06	6,04	58	29	3,13	+
male	52	32,23	4,5	5,54	1,13	2,00	6,35	41	64	1,98	+
female	48	31,05	7,0	5,16	1,17	1,93	5,17	NA	37	2,08	+
female	45	21,26	5,1	4,67	1,88	0,69	4,94	9	12	0,30	o
male	50	30,06	5,6	5,45	1,30	2,17	6,30	NA	54	2,03	+
male	55	37,91	6,5	4,78	1,52	0,68	6,18	44	42	6,09	o
female	46	26,70	6,5	4,82	2,12	0,86	5,16	14	16	1,40	o

male	46	25,24	5,2	5,30	1,97	0,58	4,91	17	18	0,64	+
male	52	30,23	5,6	5,13	1,35	1,67	6,76	19	23	0,59	+
male	51	23,25	5,6	5,86	1,98	0,70	5,26	17	23	0,70	+++
female*	50	23,05	6,0	4,93	1,43	0,54	4,85	27	18	1,01	+
female	49	37,73	8,3	6,10	1,22	2,89	6,09	36	31	4,86	+
male	52	32,20	5,6	7,10	1,53	1,43	6,69	30	28	0,67	++
female	50	30,10	5,8	6,30	2,09	0,86	5,65	29	46	5,08	o
male	55	29,14	5,4	5,68	1,62	1,44	5,62	99	60	0,51	o

BMI Body Mass Index, WBC White Blood Cells, HDL High Density Lipoprotein, TG Triglycerides, GGT Gamma Glutamyl Transferase, GTP Glutamate Pyruvate Transaminase, CRP C Reactive Protein.

*: taking oral contraceptives

+: alcohol consumption (+ 1, ++ 2, +++ 4 glass of wine per day)

NA: not available

Table 2. Drug metabolizing enzymes and transcription factors described in peripheral blood mononuclear cells of healthy subjects during the last ten years.

Authors, year	Cell type	Blood Quantities (ml)	Number of healthy subjects	Phenotype	Inducing compounds	DME and TF
(Raucy et al., 1997)	Lymphocytes	320	22	Immunoblot (microsomes) RT-PCR Chlorzoxazone - clearance	alcohol	CYP2E1
(Baron et al., 1998)	Monocytes and culture	-	10	RT-PCR Immunoblot PNP-hydroxylase	Benzanthracene Dexamethasone Cyclosporine Phenobarbital Tetradecanoyl-	CYP1A1, 1B1, 2B6, 2E1, 3A3/4

					phorbol Liposaccharide	
(Dassi et al., 1998)	PBMC	?	75	RT-PCR	Tobacco	CYP1B1
(Spencer et al., 1999)	PBMC and culture	60	10	RT-PCR	Dioxins	CYPB1
(Starkel et al., 1999)	Neutrophils and lymphocytes	100	8	Immunoblot 6βOH-cortisol	Rifampicin	CYP3A
(Takeda et al., 1999)	PBMC	?	10	RT-PCR Southern-blot		CYP11B2
(Boucher et al., 2000)	PBMC	?	17	Flow cytometry RT-PCR	Fat diet	CYP2E1 protein and mRNA
(Krovat et al., 2000)	PBMC	40	10	RT-PCR	-	CYP1A1, 2D6, 2E1, Epoxyde hydrolase, CYP2F1 (very low)
(Nakamoto et al.,	Lymphocyte	10	8	RT-PCR	Rifampicine 3	CYP3A4

2000)	s				weeks (tuberculosis)	
(Nguyen et al., 2000)	PBMC	?	10	DNA arrays	Interferon	CYP4A11, 2J2, 2E1,2A6, 1A1, 2B6, 4B1, 2C8, 3A, 1B1, 2C9, 2C19
(Smart and Daly, 2000)	PBMC and culture	20	30	EROD assay Immunoblot	-	CYP1A1, AHR
(Finnstrom et al., 2001)	Leucocytes	?	13	RT-PCR	-	CYP2E1, 1B1, 1A2, 2E1, 3A4
(Hannon-Fletcher et al., 2001)	Lymphocytes	15	8	Immunoblot (microsomes)	(diabetes)	CYP2E1
(Asghar et al., 2002)	PBMC	25	50	RT-PCR	Rifampicin	CYP2C8, 2E1, 3A5, 3A7, 4A11, 4B11, ABCB1
(Finnstrom et al.,	Leucocytes	?	19	RT-PCR	-	CYP1A2, 1B1, 2E1,

2002)						3A4
(Carcillo et al., 2003)	PBMC	8	9 86	Debrisoquine clearance RT-PCR	-	CYP2D6
(Gashaw et al., 2003)	Leucocytes	8	96	RT-PCR Alprazolam clearance	Rifampicin 5 days	CYP3A4
(Landi et al., 2003)	PBMC and culture	50	62/59	RT-PCR EROD assay	Dioxins	AHR, CYP1B1, ARNT and CYP1A1 only after cultures
(Lin et al., 2003)	PBMC and culture	20	32	RT-PCR	Benzanthracene Tobacco	CYP1A1, 1B1, AHR, ARNT
(Toide et al., 2003)	Leucocytes	-	72	RT-PCR	Dioxins	CYP1B1
(Tuominen et al., 2003)	PBMC	8	16	RT-PCR	Formylindolocarbazole	CYP1A1, 1B1
(Furukawa et al., 2004)	Buffy coat	10	20	RT-PCR	(Cancer)	CYP1A1, 1A2, 1B1, 2A6, 2B6, 2E1
(Lampe et al., 2004)	PBMC	10	85	Microarrays	Tobacco	CYP1B1

(Yamamoto et al., 2004)	PBMC	?	13	RT-PCR	Methylcholanthrene	CYP1A1, AHR, AHR repressor gene, ARNT
(Haas et al., 2005)	Lymphocytes	8	12	Caffeine, debrisoquine, omeprazole, midazolam clearances RT-PCR	Rifampicin	CYP3A4, 1A2, 2D6, 2C19 (very low)
(Landi et al., 2005)	PBMC and culture	50	62/59	RT-PCR EROD assay	Dioxins TCDD/nitrogen	CYP1A1, 1B1
(Liangpunsakul et al., 2005)	PBMC	25	20	RT-PCR	Alcohol	CYP2E1, 3A4
(Miura et al., 2006)	PBMC culture	?	7	RT-PCR	Angiotensin II	CYP11B2 and mineralocorticoid receptor

FIGURE 1

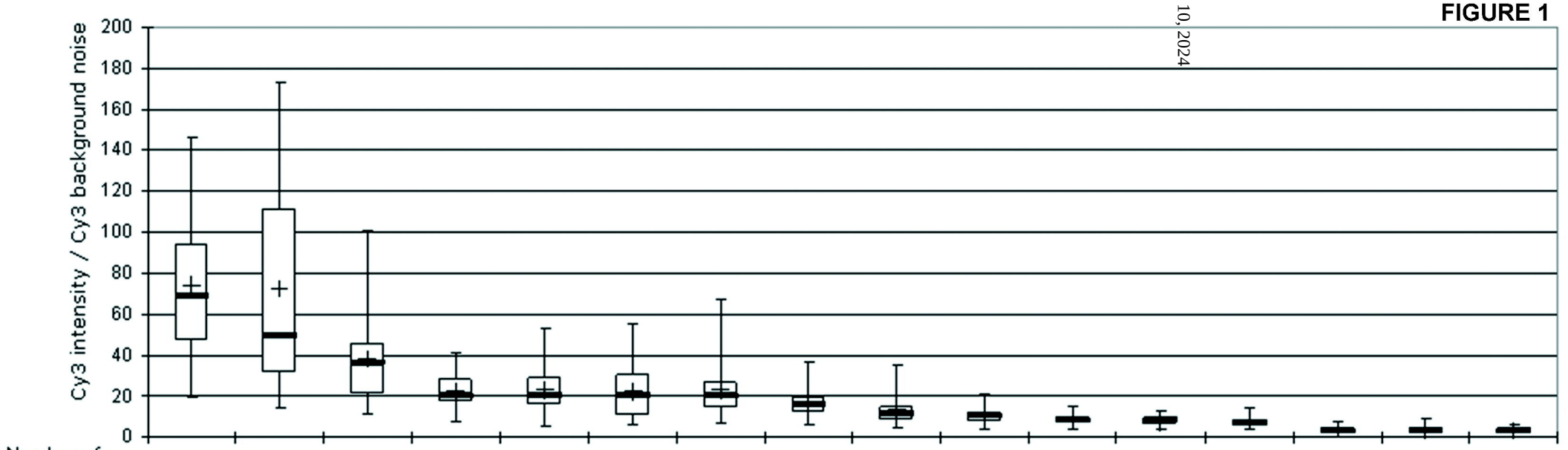
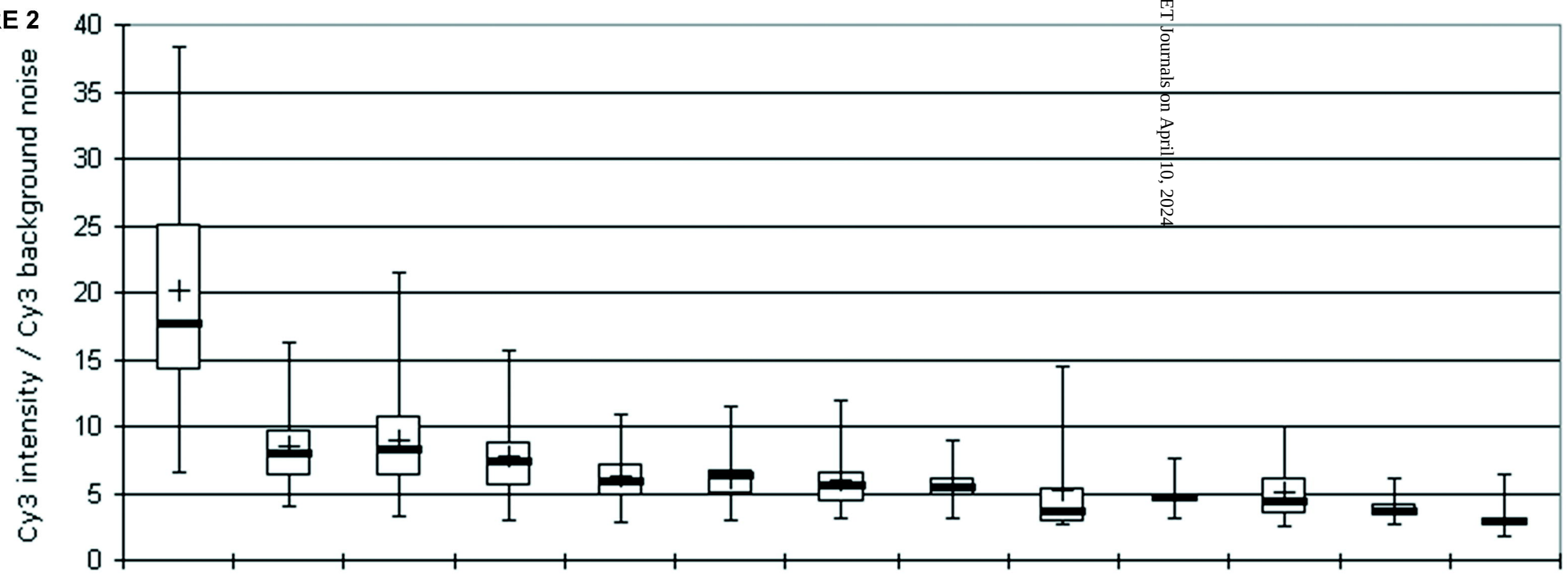

Cy3 intensity / Cy3 background noise
200
180
160
140
120
100
80
60
40
20
0
Number of Positive subjects
CV (%)
CYP2C19 20 45.8%
GSTP1 20 68.5%
CYP2C9 20 59.4%
CYP2J2 20 42.1%
CYP2D6 20 49.6%
CYP1A1 20 59.0%
CYP4F2 20 62.3%
CYP4A11 20 47.5%
CYP2E1 19 56.7%
CYP11B2 20 39.6%
CYP2C18 18 30.3%
CYP2A6 19 27.9%
GSTT1 17 35.2%
GSTM1 4 35.3%
CYP3A5 0
CYP3A4 0

FIGURE 2

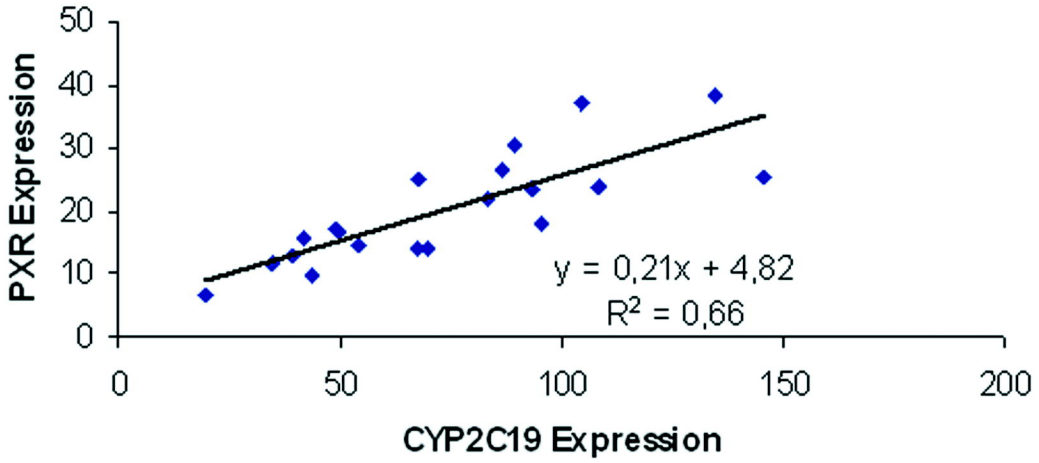


	PXR	MEF2A	VDR	LXRα	AHR	TCF7	CAR	ARNT	GR	PPARγ	LXRβ	PPARα	FXR
Number of positive subjects	19	18	17	18	16	16	13	17	8	11	7	1	0
CV (%)	43.0%	38.8%	45.9%	41.0%	32.3%	31.4%	36.0%	24.3%	67.3%	22.3%	45.9%		

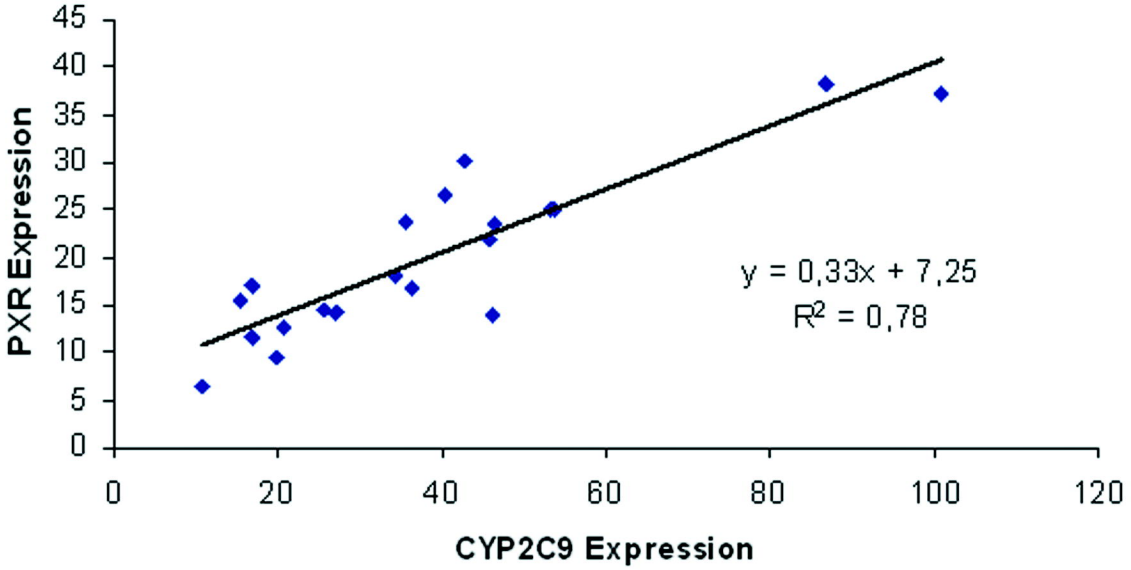
DMD Fast Forward. Published on October 16, 2007 as DOI: 10.1124/dmd.107.017172

FIGURE 3

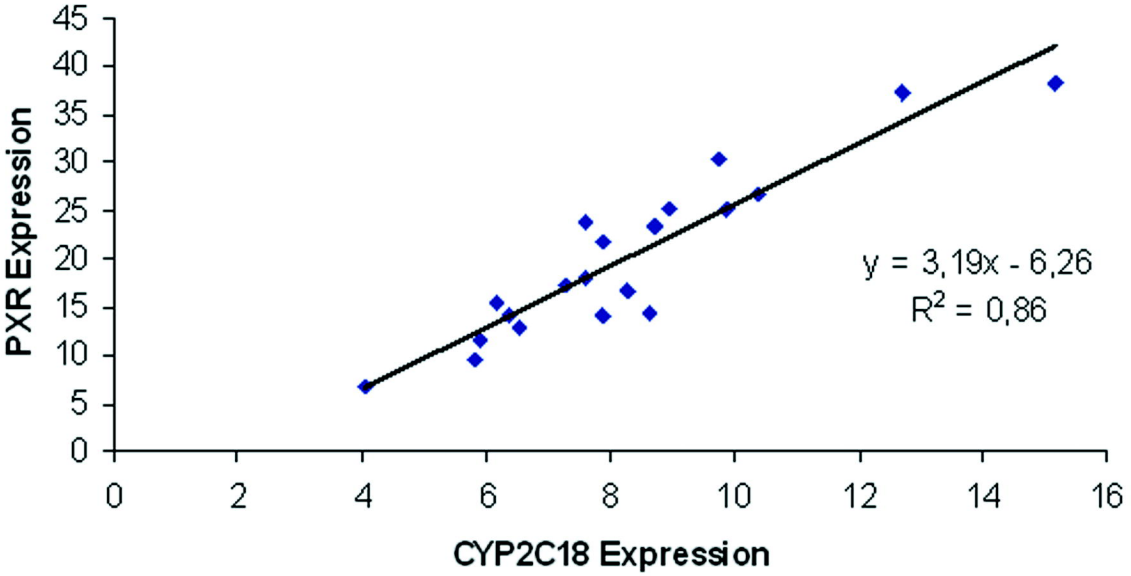
A)



B)



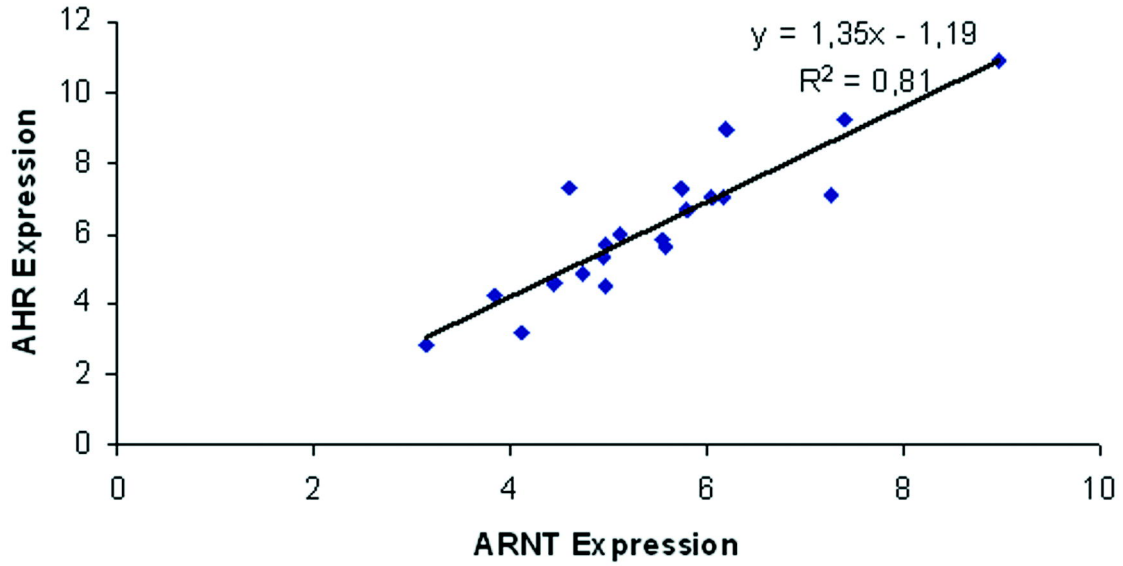
C)



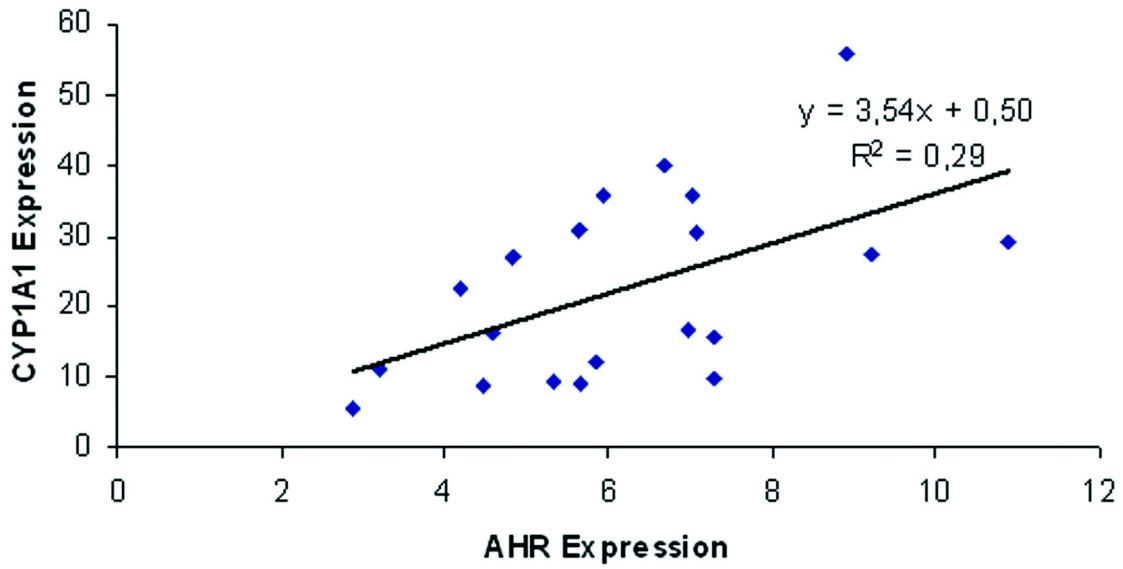
DMD Fast Forward. Published on October 16, 2007 as DOI: 10.1124/dmd.107.018228

FIGURE 4

A)



B)



C)

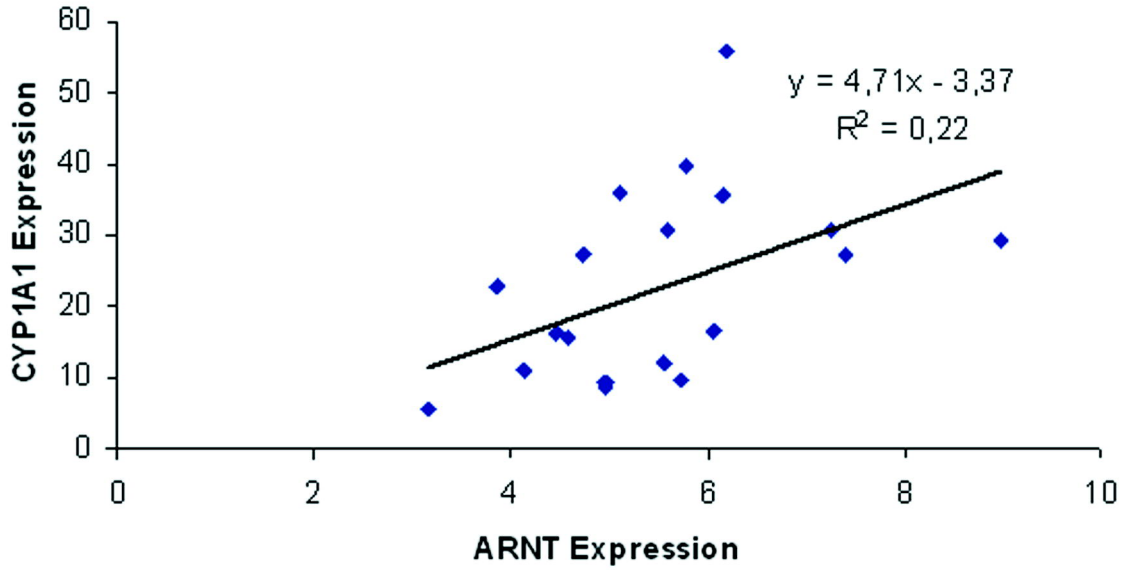
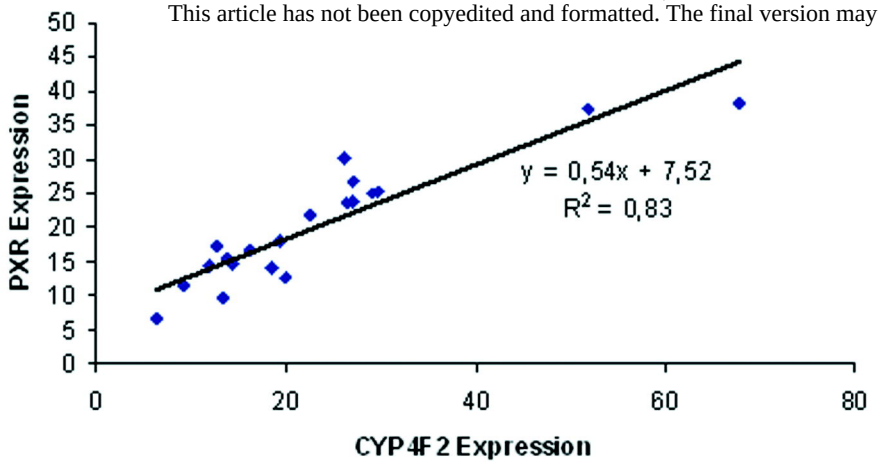
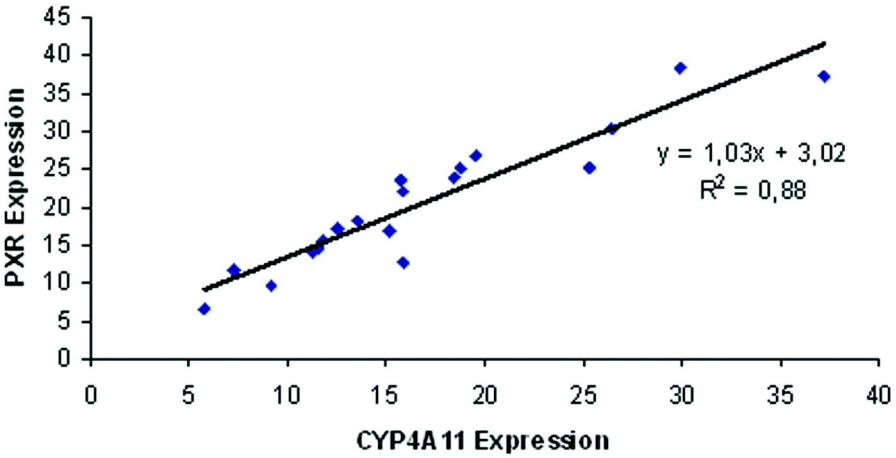


FIGURE 5

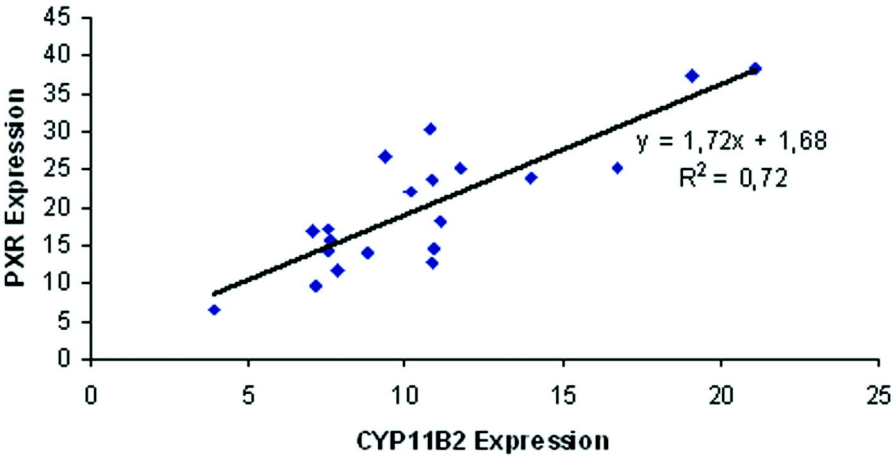
A)



B)



C)



D)