Profiling the expression of cytochrome P450 in breast cancer

Graeme I Murray[1], Siva Patimalla[1], Keith N Stewart[1], Iain D Miller[2], Steven D Heys[1].

[1]Division of Applied Medicine, School of Medicine and Dentistry, University of Aberdeen, Aberdeen, AB25 2ZD. [2]Department of Pathology, NHS Grampian, Foresterhill, Aberdeen, AB25 9ZD.

Address correspondence to: Professor Graeme I Murray, Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen, AB25 2ZD.

Tel. 01224 553794
Fax 01224 663002
e-mail: g.i.murray@abdn.ac.uk

Running title: Cytochrome P450 and breast cancer

Key words: breast cancer, cytochrome P450, immunohistochemistry, prognosis, survival, tissue microarray

Abstract

Aims

The cytochrome P450s (P450) are key oxidative enzymes which metabolise many carcinogens and anti-cancer drugs. Thus these enzymes influence tumour development, tumour response to therapy and are putative tumour biomarkers. The purpose of this study was to define the P450 expression profile in breast cancer and establish the significance of P450 expression in this tumour type.

Methods and results

A tissue microarray containing 170 breast cancers of no special type was immunostained for a panel of twenty-one P450s. The highest percentage of strongly positive staining in breast cancers was seen for CYP4X1 (50.8%), CYP2S1 (37.5%) and CYP2U1 (32.2%) respectively, while CYP2J (98.6%) and CYP3A43 (70.7%) were the P450s that most frequently displayed no immunoreactivity respectively. CYP4V2 (p=0.01), CYP4X1 (p=0.01) and CYP4Z1 (p=0.01) showed correlations with tumour grade. CYP1B1 (p=0.001), CYP3A5 (p=0.001) and CYP51 (p=0.005) showed the most significant correlations with oestrogen receptor status. Correlations with survival were identified for CYP2S1 (p=0.03), CYP3A4 (p=0.025), CYP4V2 (p=0.026) and CYP26A1 (p=0.03), although none of these P450s were independent markers of prognosis.

Conclusions

This study has defined the expression profile of cytochrome P450 in breast cancer and may offer the potential application as biomarkers to aid decisions regarding optimal adjuvant hormonal therapy.

Introduction

Breast cancer is the commonest type of cancer in women with approximately 45,000 new cases per annum in the United Kingdom [1]. The overall survival, however, has increased substantially. At present almost 80% of patients will survive for 5 years with major developments having occurred in the adjuvant therapy for patients with breast cancer [2]. These adjuvant therapies have focused on different types of hormonal therapy for those with hormone receptor positive tumours, chemotherapy for patients at increased and different risks of recurrence and the use of trastuzumab in some of those patients whose tumours overexpress her-2 receptors [3]. However, substantial numbers of patients still develop metastatic disease and further improvements in survival are dependent on a better understanding and identification of cellular targets within the malignant cell for novel therapy development and for targeting of optimal therapies.

One such possibility that has attracted considerable interest in other tumour types has been understanding the functions and potential therapeutic target of the cytochrome P450 system of enzymes. The cytochromes P450 are a multi-gene superfamily of constitutive and inducible enzymes which oxidatively metabolise a structurally diverse range of environmental and endogenous chemical entities. These enzymes have a very broad substrate specificity including environmental toxins, therapeutic drugs and several different groups of endogenous compounds including steroid hormones, eicosanoids and retinoids. Thus this group of enzymes are implicated in several different biological processes including carcinogenesis, determining response to drugs and cell signalling [4-6].

There is particular interest in determining the expression of these enzymes in tumours. There is evidence to indicate specific forms of cytochrome P450 are selectively expressed in tumours and that some forms may show tumour type selectivity. Furthermore, some of the cytochrome P450s are involved in the metabolism of chemotherapeutic agents, especially the

taxanes, docetaxel and paclitaxel, which have become key agents used in the treatment of breast cancer. Therefore, the consequences of this are that cytochrome P450s may represent valuable therapeutic targets, may influence the response of tumours to current and new chemotherapeutic agents and also to several established and postulated carcinogens [4-9]. Indeed, there have been developments in terms of therapy based on cytochrome P450 targets such as modification of expression, cytochrome P450 vaccines, cytochrome P450 pro-drug activation and inhibition [10-14].

Whilst there has been extensive investigation of cytochrome P450 profiling on certain types of human cancers [15-17], e.g. colorectal cancer, less detailed investigation have been undertaken in breast tumours. Therefore, in this study we have carried out a comprehensive investigation into the expression of a wide range of cytochrome P450s in breast cancer and determined the clinico-pathological significance of expression of these enzymes including their relationship to survival.

Materials and methods

Tissue and tissue microarray

Breast cancer tissue was obtained from a series of 170 patients with invasive breast cancer of no special type who were treated at Aberdeen Royal Infirmary between 2000 and 2003. Patients selected were those who were surgically treated by either mastectomy or breast conservation surgery, together with appropriate axillary surgery (sample or clearance as at the discretion of the responsible consultant surgeon) and had not received neoadjuvant chemotherapy prior to surgery. Complete follow up data was available for all patients. There were 17 deaths during follow-up with a mean survival of greater than 92 months. The histopathology of all the breast tumour were reported according Royal College of Pathologists criteria and the clinico-pathological parameters of the patients and their tumours included in this study are summarised in Table 1. All patients who had oestrogen receptor positive tumours received adjuvant tamoxifen for 5 years which was the standard treatment during this period.

Areas of tumour to be sampled were first identified and marked on the corresponding haematoxylin and eosin stained slide by a consultant breast pathologist (IDM). Two 1mm cores were taken from these areas of the wax embedded tumour blocks using a Beecher Instruments Microarrayer and placed in a recipient paraffin block. Following transfer, the recipient array block was heated to 37^{o}C, and a glass slide was used to carefully press down the cores to ensure they were all at the same level. Microarray blocks were sectioned using a standard microtome (Leica RM2125) producing sections at a thickness of 4μm, collected onto charged slides (Menzel Glaser Superfrost) before being dried at 70^{o}C for 30 mins. The project had the approval of The North of Scotland Research Ethics Service (ref. no. 07/S0801/6).

Antibodies

A panel (n=21) of cytochrome P450 antibodies was used in this study (Table 2). Eighteen of the antibodies were produced in GIM's laboratory and their development and characterisation has been described previously [15, 16]. Polyclonal antibodies to CYP1A1 and CYP2C were purchased from Chemicon Europe (Chandlers Ford, UK) while a polyclonal antibody to CYP2E1 was bought from Oxford Biomedical Research (Oxford, MI, USA). An antibody to cytochrome P450 reductase was obtained from Chemicon.

Immunohistochemistry

Immunohistochemistry for each antibody was performed with the Dako Envision system using a Dako autostainer (Dako, Ely, UK) as previously described [18,19]. Sections of the tissue microarray were dewaxed, rehydrated and an antigen retrieval step performed when required. The antigen retrieval step consisted of microwaving the sections in 0.01M citrate buffer at pH6.0 for 20 minutes in an 800W microwave oven operated at full power. The sections were then allowed to cool to room temperature. Primary antibody appropriately diluted (Table 2) in antibody diluent (Dako) was applied for 60 minutes at room temperature, washed with buffer (Dako) followed by peroxidase blocking 5 minutes (Dako) followed by a single 2 minute buffer wash. Pre-diluted peroxidase-polymer labelled goat anti-mouse/rabbit secondary antibody (Envision™, Dako) was applied for 30 minutes at room temperature, followed by further washing with buffer to remove unbound antibody. Sites of peroxidase activity were then demonstrated with diaminobenzidine as the chromogen applied for 3 successive 5 minute periods. Finally sections were washed in water, lightly counterstained with haematoxylin, dehydrated and mounted. Tissues known to express the relevant cytochrome P450 were used as positive controls [15, 16]. Omitting the primary antibody from

the immunohistochemical procedure and replacing it either with antibody diluent or non-immune rabbit serum as appropriate acted as negative controls.

The sections were evaluated by light microscopic examination and the intensity of immunostaining in each core assessed independently by two observers (KNS and GIM) using a scoring system previously described for the assessment of cytochrome P450 expression in tumour microarrays [15,16]. The intensity of immunostaining in each core was scored as negative, weak, moderate or strong. Any tumour immunoreactivity was regarded as positive and in each of those cases uniform tumour immunostaining was observed. Intra-tumoural heterogeneity (either within individual cores or between cores) was not identified which is consistent with our previous studies of the presence of several cytochrome P450s in whole sections of breast cancer [17,20] and also in our studies of multiple cytochrome P450 expression in tumour microarrays [15,16]. Any discrepancies in the evaluation of cytochrome P450 expression between the two observers were resolved by simultaneous re-evaluation.

Statistics

Statistical analysis including chi-squared test, Kaplan-Meier survival analysis and Cox multi-variate analysis were performed using SPSS v17 for Windows XP™ (SPSS UK, Ltd, Woking, UK). The log rank test was used to determine survival differences between individual groups. We regarded $p<0.05$ as significant

Results

Expression of individual cytochrome P450s in breast cancer and relationship of cytochrome P450 and clinico-pathological variables

The individual cytochrome P450s studied showed variable staining in individual breast cancers (Figure 1). The highest percentage of strongly positive cytochrome P450 staining was seen for CYP4X1 (50.8%), CYP2S1 (37.5%) and CYP2U1 (32.2%), respectively, while CYP2J (98.6%), CYP3A43 (70.7%) and CYP2A/2B (66.3%) showed the highest proportion of breast cancers that displayed no immunoreactivity, respectively (Figure 2). Immunoreactivity for each cytochrome P450 was identified in the cytoplasm of tumour cells and intra-tumour heterogeneity was not observed (Figure 1). There was no nuclear or cell surface membrane staining for any of the cytochrome P450s.

Several cytochrome P450s including CYP2U1 (χ^2=11.83, p=0.02), CYP4V2 (χ^2=17.14, p=0.01), CYP4X1 (χ^2=16.37, p=0.01) and CYP4Z1 (χ^2=16.5, p=0.01) showed correlations with tumour grade (table 3). CYP2U1, CYP4X1 and CYP4Z1 were associated with increasing tumour grade while expression of CYP4V2 was associated with lower tumour grade.

CYP1B1 (χ^2=16.67, p=0.001), CYP3A5 (χ^2=17.04, p=0.001) and CYP51 (χ^2=12.76, p=0.005) were the cytochrome P450s which showed the most significant correlations with oestrogen receptor status (table 4). CYP1B1 (χ^2=20.02, p<0.001), CYP2U1 (χ^2=10.64, p=0.005) and CYP51 (χ^2=18.65, p<0.001) were the cytochrome P450s which showed the most significant correlations with progesterone receptor status (table 4). There was no significant relationship between any of the cytochromes P450 and the Nottingham prognostic Index or lymph node status.

Associations with survival were identified for CYP2S1 (χ^2=4.55, p=0.03), CYP3A4 (χ^2=5.03, p=0.025), CYP4V2 (χ^2=4.98, p=0.026) and CYP26A1 (χ^2=4.67, p=0.03) in each case absence of expression being associated with better patient survival (Figure 3a-d). The

mean survival for patients with tumours that showed low/negative CYP2S1 was 96 months (95% CI 92-100 months) while the mean survival for patients with tumours that showed moderate/strong CYP2S1 was 77 months (95% CI 71-82 months. For patients with tumours that showed low/negative CYP3A4 immunoreactivity the survival was 79 months (95% CI 77-81 months) while for patients with tumours that showed moderate/strong CYP3A4 immunoreactivity the mean survival was 86 months (95% CI 79-93 months). For patients with tumours that showed negative CYP4V2 immunoreactivity had a mean survival of 96 months (95% CI 92-100 months) while for patients with tumours that showed positive CYP4V2 immunoreactivity the mean survival was 76 months(95% CI 69-82 months). For patients with tumours that showed CYP26A1 low/negative immunoreactivity the mean survival was 95 months (95% CI 91-99 months) while for patients with tumours that showed moderate/strong immunoreactivity the mean survival was 75 months(95% CI 67-82 months). In univariate survival analysis overall patient survival was also associated with Nottingham Prognostic Index (χ^2=19.15, p<0.001), tumour grade (χ^2=13.93, p=0.001) and lymph node status (χ^2=4.18, p=0.041). In Cox multi-variate analysis the Nottingham Prognostic Index remained independently significant (p=0.01) while none of cytochromes P450s were independent markers of prognosis.

Analysis of those patients who had received adjuvant tamoxifen identified a continued association with survival for CYP2S1 (χ^2=4.69, p=0.03), CYP3A4 (χ^2=6.52, p=0.01), and CYP26A1 (χ^2=6.39, p=0.01) was observed with in each case absence of expression being associated with better patient survival (Figure 3e-g). The mean survival for patients with tumours that showed low/negative CYP2S1 was 85 months (95% CI 81-88 months) while the mean survival for patients with tumours that showed moderate/strong CYP2S1 was 69 months (95% CI 62-74 months. For patients with tumours that showed low/negative CYP3A4 immunoreactivity the survival was 79 months (95% CI 77-81 months) while for

patients with tumours that showed moderate/strong CYP3A4 immunoreactivity the mean survival was 74 months (95% CI 66-83 months). For patients with tumours that showed CYP26A1 low/negative immunoreactivity the mean survival was 85 months (95% CI 82-88 months) while for patients with tumours that showed moderate/strong immunoreactivity the mean survival was 65 months(95% CI 67-82 months).

Discussion

This study has defined the expression profile of cytochrome P450 in breast cancer and correlated expression of individual cytochrome P450s with some key clinico-pathological factors namely tumour grade, oestrogen receptor status, lymph node status and overall patient survival in a large and well characterised set of patients with breast cancer of no special type. The cytochromes P450 are a multi-gene superfamily of enzymes which oxidatively metabolise a wide range of environmental and endogenous substrate. These enzymes have a very broad substrate specificity including environmental toxins, therapeutic drugs and several different groups of biologically active endogenous compounds particularly steroid hormones and eicosanoids. Thus this group of enzymes are implicated in several different biological processes including carcinogenesis, determining response to drugs and cell signalling [4-9]. There is particular interest in determining the expression of these enzymes in tumours. There is evidence to indicate that specific forms of cytochrome P450 are selectively expressed in tumours, that some forms of cytochrome P450 may show tumour-type selectivity and the consequences of this are that individual cytochrome P450s are potential tumour biomarkers and in particular may represent valuable therapeutic targets. There is also evidence to indicate that intra-tumoural expression of cytochrome P450s may also influence the response of tumours to established anti-cancer drugs [17-21].

A comprehensive panel of twenty-one cytochrome P450s were studied by immunohistochemistry in this breast cancer cohort and evaluated in relation to key patho-biological parameters. There have been several previous studies of the expression of cytochrome P450 in breast cancer using a range of investigative techniques including immunohistochemistry, reverse transcriptase PCR and activity assays. The largest previous study in terms of the number of breast cancers studied was the investigation of Haas et al [23]. In that study breast cancers from 393 patients (including 270 breast cancers of no special

type) were analysed by immunohistochemistry for five cytochrome P450s namely CYP1A1, CYP1A2, CYP1B1, CYP2E1 and CYP3A4/5. That study found that expression of CYP3A4/5 was significantly associated with lymph node metastases while CYP1B1 was associated with poor tumour differentiation. Bieche et al [24] analysed a series of 97 oestrogen receptor positive breast cancers from post-menopausal women for two cytochrome P450s (CYP2A6 and CYP2B6) which had been identified from a preliminary screen of nine cytochrome P450s (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYO2E1, CYP3A4) in a small series of oestrogen receptor positive and negative breast cancers. Neither CYP2A6 nor CYP2B6 showed any relationship with clinico-pathological parameters or survival. Previously Albin et al.[25] analysed 12 breast cancers for activity for CYP1A1, CYP2B, CYP2C, CYP2E1 and CYP3A4 and found an absence of activity for all the cytochrome P450s assayed. This finding most probably represents a lack of sensitivity of the assays used to measure activity of these cytochrome P450s in tumour samples. A survey of four cytochrome P450 mRNAs (CYP1A1, CYP1B1, CYP2C and CYP3A4) by RT-PCR was undertaken in a small series of breast cancers and found variable expression of the individual P450s with CYP1B1 mRNA being present in most breast cancer samples [26]. This is consistent with our previous study of CYP1B1 mRNA in breast cancer [27]. A previous study from this laboratory has investigated the presence of two cytochrome P450s (CYP1A and CYP3A) in 54 breast cancers of no special type and showed more frequent expression of CYP1A compared with CYP3A in this series of breast cancers and there was no correlation of expression with either tumour grade or oestrogen receptor status [25].

The main anti-cancer drugs (tamoxifen, paclitaxel, docetaxel) used in the clinical treatment of breast cancer all undergo cytochrome P450 mediated metabolism mainly deactivation. While quantitatively the majority of systemic metabolism of these drugs will occur in the liver since most of the cytochrome P450s involved in the metabolism of these

drugs are present in the liver. However, intra-tumoural cytochrome P450 mediated metabolism is emerging as an important source of modulation of the local tumour activity of these drugs especially since the profile of cytochrome P450 expression in tumours is distinct from that in liver as is the expression of other drug metabolism enzyme which can have a further influence on the outcome in relative terms of activation and deactivation [28-34].

Several of the cytochrome P450s that correlated with oestrogen receptor expression are cytochrome P450s that are known to be involved in either the oestrogen biosynthetic or metabolic pathways. CYP1B1 which was most strongly correlated with oestrogen receptor expression is responsible for the 4-hydroxylation of oestrogen while CYP51 which was the cytochrome P450 next most strongly correlated with oestrogen receptor is a key enzyme in the sterol biosynthetic pathway. Expression of both CYP1B1 and CYP51 were also correlated with progesterone receptor status.

Several cytochrome P450s (CYP2S1, CYP3A4, CYP4V2 and CYP26A1) were significantly associated with survival, although none of cytochromes P450s were independent markers of prognosis. Each of these cytochrome P450s showed very similar survival characteristics (χ^2 value, p value and mean survival) and visual inspection of the survival curves for each of these cytochrome P450s showed very similar shapes to the individual curves and investigation of the expression of these four cytochrome P450s showed that they strongly correlated with each other. Therefore any one of these cytochrome P450s could be used as a surrogate marker for the others in the context of these cytochrome P450s being prognostic markers.

In conclusion this study has defined the expression profile of cytochrome P450 in breast cancer and shown that several cytochrome P450s are frequently expressed in breast cancer. The expression of individual cytochrome P450s has been correlated with the major clinico-pathological parameters and also shown that several cytochrome P450s are associated

with outcome in breast cancer and thus they require further study as potential biomarkers to indicate patients at higher risk of disease recurrence and for whom targeted adjuvant therapy might be indicated.

Acknowledgements

This research was supported by a research grant from endowment funds of NHS Grampian.

References

1. Cancer Research UK. Breast Cancer. http://info.cancerresearchuk.org/cancerstats/types/breast/?a=5441

2. Bedard PL, Cardoso F. Recent advances in adjuvant systemic therapy for early-stage breast cancer. *Ann Oncol*. 2008;**19**(Suppl 5):122-127.

3. Dahabreh IJ, Linardou H, Siannis F, Fountzilas G, Murray S. Trastuzumab in the adjuvant treatment of early stage breast cancer: a systematic review and meta-analysis of randomized controlled trials. *Oncologist*. 2008; **13**: 620-630.

4. Murray GI. The role of cytochrome P450 in tumour development and progression and its potential in therapy. *J. Pathol.* 2000;**192**:419-426.

5. Rooney PH, Telfer C, McFadyen MCE, Melvin WT, Murray GI. The role of cytochrome P450 in cytotoxic bioactivation: Future therapeutic directions. *Curr. Cancer Drug Targets* 2004;**4**:257-265.

6. McFadyen MCE, Melvin WT, Murray GI. Cytochrome P450 enzymes: Novel options for cancer therapeutics. *Mol. Cancer. Ther.* 2004;**3**:363-371.

7. Patterson LH, Murray GI. Tumour cytochrome P450 and drug activation. *Curr. Pharm. Des.* 2002;**8**:1335-1347.

8. Bruno RD, Njar VC. Targeting cytochrome P450 enzymes: a new approach in anti-cancer drug development. *Bioorg. Med. Chem.* 2007;**15**:5047-5060.

9. Michael M, Doherty MM. Drug metabolism by tumours: its nature, relevance and therapeutic implications. *Expert Opin. Drug Metab. Toxicol.* 2007;**3**:783-803.

10. Fujita K. Cytochrome P450 and anticancer drugs. *Curr. Drug Metab.* 2006;**7**:23-37.

11. Downie D, Rooney PH, McFadyen MCE, Murray GI. Pharmacogenomics of cytochrome P450 enzymes in tumours. *Curr. Pharmacogenomics* 2004;**2**:243-254.

12. McFadyen MCE, Murray GI. Cytochrome P450 1B1: A novel anti-cancer therapeutic target. *Future Oncol.* 2005;**1**:259-263.

13. Luby TM. Targeting cytochrome P450 CYP1B1 with a therapeutic cancer vaccine. *Expert Rev. Vaccines.* 2008;**7**:995-1003.

14. Rodriguez-Antona C, Ingelman-Sundberg M. Cytochrome P450 pharmacogenetics and cancer. *Oncogene.* 2006;**25**:1679-1691.

15. Kumarakulasingham M, Rooney PH, Dundas SR, *et al*. Cytochrome P450 profile of colorectal cancer: Identification of markers of prognosis. *Clin. Cancer Res.* 2005;**11**:3759-3764.

16. Downie D, McFadyen MCE, Rooney PH, *et al*. Profiling cytochrome P450 expression in ovarian cancer: Identification of prognostic markers. *Clin. Cancer Res*. 2005;**11**:7369-7375.

17. Murray GI, Taylor MC, McFadyen MC, *et al*. Tumour specific expression of cytochrome P450 CYP1B1. *Cancer Res.* 1997;**57**:3026-3031.

18. Dundas SR, Lawrie LC, Rooney PH, Murray GI. Mortalin is overexpressed by colorectal cancer and correlates with poor survival. *J. Pathol.* 2005;**205**:74-81.

19. Coghlin C, Carpenter B, Dundas SR, Lawrie LC, Telfer C, Murray GI. Characterisation and over-expression of chaperonin T-complex proteins in colorectal cancer. *J. Pathol.* 2006;**210**:351-357.

20. Murray GI, Weaver RJ, Paterson PJ, Ewen SWB, Melvin WT, Burke MD. Expression of xenobiotic metabolising enzymes in breast cancer. *J. Pathol*. 1993;**169**:347-353.

21. Murray GI, Melvin WT, Greenlee WF, Burke MD. Regulation, function and tissue-specific expression of cytochrome P450 CYP1B1. *Annu. Rev. Pharmacol. Toxicol.* 2001;**41**:297-316.

22. Rieger MA, Ebner R, Bell DR, *et al*. Identification of a novel mammary-restricted cytochrome P450, CYP4Z1, with overexpression in breast carcinoma. *Cancer Res.* 2004;**64**:2357-2364.

23. Haas S, Pierl C, Harth V, Pesch B, *et al*. Expression of xenobiotic and steroid hormone metabolizing enzymes in human breast carcinomas. *Int J Cancer*. 2006;**119**:1785-1791.

24. Bièche I, Girault I, Urbain E, Tozlu S, Lidereau R. Relationship between intratumoral expression of genes coding for xenobiotic-metabolizing enzymes and benefit from adjuvant tamoxifen in estrogen receptor alpha-positive postmenopausal breast carcinoma. *Breast Cancer Res.* 2004;**6**:R252-263.

25. Albin N, Massaad L, Toussaint C, *et al*. Main drug-metabolizing enzyme systems in human breast tumors and peritumoral tissues. *Cancer Res.* 1993;**53**:3541-3546.

26. Huang Z, Fasco MJ, Figge HL, Keyomarsi K, Kaminsky LS. Expression of cytochromes P450 in human breast tissue and tumors. *Drug Metab. Dispos.* 1996;**24**:899-905.

27. McKay JA, Melvin WT, Ah-See AK, Ewen SWB, Greenlee WF, Marcus CB, Burke MD, Murray GI. Expression of cytochrome P450 CYP1B1 in breast cancer. *FEBS Lett.* 1995;**374**;270-272

28. Rochat B. Role of cytochrome P450 activity in the fate of anticancer agents and in drug resistance: focus on tamoxifen, paclitaxel and imatinib metabolism. *Clin. Pharmacokinet.* 2005;**44**:349-366.

29. Boocock DJ, Brown K, Gibbs AH, Sanchez E, Turteltaub KW, White IN. Identification of human CYP forms involved in the activation of tamoxifen and irreversible binding to DNA. *Carcinogenesis*. 2002;**23**:1897-1901.

30. Crewe HK, Notley LM, Wunsch RM, Lennard MS, Gillam EM. Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. *Drug Metab. Dispos.* 2002;**30**:869-874.

31. García-Martín E, Pizarro RM, Martínez C, *et al*.. Acquired resistance to the anticancer drug paclitaxel is associated with induction of cytochrome P450 2C8. *Pharmacogenomics.* 2006;**7**:575-585.

32. Miyoshi Y, Ando A, Takamura Y, Taguchi T, Tamaki Y, Noguchi S. Prediction of response to docetaxel by CYP3A4 mRNA expression in breast cancer tissues. *Int. J. Cancer.* 2002;**97**:129-132.

33. Martinez VG, O'Connor R, Liang Y, Clynes M. CYP1B1 expression is induced by docetaxel: effect on cell viability and drug resistance. *Br. J. Cancer.* 2008;**98**:564-570.

34. Marsh S, Somlo G, Li X, *et al*. Pharmacogenetic analysis of paclitaxel transport and metabolism genes in breast cancer. *Pharmacogenomics J.* 2007;**7**:362-365.

Table 1.

Clinico-pathological characteristics of patients in this study

Characteristic	Percent (number)	Relationship with survival
Age		χ^2=2.12, p=0.154
Range	36-92	
Mean	62 years	
Tumour grade		χ^2=13.93, p=0.001
1	24 % (n=42)	
2	39 % (n=65)	
3	37% (n=63)	
Tumour stage		χ^2=5.41, p=0.11
T1	68% (n=116)	
T2	26% (n=45)	
T3-4	6% (n=9)	
Lymph node status		χ^2=4.19, p=0.041
Negative	76% (n=129)	
Positive	23.5% (n=40)	
No lymph node dissection	0.5% (n=1)	
Oestrogen receptor status		χ^2=1.068, p=0.301
Positive	74.1% (n=126)	
Negative	24.7% (n=42)	
Not analysed	1.2% (n=2)	
Progesterone receptor status		χ^2=0.103, p=0.748
Positive	78.2% (n=133)	
Negative	20.6% (n=35)	
Not analysed	1.2% (n=2)	
Her 2 status		χ^2=4.166, p=0.244
0	60.1% (n=101)	
+	9.5% (n=16)	
++	14.9% (n=25)	
+++	15.5% (n=26)	
Not analysed	1.2% (n=2)	
Nottingham Prognostic Index		χ^2=19.15, p<0.001
Good<3.4	48% (n=81)	
Medium 3.4-5.4	41% (n=69)	
Poor >5.4	11% (n=18)	

Table 2

Details of the cytochrome P450 antibodies used in this study

P450 antibody	Source	Type	Antigen retrieval[1] and antibody dilution for immunohistochemistry
CYP1A1	Chemicon	Polyclonal	20 minutes, 1/1000
CYP1A2	Own laboratory	Monoclonal	20 minutes, undiluted tissue culture supernatant
CYP1B1	Own laboratory	Monoclonal	20 minutes, undiluted tissue culture supernatant
CYP2A6/2B6	Own laboratory	Monoclonal	20 minutes, undiluted tissue culture supernatant
CYP2C8/9/19	Chemicon	Polyclonal	No antigen retrieval, 1/500
CYP2E1	Oxford Biomedical Research	Polyclonal	20 minutes, 1/2000
CYP2F1	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP2J2	Own laboratory	Polyclonal	20 minutes, 1/200
CYP2R1	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP2S1	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP2U1	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP3A4	Own laboratory	Monoclonal	20 minutes, undiluted tissue culture supernatant
CYP3A5	Own laboratory	Monoclonal	20 minutes, undiluted tissue culture supernatant
CYP3A43	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP4V2	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP4X1	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP4Z1	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP24	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP26A1	Own laboratory	Monoclonal	20 minutes, 1/10
CYP39	Own laboratory	Polyclonal	20 minutes, 1/1000
CYP51	Own laboratory	Polyclonal	20 minutes, 1/1000

[1] The antigen retrieval step consisted of microwaving the sections in 0.01M citrate buffer pH6.0 for 20 minutes in an 800W microwave oven operated at full power.

Table 3

The correlation of cytochrome P450 expression and tumour grade

P450	χ^2	p value
CYP1A1	5.82	ns
CYP1A2	1.91	ns
CYP1B1	6.97	ns
CYP2A/2B	2.93	ns
CYP2C	5.90	ns
CYP2E	8.98	ns
CYP2F	3.66	ns
CYP2J	1.31	ns
CYP2R1	13.39	0.04
CYP2S1	11.70	ns
CYP2U1	11.83	0.02
CYP3A4	8.10	ns
CYP3A5	6.46	ns
CYP3A43	4.90	ns
CYP4V2	17.14	0.01
CYP4X1	16.37	0.01
CYP4Z1	16.50	0.01
CYP24	5.73	ns
CYP26A1	3.20	ns
CYP39	9.56	ns
CYP51	12.13	ns
P450 reductase	5.57	ns

Table 4

The correlation of cytochrome P450 expression, oestrogen receptor and progesterone receptor status

	Oestrogen receptor		Progesterone receptor	
P450	χ^2	p value	χ^2	p value
CYP1A1	3.87	ns	5.98	ns
CYP1A2	1.53	ns	2.41	ns
CYP1B1	16.67	0.001	20.02	<0.001
CYP2A/2B	1.96	ns	0.13	ns
CYP2C	4.33	ns	2.93	ns
CYP2E	6.00	ns	6.13	ns
CYP2F	3.32	ns	7.62	ns
CYP2J	0.50	ns	0.27	ns
CYP2R1	11.96	0.007	8.83	0.03
CYP2S1	12.62	0.006	12.63	0.006
CYP2U1	3.52	ns	10.64	0.005
CYP3A4	10.27	0.016	7.28	ns
CYP3A5	17.04	0.001	5.56	ns
CYP3A43	3.80	ns	6.33	0.04
CYP4V2	6.00	ns	12.76	0.005
CYP4X1	4.16	ns	3.58	ns
CYP4Z1	10.83	0.013	13.87	0.003
CYP24	1.52	ns	0.48	ns
CYP26A1	5.43	ns	2.84	ns
CYP39	6.27	ns	12.48	0.006
CYP51	12.76	0.005	18.65	<0.001
P450 reductase	6.53	ns	5.51	ns

Figure legends.

Figure 1

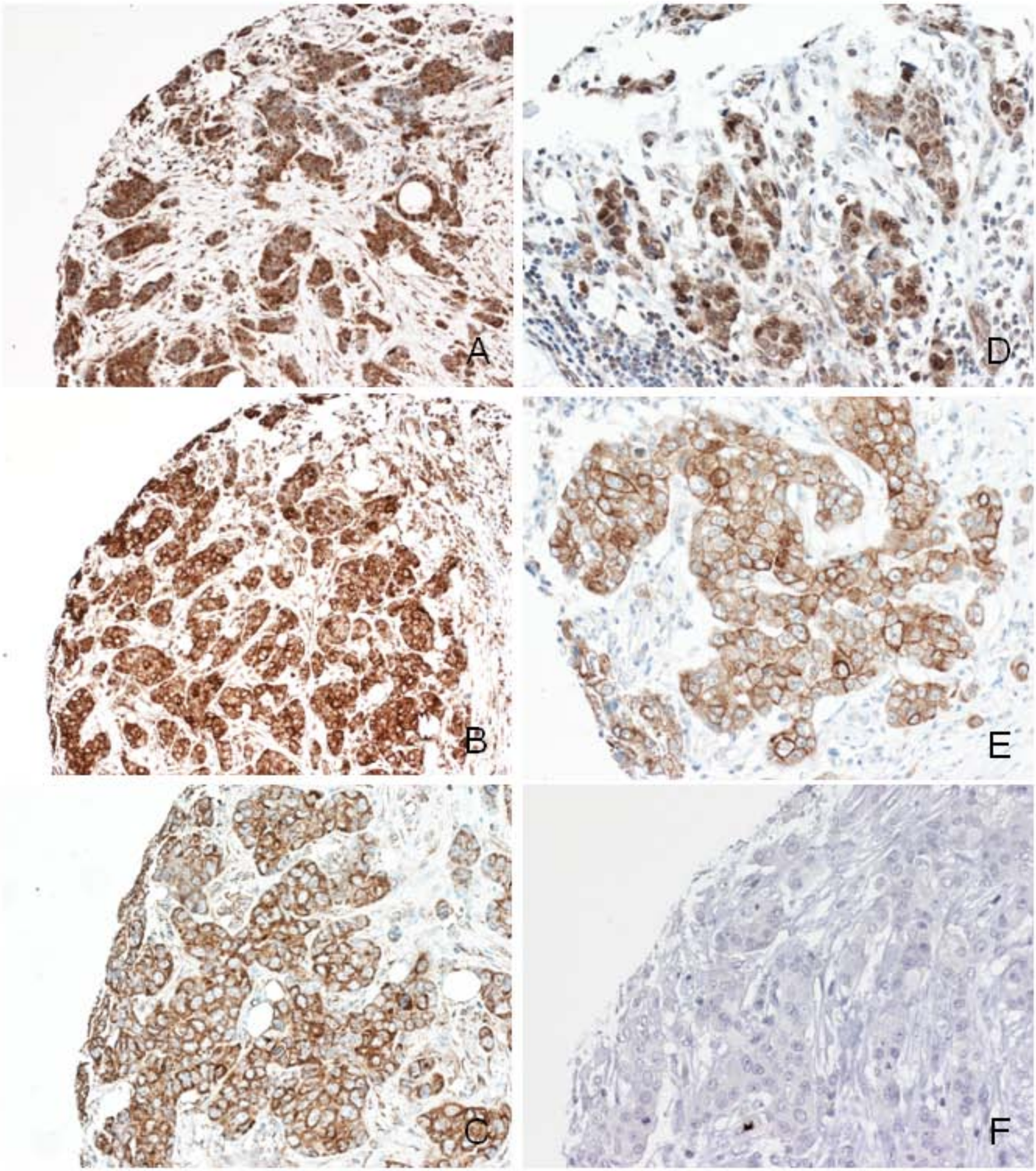
The immunohistochemical localisation of cytochrome P450 in breast cancer A. CYP2R1, B. CYP2S1, C. CYP3A4, D. CYP4V2, E. CYP26A1 and F. negative control. There is strong cytoplasmic expression in tumour cells for the individual cytochrome P450s.

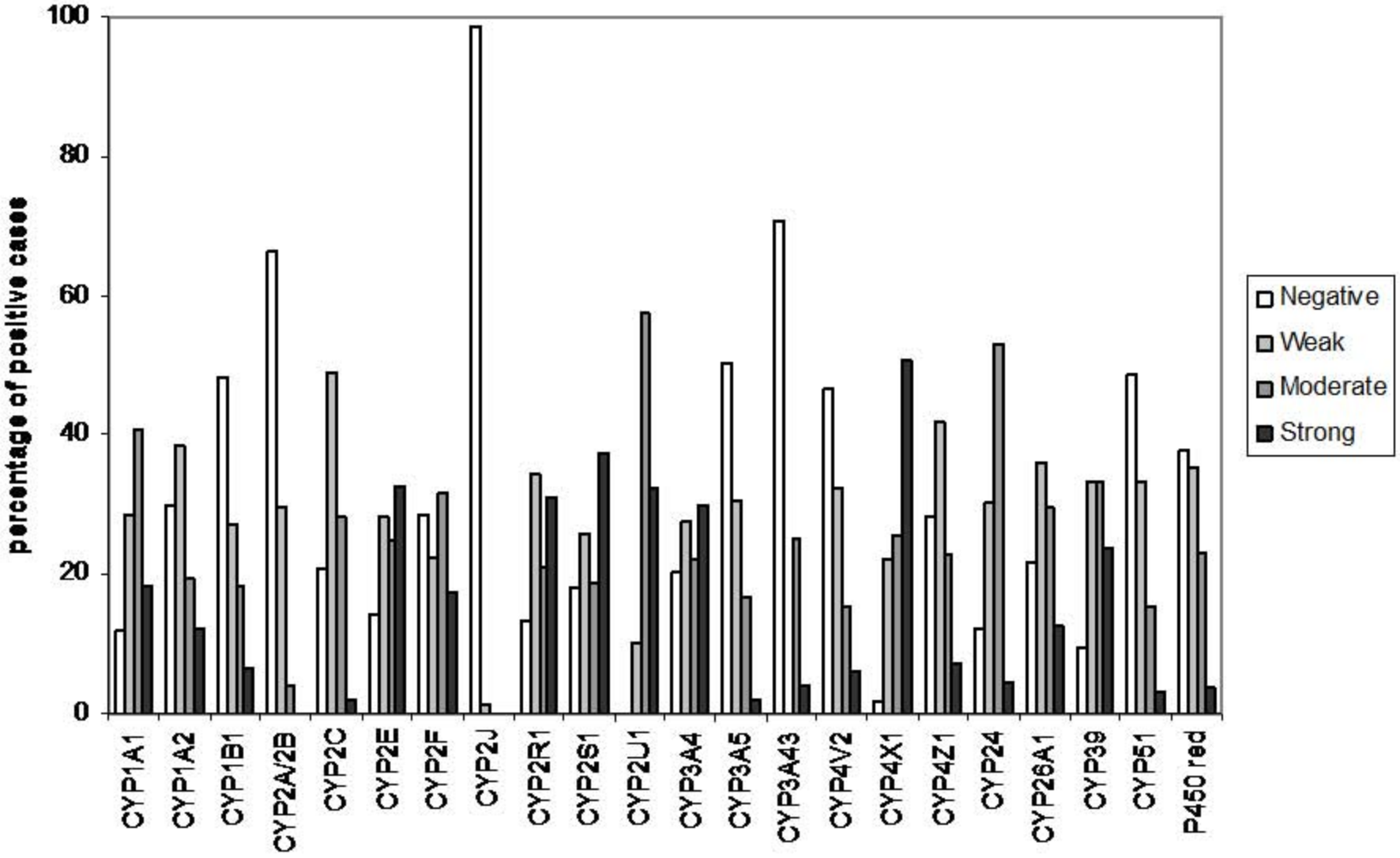
Figure 2

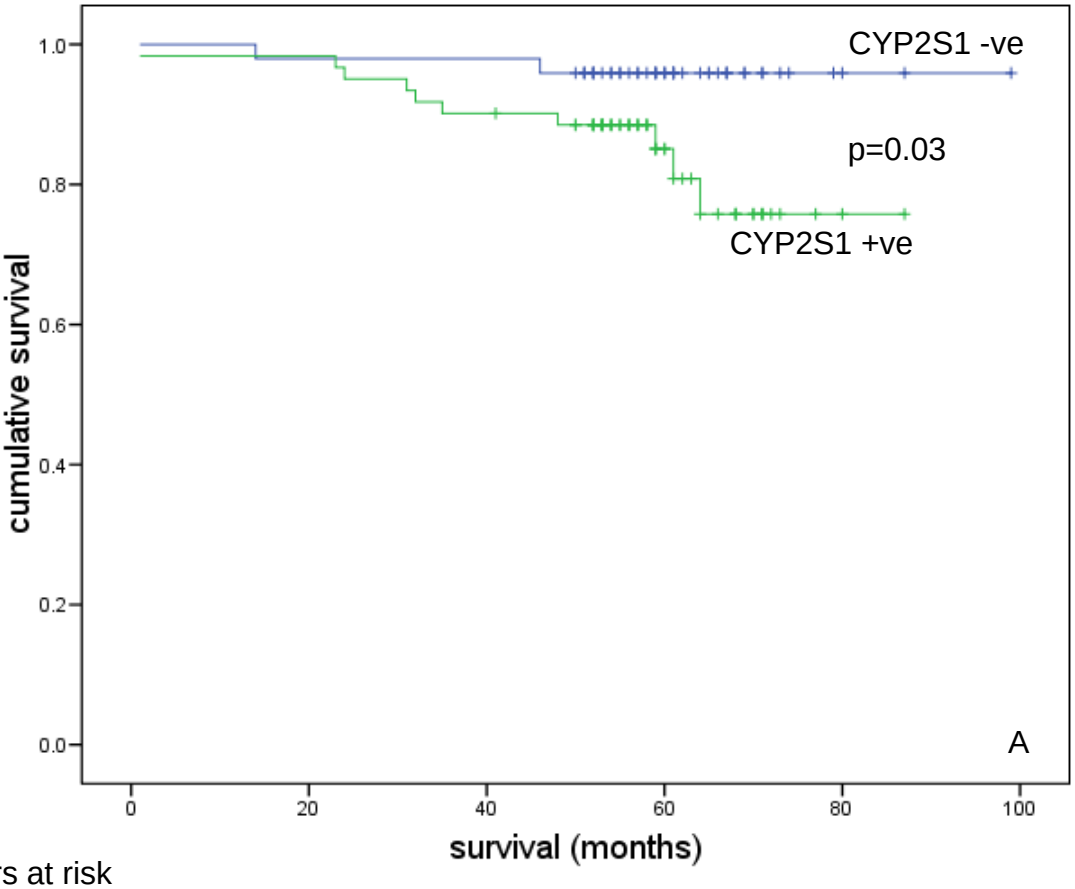
The cytochrome P450 expression frequencies in breast cancer.

Figure 3

Cytochrome P450 expression and survival in breast cancer. A-D is the complete patient cohort while E-G are those patients who have received adjuvant tamoxifen therapy. Strong expression of each of these cytochrome P450s was each associated with poorer survival.

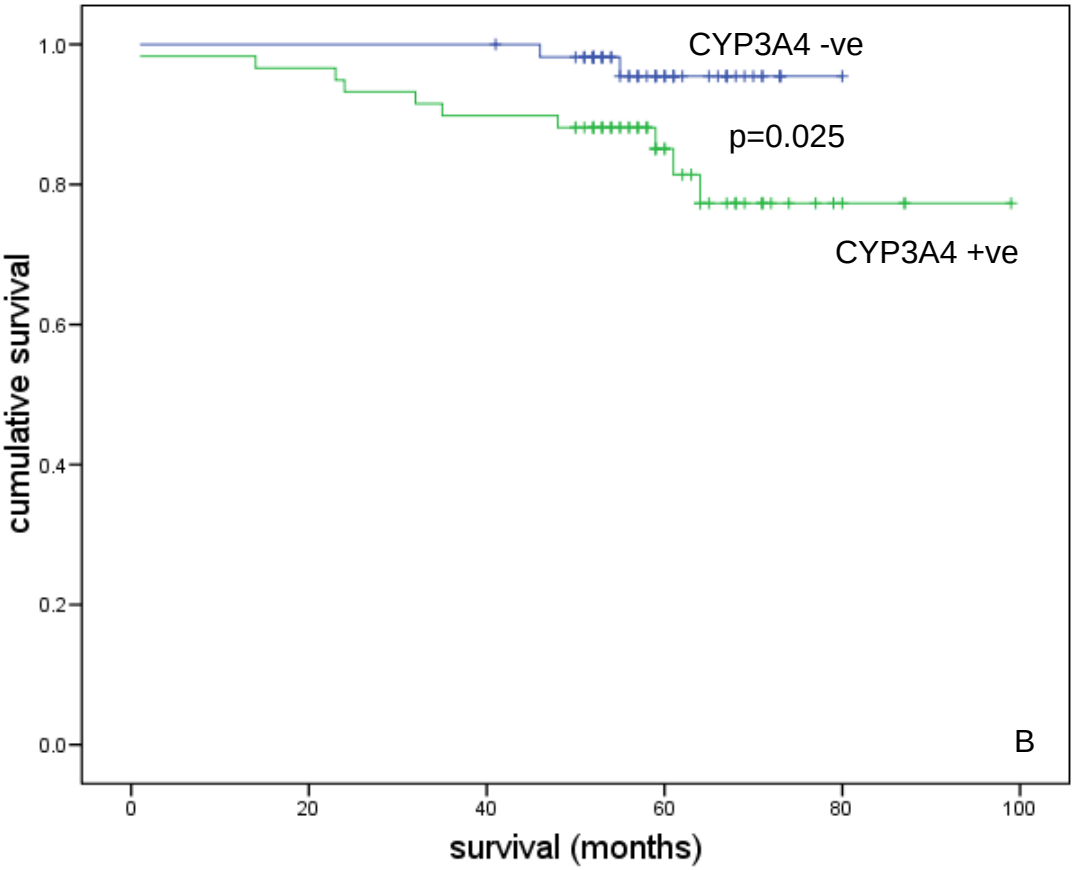

A
D
B
E
C
F


percentage of positive cases
100
80
60
40
20
0
CYP1A1
CYP1A2
CYP1B1
CYP2A/2B
CYP2C
CYP2E
CYP2F
CYP2J
CYP2R1
CYP2S1
CYP2U1
CYP3A4
CYP3A5
CYP3A43
CYP4V2
CYP4X1
CYP4Z1
CYP24
CYP26A1
CYP39
CYP51
P450 red
Negative
Weak
Moderate
Strong



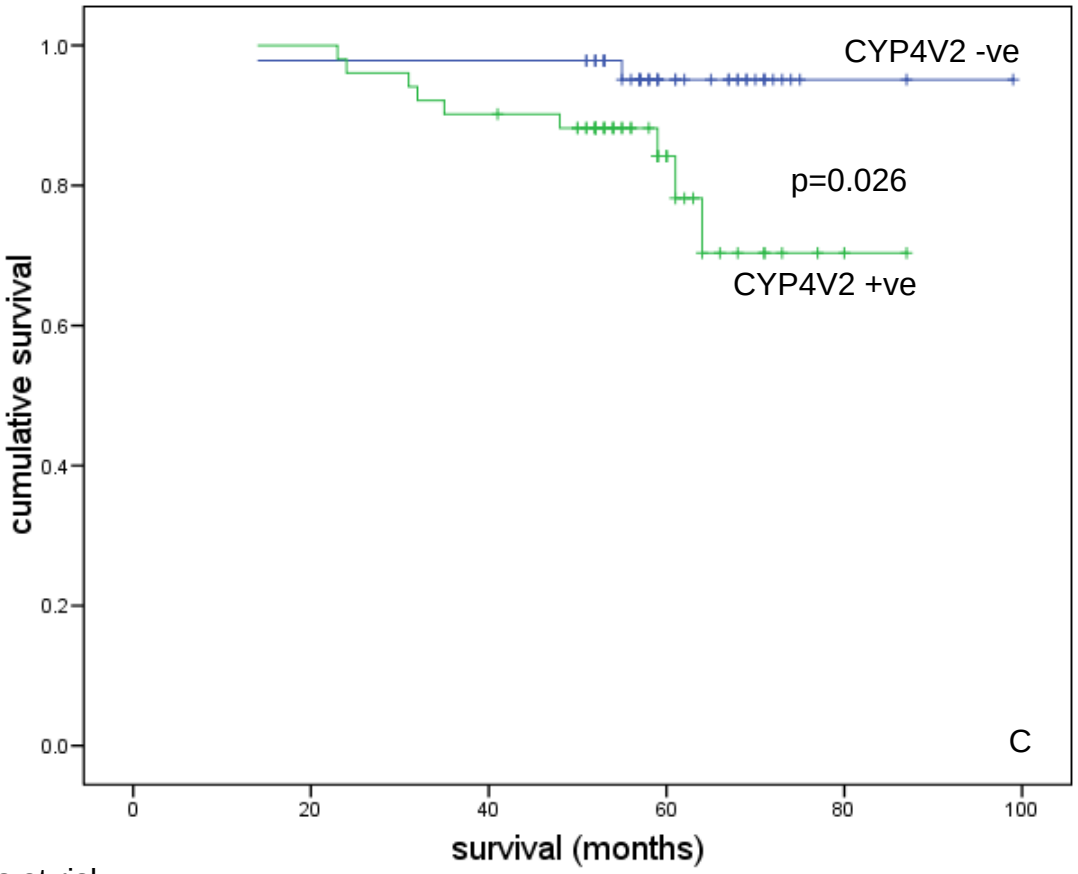
Numbers at risk

CYP2S1 –ve	49	48	48	23	3
CYP2S1 +ve	61	60	55	20	1



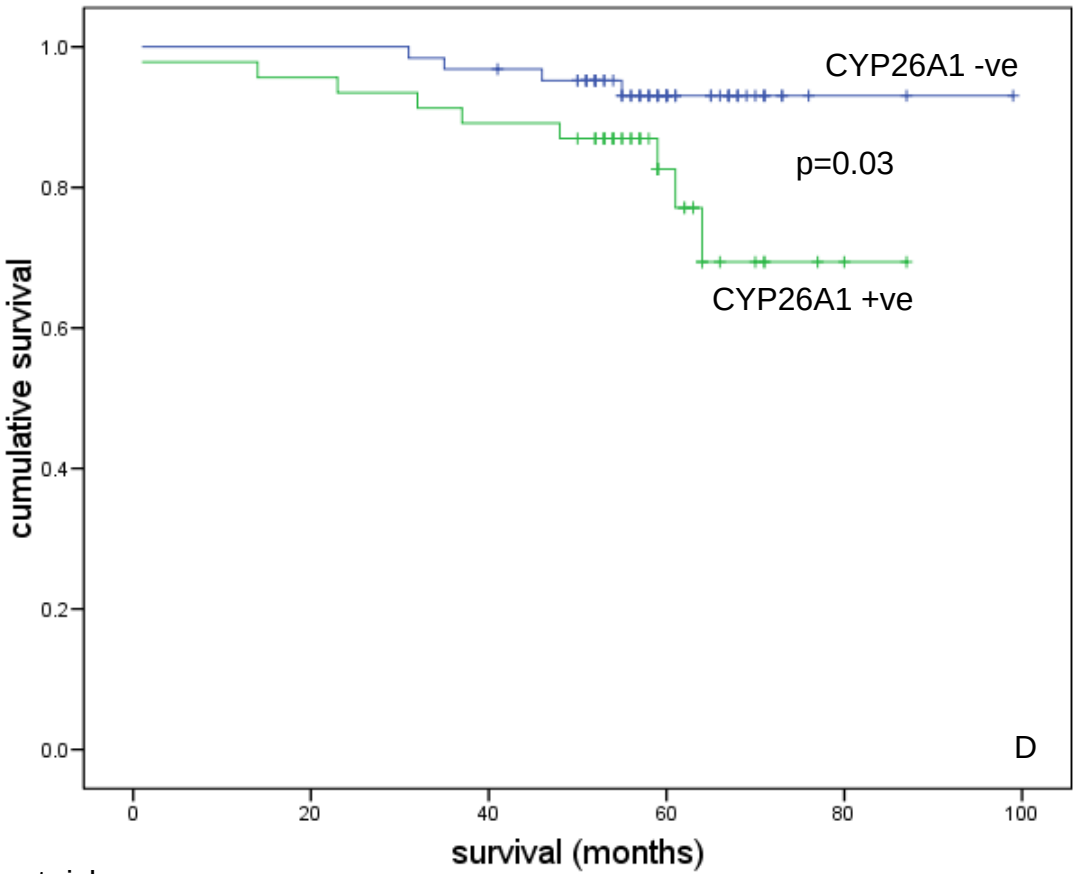
Numbers at risk

CYP3A4 –ve	56	56	56	20	0
CYP3A4 +ve	59	57	57	22	2



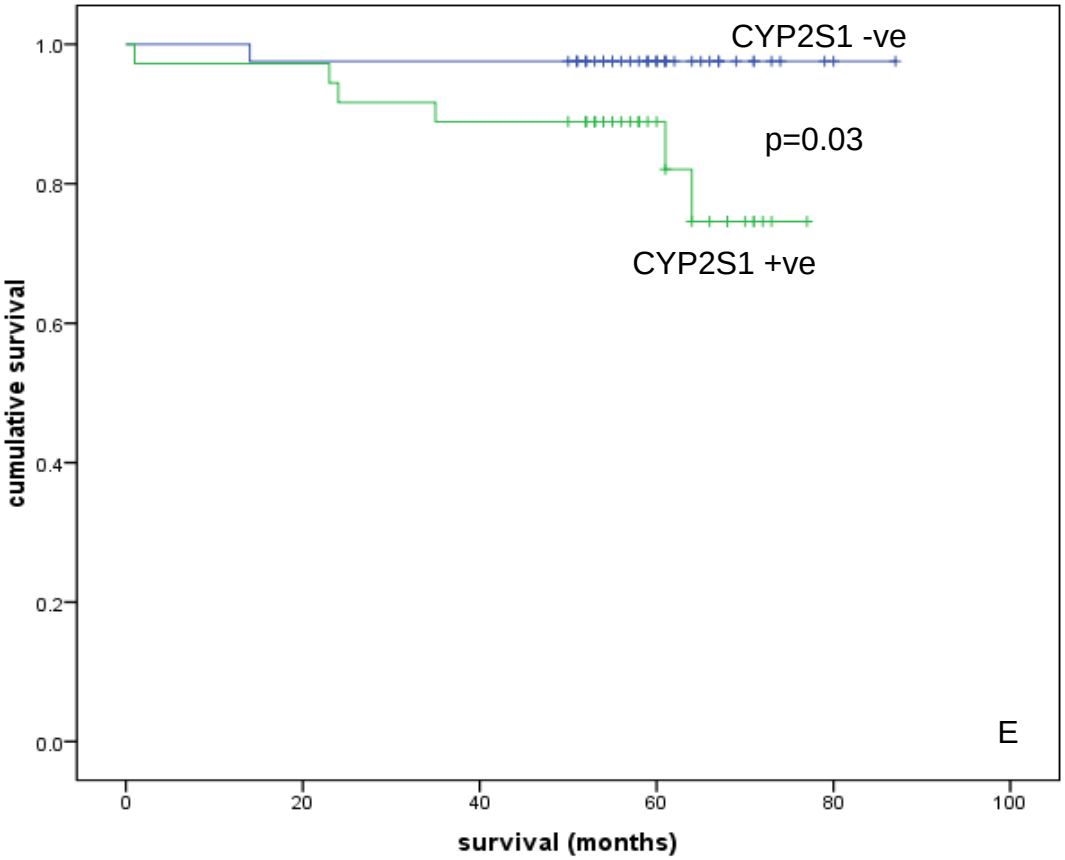
Numbers at risk

CYP4V2 –ve	46	45	45	20	2
CYP4V2 +ve	51	51	46	14	1



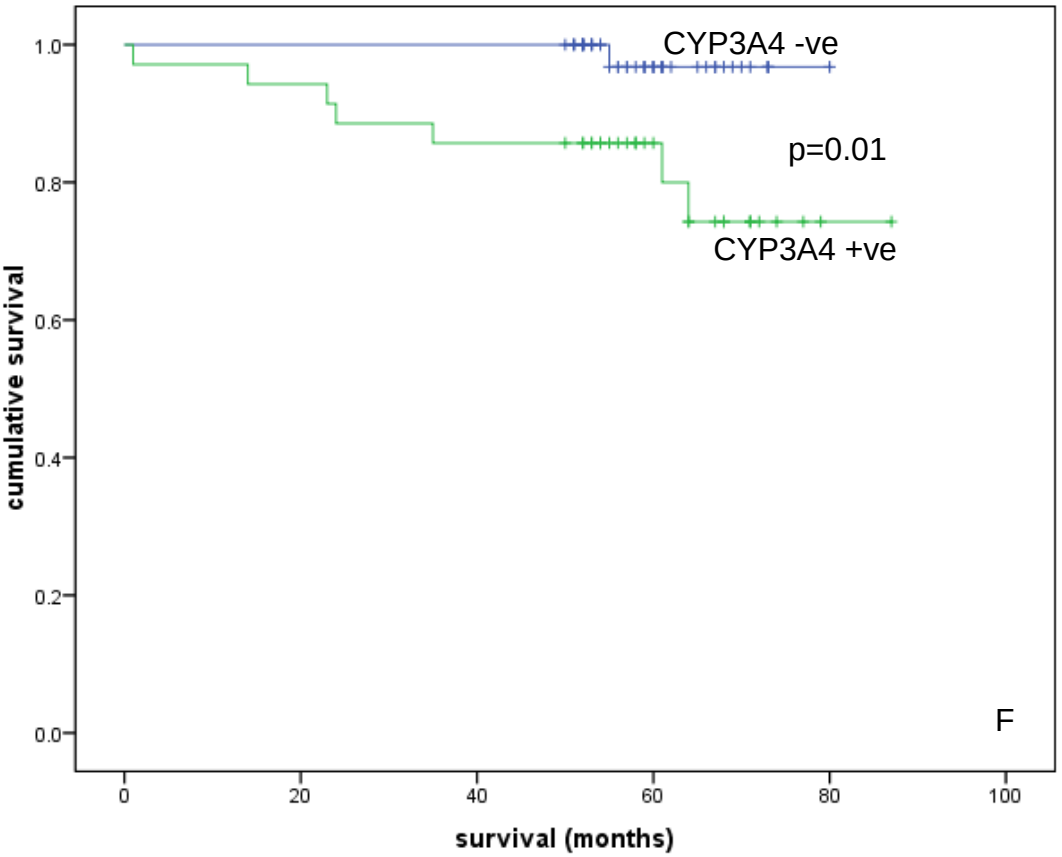
Numbers at risk

CYP26A1 –ve	63	62	61	23	2
CYP26A1 +ve	46	44	41	15	2



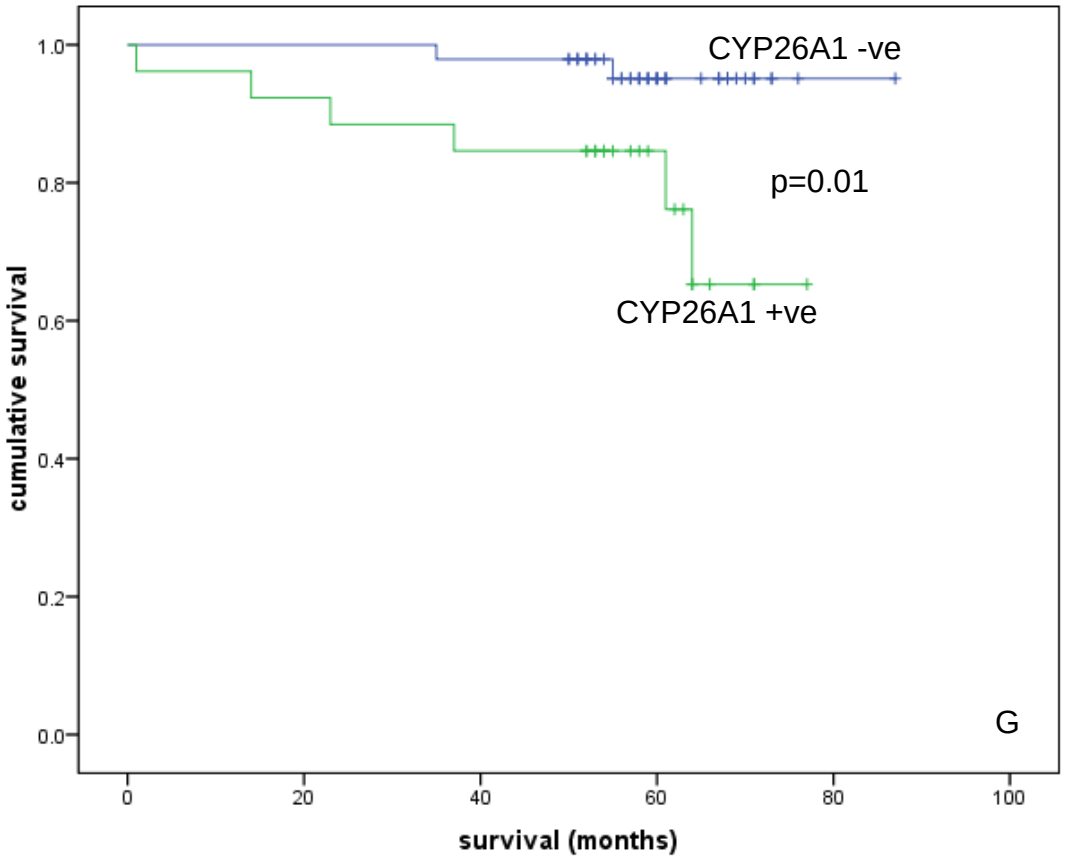
Numbers at risk

CYP2S1 –ve	41	40	40	20	1
CYP2S1 +ve	36	35	32	13	0



Numbers at risk

CYP3A4 –ve	48	30	30	21	0
CYP3A4 +ve	35	33	33	15	1



Numbers at risk

CYP26A1 –ve	48	48	47	18	1
CYP26A1 +ve	26	23	22	10	0