DIFFERENCES IN CYTOCHROME P450 (CYP3A4) EXPRESSION ALONE DO NOT EXPLAIN INTER-INDIVIDUAL VARIABILITY IN CYP3A MEDIATED HEPATIC TESTOSTERONE 6ß HYDROXYLASE (TST6ßOH) ACTIVITY

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INTRODUCTION

- The intrinsic activity of CYP450 isoforms expressed in a variety of recombinant systems has been shown to vary considerably due to differences in levels of the accessory proteins, cytochrome b5 (b5) and NADPH cytochrome P450 reductase (NCR) [1].
- The importance of these factors in explaining inter-individual variability in CYP3A4 related metabolism has only been assessed in a limited numbers of livers [2].
- Previously, using TST6βOH activity, we have demonstrated considerable variability (19 fold) in activity per unit enzyme (CYP3A4 k_{cat}) across a population of 51 livers [3] (Figure 1).
- We now report the contribution of inter-individual variability in b5 and NCR to CYP3A4 related metabolism in these same livers.

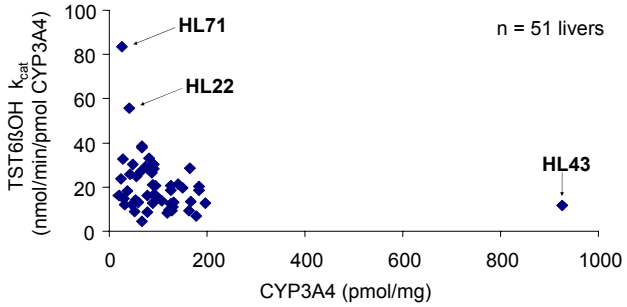

Figure 1: Relationship between CYP3A4 protein and k_{cat} (nmol/min/pmol CYP3A4)

MATERIALS & METHODS

- Microsomal CYP3A4 abundance and TST6ßOH (CYP3A) activity per mg microsomal protein (/mmp) were determined in microsomal samples from 51 livers held within the liver bank at the Unit of Clinical Pharmacology (University of Sheffield), as described previously [3].
- NCR activity was determined in all livers by reduction of the exogenous substrate cytochrome c [4].
- In a subset of 36 livers, levels of b5 holoprotein were measured by dithionite difference spectroscopy [5].

RESULTS AND DISCUSSION

- No significant relationship was found between b5 and TST6βOH activity (p = 0.84).
- However, multiple linear regression (MLR) analysis showed NCR (p = 0.04) (Figure 2) and CYP3A4 (p<0.001) to be significant covariates of TST6βOH activity.

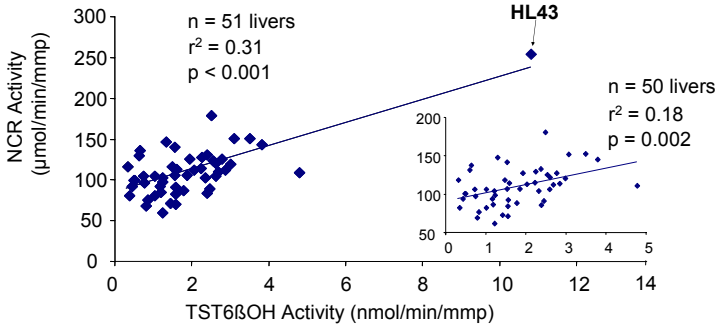

Figure 2: Relationship between TST6ßOH activity (/mmp) and NCR activity (/mmp)

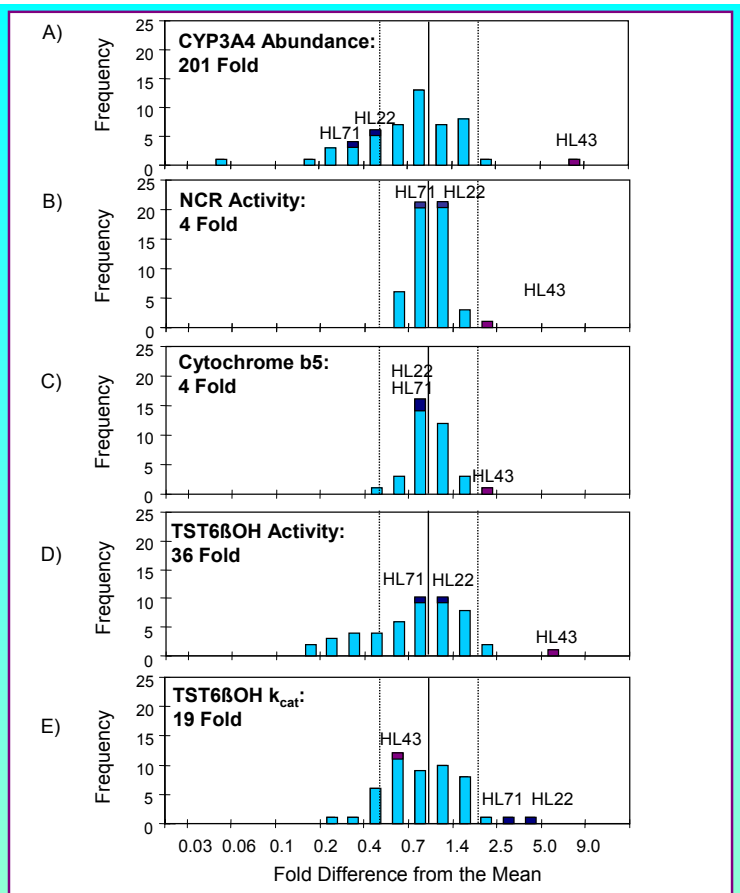

Figure 3: Fold variation in protein abundance and enzyme activity in microsomes from 51 human liver samples.
(Sample mean —— ± 2-fold ——)

- **A) CYP3A4 abundance** range 5 – 949 pmol/mmp (201-fold or 42- fold excluding HL43).
- **B) NCR activity** range 62 – 255 nmol/min/mmp) (4-fold or 3-fold excluding HL43).
- **C) b5 abundance** range 0.15 – 0.59 nmol/mmp (4-fold or 3-fold excluding HL43).
- **D) TST6βOH activity** range 0.3-10.8 nmol/min/mmp (36-fold or 16-fold excluding HL43).
- **E) TST6βOH k_{cat}** range 4 - 83 pmol/min/pmol CYP3A4 (19-fold with or without exclusion of HL43).

- HL22 and HL71 expressed relatively low levels of CYP3A4 (Figure 3A). Nevertheless, they had high catalytic activity and similar levels of b5 and NCR activity to the sample mean (Figure 3B, C, E).
- Particularly high levels of CYP3A4, b5, NCR activity, and thus CYP3A activity / mmp, were observed in a sample (HL43) obtained from an individual exposed to phenytoin, a CYP3A inducer (Figure 1 and 3).
- Correction for CYP3A4 abundance in sample HL43 produced a value of CYP3A k_{cat} within 2-fold of the mean for all livers (Figure 3).
- A less variable k_{cat} has recently been reported for midazolam by CYP3A4 [6], indicating a need to investigate the variability of k_{cat} across a range of CYP3A substrates.

CONCLUSIONS

Differences in the level of expression of CYP3A4 alone do not explain variability in TST6βOH activity. Although differences in the level of the accessory protein, NCR, also accounts for a proportion of the variability, other sources remain to be identified.

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