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Title page

Title: Recovery of CYP3A Phenotype Following Kidney Transplantation

Authors: Kristine Hole, Elisabet Størset, Ane Olastuen, Tore Haslemo, Grete Birkeland Kro, Karsten Midtvedt, Anders Åsberg, Espen Molden

Affiliations: *Center for Psychopharmacology, Diakonhjemmet Hospital (K.H., T.H., E.M.); Department of Transplantation Medicine, Oslo University Hospital Rikshospitalet (E.S., K.M., A.Å.); Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo (A.O., A.Å., E.M.); and Department of Microbiology, Oslo University Hospital Rikshospitalet (G.B.K.), Oslo, Norway.*

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CYP3A Phenotype After Kidney Transplantation

Corresponding author:

Kristine Hole, Center for Psychopharmacology, Diakonhjemmet Hospital, PO Box 23
Vinderen, 0319 Oslo, Norway. E-mail: kristine.hole@diakonsyk.no. Phone number: +47
22454656. Fax: +47 22454698.

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ABBREVIATIONS: 4 β OHC, 4 β -hydroxycholesterol; CYP, cytochrome P450; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; LLOQ, lower limit of quantification.

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Abstract

End-stage renal disease (ESRD) impairs drug metabolism via cytochrome P450 (CYP) 3A. However, it is unclear whether CYP3A activity recovers following kidney transplantation. Therefore, the aim of this study was to evaluate the change in CYP3A activity measured as 4 β -hydroxycholesterol (4 β OHC) concentration after kidney transplantation. In total, data from 58 renal transplant recipients with 550 prospective 4 β OHC measurements were included in the study. One sample per patient was collected before transplantation, and 2-12 samples per patient were collected 1-82 days after transplantation. The measured pre-transplant 4 β OHC concentrations ranged >7-fold with a median value of 22.8 ng/mL. Linear mixed model analysis identified a 0.16 ng/mL increase in 4 β OHC concentration per day after transplantation ($p<0.001$), indicating a regain in CYP3A activity. Increasing estimated glomerular filtration rate after transplantation was associated with increasing 4 β OHC concentration ($p<0.001$), supporting that CYP3A activity increases with recovering uremia. In conclusion, this study indicates that CYP3A activity is regained subsequent to kidney transplantation.

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Introduction

End-stage renal disease (ESRD) impairs not only renal drug clearance, but also non-renal clearance via impairment of cytochrome P450 (CYP) enzymes and drug transport proteins (Ladda and Goralski, 2016). A number of CYP enzymes display reduced activity in ESRD patients (Ladda and Goralski, 2016). The mechanism behind this is not fully understood, but it might be related to direct inhibition of CYP metabolism or reduced protein expression caused by uremic toxins (Guevin et al., 2002; Barnes et al., 2014).

The CYP3A subfamily is involved in the metabolism of 30% of clinically used drugs, and CYP3A4 and CYP3A5 are the most relevant enzymes (Zanger and Schwab, 2013). Both enzymes display genetic polymorphisms, but the functional relevance is most evident for CYP3A5, where the *CYP3A5**3 variant allele – being the most frequent allele in most populations – encodes for a non-functional CYP3A5 enzyme (Kuehl et al., 2001). For CYP3A4, the *CYP3A4**22 variant allele is related to reduced enzyme activity (Wang et al., 2011), and has been associated with an approximately 30% reduced tacrolimus dose requirement (Elens et al., 2011). However, the general relevance of *CYP3A4**22 on CYP3A phenotype is unclear.

Due to the extensive and mainly non-genetic interindividual variability in CYP3A enzyme activity, phenotyping is likely to be more clinically useful than genotyping for dose individualization. Midazolam is considered the gold standard probe drug for measuring CYP3A4 phenotype, but endogenous phenotype markers have the advantage of not needing probe drug administration. 4 β -Hydroxycholesterol (4 β OHC) is metabolized from cholesterol by CYP3A4 and CYP3A5 (Bodin et al., 2001; Diczfalusy et al., 2008), and is a promising CYP3A phenotype marker. Plasma concentration of 4 β OHC has proven sensitive to CYP3A induction and inhibition (Josephson et al., 2008; Lutjohann et al., 2009; Mangold et al., 2016), and correlates with oral and intravenous midazolam clearance (Tomalik-Scharte et al., 2009).

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Several studies have shown reduced CYP3A metabolism in patients with impaired renal function (Dowling et al., 2003; Kirwan et al., 2009; Kirwan et al., 2012). Hemodialysis has been reported to rapidly improve hepatic CYP3A4 activity, as measured by the ¹⁴C-erythromycin breath test (Nolin et al., 2006). However, there are conflicting study results regarding change in CYP3A phenotype after kidney transplantation, with reports of increased 4βOHC concentration (Suzuki et al., 2013) and decreased midazolam clearance (de Jonge et al., 2015). Furthermore, Suzuki *et al.* reported that post-transplant change in 4βOHC concentration was dependent on *CYP3A5* genotype, since only *CYP3A5*1* carriers – and not *CYP3A5*3/*3* homozygotes – experienced increased enzyme activity after transplantation (Suzuki et al., 2015). Thus, further elucidation of CYP3A-metabolizing status following kidney transplantation is needed.

The primary aim of this study was to evaluate the change in 4βOHC concentration after kidney transplantation in patients with ESRD. Furthermore, we wanted to assess the impact of *CYP3A4* and *CYP3A5* genotypes, demographic factors, pre-transplant dialysis status and post-transplant creatinine levels on the potential change in 4βOHC concentration following transplantation.

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Materials and Methods

Patients. This study included patients from a previously described randomized controlled trial evaluating computerized tacrolimus dosing in renal transplant recipients (www.ClinicalTrials.gov, NCT02010320) (Storset et al., 2015). The patients underwent kidney transplantation at Oslo University Hospital Rikshospitalet between January and June 2014. All patients were >18 years and treated with a triple therapy immunosuppressive regime consisting of tacrolimus, mycophenolate mofetil and oral prednisolone (Storset et al., 2015). Induction therapy consisted of 20 mg basiliximab on day 0 and 4, and intravenous methylprednisolone on day 0. None of the included patients used CYP3A inducers or inhibitors during the study period, except for corticosteroids. Acute rejection episodes and concurrent methylprednisolone treatment was registered. The study was approved by the Regional Committee for Medical Research Ethics. All participants gave written informed consent.

Genotype analyses. Analyses of *CYP3A4**22 (rs35599367) and *CYP3A5**3 (rs776746) variant alleles were performed by real-time polymerase chain reaction and melt curve analysis with hybridization probes on the LightCycler® 480 instrument (Roche Applied Science, Penzberg, Germany).

4 β -hydroxycholesterol analyses. Plasma concentrations of 4 β OHC were determined by a previously described ultra-performance liquid chromatography tandem mass spectrometry method using atmospheric pressure chemical ionization (Gjestad et al., 2016), with an added filtration step (Størset et al., 2017). A quality control sample produced at the time of the oldest samples (January 2014) was included in each run to ensure stability during storage. Duplicate measurements were performed for each sample, and mean values of duplicates were used for statistical analyses. The lower limit of quantification (LLOQ) was 10 ng/mL, and 4 β OHC measurements below LLOQ were excluded from statistical analysis. Intra- and interday

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precision for the method was <8% at 10 ng/mL and <4% at 644 ng/mL ($n = 6$), while the corresponding intra- and interday accuracies were <15% and <2%, respectively ($n = 6$).

Creatinine analyses. Plasma creatinine was analyzed in fresh samples on the COBAS 8000 platform using the Roche CREP2 kit, a colorimetric based method (no. 05168589190, Roche, Basel Switzerland). The intra- and interpatient coefficients of variation were 5.3% and 14.2%, respectively.

Endpoints and statistical analyses. Kruskal-Wallis and Mann-Whitney U tests were used for comparison of pre-transplantation 4 β OHC concentrations between subgroups. Correlations were evaluated using Spearman's signed rank test. Linear mixed modeling with random intercept and random slope was used to evaluate the effects of covariates on variation in 4 β OHC concentration. An unstructured covariance type was used, and calculation of estimates was based on restricted maximum likelihoods. Evaluated fixed effects included *CYP3A4* and *CYP3A5* genotypes, days since transplantation, estimated glomerular filtration rate (eGFR) calculated by the Cockcroft-Gault-formula (Cockcroft and Gault, 1976), bodyweight, sex, age, whether the patient was subject to dialysis before transplantation (independent of previous transplantations), and whether the transplant originated from living or deceased donor. Two separate mixed model analyses were performed; one including samples from pre- and post-transplantation, where bodyweight was included as a covariate but not eGFR, and one including samples only from post-transplantation, where eGFR was included as a covariate but not bodyweight. Since bodyweight is incorporated in eGFR, we did not use both covariates in the same analyses. The final mixed models were chosen by Akaike's information criterion, and only statistically significant covariates were included. The impact of genotype on change in 4 β OHC concentration over time was assessed by comparing the slope of change in 4 β OHC concentration between genotype subgroups. Statistical significance was considered as $p < 0.05$. Statistical analyses were performed using the IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY). SPSS and

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GraphPad Prism for Windows, version 6.01 (GraphPad Software, La Jolla, CA), were used for graphical presentations.

Results

Patient characteristics. One patient was carrier of both *CYP3A4**22 and *CYP3A5**1 alleles, and was excluded from the study. Clinical and demographic characteristics of the 58 remaining patients are listed in Table 1. Thirty-nine patients were male (67%) and median age was 56 years (range 24 to 76). This was the first kidney transplantation for 54 of the patients (93%). There were 40 patients (69%) who had been subject to dialysis treatment prior to transplantation, of which one patient was receiving a re-transplant. Regarding genotype distributions in the population, 5 patients (9%) had the *CYP3A4**1/*22 genotype, while 12 patients (21%) had the *CYP3A5**1/*3 genotype. The remaining 41 patients (71%) were *CYP3A4**1/*1 and *CYP3A5**3/*3 homozygotes. Three of the patients carrying the *CYP3A5**1 allele were of non-Caucasian origin.

Pre-transplantation 4 β OHC concentration. One 4 β OHC measurement was available from the period immediately prior to transplantation (1-10 days) for all but 3 patients; two patients lacked measurements due to shortage of plasma, and one measurement was excluded due to level below LLOQ. Pre-transplant 4 β OHC concentrations of the remaining 55 patients ranged >7-fold (10.0 to 73.1 ng/mL) with a median value of 22.8 ng/mL. The median pre-transplantation 4 β OHC concentration in *CYP3A4**22 carriers was 16.1 ng/mL (range 12.8-27.7 ng/mL, $n=5$), in combined *CYP3A4**1/*1 and *CYP3A5**3/*3 homozygotes it was 22.3 ng/mL (10.3-71.2 ng/mL, $n=39$), and in *CYP3A5**1 carriers it was 33.5 ng/mL (10.0-73.1 ng/mL, $n=11$) (Kruskal-Wallis $p=0.04$). The Mann-Whitney U test revealed a significant difference in 4 β OHC concentration between *CYP3A5**1 carriers and patients with combined *CYP3A4**1/*1 and *CYP3A5**3/*3 genotypes ($p=0.03$), but not between *CYP3A4**22 carriers and patients with combined *CYP3A4**1/*1 and *CYP3A5**3/*3 genotypes ($p=0.3$) (Figure 1A). As shown in Figure 1B, female patients had a median 4 β OHC concentration of 31.3 ng/mL (11.1 to 71.2 ng/mL) compared to 22.3 ng/mL in male patients (10.0 to 73.1 ng/mL)

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(Mann-Whitney $p=0.049$). Pre-transplantation 4 β OHC concentration was not different between dialysis and non-dialysis patients (Mann-Whitney $p=0.5$). Pre-transplant bodyweight correlated with 4 β OHC concentration (Spearman's $r = -0.58, p<0.001$).

Change in 4 β OHC concentration after transplantation. A median of nine 4 β OHC measurements per patient (range 2 to 12) were available from the post-transplantation period (1-82 days). Nine post-transplant 4 β OHC measurements were below LLOQ, and were not included in the statistical analysis. In total 550 pre- and post-transplantation 4 β OHC measurements from 58 patients were included in the study (Figure 2). There were 39 missing bodyweight measurements and 27 missing creatinine concentration measurements during follow-up after transplantation.

As shown in Figure 3, correlation between plasma creatinine and 4 β OHC concentrations from week one post-transplantation was borderline significant (Spearman's $r = -0.36, p=0.053, n=30$). Linear mixed modeling analysis including both pre- and post-transplantation samples identified 'days since transplantation', bodyweight and *CYP3A5* genotype as significant covariates with impact on 4 β OHC concentration (Table 2). The final model predicted a 0.16 ng/mL increase in 4 β OHC per day after transplantation ($p<0.001$). This corresponds to a 13.1 ng/mL increase in 4 β OHC concentration from before transplantation to day 82 after transplantation, or a 46% increase for a 70kg person. A bodyweight increase of 1 kg predicted a 0.4 ng/mL reduction in 4 β OHC concentration ($p<0.001$), which corresponds to a pre-transplantation 4 β OHC concentration of 33 ng/mL for a 60kg patient, and 16 ng/mL for a 100kg patient who did not express *CYP3A5*. Carrying the *CYP3A5*1* allele indicated a 7.8 ng/mL higher 4 β OHC level than being homozygous for *CYP3A5*3/*3* ($p=0.02$). Being carrier of the *CYP3A4*22* allele did not have a significant impact on 4 β OHC levels ($p=0.5$), nor did sex, age, pre-transplant dialysis status or whether the transplanted kidney originated from a living or deceased donor ($p>0.2$). The slope of change in 4 β OHC concentration according to

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days after transplantation was not significantly different between the genotype subgroups ($p>0.5$). When excluding all samples from patients after a rejection episode and concurrent methylprednisolone treatment, the results of the mixed model analysis were essentially the same (no differences in significance levels of the tested variables, results not shown). A linear mixed model analysis based solely on post-transplantation samples further identified an association between $4\beta\text{OHC}$ concentration and eGFR (Table 3). A 10 mL/min increase in eGFR was associated with a 1.0 ng/mL increase in $4\beta\text{OHC}$ levels ($p<0.001$).

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Discussion

Our study of 58 kidney transplant recipients showed a gradual increase in CYP3A activity from immediately before to 82 days after kidney transplantation, suggesting that CYP3A impairment due to ESRD is recovered subsequent to transplantation. Furthermore, post-transplantation increase in 4 β OHC concentration was associated with increasing eGFR, supporting that uremia or uremic toxins impairs CYP3A activity. However, the degree of increase in CYP3A activity was not statistically associated with *CYP3A4* or *CYP3A5* genotypes.

The gradual increase in 4 β OHC concentration with increasing time after transplantation coincides with findings in Japanese kidney transplant recipients, where a 30-50% increase in 4 β OHC concentration was found on day 90 and 180 after transplantation compared with pre-transplant 4 β OHC concentration (Suzuki et al., 2013; Suzuki et al., 2015). Furthermore, *in vitro* studies of rat hepatocytes incubated with serum from chronic renal failure patients reported a reduction of CYP3A activity and enzyme expression prior to, but not after, transplantation (Michaud et al., 2005). Clinical implications could be that dose reductions of CYP3A metabolized drugs in ESRD patients may prevent overdosing and concentration-dependent side effects, and correspondingly that post-transplantation dose increase of CYP3A drugs may prevent therapeutic failure. However, it should be mentioned that one study actually reported decreased clearance of the CYP3A substrate midazolam 1 to 12 months after transplantation compared to 7 days after transplantation (de Jonge et al., 2015). Although midazolam is considered a gold standard CYP3A probe drug, it is also highly bound to plasma proteins (Kirwan et al., 2010). Albumin levels are reported to be reduced in patients with chronic kidney disease and after recent surgery (Meijers et al., 2008; Hubner et al., 2016), and increase with time after transplantation (Størset et al., 2014). Thus, it is possible that the reported decreasing midazolam plasma clearance mainly reflects increasing protein

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binding rather than decreasing CYP3A activity. Nevertheless, further studies are needed to elucidate the change in CYP3A activity after kidney transplantation, and the potential clinical relevance of such a change.

The median value of pre-transplantation 4 β OHC concentration in the present population was 22.8 ng/mL, which is approximately 10% lower than the reported median concentration in a Norwegian patient population of psychiatric patients (Hole et al., 2017), and approximately 15-25% lower than mean concentrations in healthy Scandinavian volunteers (Bodin et al., 2001; Diczfalusy et al., 2008). This supports that CYP3A activity is impaired in ESRD patients. The mechanism through which ESRD impairs CYP3A activity is not fully elucidated, but is considered to involve uremic toxins (Ladda and Goralski, 2016). Possible mechanisms include direct enzymatic inhibition and/or reduced CYP gene expression by uremic toxins (Barnes et al., 2014; Ladda and Goralski, 2016). Suzuki *et al.* reported a negative correlation of plasma indoxyl sulfate with 4 β OHC concentration in stable kidney transplant recipients (Suzuki et al., 2014), indicating that a post-transplant decline of uremic toxin was linked to regain of CYP3A activity. This coincides with our findings that a post-transplantation increase in eGFR was associated with an increase in 4 β OHC concentration. We observed a significant impact of *CYP3A5*, but not *CYP3A4* genotype on pre-transplant 4 β OHC concentration. There were only 5 *CYP3A4**22 carriers, which may explain why the 4 β OHC concentration was not significantly lower in this group compared to the other genotype groups. It is also possible that non-genetic factors such as inflammation and uremic toxins mask the effects of *CYP3A4* genotype on CYP3A phenotype in this population (Morgan, 1997; Suzuki et al., 2014). Suzuki *et al.* reported identical pre-transplantation 4 β OHC levels in *CYP3A5**1 carriers and non-carriers, with a mean concentration of 38 ng/mL (Suzuki et al., 2015). They further reported a post-transplantation increase in 4 β OHC concentration only among *CYP3A5**1 carriers, and not among *CYP3A5**3/*3 homozygotes

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(Suzuki et al., 2015). We found no difference in the post-transplantation increase in 4 β OHC concentration when comparing *CYP3A4**22 carriers, *CYP3A4**1/*1 and *CYP3A5**3/*3 homozygotes, and *CYP3A5**1 carriers, indicating a recovery of CYP3A activity independent of genotype. The inconsistent findings regarding impact of *CYP3A4/5* genotype on metabolizing phenotype both before and after kidney transplantation might be related to ethnic differences, but this is a speculation which needs to be investigated in further studies. In this study we used 4 β OHC concentrations as a measure of *in vivo* CYP3A activity without adjusting for total cholesterol levels. Total cholesterol has only minor impact on the variation in 4 β OHC concentration (Diczfalusy et al., 2008), except for in statin treated patients (Bjorkhem-Bergman et al., 2016). None of the patients included in our study received statins. Thus, it is not expected that using 4 β OHC:cholesterol ratio would have led to different conclusions. The elimination half-life of 4 β OHC is considered to be between 3 and 17 days (Bodin et al., 2002; Diczfalusy et al., 2009; Ngaimisi et al., 2014), which is a limitation when studying changes in CYP3A activity over short time periods. However, the fact that use of a CYP3A inducer for a week doubles 4 β OHC concentration indicates that this biomarker rapidly captures increased enzyme activity (Marschall et al., 2005; Wide et al., 2008). We are therefore confident that 4 β OHC can reflect recovery in CYP3A activity within the time span of our study.

Studying dynamic changes early after kidney transplantation is complex, and there are several factors that were not accounted for in our analysis, such as delayed graft function, albumin levels, and corticosteroid doses. All patients received methylprednisolone on the day of transplantation, and prednisolone in the immunosuppressive regimen. CYP3A induction by corticosteroids is reported in the literature, most potently by dexamethasone, but also by prednisolone and methylprednisolone (Matoulkova et al., 2014). Clinical observations of increasing tacrolimus concentrations concurring with tapering of corticosteroid dosing has

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been interpreted as indirect evidence that corticosteroid induction of CYP3A and P-glycoprotein is of clinical relevance. However, we recently showed that CYP3A activity measured as 4 β OHC did not account for the increasing tacrolimus concentration with time after kidney transplantation (Størset et al., 2017). The conclusions regarding corticosteroid CYP3A induction are conflicting, and the clinical evidence limited (Matoulkova et al., 2014). Therefore, we do not suspect that the post-transplant increase in CYP3A activity is due to corticosteroid CYP induction, but both oral and intravenous corticosteroids given after transplantation may still have been an interfering factor on 4 β OHC levels that our study did not completely account for. In future studies, it is important to account for interfering factors, such as corticosteroid use, on post-transplant regain of CYP3A phenotype.

CYP enzymes, including CYP3A isoforms, are known to be expressed in kidneys (Aleksa et al., 2005). The increased metabolic activity following transplantation might therefore in theory reflect higher renal CYP3A activity in the donor kidney than in the impaired kidney. CYP3A5 expression has been shown to be lower in renal transplant patients with calcine urine-induced nephrotoxicity compared to controls, which could support that metabolic activity is declined during renal failure (Joy et al., 2007). However, Aleksa *et al.* have reported that total expression of CYP enzymes in the kidneys is 80-90% lower than in the liver (Aleksa et al., 2005). Overall, we therefore consider it likely that the raise in 4 β OHC level following transplantation mainly reflects a gradual regain of CYP3A metabolism in liver/intestine, but it is possible that higher metabolic activity in the donor vs. the failing kidney may have contributed to the observed increase in 4 β OHC concentration. Regarding causality behind the post-transplant increase in 4 β OHC concentrations, it is necessary with further investigation to clarify the possible mechanisms behind the suppression of CYP3A phenotype in ESRD patients. Another limitation in our study is the low number of *CYP3A4**22 carriers. Since the *CYP3A4**22 allele codes for a reduced function enzyme, and patients rarely are homozygous, a larger number of patients would probably be needed to find significant impact on CYP3A phenotype. Furthermore, there were no *CYP3A5**1/*1

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homozygotes, so we could not investigate whether there was a stepwise increase in 4 β OHC concentration according to number of *CYP3A5*1* alleles. The main strength of our study is the high number of samples from the early post-transplant period, which allowed us to investigate intra- and interindividual change in 4 β OHC concentration after transplantation. The study would have been further strengthened by investigating the clinical effect of change in 4 β OHC concentration on clearance of CYP3A metabolized drugs. We have previously reported that 4 β OHC concentration did not improve tacrolimus dose predictions (Størset et al., 2017). However, it would be interesting to see whether 4 β OHC concentration in ESRD patients and early after transplantation is associated with clearance of other CYP3A drugs with less complex pharmacokinetic profiles than tacrolimus.

In conclusion, we found that CYP3A activity increased after kidney transplantation, indicating a regain of enzyme activity impaired by ESRD. Clinically, this implicates that dosing of CYP3A-metabolized drugs may need to be reduced in patients with ESRD, and increased following kidney transplantation.

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Authorship contributions

Participated in research design: Hole, Størset, Olastuen, Midtvedt, Åsberg, Molden.

Contributed to acquisition of data: Hole, Størset, Olastuen, Kro, Midtvedt, Åsberg, Molden.

Performed data analysis: Hole, Haslemo.

Wrote or contributed to the writing of the manuscript: Hole, Størset, Olastuen, Haslemo, Kro, Midtvedt, Åsberg, Molden.

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References

- Aleksa K, Matsell D, Krausz K, Gelboin H, Ito S, and Koren G (2005) Cytochrome P450 3A and 2B6 in the developing kidney: implications for ifosfamide nephrotoxicity. *Pediatric nephrology (Berlin, Germany)* **20**:872-885.
- Barnes KJ, Rowland A, Polasek TM, and Miners JO (2014) Inhibition of human drug-metabolising cytochrome P450 and UDP-glucuronosyltransferase enzyme activities in vitro by uremic toxins. *European journal of clinical pharmacology* **70**:1097-1106.
- Bjorkhem-Bergman L, Nylen H, Eriksson M, Parini P, and Diczfalusy U (2016) Effect of Statin Treatment on Plasma 4beta-Hydroxycholesterol Concentrations. *Basic & clinical pharmacology & toxicology* **118**:499-502.
- Bodin K, Andersson U, Rystedt E, Ellis E, Norlin M, Pikuleva I, Eggertsen G, Bjorkhem I, and Diczfalusy U (2002) Metabolism of 4 beta -hydroxycholesterol in humans. *The Journal of biological chemistry* **277**:31534-31540.
- Bodin K, Bretilon L, Aden Y, Bertilsson L, Broome U, Einarsson C, and Diczfalusy U (2001) Antiepileptic drugs increase plasma levels of 4beta-hydroxycholesterol in humans: evidence for involvement of cytochrome p450 3A4. *The Journal of biological chemistry* **276**:38685-38689.
- Cockcroft DW and Gault MH (1976) Prediction of creatinine clearance from serum creatinine. *Nephron* **16**:31-41.
- de Jonge H, Vanhove T, de Loo H, Verbeke K, and Kuypers DRJ (2015) Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing haematocrit. *British journal of clinical pharmacology* **80**:548-559.
- Diczfalusy U, Kanebratt KP, Bredberg E, Andersson TB, Bottiger Y, and Bertilsson L (2009) 4beta-hydroxycholesterol as an endogenous marker for CYP3A4/5 activity. Stability and half-life of elimination after induction with rifampicin. *British journal of clinical pharmacology* **67**:38-43.
- Diczfalusy U, Miura J, Roh HK, Mirghani RA, Sayi J, Larsson H, Bodin KG, Allqvist A, Jande M, Kim JW, Aklillu E, Gustafsson LL, and Bertilsson L (2008) 4Beta-hydroxycholesterol is a new endogenous CYP3A marker: relationship to CYP3A5 genotype, quinine 3-hydroxylation and sex in Koreans, Swedes and Tanzanians. *Pharmacogenetics and genomics* **18**:201-208.
- Dowling TC, Briglia AE, Fink JC, Hanes DS, Light PD, Stackiewicz L, Karyekar CS, Eddington ND, Weir MR, and Henrich WL (2003) Characterization of hepatic cytochrome p4503A activity in patients with end-stage renal disease. *Clinical pharmacology and therapeutics* **73**:427-434.
- Elens L, Bouamar R, Hesselink DA, Haufroid V, van der Heiden IP, van Gelder T, and van Schaik RH (2011) A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. *Clinical chemistry* **57**:1574-1583.
- Gjestad C, Huynh DK, Haslemo T, and Molden E (2016) 4beta-hydroxycholesterol correlates with dose but not steady-state concentration of carbamazepine: indication of intestinal CYP3A in biomarker formation? *British journal of clinical pharmacology* **81**:269-276.
- Guevin C, Michaud J, Naud J, Leblond FA, and Pichette V (2002) Down-regulation of hepatic cytochrome p450 in chronic renal failure: role of uremic mediators. *Br J Pharmacol* **137**:1039-1046.
- Hole K, Gjestad C, Heitmann KM, Haslemo T, Molden E, and Bremer S (2017) Impact of genetic and nongenetic factors on interindividual variability in 4beta-hydroxycholesterol concentration. *European journal of clinical pharmacology* **73**:317-324.
- Hubner M, Mantziari S, Demartines N, Pralong F, Coti-Bertrand P, and Schafer M (2016) Postoperative Albumin Drop Is a Marker for Surgical Stress and a Predictor for Clinical Outcome: A Pilot Study. *Gastroenterology research and practice* **2016**:8743187.
- Josephson F, Bertilsson L, Bottiger Y, Flamholz L, Gisslen M, Ormaasen V, Sonnerborg A, and Diczfalusy U (2008) CYP3A induction and inhibition by different antiretroviral regimens reflected by changes in plasma 4beta-hydroxycholesterol levels. *European journal of clinical pharmacology* **64**:775-781.

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- Joy MS, Hogan SL, Thompson BD, Finn WF, and Nickleit V (2007) Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity. *Nephrology Dialysis Transplantation* **22**:1963-1968.
- Kirwan C, MacPhee I, and Philips B (2010) Using drug probes to monitor hepatic drug metabolism in critically ill patients: midazolam, a flawed but useful tool for clinical investigation of CYP3A activity? *Expert opinion on drug metabolism & toxicology* **6**:761-771.
- Kirwan CJ, Lee T, Holt DW, Grounds RM, MacPhee IA, and Philips BJ (2009) Using midazolam to monitor changes in hepatic drug metabolism in critically ill patients. *Intensive care medicine* **35**:1271-1275.
- Kirwan CJ, MacPhee IA, Lee T, Holt DW, and Philips BJ (2012) Acute kidney injury reduces the hepatic metabolism of midazolam in critically ill patients. *Intensive care medicine* **38**:76-84.
- Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, and Schuetz E (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nature genetics* **27**:383-391.
- Ladda MA and Goralski KB (2016) The Effects of CKD on Cytochrome P450-Mediated Drug Metabolism. *Advances in chronic kidney disease* **23**:67-75.
- Lutjohann D, Marinova M, Schneider B, Oldenburg J, von Bergmann K, Bieber T, Bjorkhem I, and Diczfalusy U (2009) 4beta-hydroxycholesterol as a marker of CYP3A4 inhibition in vivo - effects of itraconazole in man. *International journal of clinical pharmacology and therapeutics* **47**:709-715.
- Mangold JB, Wu F, and Rebello S (2016) Compelling Relationship of CYP3A Induction to Levels of the Putative Biomarker 4beta-Hydroxycholesterol and Changes in Midazolam Exposure. *Clinical pharmacology in drug development* **5**:245-249.
- Marschall HU, Wagner M, Zollner G, Fickert P, Diczfalusy U, Gumhold J, Silbert D, Fuchsbichler A, Benthin L, Grundstrom R, Gustafsson U, Sahlin S, Einarsson C, and Trauner M (2005) Complementary stimulation of hepatobiliary transport and detoxification systems by rifampicin and ursodeoxycholic acid in humans. *Gastroenterology* **129**:476-485.
- Matoulkova P, Pavek P, Maly J, and Vlcek J (2014) Cytochrome P450 enzyme regulation by glucocorticoids and consequences in terms of drug interaction. *Expert opinion on drug metabolism & toxicology* **10**:425-435.
- Meijers BK, Bammens B, Verbeke K, and Evenepoel P (2008) A review of albumin binding in CKD. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **51**:839-850.
- Michaud J, Dubé P, Naud J, Leblond FA, Desbiens K, Bonnardeaux A, and Pichette V (2005) Effects of serum from patients with chronic renal failure on rat hepatic cytochrome P450. *British Journal of Pharmacology* **144**:1067-1077.
- Morgan ET (1997) Regulation of cytochromes P450 during inflammation and infection. *Drug metabolism reviews* **29**:1129-1188.
- Ngaimisi E, Minzi O, Mugusi S, Sasi P, Riedel KD, Suda A, Ueda N, Bakari M, Janabi M, Mugusi F, Bertilsson L, Burhenne J, Aklillu E, and Diczfalusy U (2014) Pharmacokinetic and pharmacogenomic modelling of the CYP3A activity marker 4beta-hydroxycholesterol during efavirenz treatment and efavirenz/rifampicin co-treatment. *The Journal of antimicrobial chemotherapy* **69**:3311-3319.
- Nolin TD, Appiah K, Kendrick SA, Le P, McMonagle E, and Himmelfarb J (2006) Hemodialysis acutely improves hepatic CYP3A4 metabolic activity. *Journal of the American Society of Nephrology : JASN* **17**:2363-2367.
- Storset E, Asberg A, Skauby M, Neely M, Bergan S, Bremer S, and Midtvedt K (2015) Improved Tacrolimus Target Concentration Achievement Using Computerized Dosing in Renal Transplant Recipients--A Prospective, Randomized Study. *Transplantation* **99**:2158-2166.

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- Størset E, Hole K, Midtvedt K, Bergan S, Molden E, and Åsberg A (2017) The CYP3A biomarker 4 β -hydroxycholesterol does not improve tacrolimus dose predictions early after kidney transplantation. *British journal of clinical pharmacology* **83**:1457-1465.
- Størset E, Holford N, Midtvedt K, Bremer S, Bergan S, and Åsberg A (2014) Importance of hematocrit for a tacrolimus target concentration strategy. *European journal of clinical pharmacology* **70**:65-77.
- Suzuki Y, Fujioka T, Sato F, Matsumoto K, Muraya N, Tanaka R, Sato Y, Ohno K, Mimata H, Kishino S, and Itoh H (2015) CYP3A5 polymorphism affects the increase in CYP3A activity after living kidney transplantation in patients with end stage renal disease. *British journal of clinical pharmacology* **80**:1421-1428.
- Suzuki Y, Itoh H, Fujioka T, Sato F, Kawasaki K, Sato Y, Sato Y, Ohno K, Mimata H, and Kishino S (2014) Association of plasma concentration of 4beta-hydroxycholesterol with CYP3A5 polymorphism and plasma concentration of indoxyl sulfate in stable kidney transplant recipients. *Drug metabolism and disposition: the biological fate of chemicals* **42**:105-110.
- Suzuki Y, Itoh H, Sato F, Kawasaki K, Sato Y, Fujioka T, Sato Y, Ohno K, Mimata H, and Kishino S (2013) Significant increase in plasma 4beta-hydroxycholesterol concentration in patients after kidney transplantation. *Journal of lipid research* **54**:2568-2572.
- Tomalik-Scharte D, Lutjohann D, Doroshenko O, Frank D, Jetter A, and Fuhr U (2009) Plasma 4beta-hydroxycholesterol: an endogenous CYP3A metric? *Clinical pharmacology and therapeutics* **86**:147-153.
- Wang D, Guo Y, Wrighton SA, Cooke GE, and Sadee W (2011) Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *The pharmacogenomics journal* **11**:274-286.
- Wide K, Larsson H, Bertilsson L, and Diczfalusy U (2008) Time course of the increase in 4beta-hydroxycholesterol concentration during carbamazepine treatment of paediatric patients with epilepsy. *British journal of clinical pharmacology* **65**:708-715.
- Zanger UM and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & therapeutics* **138**:103-141.

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Footnotes

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Address correspondence to:

Kristine Hole, Center for Psychopharmacology, Diakonhjemmet Hospital, PO Box 23 Vinderen, 0319 Oslo, Norway. E-mail: kristine.hole@diakonsyk.no.

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Figure Legends

Fig. 1. Pre-transplantation 4 β -hydroxycholesterol concentrations in A) *CYP3A4**22 carriers ($n=5$), *CYP3A4**1/*1 and *CYP3A5**3/*3 homozygotes ($n=39$), and *CYP3A5**1 carriers ($n=11$), and B) males ($n=36$) and females ($n=19$). Medians are expressed as solid lines, and p -values are derived from Mann-Whitney U-Tests.

Fig. 2. Measured 4 β -hydroxycholesterol concentrations in all patients according to days after transplantation and stratified by *CYP3A4* and *CYP3A5* genotypes.

Fig. 3. Correlation between 4 β -hydroxycholesterol concentration and creatinine concentration one week after transplantation ($n=30$). P - and r -values are estimated from Spearman's test. Linear trend line is added for visual purposes.

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TABLE 1
 Clinical and demographic patient characteristics

	Patients with <i>CYP3A4</i> *1/*22	Patients with <i>CYP3A4</i> *1/*1 and <i>CYP3A5</i> *3/*3	Patients with <i>CYP3A5</i> *1/*3
Number of patients	5	41	12
Sex (male / female)	4 / 1	27 / 14	8 / 4
Age, years	63 (47-66)	54 (24-76)	61 (30-74)
Bodyweight before transplantation, kg	89 (76-104)	76 (58-116)	79 (63-118)
Ethnicity (Caucasian / other)	5 / 0	40 / 1	9 / 3
Donor (living / deceased)	1 / 4	12 / 29	3 / 9
Previous transplantations (0 / 1 / 2)	5 / 0 / 0	37 / 3 / 1	12 / 0 / 0
Pre-transplant dialysis (HD / PD / none)	2 / 1 / 2	19 / 9 / 13	7 / 2 / 3

CYP, cytochrome P450; HD, haemodialysis; PD, peritoneal dialysis. Data are expressed as numbers or median (range).

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TABLE 2

Linear mixed model estimates for predictors of 4 β -hydroxycholesterol concentration (ng/mL)
including pre- and post-transplantation samples

Variables	Estimate	95% confidence interval		<i>p</i> -value
		Lower bound	Upper bound	
Intercept	59.58	46.78	72.38	<0.001
Time since transplantation, days	+0.16	+0.11	+0.22	<0.001
Bodyweight, kg	-0.44	-0.59	-0.29	<0.001
<i>CYP3A5*1</i> carrier ^a	+7.78	+1.23	+14.33	0.02

^aVs. non-carrier

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TABLE 3

Linear mixed model estimates for predictors of 4 β -hydroxycholesterol concentration (ng/mL)
including solely post-transplantation samples

Variables	Estimate	95% confidence interval		<i>p</i> -value
		Lower bound	Upper bound	
Intercept	15.64	11.25	20.03	<0.001
Time since transplantation, days	+0.18	+0.12	+0.24	<0.001
Estimated glomerular filtration rate, mL/min	+0.10	+0.05	+0.16	<0.001
<i>CYP3A5*1</i> carrier ^a	+10.43	+3.65	+17.20	0.003

^aVs. non-carrier

Figures

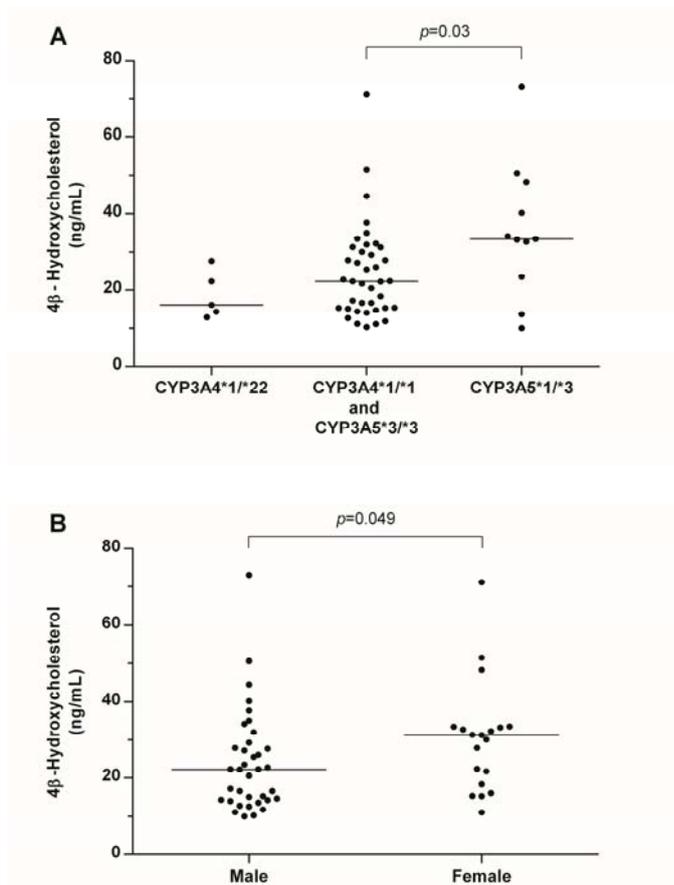


Fig. 1.

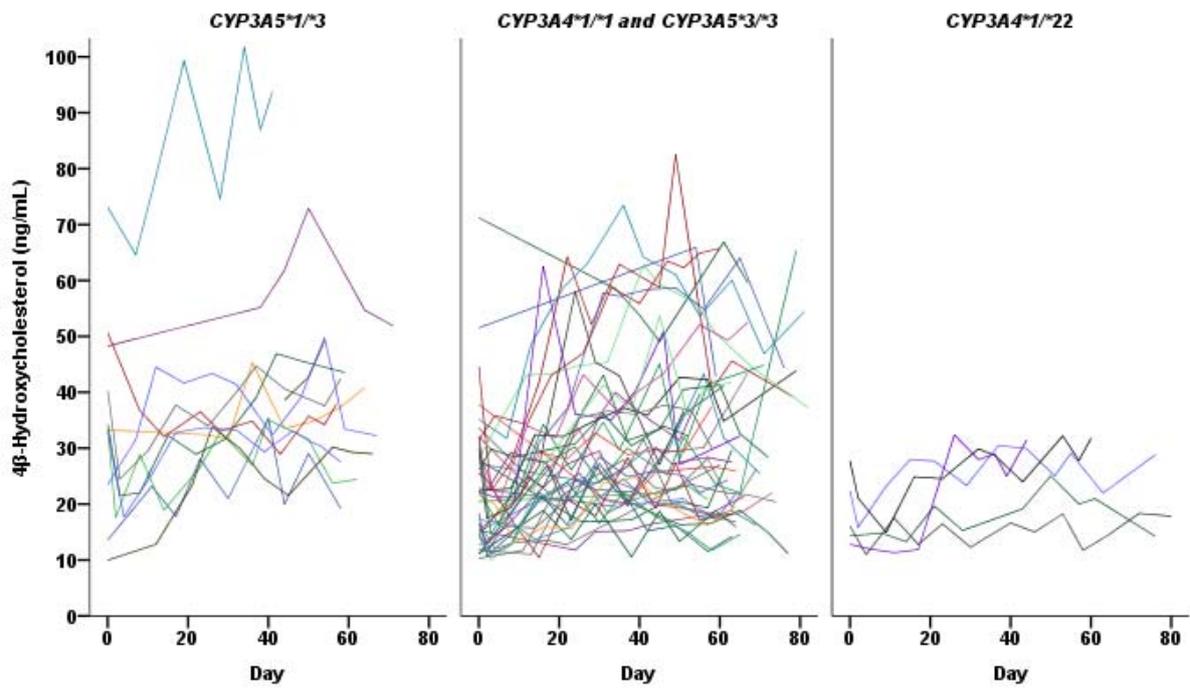


Fig. 2.

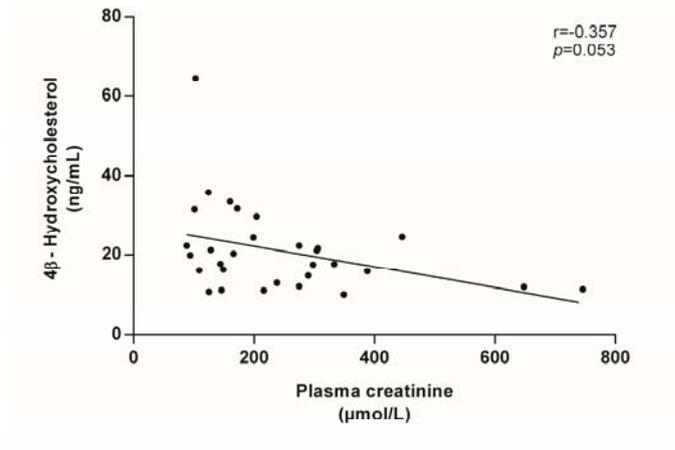


Fig. 3.