

## Accelerated Communication

# Seasonal Variation in Blood Drug Concentrations and a Potential Relationship to Vitamin D

Jonatan D. Lindh, Marine L. Andersson, Erik Eliasson, and Linda Björkhem-Bergman

Karolinska Institutet, Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska University Hospital Huddinge, Stockholm, Sweden

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### ABSTRACT:

The most important enzyme in hepatic drug metabolism is cytochrome P450 3A4. Published in vitro data indicate that vitamin D may up-regulate the expression of the CYP3A4 gene. Individual vitamin D levels are highly dependent on sunlight exposure and show great seasonal variability in northern countries. The aim of the present study was to investigate whether plasma concentrations of CYP3A4 drug substrates exhibit seasonal changes compatible with a stimulatory effect of vitamin D on drug metabolism. Three immunosuppressants (tacrolimus, sirolimus, and cyclosporine) were analyzed, because these CYP3A4 drug substrates are subject to long-term use and repeated concentration determinations. In addition, mycophenolic acid was included in the analysis as a control drug independent of CYP3A4 metabolism. Concentration-to-dose ratios were extracted from the Karolinska Therapeutic Drug Monitoring database and compared between the 3-month

periods of lowest and highest vitamin D levels. Sirolimus and tacrolimus levels showed seasonal variability that was highly consistent with changes in vitamin D; for example, significantly lower drug concentrations in July to September than in January to March. As expected, no significant difference was evident for mycophenolic acid, but this result was also the case with cyclosporine, possibly due to cross-reactivity of CYP3A4-mediated metabolites with the immunoassay used for quantification. In conclusion, there is cyclic variation in blood levels of important immunosuppressants throughout the year that correlates with UV light-dependent changes in vitamin D levels. Even though a causal relationship remains to be established, it is suggested that individual differences in vitamin D may contribute to variability in drug metabolism and disposition.

### Introduction

CYP3A4 is the most important drug-metabolizing cytochrome P450 enzyme with regards to hepatic and intestinal expression, as well as the number of identified drug substrates (Evans and Relling, 1999; Daly, 2006). The activity of this enzyme is known to show a significant variability not only between different individuals but also within the same individual at different time points. The reason for this activity is unknown, but a recent study on the upstream regulatory region of the CYP3A4 gene suggests an extraordinary potential for interactions with exogenous and endogenous ligands of different nuclear receptors (Qiu et al., 2010).

In vitro studies have indicated that vitamin D may induce the expression of CYP3A4 by a vitamin D receptor-mediated increase in gene transcription (Schmiedlin-Ren et al., 1997; Fan et al., 2009), and

this activity would result in increased metabolism of CYP3A4 drug substrates. Indeed, these molecular findings suggest a potential influence of vitamin D on the turnover of many drugs, but the relevance for drug exposure in patients has not been explored. Vitamin D is not a true vitamin because it can be formed in vivo from endogenous 7-dehydrocholesterol. This synthesis takes place in the skin and is dependent on sunlight (UV-B) exposure. Because the amount of sunlight varies widely between summer and winter, especially in countries closer to the arctic circles, plasma levels of vitamin D exhibit cyclic changes throughout the year (Landin-Wilhelmsen et al., 1995; Virtanen et al., 2010). Compared with the endogenous synthesis of vitamin D, the dietary intake of vitamin D is quantitatively less important and does not abolish the seasonal variation in serum vitamin D levels (Lund and Sorensen, 1979; Virtanen et al., 2010).

The aim of the present study was to test the hypothesis that plasma concentrations of typical CYP3A4 drug substrates exhibit seasonal variations compatible with enzyme induction by vitamin D. Focus on the immunosuppressants tacrolimus, sirolimus, and cyclosporine was motivated by their CYP3A4-dependent metabolism, and that most patients on these drugs are subject to long-term use and repeated concentration determinations.

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**ABBREVIATIONS:** TDM, therapeutic drug monitoring; C/D, concentration-to-dose; LC/MS/MS, liquid chromatography/tandem mass spectrometry; P-gp, P-glycoprotein.

## Materials and Methods

**Drug Concentrations.** Study data were extracted from a large routine therapeutic drug monitoring (TDM) service database within Clinical Pharmacology, Karolinska University Hospital Laboratory. This laboratory is quality-assured by Swedish authorities (SWEDAC, Borås, Sweden) and participates in external quality control programs. All routine blood concentration measurements of tacrolimus, sirolimus, cyclosporine (based on commercial immunoassays), or serum concentrations of mycophenolic acid (based on chromatography) performed between January 1, 2000 and May 31, 2010 were screened. Samples meeting the following criteria were included in the analysis: 1) sample drawn at the end of a normal dosage interval (trough value); 2) specified dosage of the analyzed immunosuppressant; and 3) patients being at least 18 years old. Mycophenolic acid was included as a negative control, because its elimination is known to be independent of CYP3A4 (Fulton and Markham, 1996).

For each sample, the concentration-to-dose (C/D) ratio was calculated by dividing the serum concentration of the immunosuppressant by the daily dose. In the main analysis, the C/D ratio of each drug was compared between the 3 months associated with the lowest endogenous levels of vitamin D (January, February, and March) and the 3 months with the highest levels (July, August, and September) (Virtanen et al., 2010). To verify the robustness of the results, these comparisons were performed by using both unpaired and paired analysis methods. In the unpaired analysis, all samples that were drawn during any of the two time intervals were included, and the dose-corrected plasma concentrations were compared using the Mann-Whitney *U* test. If a patient contributed more than one sample in January to March or in July to September, these C/D ratios were substituted with an individual median value for the 3-month period. The paired analysis included only ratios from patients contributing samples in both time periods. In this analysis, the Wilcoxon signed-rank test was used to calculate the median within-patient change in dose-corrected drug concentration between January to March and July to September.

When analyses indicated a significant seasonal change in exposure to a drug, the temporal pattern was further investigated by calculating median C/D ratios for each of the 12 calendar months, presented graphically as deviations from the year-average. To avoid correlated samples and overweighting of patients subjected to repeated sampling, multiple values from the same calendar month were replaced with a median value, as described above.

In all analyses, *P* values <0.05 (two-sided) were considered statistically significant. Data extraction was performed by using Crystal Reports XI (Business Objects Software Ltd., Waldorf, Germany), and statistical analyses were by performed using StatsDirect statistical software version 2.7.2 (StatsDirect, Sale, Cheshire, UK).

**UV Light Data and Vitamin D Levels.** Data on UV light exposure in the Stockholm area of Sweden was obtained from The Swedish Meteorological and Hydrological Institute, and average monthly UV light exposure was calculated using data from the years 2000 to 2008.

Published data on serum vitamin D levels in a Finnish cohort of 1136 participants (aged 53–73) were generously obtained by correspondence with Virtanen et al. (2010). These data were used to identify the time period associated with the highest levels of vitamin D (July–September) and the period with the lowest levels (January–March). This procedure was also, to a great extent, in accordance with data reported from other European countries at similar latitudes; more specifically, in Scandinavian countries and in the United Kingdom (Lund and Sorensen, 1979; Finch et al., 1992; Landin-Wilhelmsen et al., 1995).

**Ethical Approval.** The study was approved by the Regional Ethics Committee at Karolinska Institutet, Stockholm (approval no. 2010/1735-31/2).

## Results

A total of 39 316 tacrolimus concentrations (from 1671 patients) meeting the inclusion criteria were identified in the database. The corresponding numbers for sirolimus, cyclosporine, and mycophenolic acid were 3239, 24,414, and 1305, from 344, 1555, and 530 patients, respectively.

The comparison of drug exposures between the 3 months with the lowest vitamin D levels and the 3 months with the highest levels is presented in Table 1. Both tacrolimus and sirolimus exhibited significantly lower dose-corrected concentrations in July to September compared with January to March. For tacrolimus, the reduction was statistically significant in both paired and unpaired analyses, with a similar magnitude of 5 to 7%. For sirolimus, the reduction was more pronounced, 17% in the unpaired comparison and 8% in the paired. However, the smaller number of included samples rendered the analysis a lower statistical power, and the change in sirolimus C/D ratio reached statistical significance only in the unpaired comparison. The large fraction of patients who actually exhibited a reduction in drug concentration between winter and summer was virtually identical for tacrolimus (62%) and sirolimus (63%). There was no evidence of a change in cyclosporine or mycophenolic acid concentration between the two time periods.

The two drugs showing a significant seasonal change in blood concentration were further analyzed by calculation of C/D ratios separately for each month (Fig. 1, C and D). Both tacrolimus and sirolimus showed a very similar pattern with above-average dose-corrected concentrations during December to April and predominantly below-average concentrations during May to September. The changes in drug concentration closely mirrored those of vitamin D in plasma (Fig. 1B), and, although the temporal pattern was very similar for both drugs, the amplitude was severalfold larger for sirolimus. Figure 1A demonstrates the monthly UV light exposure in Sweden with a peak during the summer period. Maximal vitamin D levels (Fig. 1B) are reached somewhat later due to an accumulation of vitamin D in the late summer. The half-life of vitamin D is reported to be approximately 1 month (Heaney et al., 2008), resulting in a delayed decline in vitamin D levels during the early autumn.

## Discussion

The data presented in this investigation support the hypothesis that seasonal changes in UV exposure and vitamin D have an impact on CYP3A4-dependent drug metabolism. Accordingly, the bioavailability of sirolimus and tacrolimus is reduced during the summer. The findings indicate a novel mechanism behind inter- and intraindividual differences in drug disposition that needs to be challenged by other data sets and study designs. However, the current approach to use accumulated data in a large TDM-database indeed offered enough statistical power to detect even subtle differences between different months during the year (Fig. 1).

In contrast to the seasonal variation of tacrolimus and sirolimus, the concentrations of two other immunosuppressants, cyclosporine and mycophenolic acid, did not show any significant seasonal change

TABLE 1

*Change in dose-corrected concentration (C/D ratio) of four immunosuppressants between winter (January–March) and summer (July–September)*

*n* refers to the total number of values included in the analysis (i.e., two per patient in the paired comparison).

|                   | Unpaired Test |                      |                | Paired Test |                     |                |
|-------------------|---------------|----------------------|----------------|-------------|---------------------|----------------|
|                   | <i>n</i>      | Change in C/D Ratio  | <i>P</i> Value | <i>n</i>    | Change in C/D Ratio | <i>P</i> Value |
| Tacrolimus        | 2439          | −4.9% (−8.8; −0.9)   | 0.02           | 1898        | −6.9% (−9.0; −4.9)  | <0.0001        |
| Sirolimus         | 366           | −17.2% (−25.3; −4.0) | 0.007          | 184         | −8.1% (−16.5; 0.3)  | 0.06           |
| Cyclosporine      | 2020          | 2.0% (−2.8; 4.4)     | 0.67           | 1418        | 0.7% (−1.5; 2.9)    | 0.52           |
| Mycophenolic acid | 397           | 1.2% (−12.4; 19.3)   | 0.71           | 106         | −6.1% (−27.4; 14.5) | 0.55           |

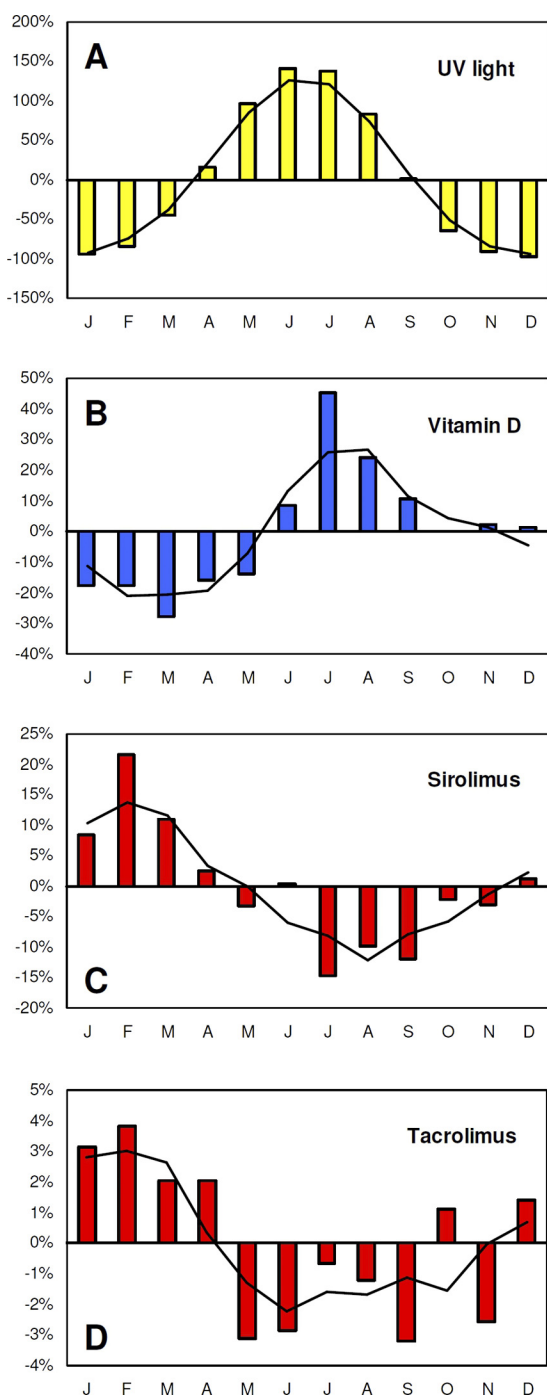


FIG. 1. A, monthly UV radiation in Stockholm, Sweden. B, monthly serum levels of vitamin D (25OH vitamin D) in a Finnish cohort ( $n = 1136$ ). Raw data was obtained from Virtanen et al. (2010). C, monthly sirolimus C/D ratios in patients monitored at Karolinska ( $n = 344$  patients). D, monthly tacrolimus C/D ratios in patients monitored at Karolinska ( $n = 1671$  patients). All values are presented as deviations from the yearly average. Lines represent moving average of three adjacent months.

from winter to summer. While being used essentially by the same patient category as the other three drugs, mycophenolic acid is metabolized primarily by glucuronidation and not by CYP3A4 (Fulton and Markham, 1996). Hence, it was included to serve as a negative control.

One possible explanation for the diverging effect of vitamin D on measured cyclosporine, tacrolimus, and sirolimus concentrations is that the immunoassays used for quantification may differ with regard

to analyte specificity. Commercially available cyclosporine assays have been shown to cross-react with major cyclosporine metabolites, and codetection of CYP3A4-mediated metabolites could effectively obscure the association between enzyme induction and measured drug concentrations (Stettin et al., 2006). To examine whether different contributions of drug metabolites in the three analyses could explain the seemingly heterogeneous effects of sun exposure, we used validation data collected at the laboratory as the cyclosporine, tacrolimus, and sirolimus immunoassays were replaced by highly specific liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods in June 2010. In a series of cyclosporine samples analyzed with both the immunoassay and the LC/MS/MS method, the former resulted in concentrations 35% higher than the latter, indicating a substantial cross-reactivity with cyclosporine metabolites. For tacrolimus, the corresponding value was +20%, whereas there was no indication of cross-reactivity with sirolimus metabolites (S. Rosenberg, unpublished data). These differences in assay specificity are in good correspondence with the differential influence of sun exposure and indicate that the cyclosporine and tacrolimus results may underestimate the true effect of CYP3A4 induction. To evaluate the impact of the assay performance, we repeated the unpaired main analysis in cyclosporine samples analyzed with the newly introduced LC/MS/MS method and found a seasonal effect very similar to that of sirolimus in the original analysis, with 17.6% lower C/D ratios in the summer compared with the winter ( $P = 0.028$ ). Although this result clearly supports the notion that codetection of cyclosporine metabolites could have diluted the results obtained by the immunologic method, the reanalysis was based on a small number of samples ( $n = 272$ ) and larger materials are required to allow firm conclusions.

Another factor that could have contributed to the lack of effect on cyclosporine concentrations might simply relate to the route of drug administration, and that cyclosporine more often is administered intravenously compared with tacrolimus and sirolimus (Swedish on line Medical Products Agency, <http://sweweb.mpa.se/swedisii/default.asp?url=>). A predominant effect of vitamin D on first-pass elimination in the intestinal wall and in the liver would then be lost, and this could have influenced the results. Indeed, a post hoc reanalysis of only those cyclosporine samples in which oral administration could be verified indicated a trend toward the seasonal shifts noticed for tacrolimus and sirolimus, albeit not statistically significant (data not shown).

In addition to differences in assay performance and drug administration routes, the individual pharmacokinetic properties of cyclosporine, sirolimus, and tacrolimus may have contributed to the heterogeneous results. Although all three drugs are primarily metabolized by CYP3A4 (Lampen et al., 1998; Iwasaki, 2007; Staatz et al., 2010), they are also substrates of the drug efflux pump P-glycoprotein (P-gp), which limits oral bioavailability by pumping absorbed drug molecules back to the intestinal lumen (Lo and Burckart, 1999). Similar to CYP3A4, P-gp is induced by vitamin D, but at the vitamin D concentration investigated (1,25(OH)<sub>2</sub>vitamin D<sub>3</sub> 100 nM) the level of P-gp induction is much lower than that of CYP3A4 (Fan et al., 2009). If the relative influence of CYP3A4 and P-gp on the overall bioavailability varies among cyclosporine, sirolimus, and tacrolimus, this could affect their sensitivity to vitamin D-associated enzyme induction. Although the relative contribution of CYP3A4 and P-gp has not been well characterized for these drugs, their dissimilar bioavailabilities (cyclosporine 30–60%, sirolimus 14%, tacrolimus 20–25%) indicate potentially important differences with regard to first-pass metabolism and/or drug efflux [AstellasPharma (2010) Summary of Product Characteristic: Prograf, <http://www.medicines.org.uk/emc/document.aspx?documentId=11102>; Novartis (2010) Summary of



Product Characteristic: Neoral, <http://www.medicines.org.uk/emc/medicine/1307/SPC/>; Wyeth (2011) Summary of Product Characteristic: Rapamune, <http://www.medicines.org.uk/EMC/medicine/5747/SPC/Rapamune/>. Furthermore, at therapeutic doses cyclosporine is an inducer of CYP3A4 and an inhibitor of P-gp gene expression, and, theoretically, this could have limited the potential for further induction of its first-pass metabolism by vitamin D (Lo and Burckart, 1999; Staatz et al., 2010).

Compared with tacrolimus and sirolimus, the blood concentrations achieved at therapeutic doses of cyclosporine are approximately 10 times higher (100 versus 10 nM for the former drugs), a difference that could have implications for the molecular interactions with drug-metabolizing enzymes and drug transporters. Although the blood drug concentrations are well below the associated  $K_m$  for CYP3A4 (4–7  $\mu$ M for cyclosporine, tacrolimus, and sirolimus) (Lampen et al., 1995, 1996, 1998) and P-gp (8  $\mu$ M for cyclosporine) (Saeki et al., 1993), the intracellular concentrations in enterocytes and hepatocytes may be higher, resulting in a transition to nonlinear kinetics for cyclosporine.

It should be noted that cyclosporine, tacrolimus, and sirolimus are not only metabolized by CYP3A4 but also by CYP3A5. Although CYP3A5 is induced by vitamin D, the induction is less pronounced than that of CYP3A4 (Schmiedlin-Ren et al., 1997), and the CYP3A5-mediated metabolism might be devoid of a pronounced seasonal variation related to UV light exposure. However, the majority of the Swedish, primarily Caucasian population do not express the CYP3A5 gene, and this pathway is expected to be of little importance for the first-pass metabolism and elimination of immunosuppressants in the included patients (Mirghani et al., 2006). On the other hand, patients of African descent—a group at high risk of vitamin D deficiency during periods of low sunlight exposure—commonly express functional CYP3A5, and theoretically this additional pathway could curb the influence of UV light exposure on the turnover of immunosuppressants (Mirghani et al., 2006).

In vitro, CYP3A4 induction has been demonstrated at 25-(OH)-vitamin D<sub>3</sub> concentrations from 250 nM and reaches a maximum at 5  $\mu$ M (Schmiedlin-Ren et al., 1997). In human blood, the concentration of 25-(OH)-vitamin D<sub>3</sub> is generally lower, with population mean values ranging from 65 to 100 nM in samples collected during the summer (Lund and Sorensen, 1979; Burgaz et al., 2009; Christensen et al., 2010; Virtanen et al., 2010). However, it is generally difficult to draw reliable conclusions regarding concentration-effect relationships from in vitro studies, and the apparent concentration discrepancy does not exclude the possibility of enzyme induction in vivo. For example, the blood concentration may differ substantially from that in hepatocytes and enterocytes where the induction processes take place. In addition, in vitro experiments have addressed the inducing properties of 25-(OH)-vitamin D<sub>3</sub> and 1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> (a more potent inducer of CYP3A4) separately, whereas the induction in vivo reflects the combined effect of the two forms of vitamin D.

An obvious limitation of this study is that seasonal vitamin D data originated from a geographically related but still different population than the patients in the TDM database. Hence, confirmation of a direct relationship between vitamin D levels in plasma and drug exposure requires further studies in which intraindividual comparisons are made between vitamin D and drug levels or other biomarkers of drug metabolic capacity. In addition, the possible effect of exogenous vitamin D intake on drug metabolism deserves further attention.

The clinical significance of the observed seasonal change in C/D ratios of tacrolimus and sirolimus is unclear at this stage. The relative effect, despite being statistically significant, is limited, and these immunosuppressants are also subject to routine monitoring by con-

centration determinations. However, a seasonal change in drug exposure might be of greater importance in other conditions where TDM is rarely used, such as cardiovascular disease or diabetes. This notion gains some support from a small study by Negero et al. (2007), in which increased blood pressure was noted during coadministration of activated vitamin D and the calcium channel blocker nifedipine. However, drug levels were not measured and the mechanism of the proposed interaction could not be established.

In summary, we propose a mechanism behind inter- and intraindividual variability in drug disposition that would explain the observed changes over time in blood concentrations of important immunosuppressants. Seasonal differences in UV light and the resulting impact on individual vitamin D levels may lead to seasonal changes in the activity of enzymes and transporters of major relevance for drug disposition. Clearly, this hypothesis needs to be tested by additional studies based on different methodological approaches.

#### Authorship Contributions

*Participated in research design:* Lindh, Andersson, Eliasson, and Björkhem-Bergman.

*Performed data analysis:* Lindh, Andersson, Eliasson, and Björkhem-Bergman.

*Wrote or contributed to the writing of the manuscript:* Lindh, Andersson, Eliasson, and Björkhem-Bergman.

#### References

- Burgaz A, Akesson A, Michaëlsson K, and Wolk A (2009) 25-Hydroxyvitamin D accumulation during summer in elderly women at latitude 60 degrees N. *J Intern Med* **266**:476–483.
- Christensen MH, Lien EA, Hustad S, and Almås B (2010) Seasonal and age-related differences in serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and parathyroid hormone in patients from Western Norway. *Scand J Clin Lab Invest* **70**:281–286.
- Daly AK (2006) Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet* **45**:13–31.
- Evans WE and Relling MV (1999) Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* **286**:487–491.
- Fan J, Liu S, Du Y, Morrison J, Shipman R, and Pang KS (2009) Up-regulation of transporters and enzymes by the vitamin D receptor ligands, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and vitamin D analogs, in the Caco-2 cell monolayer. *J Pharmacol Exp Ther* **330**:389–402.
- Finch PJ, Ang L, Colston KW, Nisbet J, and Maxwell JD (1992) Blunted seasonal variation in serum 25-hydroxy vitamin D and increased risk of osteomalacia in vegetarian London Asians. *Eur J Clin Nutr* **46**:509–515.
- Fulton B and Markham A (1996) Mycophenolate mofetil. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in renal transplantation. *Drugs* **51**:278–298.
- Heaney RP, Armas LA, Shary JR, Bell NH, Binkley N, and Hollis BW (2008) 25-Hydroxylation of vitamin D<sub>3</sub>: relation to circulating vitamin D<sub>3</sub> under various input conditions. *Am J Clin Nutr* **87**:1738–1742.
- Iwasaki K (2007) Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. *Drug Metab Pharmacokinet* **22**:328–335.
- Lampen A, Christians U, Bader A, Hackbarth I, and Sewing KF (1996) Drug interactions and interindividual variability of ciclosporin metabolism in the small intestine. *Pharmacology* **52**:159–168.
- Lampen A, Christians U, Guengerich FP, Watkins PB, Kolars JC, Bader A, Gonschior AK, Dralle H, Hackbarth I, and Sewing KF (1995) Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metab Dispos* **23**:1315–1324.
- Lampen A, Zhang Y, Hackbarth I, Benet LZ, Sewing KF, and Christians U (1998) Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. *J Pharmacol Exp Ther* **285**:1104–1112.
- Landin-Wilhelmsen K, Wilhelmsen L, Wilks J, Lappas G, Rosén T, Lindstedt G, Lundberg PA, and Bengtsson BA (1995) Sunlight increases serum 25(OH) vitamin D concentration whereas 1,25(OH)<sub>2</sub>D<sub>3</sub> is unaffected. Results from a general population study in Göteborg, Sweden (The WHO MONICA Project). *Eur J Clin Nutr* **49**:400–407.
- Lo A and Burckart GJ (1999) P-Glycoprotein and drug therapy in organ transplantation. *J Clin Pharmacol* **39**:995–1005.
- Lund B and Sørensen OH (1979) Measurement of 25-hydroxyvitamin D in serum and its relation to sunshine, age and vitamin D intake in the Danish population. *Scand J Clin Lab Invest* **39**:23–30.
- Mirghani RA, Sayi J, Aklilu E, Allqvist A, Jande M, Wennerholm A, Eriksen J, Herben VM, Jones BC, Gustafsson LL, et al. (2006) CYP3A5 genotype has significant effect on quinine 3-hydroxylation in Tanzanians, who have lower total CYP3A activity than a Swedish population. *Pharmacogenet Genomics* **16**:637–645.
- Negero S, Izumi S, Furukubo T, Satoh M, Matsunaga C, Yamakawa T, Ikegawa S, Nakanishi H, Kadowaki D, and Hirata S (2007) Interaction between activated VD<sub>3</sub> and Ca channel blockers in patients undergoing hemodialysis. *Int J Clin Pharmacol Ther* **45**:186–187.
- Qiu H, Mathias M, Nestler S, Bengel C, Nem D, Gödel-Armbrust U, Lang T, Taudien S, Burk O, and Wojnowski L (2010) The unique complexity of the CYP3A4 upstream region suggests a nongenetic explanation of its expression variability. *Pharmacogenet Genomics* **20**:167–178.
- Saeki T, Ueda K, Tanigawara Y, Hori R, and Komano T (1993) Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* **268**:6077–6080.
- Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, Lown KS, and Watkins PB (1997)

Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub>. *Mol Pharmacol* **51**:741–754.

Staatz CE, Goodman LK, and Tett SE (2010) Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. *Clin Pharmacokinet* **49**:141–175.

Stettin M, Halwachs-Baumann G, Genser B, Frühwirth F, März W, and Khoschror GA (2006) Determination of cyclosporine A in whole blood: comparison of a chromatographic method with three different immunological methods. *Talanta* **69**:1100–1105.

Virtanen JK, Nurmi T, Voutilainen S, Mursu J, and Tuomainen TP (2010) Association of serum

25-hydroxyvitamin D with the risk of death in a general older population in Finland. *Eur J Nutr* doi:10.1007/s00394-010-0138-3.

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**Address correspondence to:** Erik Eliasson, Karolinska Institutet, Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska University Hospital, Huddinge, SE 141 86 Stockholm, Sweden. E-mail: erik.eliasson@ki.se

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