The cytochrome P450 3AP1 genotype shows correlation with clearance (CL/F) in a renal transplant population

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The cytochrome P_{450} 3A (CYP3A) series is important for the metabolism of immunosuppressive agents [1]. The influence of the CYP3AP1 pseudogene variant for sirolimus has not been elucidated. Polymorphisms in these genes affect the production of the enzymes and hence possibly the pharmacokinetics and may prove a powerful means of individualizing dosage strategies in posttransplantation therapy [2].

PURPOSE

To study the relationship between CYP3AP1 genotype and surrogates of clearance, and also the parameter itself estimated with Bayesian methods, of Rapamune® sirolimus (SRL) in renal transplant patients.

METHODS

Blood samples (n = 768) from renal transplant recipients (n = 41) at Paraskevaidion Transplantation Center in Cyprus were analyzed retrospectively. The samples were routine monitoring predose troughs (Cmin) for SRL which was administered orally once daily (08:00) with cyclosporine 6 hours later and twice daily. Cmin is assumed to be a surrogate for exposure of the area under the concentration/time curve (AUC) for SRL. Since Dose/AUC is equivalent to the systemic pharmacokinetic clearance (CL/F), the ratio is often used as a phenotype in genotypic analysis.

Polymerase chain reaction (PCR) was performed to differentiate polymorphisms located in the CYP3AP1 pseudogene (A/G-44). The amplified products were subjected to cleavage with a restriction enzyme and the cleavage reaction was analysed by gel electrophoresis (RFLP). The allelic variants differentiated for the CYP3AP1 pseudogene were CYP3AP1*1 and CYP3AP1*3. The wildtype (-/-) was carried by most patients (83%) compared to the mutation in one allele (+/-) (17%).

Initially, relationships between genotype code (GCOD) and variables (Cmin, Dose, Cmin/ Dose, weight, sex) were explored visually then with principal component and factor analysis, and with linear and logistic regression. Inference was aided by SPSS (SPSS Inc., Chicago, IL). No significant relationship was found between GCOD and the variables.

The PREDPP facility in NONMEM (nonlinear mixed effect modeling, NONMEM Project Group, University of California at San Francisco, CA) was used for regressing the categorical variable GCOD on Cmin/Dose. Then the NMTRAN facility was used with a two-compartment model (literature values [3], except for CL) in the POSTHOC (MAP Bayesian) method, to extract the individual patient CL and compare this with GCOD. Comparison was performed with the Xpose package [4] as implemented in SPLUS (Insightful Corp., Seattle, WA).

Table I. Characteristics of renal transplant patients (n = 51) with sirolimus (also with cyclosporine and prednisone).

Variable	Mean	Range
Weight [kg]	67.2	27 - 122
Cmin [μg/L]	8.3	3.6 – 21.9
Dose [mg]	2.2	1 – 10
Cmin/ Dose [(1000xL)$^{-1}$]	3.8	0.26 – 15.6
CL/F [L/h]	6.9	1.7 – 15.4

RESULTS

Linear regressions of Cmin/ Dose and Dose alone *versus* genotype code, weight and sex were performed and gcode and body weight were significant covariables. Factor analysis showed that Gcode, Cmin and weight were significant components of nearly equal importance (Fig. 1).

Due to the large variability and all components a mixed effects analysis was performed. Mixed effects modeling of Cmin/ Dose showed a significant difference between the two subgroups in this variable (mean ± SD) (3.63 ± 1.17 [1000 x L] $^{-1}$ and 2.86 ± 0.72 [1000 x L] $^{-1}$, respectively; $p < 0.0001$) and insignificant effects for covariates weight and sex.

RESULTS - continued

Two compartment empirical parameters were obtained for SRL based on literature values [3] of prior means and using uninformed (flat) Bayes priors. The oral pharmacokinetic systemic clearance (CL/F) values, one for each patient, showed a significant relationship in general additive modeling with genotype code 1 or 2 (for homozygotes and heterozygotes, respectively) (Fig. 2). A bootstrap method was used to test the model for stability but failed, most likely due to the limited size of the sample.

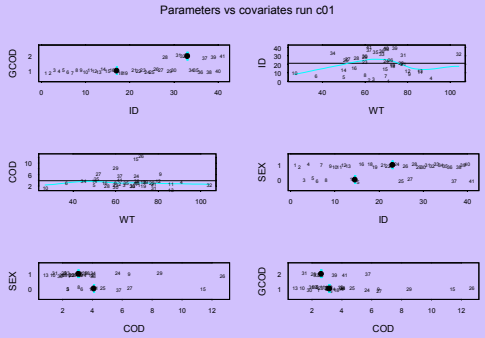

Figure 1. Variable co-plot for patient serial number (ID), genotype code (GCOD), Cmin/ Dose (COD), sex and body weight (WT)

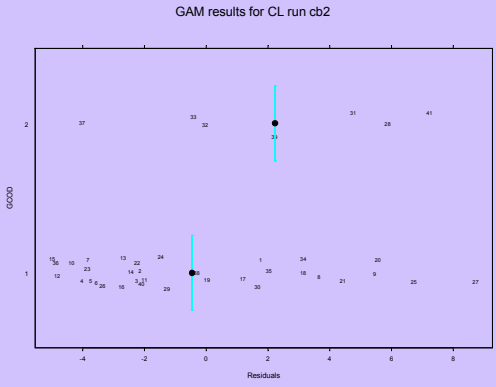

Figure 2. CYP3AP1 genotype code (GCOD) (1 = homozygotes; 2 = heterozygotes) in 41 renal transplant patients versus residuals for systemic CL/F of sirolimus (CLmedian - CL individual).

CONCLUSIONS

Polymorphisms in the CYP3AP1 gene appear to associate with different subpopulations of systemic SRL clearance (CL/F) or of Cmin/Dose in renal transplant patients. However, prospective testing in larger patient groups is needed to verify this result, preferably with estimation of the complete AUC in a population analysis setting.

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