Research Highlights

Highlights from the latest articles in transplantation pharmacogenomics

Pharmacogenetics in solid organ transplantation: a transition from kinetics to dynamics

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The focus of pharmacogenetic research in solid-organ transplantation has shifted from 'kinetics' to 'dynamics'. The impact of genetic variants encoding metabolizing enzymes or transporter proteins of immunosuppressive compounds on blood drug concentrations has been intensively studied in the last decade. Today, genetic polymorphisms coding for drug target or signaling proteins involved in pharmacodynamics pathways are intensively explored in renal disease states and translated into clinically relevant outcome indicators: efficacy and toxicity (i.e., adverse events).

The reasons for this transition in pharmacogenetic research are obvious: although important genetic determinants of immunosuppressive drug exposure have been identified (e.g., *CYP3A5* and *UGT1A9*), the clinical use of this information for optimization of drug dosing has not yet succeeded to replace concentration-controlled drug dosing in terms of increased efficacy and/or reduced toxicity [1]; although most immunosuppressive drugs require concentration-controlled dosing owing to their narrow therapeutic window, the upper limit concentrations indicating increased risk for adverse events have not been established. Indeed, despite controlled efforts to minimize drug exposure, most

immunosuppressive drug combinations tend to retain their specific toxicity profiles [2]. Conversely, the minimum drug concentrations needed to maintain efficacy (avoidance of acute rejection) have not been unequivocally determined for the different immunosuppressive drug combinations [3,4].

References

- 1 Thervet E, Lloriot MA, Barbier S *et al.* Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin. Pharmacol. Ther.* 87(6), 721–726 (2010).
- 2 Ekberg H, Bernasconi C, Nöldeke J *et al.* Cyclosporine, tacrolimus and sirolimus retain their distinct toxicity profiles despite low doses in the Symphony study. *Nephrol. Dial. Transplant.* 25(6), 2004–2010 (2010).
- 3 Kuypers DR, Le Meur Y, Cantarovich M *et al.*; Transplantation Society (TTS) Consensus Group on TDM of MPA. Consensus report on therapeutic drug monitoring of mycophenolic acid in solid organ transplantation. *Clin. J. Am. Soc. Nephrol.* 5(2), 341–358 (2010).
- 4 Wallemacq P, Armstrong VW, Brunet M *et al.* Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther. Drug Monit.* 31(2), 139–152 (2009).

Pharmacogenetics of proliferation signal inhibitors (mTOR inhibitors) in renal transplantation

Evaluation of: Woillard JB, Kamar N, Rousseau A, Rostaing L, Marquet P, Picard N. Association of sirolimus adverse effects with m-TOR, p70S6K or Raptor polymorphisms in kidney transplant recipients. *Pharmacogenet. Genomics* 22(10), 725–732 (2012).

Woillard *et al.* studied the associations between several candidate polymorphisms in the *mTOR*, Raptor and *p70S6K* genes and the respective sirolimus (SRL) dose/exposure and time-dependent adverse events (AEs; anemia, hyperlipidemia, cutaneous lesions, edemas and infections) [1]. SRL exerts its immunosuppressive effects through inhibition of the mammalian target of rapamycin complex (mTORC1), a protein kinase that modulates cell growth and proliferation as well as metabolic homeostasis. As Raptor regulates mTOR activity and the activation of p70S6K mediates some of SRL actions, SNPs of the genes encoding the latter proteins, were obvious candidates for having a potential role in drug-related AEs [2]. A first exploratory retrospective analysis was performed in a ‘discovery population’ of patients switched to SRL from a calcineurin inhibitor (era 2000–2008) at a mean of 5.9 ± 5.9 years (0.3–25.9 years) post-transplantation. Only patients who had not prematurely discontinued SRL therapy because of AEs in the 6 months following conversion were included in the analysis ($n = 113$). Subsequently, the analysis was repeated in a smaller ‘validation population’, which comprised a subset of recipients that participated in an open-label randomized multicenter trial (CONCEPT study) and were switched at 3 months post-transplantation (mean of 90.8 ± 8 days; 77–116 days) from cyclosporine to SRL and mycophenolate mofetil ($n = 66$) [3]. Clinical data and covariates and information on

SRL-related AEs were collected from the patient medical files and clinical research files, respectively, and incorporated into a multilinear or logistic regression imbedded in linear mixed-effect models together with the candidate *mTOR*, Raptor and *p70S6K* gene polymorphisms. In the discovery population, the mTOR AGAAA haplotype (rs1770345/rs2300095/rs2076655/rs1883965/rs12732063; frequency = 0.055), blood trough SRL concentrations and the time to SRL conversion were significantly associated with a decrease in hemoglobin (Hb) level (as well as mean corpuscular volume). Intriguingly, while the mTOR AGAAA haplotype and time to SRL conversion remained significantly associated with decreased Hb levels in the validation population, trough SRL concentrations were associated with increased Hb levels in the validation set. No associations were found between total and low-density lipoprotein cholesterol concentrations, triglyceride concentrations, cutaneous AEs, edemas or infections and *mTOR*, Raptor or *p70S6K* SNPs or haplotypes. Additionally, this study confirmed the findings of earlier reports showing an association between trough SRL concentrations and total cholesterol and triglyceride concentrations [4]. Associations between trough SRL levels and edemas and cutaneous AEs observed in the discovery population could not be confirmed in the validation study.

The results of this interesting study illustrate a few important caveats of studies examining the direct association between SNPs encoding immunosuppressive drug target proteins and their pharmacodynamics or adverse effects:

- A very precise and accurate phenotypic definition is absolutely mandatory. While an extensive data set was obtained in this study, important clinical determinants of SRL-related AEs were not incorporated in the analysis. For example, anemia was not

corrected for donor age, presence of chronic allograft injury, hyperparathyroidism, use of concomitant drugs that hamper erythropoiesis (e.g., angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, trimethoprim/sulfamethoxazole and azathioprine) and most importantly, exposure to mycophenolic acid, which is highly variable and a major determinant of anemia after transplantation [5];

- Training (discovery) and validation (confirmation) populations need to be optimally and closely matched [6]. Not only in terms of absolute numbers, but also in terms of timing (and era), selection (patients remaining on SRL for at least 6 months post-conversion vs per protocol randomization), indication for intervention (clinically driven SRL switch vs per protocol conversion), organ status (failing or ‘troubled’ grafts vs *de novo* grafts), absolute number of (adverse) events, concomitant drugs;
- A prospective design is necessary to precisely delineate the complex time course of (adverse) events and dose modifications and to correctly identify cause and effect relationships. Despite comprehensive retrospective data collection and adequate time-dependent statistical methods, false-positive findings or reversed associative findings are potential risks as illustrated by the alternating association between trough SRL concentrations and Hb levels observed in this study;
- The candidate SNPs or haplotypes need to be sufficiently expressed in the study populations. In order to justify routine identification of genetic variants that predispose to AEs, the latter need to be serious, life-threatening or difficult to correct so that potential genotyping of a total population remains cost effective.

The study by Woillard *et al.* provides important learning points for the development of larger prospective studies addressing the value of genotyping solid-organ recipients as well as for risk stratification of AEs related to immunosuppressive drugs.

References

- 1 Woillard JB, Kamar N, Rousseau A, Rostaing L, Marquet P, Picard N. Association of sirolimus adverse effects with m-TOR, p70S6K or Raptor polymorphisms in kidney transplant recipients. *Pharmacogenet. Genomics* 22(10), 725–732 (2012).
- 2 Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat. Rev. Discov.* 10(11), 868–880 (2011).
- 3 Lebranchu Y, Thierry A, Toupance O *et al.* Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am. J. Transplant.* 9(5), 1115–1123 (2009).
- 4 Kahan BD, Napoli KL, Kelly PA *et al.* Therapeutic drug monitoring of sirolimus: correlations with efficacy and toxicity. *Clin. Transplant.* 14(2), 97–109 (2000).
- 5 Yabu JM, Winkelmayer WC. Posttransplantation anemia: mechanisms and management. *Clin. J. Am. Soc. Nephrol.* 6(7), 1794–1801 (2011).
- 6 Marian AJ. Molecular genetic studies of complex phenotypes. *Transl. Res.* 159(2), 64–79 (2012).

Do inosine monophosphate dehydrogenase SNPs affect clinical outcome after kidney transplantation?

Evaluation of: Shah S, Harwood SM, Döhler B, Opelz G, Yaqoob MM. Inosine monophosphate dehydrogenase polymorphisms and renal allograft outcome. *Transplantation* 94(5), 486–491 (2012).

A more quantitative approach to assess the impact of genetic variants of pharmacodynamic target molecules on clinical outcome parameters was explored in a recent study of the Collaborative Transplant Study (CTS) [1]. As inosine monophosphate dehydrogenase (IMPDH) activity varies tenfold between patients listed for renal transplantation, and as part of this variation is related to SNPs in the *IMPDHI* and *II* genes, it is hypothesized that personalized mycophenolate mofetil (MMF; an inhibitor of IMPDH) dosing would enhance efficacy and reduce toxicity. Indeed, two SNPs in intron 7 of *IMPDHI* (rs2278293; C>T and rs2278294; G>A) and the A>G variant in *IMPDHII* (rs17006052) were found to be significantly associated with a reduced and increased 1-year acute rejection incidence in clinical studies, respectively [2–4]. However, these findings could not be consistently reproduced in subsequent trials. Therefore, Shah *et al.* examined the effect of these three *IMPDHII/III* SNPs on acute rejection rates, graft function, graft

survival and MMF dosing in a random sample of 1040 renal recipients from the CTS DNA bank. Patients were transplanted between 1995 and 2007 in Europe and the USA with a calcineurin inhibitor-based immunosuppressive regimen. Only deceased-donor transplantations from white donors to white recipients were included in order to reduce population factors on gene frequency. None of the examined *IMPDHI* SNPs (rs2278293; rs2278294; G>A) or the *IMPDHII* SNP (rs17006052) had any effect on death-censored 5-year graft and patient survival, 1-year acute rejection rates and graft function (measured as the time-related proportion of patients with a serum creatinine level greater than 130 μ M). These results were observed irrespective of the type of calcineurin-inhibitor used (tacrolimus versus cyclosporine). The MMF doses tolerated at 1 and 3 years post-transplantation were not different between genotypes, but were, as expected, consistently higher in cyclosporine-treated patients compared with tacrolimus-treated recipients [5]. Interestingly, 2-year death-censored graft survival was significantly ($p = 0.027$) worse in recipients carrying the GG variant of *IMPDHI* G>A (rs2278294) compared with GA and AA carriers but this difference completely disappeared with prolonged follow-up. Based on this large cohort analysis, previous findings of several smaller studies demonstrating an effect of *IMPDHI* and *II* SNPs on

acute allograft rejection rates could not be confirmed. Moreover, relevant clinical end points, such as graft and patient survival and graft function, were not influenced by *IMPDH* SNPs. This study also indirectly suggested that MMF-related adverse effects (e.g., leukopenia and gastrointestinal symptoms) were not significantly influenced by *IMPDH* SNPs as no differences in 1- and 3-year MMF dosing (adjusted for type of calcineurin inhibitor) were observed between genotypes. While the large-scale approach used in this study had several advantages compared with previous smaller studies in terms of patient selection, prolonged follow-up and reproducibility, it was limited to one type of donor (deceased), white donor to white recipient transplantations only and provided no details on the type and severity of drug-related AEs. In addition, baseline demographic differences were observed between *IMPDHI* genotype groups in this random sample of 1040 recipients, for example geographical location (Europe vs USA), donor age and cold ischemia time. Despite these limitations and the lack of prospectively collected data, the results of this analysis do not support routine screening of patients for *IMPDH* SNPs as they do not seem to affect renal graft or patient outcome. Whether polymorphisms in the genes encoding enzymes involved in mycophenolic acid metabolism (e.g., *UGT1A9* -275T>A

[rs6714486] and -2152C>T [rs17868320]), which affect MMF dosing [6], are more important for graft outcome, needs to be examined prospectively in similar large cohorts, preferably in combination with the *IMPDH1* and *II* genotypes.

References

- Shah S, Harwood SM, Döhler B, Opelz G, Yaqoob MM. Inosine monophosphate dehydrogenase polymorphisms and renal allograft outcome. *Transplantation* 94(5), 486–491 (2012).
- Wang J, Yang JW, Zeevi A *et al.* *IMPDH1* gene polymorphisms and association with acute rejection in renal transplant patients. *Clin. Pharmacol. Ther.* 83(5), 711–717 (2008).
- Gensburger O, Van Schaik RH, Picard N *et al.* Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolatemofetil. *Pharmacogenet. Genomics* 20(9), 537–543 (2010).
- Grinyó J, Vanrenterghem Y, Nashan B *et al.* Association of four DNA polymorphisms with acute rejection after kidney transplantation. *Transpl. Int.* 21(9), 879–891 (2008).
- Kobayashi M, Saitoh H, Kobayashi M, Tadano K, Takahashi Y, Hirano T. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. *J. Pharmacol. Exp. Ther.* 309(3), 1029–1035 (2004).
- Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (*UGT1A9*) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in *de novo* renal allograft recipients. *Clin. Pharmacol. Ther.* 78(4), 351–361 (2005).

Genetics of renal dysfunction after pediatric heart transplantation: the unequal struggle of a maturing kidney

Evaluation of: Feingold B, Brooks MM, Zeevi A *et al.* Renal function and genetic polymorphisms in pediatric heart transplant recipients. *J. Heart Lung Transplant.* 31(9), 1003–1008 (2012).

A subset of the Pediatric Heart Transplantation Study (PHTS) database was interrogated to examine the effects of a panel of prespecified polymorphisms, including genes coding for drug metabolizing proteins, cytokines and growth factors, on renal function after cardiac transplantation [1]. Nineteen functional SNPs in 14 candidate genes involved in renal dysfunction after solid-organ transplantation (*CYP3A5*, *ABCB1*, *TGF-β1* and *ACE*), end-stage renal disease (*TNF-α*, *IL-6*, *IL-4*, *IL-1RN* and *IL-10*) and kidney injury (*HO-1*, *NOS2*, *FAS*, *FASL* and *IL-15RA*) were tested against repeated annual glomerular filtration rate (eGFR) outcome measures (multivariable linear mixed models) and the onset of renal dysfunction, defined as eGFR <60 ml/min/1.73m², after cardiac transplantation (Cox proportional hazard modeling). A total of 453 pediatric recipients (mean age: 6.2 ± 6.1 years; 33%

aged <1 year) were followed for a mean of 5.1 ± 2.5 years (29.8% of patients had 8-year follow-up data). Both the *FASL* (-844C>T) T allele and *HO-1* (326A>G) G allele were associated with decreased renal function after heart transplantation and were more frequently observed in black recipients. However, after adjusting for the recipient's age at time of transplantation, race and gender, these associations were no longer significant. When prespecified genotypes and haplotypes that had previously been identified in relation to renal disease were examined, no associations were found with renal (dys) function in this cohort of cardiac recipients. This study failed to confirm earlier reports linking *TGF-β1* SNPs (rs1800471: TGF-β high-producer phenotype) and the *CYP3A5**3 (rs776746) SNP to worse renal function after (predominantly) adult cardiac transplantation. The reasons for this discrepancy are not completely clear. While the current study only included pediatric patients with an eGFR ≥60 ml/min/1.73m² at 1 year post-transplantation (in order to exclude pre-existing moderate-to-severe renal insufficiency), many other variables potentially affecting renal function after grafting were not retained: episodes of

acute kidney injury, proteinuria and/or hematuria as indicator of pre-existing renal disease, calcineurin inhibitor doses and trough concentrations and concomitant medication. While it is recognized that chronic renal allograft dysfunction is a very complex and multifactorial problem [2], chronic renal insufficiency after nonrenal organ transplantation is an equally intricate complex process as confirmed by recent renal histology studies in liver and other nonrenal transplant recipients [3,4]. Histological evidence of calcineurin inhibitor-associated nephrotoxicity was observed in the latter studies in 16–35.5% of biopsies [3,4]. That fact that primary and secondary renal injuries were more frequently observed than calcineurin inhibitor-associated nephrotoxicity, illustrates that correct phenotyping of the true causes of renal dysfunction after nonrenal organ transplantation is equally important as it is in the setting of kidney transplantation. Age-dependent renal development and (enzymatic) maturation is another important variable when assessing the effects of these genetic variants and clinical covariables on kidney function in pediatric organ recipients. Postnatal renal development and the maturation of renal drug elimination pathways are

gradual and continuous processes from birth to adolescence with peak maturation between 1 and 3 years [5,6]. It is clear that the (cumulative) impact of different types of renal injuries (e.g., drug toxicity, [allo-] immunological and inflammatory/repair responses) will differ with biological age (shown in this analysis), but also with the renal developmental stage, which is more difficult to identify in the individual patient. The different results between adult and pediatric studies on genetic variants could, at least partly, be explained by this phenomenon. The interesting study of Feingold *et al.* would have benefited from renal histology data, ideally obtained from a kidney biopsy performed at the moment

of/or prior to cardiac transplantation or alternatively at the time of renal dysfunction.

References

- 1 Feingold B, Brooks MM, Zeevi A *et al.* Renal function and genetic polymorphisms in pediatric heart transplant recipients. *J. Heart Lung Transplant.* 31(9), 1003–1008 (2012).
- 2 Nankivell BJ, Kuypers DR. Diagnosis and prevention of chronic kidney allograft loss. *Lancet* 378(9800), 1428–1437 (2011).
- 3 Kim JY, Akalin E, Dikman S *et al.* The variable pathology of kidney disease after liver transplantation. *Transplantation* 89(2), 215–221 (2010).
- 4 Kubal C, Cockwell P, Gunson B *et al.* Chronic kidney disease after nonrenal solid organ transplantation: a histological assessment and utility of chronic allograft damage index scoring. *Transplantation* 93(4), 406–411 (2012).
- 5 Charlton JR, Norwood VF, Kiley SS, Gurka MJ, Chevalier RL. Evolution of the urinary proteome during human renal development and maturation: variations with gestational and postnatal age. *Pediatr. Res.* 72, 179–185 (2012).
- 6 Tayman C, Rayyan M, Allegaert K. Neonatal pharmacology: extensive interindividual variability despite limited size. *J. Pediatr. Pharmacol. Ther.* 16(3), 170–184 (2011).