Research Highlights

Genetics in renal transplantation: a recent tale of chemokines, enzymes, drugs and HLA

Donor chemokine receptor 5 polymorphisms and renal allograft rejection

Evaluation of: Cha RH, Yang SH, Kim HS *et al.*: Genetic interactions between the donor and the recipient for susceptibility to acute rejection in kidney transplantation: polymorphisms of *CCR5*. *Nephrol. Dial. Transplant.* 24(9), 2919–2925 (2009).

Cha and co-workers recently not only confirmed that the common 59029G>A polymorphism of the chemokine receptor 5 (*CCR5*) gene in Korean kidney transplant recipients was associated with early acute allograft rejection and repeated acute rejection episodes, but also demonstrated that when combining both recipient and donor *CCR5* genotype, early rejection episodes were more prevalent as the combined A allelic number increased [1]. This relationship remained significant after adjustment for gender, human leukocyte antigen (HLA) mismatch, type of donor and recipient age. However, despite these interesting findings, the lack of prospective data captured in this study, for example, the sometimes insufficient data (e.g., only 40% of all cold ischemia times were available), the effect of era (recruitment from 1982 through to 2006) and the lack of surveillance renal graft histology (protocol biopsies), warrant against premature extrapolation of the current observations. The *CCR5* 59029G>A polymorphism, which causes increased transcriptional activity and protein levels, is thought to be implicated in the progression of diabetic nephropathy, chronic allograft nephropathy and acute rejection phenomena in liver, kidney and heart transplantation [2,3]. While *CCR5* is predominantly expressed in monocytes, dendritic cells and T-memory cells, it serves as a receptor

for many cytokines involved in the clinical and subclinical alloimmune response, such as RANTES/CCL5, MIP-1 and MCP-2. This study elegantly demonstrates that, despite evidence of chimerism occurring after kidney transplantation, albeit in a limited number of cell types, local alloimmune reactivity after renal transplantation remains, in part, determined by donor characteristics, including cytokine genotypes [4]. Interestingly, the authors found a poorer graft survival rate in recipients carrying grafts from A allele positive donors compared with patients receiving a kidney from A allele negative donors, while graft survival was not affected by the 59029G>A *CCR5* genotype of the recipient. Again, these findings highlight the persistent importance of donor-associated genetic characteristics in long-term renal allograft outcome. The next steps following the description of these intriguing relationships would include the demonstration and location of increased expression of *CCR5* in renal allografts according to donor and recipient genotype, the quantification of *CCR5* in renal tissue and blood, preferably at regular time points after grafting, and the concurrent assessment of other key cytokines and chemokines involved in the intricate alloimmune response network, such as TNF- α , Fas ligand, TGF- β , IFN- γ , perforin, granzyme B, IL-2R, IL-6 and IL-10. A simultaneous thorough study of the different regulatory and effector cells involved in the alloimmune response (e.g., macrophages, CD4, CD25, FoxP3, T-regulatory cells, memory T cells and dendritic cells) would potentially enable the discovery of novel mechanistic pathways as a basis for future therapies, as was recently shown by the successful use of *CCR5* and *CXCR3* blockade (by TAK-779) in murine cardiac allograft vasculopathy [5].

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Financial & competing interests disclosure

Dirk RJ Kuypers is co-author of the manuscript by van Schaik R *et al.*: UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. *Clin. Pharmacol. Ther.* 86(3), 319–327 (2009). In relation to the clinical study described in this manuscript, Dirk RJ Kuypers has received honoraria from F Hoffmann-La Roche. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Glutathione S-transferases genotypes and their role in renal alloimmune and ischemia-reperfusion injury

Evaluation of: Singh R, Manchanda PK, Kesarwani P, Srivastava A, Mittal RD: Influence of genetic polymorphisms in *GSTM1*, *GSTM3*, *GSTT1* and *GSTP1* on allograft outcome in renal transplant recipients. *Clin. Transplant.* 23(4), 490–498 (2009).

Another family of cytosolic enzymes are glutathione S-transferases (GSTs), which are responsible for tissue protection against oxidative stress and xenobiotics and involved in transplantation-related ischemia-reperfusion injury and alloimmune activation. Singh and co-workers demonstrated that genetic polymorphisms in the *GSTM1*, *GSTM3*, *GSTT1* and *GSTP1* genes (encoding different isoforms of GST) are associated with renal allograft outcome in a population of 273 kidney transplant recipients [1]. Various GST isoforms are promptly released into the urine in response to renal tubular damage, and this process occurs not only prior to any significant changes in serum creatinine concentration, but also according to the specific site of injury [2]. For example, the *GSTT1* null genotype, associated with decreased enzymatic activity, has been related to the development of end-stage renal disease in diabetic and hypertensive patients [3]. In the current study in Indian-organ recipients, the *GSTM1* null genotype was more prevalent amongst recipients who

suffered from graft rejection (64.9%) or delayed graft function (53.1%) compared with patients with stable kidney function (46.7%), indicating a more than threefold risk in the former patients. In addition, the *GSTM1* null genotype was associated with a shorter time to first rejection; co-inheritance of the *GSTM3* AB and BB genotype further increased the risk for rejection and also reduced the mean time to first rejection. How exactly genetically determined decreased GST activity could lead to allograft rejection is not clear; it is plausible that alterations in GST activity will not necessarily play an important role in triggering rejection phenomena, but rather determine the extent of tissue repair response to alloimmune injury. However, patients receiving a kidney from a *GSTT1* mismatched donor developed anti-GSTT1 antibodies after 32–60 months, together with histological signs of chronic antibody-mediated renal allograft rejection but without the presence of anti-HLA donor-specific antibodies, suggesting a potential direct role of GST in triggering of the alloimmune response [4]. Similarly, in the current study the frequency of the *GSTP1* variant genotype GG was higher in recipients with delayed graft function (15.6%) compared with patients with stable function (7.1%), signifying a more than sevenfold risk. Again, while delayed renal allograft function is often caused by the complex interplay of

suboptimal donor organ quality, ischemia reperfusion injury, acute tubular necrosis and calcineurin inhibitor toxicity, altered GST activity as a consequence of *GST* polymorphisms might be more important for the protection against and the recovery from delayed graft function, rather than its origination [5]. Further studies, preferably in animal models are necessary to shed more light on the exact mechanisms underlying the link between different *GST* polymorphisms and acute allograft rejection and delayed graft function. Another intriguing finding in the study of Singh *et al.* was the association between the *GSTM1* null genotype and lower early ciclosporin dose requirements in patients carrying this polymorphism. Since all GSTs are phase II drug-metabolizing enzymes, this observation demands further consideration in prospective pharmacokinetic studies.

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Mycophenolic acid pharmacogenetics and clinical efficacy in kidney transplantation

Evaluation of: van Schaik R, van Agteren M, de Fijter J *et al.*: *UGT1A9* -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. *Clin. Pharmacol. Ther.* 86(3), 319–327 (2009).

Whether mycophenolate mofetil (Cellcept™, Roche, Basel, Switzerland) should be dosed in a concentration-controlled manner in renal allograft recipients is still a matter of debate. While a clear relationship has been determined between early mycophenolic acid (MPA) exposure and avoidance of acute renal allograft rejection, the benefit of MPA therapeutic drug monitoring has not been unequivocally established by three recent prospective randomized trials in renal transplantation [1–3]. What was learned from the latter studies is that 48.8 and 23.8% of ciclosporin co-treated and tacrolimus co-treated patients, respectively, were underexposed to MPA early after transplantation (day 3) by using the currently advised fixed mycophenolate mofetil (MMF) loading doses, and were aiming for a minimal target area under the concentration–time curve (AUC) concentration threshold of 30 mg·h/l [2]. While higher MMF loading doses or early intensive MPA exposure monitoring and MMF dose adjustments could overcome this initial underexposure, it would be clinically helpful to identify patients at risk for reduced early exposure prior to transplantation. van Schaik and co-workers examined the impact of the *UGT1A8*, *UGT1A9*, *UGT2B7* and *MRP2* polymorphisms on MPA exposure and clinical outcome in a

large group of 338 *de novo* renal allograft recipients that was part of the The Fixed Dose Concentration-Controlled (FDCC) trial study population (n = 901) [4]. The authors confirmed earlier studies by showing that tacrolimus co-treated patients who were carriers of the *UGT1A9* -275T>A and/or *UGT1A9* -2152C>T polymorphism(s) displayed a lower MPA AUC_{0–12h} early post-transplantation (geometrical mean MPA AUC_{0–12h} on day 3 of 30 mg·h/l versus 44 mg·h/l for wild-type patients) but also throughout the first postoperative year [5]. In addition, in a logistic regression analysis of biopsy-proven acute allograft rejection in the first year in patients receiving a fixed daily MMF dose of 2 g and taking into account recipient age and gender, donor type, number of transplantations, induction therapy, panel-reactive antibodies, number of HLA mismatches, *CYP3A5* genotype and tacrolimus pre-dose trough concentrations, the *UGT1A9* -275T>A and/or *UGT1A9* -2152C>T polymorphism(s) were the only significant predictors of acute graft rejection (odds ratio: 13.3, 95% CI: 1.1–162.3, p = 0.042). In ciclosporin co-treated subjects, no effect of the *UGT1A9* -275T>A and/or *UGT1A9* -2152C>T polymorphism(s) on MPA exposure nor on clinical outcome was observed. These findings could potentially have clinical implications: prior genotyping of patients would enable the pre-emptive adjustment of MMF doses in tacrolimus co-treated transplant recipients in order to reduce the risk of early MPA underexposure and hence acute rejection. As stated by the authors and based on the observed 23% difference in acute rejection incidence between carriers and noncarriers of the *UGT1A9* -275T>A and/or *UGT1A9*

-2152C>T polymorphism(s) and the 12% prevalence of these SNPs, 36 patients would need to be genotyped in order to potentially prevent one acute rejection episode. While with the current limited penetration of genotyping methodology into clinical practice this 36:1 ratio might appear as a substantial investment, hopefully future expansion and facilitation of genotyping procedures could well prove these types of strategies to be very efficient in terms of cost–benefit.

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What is the link between *HLA-G* polymorphisms and post-transplantation lipid disorders?

Evaluation of: Piancatelli D, Maccarone D, Liberatore G *et al.*: *HLA-G* 14-bp insertion/deletion polymorphism in kidney transplant patients with metabolic complications. *Transplant. Proc.* 41(4), 1187–1188 (2009).

How a 14-bp insertion/deletion polymorphism at exon 8 of the 3'-UTR of the *HLA-G* gene, encoding the nonclassical HLA class I molecule HLA-G and associated with low mRNA availability, can result in less dyslipidemia after kidney transplantation is an intriguing question. Piancatelli and co-workers observed in a group of 124 renal recipients that amongst patients with dyslipidemia, the *HLA-G* -14/-14 genotype was less frequent than the heterozygous *HLA-G* +14/-14 and nondeleted homozygous *HLA-G* +14/+14 genotypes compared with patients without lipid disorders and controls ($n = 98$), and these differences persisted after correction for pharmacological therapy [1].

HLA-G expression, primarily present during pregnancy and enabling maternal–fetal immune tolerance, has also been associated with reduced alloreactive CD4⁺ T-cell responses and decreased antigen-specific CD8⁺ T-cell- and NK-cell-mediated lysis in transplantation settings, conferring protection against rejection phenomena [2,3]. However, the clinical consequences of this immunomodulating role of HLA-G could not be confirmed for the homozygous *HLA-G* +14/+14 genotypes in the current study. Why patients with the homozygous deletion (*HLA-G* -14/-14 genotype) were overrepresented in the group of kidney recipients without lipid disorders compared with the control group of nontransplanted subject, remains unexplained. Similar associations have been reported in other settings – for example, in patients with coronary artery disease where *HLA-DRB1*01* was associated with low-density lipoprotein while *HLA-B*07* was protective against coronary artery disease [4]. Whatever the

exact nature of the underlying mechanisms linking *HLA* genes with lipid disorders, confirmatory clinical observations in renal transplant patients are necessary.

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