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Pharmacogenomics and lung transplantation: clinical implications

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Introduction

The promise of drug individualization through pharmacogenomics will be evident initially in those patients who have the poorest therapeutic response or most severe adverse drug effects. In solid-organ transplantation, lung transplantation has the poorest outcome among the commonly transplanted major organs with less than 80% one-year and less than 50% five-year survival. The reasons for this poor outcome are multiple, and have been extensively reviewed elsewhere.[1–3] Some of the problems encountered after lung transplantation are unique to lungs, but other problems are shared with all solid-organ transplants. In the former category is the early development of bronchiolitis obliterans (OB) and bronchiolitis obliterans syndrome (BOS) as a manifestation of chronic rejection of the lung. In the latter category are the drug-related toxicities.

The lung transplant patient is at risk for multiple drug toxicities, manifested by nephrotoxicity, hepatotoxicity, hyperlipidemia, hypertension, and post-transplant diabetes mellitus. At the same time, the transplant patient continually runs the risk of under-immunosuppression, with loss of the graft, and over-immunosuppression, with infection and lymphoproliferative disease as the result. Therefore, considerable effort has been put into the pharmacokinetics (PK) and pharmacodynamics (PD) of the antirejection agents used in organ transplantation. Blood concentration monitoring has permitted the rational use of difficult-to-use agents such as cyclosporine and tacrolimus. The use of complex drug regimens in transplant patients has also been dependent upon monitoring of the PK of the immunosuppressants to adjust for frequent drug–drug interactions. At the same time, blood concentration monitoring alone does not prevent many of the more serious side effects of the immunosuppressants. For example, diminished renal function even in non-renal transplants is a persistent problem, and ultimately affects almost 30% of all patients on long-term cyclosporine or tacrolimus. Therefore, lung transplant patients require the individualization of immunosuppression and freedom from adverse drug effects that is promised by pharmacogenomics.

Pharmacogenetic associations now suggest that treatment algorithms for lung transplant patients can be established. The availability of new agents, such as belatacept, with radically altered toxicity profiles have been tested in renal transplant patients,[4] and specific immunosuppression with localized inhalational therapy[5] add flexibility to drug therapy choices in treatment algorithms. Pharmacogenomic monitoring of peripheral blood biomarkers has been established in cardiac transplantation, and is being tested as a more sensitive biomarker of pulmonary processes such as the initiation of chronic rejection in the transplanted lung.

The objectives of this discussion are to review what we presently understand about the pharmacogenetics of specific candidate genes in relation to their effects on both drug disposition and effect in lung transplant patients, to review the biomarkers that will allow us to establish treatment algorithms for lung transplant patients, and to describe the process by which will we will be able to integrate this information into clinical practice in the coming years.

Pharmacogenetic and pharmacogenomic studies in lung transplant patients and other transplant populations

The development of pharmacogenetic information for application to drug selection algorithms for lung transplant patients is represented by the left side of Figure 1. Our current state of knowledge is still at the stage where we are establishing clinical associations with gene polymorphisms and validating these findings in broader patient populations. As noted in Figure 1, the route to using this information for validated drug selection algorithms in lung transplant patients could require considerable more effort to examine the effect of multiple gene polymorphisms, model these findings in concert with known information on determinants of PK and PD, and extensively test drug treatment algorithms. However, if a particular gene polymorphism has sufficient weight, it may be applied directly after validation in a larger patient population. This may be the case for *ABCB1* gene polymorphisms, as discussed below.

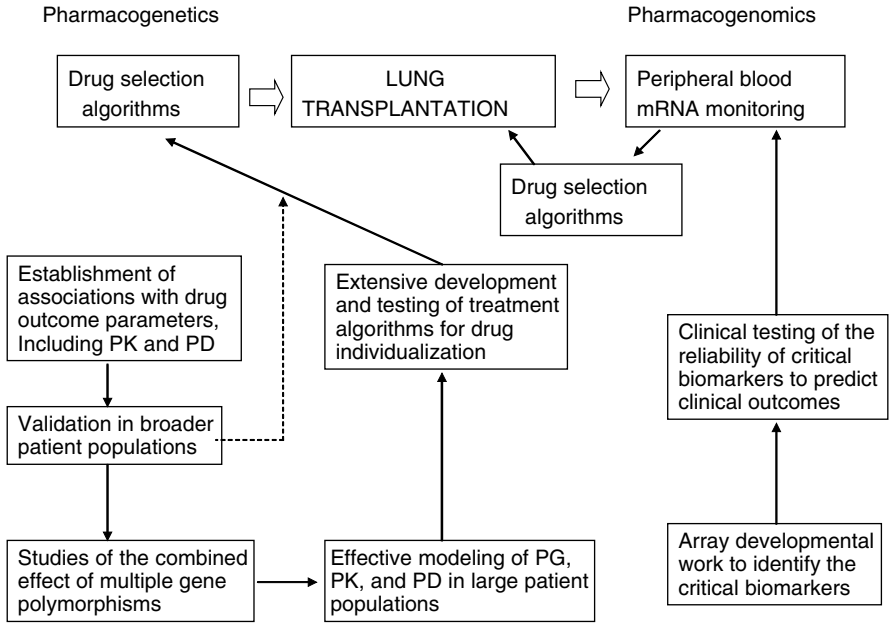

Figure 1 Graphic depiction of the steps involved in the development of pharmacogenetics and pharmacogenomics in lung transplant patients. The left side of the figure indicates the route for development of pharmacogenetic associations, and the route may be shortcut at the point of the dashed line for polymorphisms that are heavily weighted for impact on patient outcomes. The right side demonstrates the development of pharmacogenomic monitoring in lung transplant patients. Both sides come together in the treatment and monitoring of the lung transplant patient.

Table 1 Relationship between the immunosuppressive agent and the CYP3A5 and *ABCB1* gene polymorphisms that may allow prediction of drug disposition in transplant patients

Drug	*Gene polymorphism*	*Effect on drug disposition*
Tacrolimus	CYP3A5	Lung transplant patient *1 carriers have a lower drug blood level per mg dosage over the first year[14]; one report[8] suggests doubling the dose in *1 expressors
	ABCB1	Lung transplant patient haplotypes associated with high pump function have a lower level/dose in the first year
Cyclosporine	CYP3A5, *ABCB1*	Pharmacogenetics does not predict pharmacokinetics
Sirolimus	CYP3A5	Pharmacogenetics does not predict pharmacokinetics, but *1 expressors may require larger doses
	ABCB1	No association with sirolimus dosing
Corticosteroids	CYP3A5, *ABCB1*	Unknown

Our current knowledge of pharmacogenetics and transplant patients can be divided into information specifically on drug disposition and information on the drug effect and patient outcomes. Both aspects of drug management for lung transplant patients will ultimately affect the long-term outcome after transplantation.

Drug dispostion

Table 1 lists the drugs and associated drug disposition consequences for critical gene polymorphisms. A more complete discussion of these immunosuppressant agents and the associated changes in lung transplant and other solid-organ transplant patients follows.

Tacrolimus. Tacrolimus PK has extreme variability,[6,7] and yet the early attainment of blood concentrations may be critical for preventing early episodes of acute rejection.[8,9] Tacrolimus is also subject to poor bioavailability and drug–drug interactions, which has made aggressive blood concentration monitoring part of the

normal transplant patient care routine. The advent of pharmacogenetics has provided a potential opportunity to both explain the variability in tacrolimus PK, and to use pharmacogenetics to predict the tacrolimus blood concentrations post transplantation. The potential advantages of this approach for tacrolimus have been reviewed previously.[10]

Tacrolimus is a substrate for cytochrome P450 3A and for the membrane transporter P-glycoprotein (P-gp). The polymorphism associated with the gene that encodes for P-gp has been studied in a number of disease states.[11] The polymorphisms associated with CYP3A4 have a low frequency which precludes their efficient study in the transplant population, but CYP3A5 has an insertion polymorphism which leads to the non-expression of CYP3A5 activity. Huang *et al.*[12] have recently suggested that a significant contribution to hepatic CYP3A activity comes from CYP3A5. Therefore, the most extensive study of tacrolimus dosing in organ transplant patients has been with the *ABCB1* and CYP3A5 polymorphisms. The other limiting factor in studying tacrolimus in transplant patients is that blood concentrations are limited to the range established within a transplant center, and therefore the appropriate measure between patients is the blood concentration per dose of tacrolimus administered. Comparisons between centers are also limited by variations in the other medications given to the transplant patient.

Our initial studies have demonstrated a positive association between tacrolimus dosing and the CYP3A5 gene polymorphism in pediatric heart[13] and adult lung[14] transplant patients. As expected, the CYP3A5 *3/*3 non-expressor patients have a higher tacrolimus level/dose than do the CYP3A5 *1/*1 or *1/*3 enzyme expressors. The predose trough concentration of tacrolimus was used in these studies which is appropriate in that this concentration has a good relationship to the area under the blood concentration versus time curve for the drug.[7] However, there is considerable overlap between the level/dose achieved after transplantation and the CYP3A5 genotype. This variability is best demonstrated in our previous publication[14] and in the report by Hesselink *et al.*[15] Therefore, the application of this information presently in predicting the tacrolimus PK for a specific patient is very limited. MacPhee *et al.*[8] have demonstrated that CYP3A5 expressors, assessed through the linkage with CYP3AP1, have lower tacrolimus concentrations in the immediate postoperative period and take a longer period of time to achieve therapeutic range blood concentrations. There were no differences in clinical outcome between the two groups of transplant patients. They recommend that CYP3A5 expressors initially get double the dosage of tacrolimus administered to CYP3A5 non-expressors, but this proposal should be tested thoroughly before initiation in clinical practice.

The *ABCB1* gene polymorphism effect on tacrolimus PK has been difficult to distinguish in light of the CYP3A5 effect. Potentially, the *ABCB1* genotypes associated with greater pump function, such as *ABCB1* 2677 GG or 3435 CC, should be associated with poorer tacrolimus absorption and a lower level/dose. In fact, we found that this was true in our pediatric heart transplant population[13] but not initially true in our adult lung transplant population.[14] Subsequent studies in adult transplant patients[15,16] also did not find this association, but these studies, however, used a mixed population of CYP3A5 expressors and non-expressors in examining *ABCB1* polymorphism effects on tacrolimus dosing. They also did not examine *ABCB1* haplotypes, a technique that produced a positive association with cyclosporine.[17] A recent study in our adult lung transplant patient group limiting patients to CYP3A5 *3/*3 non-expressors does show that there is an association between *ABCB1* haplotype and the tacrolimus level/dose.[18] Therefore, *ABCB1* gene polymorphisms do help to explain tacrolimus disposition, and should be incorporated into future studies of the concurrent effects of multiple gene polymorphisms on tacrolimus dosage and outcome in transplant patients.

Cyclosporine. The initial observations using cyclosporine trough blood concentrations did not find any association between levels and pharmacogenetic differences in *ABCB1* or CYP3A.[19] Using an abbreviated area under the blood concentration-versus-time curve (AUC) for cyclosporine, Anglicheau *et al.*[20] did not find any relationship between cyclosporine PK parameters and either CYP3A5 genotype or *ABCB1* haplotype. Using more extensive blood sampling over 24 h in a small number of patients, Min *et al.*[21] did find that the CYP3A5*1 expressors has a lower AUC and a higher cyclosporine clearance rate than the non-expressors.

A more extensive approach to population modeling of cyclosporine in kidney and heart transplant patients in relation to *ABCB1* and CYP3A polymorphisms has recently been published.[22] This group found that African-American and Asian transplant patients had a significantly reduced oral cyclosporine clearance, but considerable overlap occurs with the Bayesian oral clearance values of Caucasian transplant patients. In the end, this report concludes that genotyping is unlikely to contribute to the accurate estimation of the initial dosing for cyclosporine.

Sirolimus. Sirolimus blood concentration monitoring is a more recent development in transplantation, but blood concentrations are associated with acute rejection episodes and adverse events.[23] Sirolimus is a substrate for both P-gp and CYP3A, and only one study has examined the effects of pharmacogenetic polymorphisms on sirolimus dosing. Anglicheau *et al.*[24] found that sirolimus trough concentrations were significantly affected by the CYP3A5 genotype when controlling for drug interactions and other significant transplant factors, such as when the drug is used for rescue therapy versus being used *de novo* from the time of transplantation. The CYP3A5*1 expressors had a lower

Table 2 The relationship between genetic polymorphisms and the drug effect and patient outcomes in lung and solid-organ transplant patients

Gene polymorphism	*Drug(s)*	*Clinical implication*
ABCB1 3435 TT	CsA, tacrolimus Steroids	Nephrotoxicity; potential to use calcineurin-sparing regimens Reduced osteonecrosis of the femoral head; identify patients at risk and initiate prophylaxis
ABCB1 2677 GG	Steroids, thymoglobulin All	Drug resistance to rejection treatment in lung transplant patients; potential to use a more aggressive immunosuppressive regimen Possibly improved patient survival after lung transplantation
IL-10 GCC/GCC (−1082, −819, −592)	All	Protective for heart, lung, and liver transplants; associated with rejection in renal transplants
TNF-α	All	Associated with acute rejection in heart and liver transplants, but no effect in lung transplants
IL-6	All	Earlier development of BOS in lung transplant
VEGF	All	Associated with acute rejection of kidney, heart, and lung transplants
TGF-β	All	Associated with OB and with lower long-term survival in lung transplants; transplant coronary artery disease and ventricular fibrosis in heart transplants; cyclosporine-induced renal dysfunction in heart and kidney transplants
IFN-γ	All	Associated with acute rejection, development of lung transplant fibrosis, and the incidence of PTLD

IL-10, interleukin 10; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; OB, bronchiolitis obliterans; BOS, bronchiolitis obliterans syndrome; IFN-γ, interferon-γ ; PTLD, post-transplant lymphoproliferative disorder.

sirolimus concentration/dose ratio as expected. *ABCB1* polymorphisms did not affect sirolimus dosing in this study.

Corticosteroids. No work has been published on the PG of corticosteroids in transplant patients. However, previous observations regarding prednisolone or methylprednisolone PK would suggest that CYP3A5 or *ABCB1* polymorphisms may play a role in mediating the effect of drug interactions or adverse effects with corticosteroids. For example, Potter *et al*.[25] found that transplant patients receiving cyclosporine had a higher 6-h prednisolone AUC, and hypothesized that cyclosporine inhibited P-gp to allow more prednisolone to be absorbed. If this is true, then *ABCB1* polymorphisms may play a role in differences observed between patients in the extent of the interaction. Similarly, a previous study has demonstrated that higher doses of prednisone are associated with a lower tacrolimus concentration/dose ratio.[26] If that effect is on the basis of the induction of CYP3A as postulated, then CYP3A5 polymorphisms could alter an individual patient's susceptibility to such an interaction.

Drug effect and patient outcomes

Table 2 summarizes the relationships between genetic polymorphisms and drug effect and patient outcomes in lung and other solid-organ transplant patients. The following discussion provides a more detailed explanation of the observed changes.

ABCB1 and transplant patient outcomes. Although the *ABCB1* gene product, P-gp, is primarily considered a membrane transporter, P-gp has been linked to a number of cellular processes in lymphocytes that may produce a much broader impact on cell function and survival than might first be assumed. For example, P-gp activity is inversely proportional to cell apoptosis in many cell systems. Previous studies in tumor cell lines have demonstrated that P-gp blockade produces caspace-dependent cell apoptosis.[27,28] Also, we have previously demonstrated that cell activation turns off P-gp function,[29] which may have significant implications for drug response in an episode of acute rejection or during T-cell activation related to infection. Finally, the continued exposure of T cells to P-gp substrates causes an upregulation of P-gp activity.[30] This upregulation of P-gp activity leads to drug resistance to oncologic agents, and steroid-resistant OB in lung transplant patients is associated with a high percentage of P-gp-positive cells.[31]

Drug resistant rejection. Lung transplant patients have frequent episodes of acute rejection during the first post-transplant year, and some of these rejection episodes persist in spite of aggressive therapy with high-dose corticosteroids or anti-T-cell therapy. In

the lung transplant population, we have documented associations between acute persistent rejection and two different gene polymorphisms. The first association is with the *ABCB1* 3435 polymorphism, and we found that 72% of patients with the C allele had acute persistent rejection in comparison to 52% for TT patients ($P=0.04$).[32] We also found that the high interleukin-10 (IL-10)-producing genotype had a lower rate of acute persistent rejection than did the IL-10 intermediate or low producers.[33] For the IL-10 high producers, 35% of patients were resistant to their rejection treatment whereas 59% of lung transplant patients were drug resistant in the IL-10 intermediate/low group. These studies suggest that a patient population who may be at risk for drug resistance may be able to be identified in the future, and could be treated with more aggressive immunosuppressive therapy.

Steroid resistance. Steroid resistance is frequently noted during treatment of acute rejection or OB, and is associated with a high percentage of P-gp-positive cells on lung biopsy.[31] Steroid resistance during periods of normal dosing is more difficult to measure in adult transplant patients because of varying steroid withdrawal protocols and other concurrent drug therapy. The exception to this is in the pediatric transplant patients, where steroid withdrawal is aggressive because of the long-term growth abnormalities associated with chronic steroid use. We have previously shown in a small group of pediatric heart transplant patients that *ABCB1* 3435 CC patients have a higher rate of requiring corticosteroid use at 1 year post transplantation than the CT and TT patients.[34] Logistic regression analysis in this same patient population incorporated CYP3A5 and six cytokine polymorphisms, but still demonstrated that *ABCB1* polymorphism was still the dominant factor in resistance to weaning the pediatric transplant patients off of corticosteroids.[35] While the response to corticosteroids is under the control of a large number of factors, *ABCB1* polymorphism is likely to have some role in this process.

Calcineurin nephrotoxicity. Several earlier publications theorized a link between cyclosporine's inhibition of P-gp activity and the drug's nephrotoxicity. In a recent study, Hauser *et al*.[36] found that the *ABCB1* 3435 TT in renal transplant donors, but not recipients, correlated with cyclosporine nephrotoxicity that was reversible when the transplant patient was switched to a calcineurin-free drug regimen. In this study, a multivariate model including nongenetic covariates still found that only the donor's *ABCB1* 3435 TT genotype was associated with cyclosporine nephrotoxicity. This result conflicts with an earlier report linking the *ABCB1* 2677T allele with a reduced risk of nephrotoxicity in liver transplant patients at 3 years post transplantation, but this study did not document the reason for the elevated serum creatinine and found the association only for male patients.[37]

Steroid-induced osteonecrosis. One study of renal transplant patients in Japan has identified an *ABCB1* genotype that is associated with steroid-induced osteonecrosis of the femoral head.[38] The investigators studied 136 renal transplant patients out of which 30 developed osteonecrosis. Out of the 30 patients, only one patient was *ABCB1* 3435 TT, and the other 29 patients had genotypes containing the C allele. The authors suggest that *ABCB1* genotyping could assist in identifying transplant patients at risk for osteonecrosis so that preventive therapy could be initiated.

Patient survival. The ultimate indicator of the pharmacologic effect or pharmacodynamics of a drug regimen in a transplant patient population is patient survival. This is particularly true of liver, lung, and heart transplant patients who cannot be maintained if their transplanted graft fails. The concept that a single gene polymorphism could affect patient survival in a complex patient population is difficult to conceive, but an earlier study by Hasida *et al*.[39] suggested that may be possible. In 47 recipients of living-donor liver transplantation, Hasida *et al*. found that the *ABCB1* mRNA content in the intestinal biopsy was associated with patient survival. Patients who had high amounts of *ABCB1* mRNA had a significantly poorer patient survival than did the patients with low amounts of *ABCB1* mRNA. The production of *ABCB1* mRNA and P-gp activity could obviously be related to *ABCB1* genotypes.

In our lung transplant patients, we have observed a different pattern. The survival after lung transplantation is approximately 50% at 4 years post transplantation. While we observe no differences in *ABCB1* genotypes up until 4 years, at that point, a marked variance occurs between the *ABCB1* 2677 GG patients and the 2677 T allele carriers who have a poorer survival. Owing to the small number of lung transplant patients available for this analysis ($n=<100$), the results to date are not significant. The process of chronic rejection is a very different pathologic process than acute rejection, and almost all lung transplant patients at 4 years post transplantation have some evidence of chronic rejection. Whether the chronic rejection process either directly or indirectly involves P-gp is unknown, but is a possibility to be explored.

Interleukin 10. Interleukin-10 is produced by a variety of cells, and is an immunoregulatory cytokine that can alter the balance of Th1 and Th2 T lymphocytes. IL-10 can function as a negative regulator of tumor necrosis factor-α (TNF-α), so has been considered an immunosuppressive cytokine. Three SNPs in the IL-10 gene promoter region (-1082A/G, -819C/T, and -592C/A) have been studied in relation to transplantation.

In the lung transplant population, the IL-10 high genotype is associated with a protective effect against acute refractory rejection.[33] In a retrospective study in pediatric liver transplant patients, we previously demonstrated that patients off immunosuppression or on a minimal level of immunosuppression displayed a low TNF-α and high/intermediate IL-10 genetic pro-

file.[40] A significant association between the IL-10 high genotype and protection from acute rejection was also described in pediatric heart transplant recipients.[41] Patients with a combination of IL-10 high and low TNF-α were at the lowest risk for acute rejection. These results suggest that the balance between an anti-inflammatory cytokine (IL-10) and a pro-inflammatory cytokine (TNF-α) may influence graft acceptance or graft rejection.

Warle *et al*.[42] carried out a meta-analysis on cytokine gene polymorphism and acute liver graft rejection. In the overall analysis, only the IL-10 polymorphism associated with the low production (−1082A allele) was identified as a genetic risk factor for acute liver allograft rejection.[42] Similar findings in two adult heart transplant studies have demonstrated that the TNF-α high, IL-10 low genotype is associated with increased acute rejection.[43,44]

The IL-10 high genotype in renal allograft recipients has been associated with acute rejection[23,45–47] in contrast to its proposed protective function in heart, lung, and liver allografts. This difference in outcome is most likely a result of IL-10-driven B-cell proliferation ultimately resulting in antibody-mediated renal graft damage. The opposite effects of the IL-10 high genotype in kidney allografts compared with other organs illustrate the importance of context when assessing the potential for a cytokine gene polymorphism to affect organ transplant rejection.

Tumor necrosis factor-α. Tumor necrosis factor-α is an inflammatory cytokine that stimulates macrophage function and has been implicated in acute and chronic rejection of various solid-organ transplants. For TNF-α, an SNP in the promoter at position −308 A/G has been associated with cardiac rejection when the patient carries the A allele (high phenotype).[41,43,44] Some heart transplant studies do not support this observation, but these discrepancies may result from differences between rejection criteria (ISHLT grade $\geqslant$2 compared to $\geqslant$3A) or a smaller population size with limited statistical power.[48–50]

In lung transplant recipients, we did not observe an increase in risk of refractory rejection associated with TNF-α high genotype.[33] In liver transplant recipients, the TNF-α high genotype was associated with acute rejection in some studies[51–53] but not in others.[54–56] The TNF-α high genotype correlates with acute rejection in multiple adult kidney transplant studies from various demographic populations.[23,45–47,57]

Interleukin-6. Interleukin-6 is derived from antigen-presenting cells and has an important function in inflammation and T-cell differentiation. For the IL-6 gene, the G allele in the promoter −174 G/C is associated with high production and earlier development of BOS in lung transplant recipients.[58] We have observed that the frequency of acute persistent lung allograft rejection was increased in patients having IL-10 GCC/ACC haplotype (intermediate phenotype) associated with IL-6 G/C haplotype.[33] In contrast to the lung transplant patients, kidney transplants with the G allele are associated with long-term allograft survival.[59]

Transforming growth factor-beta 1. Transforming growth factor-beta 1 (TGF-β1) is a pro-fibrogenic growth factor involved in normal wound healing and fibrosis. Various polymorphisms have been demonstrated, but two affecting the structure of the leader sequence at codons 10 and 25 influence protein processing and production.[60] The presence of TGF-β1 in lung and heart allografts is strongly associated with the TGF-β1 genotype.[61–63] Similarly, the development of OB and the survival of lung transplant recipients is significantly associated with TGF-β1 genotype,[64] as is fibrosis of the heart and the development of coronary vasculopathy in cardiac transplant recipients.[65,66]

Cyclosporine may increase the expression of TGF-β1 in transplant recipients.[67] The development of renal insufficiency in cardiothoracic transplant recipients treated with cyclosporine is higher in patients with TGF-β1 genotypes.[68]

Interferon-gamma. Interferon-gamma (IFN-γ)is produced by activated Th1 T cells and natural killer (NK) cells, and is associated with inflammatory responses. Several polymorphisms in the IFN-γ gene may have a phenotype of importance, especially one in the first intron that has been shown to greatly influence IFN-γ production *in vitro*.[69,70] Although the IFN-γ genotype is associated with resistance to viral infection,[71] most studies have not shown a particularly strong association between IFN-γ and acute graft rejection.[46,72,73] However, IFN-γ may play a role in driving the chronic rejection process which would explain the association observed between IFN-γ genotype and the development of OB in adult lung transplant recipients.[58,74]

Lung transplant recipients who are of the high producer IFN-γ genotype may be less likely to develop post-transplant lymphoproliferative disorder (PTLD).[75] Since PTLD has a viral etiology, this finding may relate to the anti-viral function of IFN-γ.

Vascular endothelial growth factor. Vascular endothelial growth factor (VEGF) was originally studied because of its role in the development of blood vessels. However, VEGF is a potently pro-inflammatory molecule, being vasoactive, chemoattractant for inflammatory cells and an inducer of chemokine production. Polymorphisms in the promoter of the VEGF gene are associated with the *in vitro* production of VEGF.[76] Originally, VEGF was studied because of its potential involvement in the process of chronic rejection. However, the association between VEGF genotype and acute rejection of kidney,[77] heart, and lung (unpublished data) transplants is a significant finding that will have to be incorporated into future pharmacogenetic models.

Pharmacogenomic studies and biomarkers in lung transplant patients

A major problem in lung and other solid-organ transplantation is that

the biomarkers that have been used for drug response and toxicity have not substantially changed in the past decade. Even in renal transplantation, the strategies to diagnose rejection have not changed in 20 years.[78] The clinical biomarkers for patient outcomes following lung transplantation are listed in Table 3, many of which have been inconsistently associated with the development of BOS.[79] The exceptions to the old biomarkers are a very recent study of FoxP3 in renal transplant patients[80] and the pharmacogenomic CARGO and LARGO studies. The Cardiac Allograft Rejection Gene Expression Observational (CARGO) and Lung Allograft Rejection Gene Expression Observational (LARGO) studies are the first ventures into identifying and screening large numbers of expressed mRNAs in peripheral blood as biomarkers for clinical status in cardiac and lung transplant patients, respectively.

CARGO/LARGO studies

The CARGO study was the first transplantation study to explore the use of pharmacogenomics to monitor heart transplant patients using gene expression profiling.[81] The objective of the study was really to replace the need for heart biopsy by using peripheral blood gene expression profiling, but in fact was the first use of a technique that will be refined to assist in making drug-related decisions regarding transplantation immunosuppressive drug therapy.

The CARGO study started with 7370 gene candidates, and was able to narrow down to and validate 11 genes that are monitored in peripheral

Table 3 Biomarkers for patient response in lung transplantation

Biomarker	*Clinical implication*
BOS by pulmonary function testing	Accepted biomarker for OB and chronic rejection function testing
Changes in bronchoalveolar lavage (BAL) fluid	Neutrophliia, IL-8, chemokine MCP-1/CCR2, IFN-γ have all been shown to be altered in BOS
Increased anti-HLA and non-HLA antibodies in peripheral blood	Precede or demonstrate risk for development of BOS
Increased exhaled nitric oxide	Conflicting reports have associated this with early BOS
Radiographic changes	Present studies have not consistently linked changes with BOS
Bronchial hyper-responsiveness to methacholine or histamine	May precede the onset of BOS
Gene expression profiling in peripheral blood	Potential to discriminate acute rejection, BOS, and drug toxicity

IL-10, interleukin 10; BOS, bronchiolitis obliterans syndrome; IFN-γ, interferon-γ.

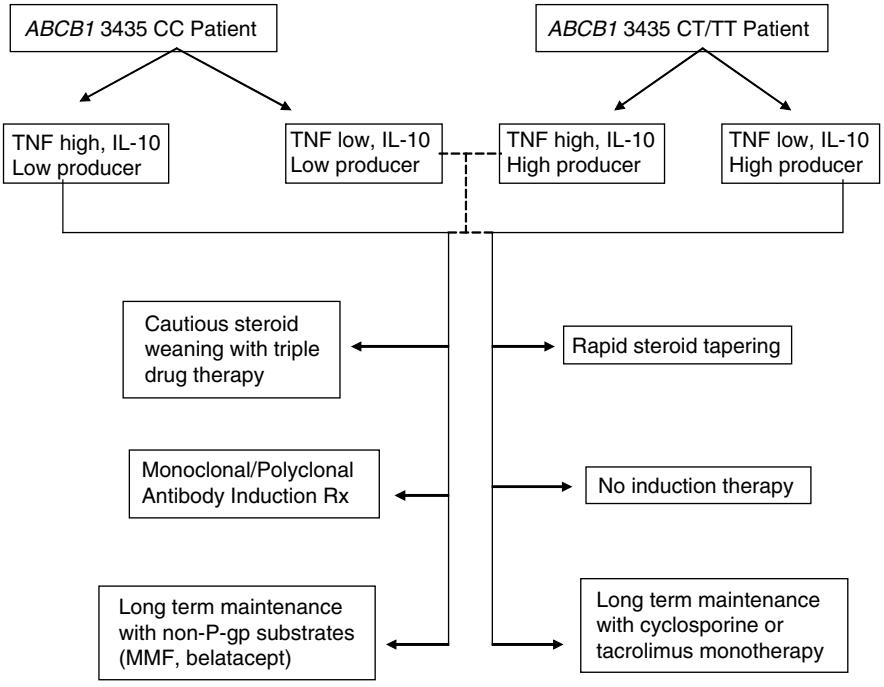

Figure 2 Hypothetical treatment algorithm for transplant patients based upon *ABCB1*, TNF-α, and IL-10 polymorphisms. The benefit of these algorithms for initiation of immunosuppressive therapy, and later for adjustment of therapy in conjunction with pharmacogenomic monitoring must be tested in lung transplant patients prospectively. The dashed line represents a decision pathway that is unclear at the present time.

blood. Among 68 genes that discriminate between cardiac rejection and quiescence, the dendrogram of genes include steroid responsive genes, hematopoiesis, platelet, and T lymphocyte activation and migration genes. The LARGO study used a similar gene set in lung transplant patients.[82] In the LARGO study, samples associated with airway inflammation demonstrated upregulation of membrane metalloendopeptidase ($P<0.04$) and a similar trend for TNFSF6 (FasL), CXCR3, and S100A, suggesting potential roles for matrix degradation, apoptosis, and cell trafficking in bronchiolar remodeling. Therefore, it appears that the pharmacogenomics approach to lung transplant patient monitoring using peripheral blood will be successful at predicting clinical events related to immune activation and inflammation.

The LARGO study also supports the hypothesis that peripheral blood gene expression profiles could be developed for the adverse drug effects that plague lung transplant patients. Different gene signatures would be expected for infectious diseases, renal toxicity from cyclosporine or tacrolimus, and tissue damage from metabolic diseases such as hyperlipidemia or post-transplant diabetes mellitus. The right side of Figure 1 demonstrates how pharmacogenomic monitoring techniques will provide information that can be used for making decisions related to altering drugs or dosages in lung transplant patients. If we want to push the drug selection process into clinical practice, Figure 2 demonstrates how such an algorithm might appear using the *ABCB1* C3435T polymorphism as the pinion on which to base a decision for the initial management of the transplant patient. This algorithm has not been tested at the present time, but is logical based upon the information presented here. Additional polymorphisms, such as the TNF-α and the IL-10 polymorphisms must be considered and their importance must be given a weight in making clinical decisions. With pharmacogenomic monitoring of patients post lung transplantation, additional algorithms would have to be developed to address the issues of drug resistant rejection episodes, patients with concurrent infection and rejection, and other complex patient management issues.

Conclusions and future considerations

Pharmacogenetics and pharmacogenomics in organ transplant patients are at an early stage in its evolution to a clinically useful tool. Nevertheless, clinical applications of these tools in lung transplant patients are being implemented more quickly than in other solid organ transplant patients, and will have an impact on patient outcomes. These clinical applications will evolve as studies of pharmacogenetic polymorphisms in lung transplant patients are expanded, and drug selection algorithms are tested. At the same time, new biomarkers for drug response and toxicity will be generated through pharmacogenomic studies of gene profiling in peripheral blood. Combined together to provide drug selection with a wider variety of immunosuppressive agents along with monitoring of the pathophysiological processes related to lung transplant rejection or infection, pharmacogenetics and pharmacogenomics will have a major clinical impact on the care of lung transplant patients.

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Duality of interest

None declared.

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