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Genetic polymorphisms and the fate of the transplanted organ

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Abstract

There has been an abundance of publications describing genetic variability in molecules affecting innate and adaptive immunity, pharmacogenetics, and other nonimmunological factors like the renin-angiotensin aldosterone system, coagulation, and fibrosis markers. Studies indicated some associations between polymorphisms in these candidate genes with outcomes in organ transplantation and underlined a potential role of genetic variability in transplantation. To be clinically applicable, large prospective studies must be performed to better define the potential benefits of genotyping on these genetic markers and clinical outcomes. The purposes of this review are to summarize recent data describing associations of polymorphisms in both immunological and nonimmunological molecules with transplant outcomes, with a particular emphasis on renal transplantation, and discuss limitations and clinical implications.

1. Introduction

Despite improved immunosuppressive regimens, newer organ preservation techniques, and decreased rejection rates, the improvement in long-term kidney allograft survival has been modest [1]. The major causes of renal transplant loss are accounted for by death with a functioning graft (from cardiovascular, infectious, or malignant disease) and loss of the allograft from chronic renal dysfunction. Up to 30% of patients awaiting renal transplantation had a previous failed kidney allograft [2,3]. The lack of a more substantial improvement in long-term survival may to some extent be accounted for by the increased use of extended criteria donor kidneys and the growing number of older transplant recipients. Most transplant centers determine individual transplant patients' immunological status based on immunological risk factors such as panel reactive antibody titers, HLA compatibility, previously failed transplant, age, and ethnicity. Despite the indisputable success of these protocols they ignore the vast genetic diversity within transplant recipients that may lead to under- or overimmunosuppression.

Genetic analysis in transplantation has the potential to individualize immunosuppressive regimens by identifying risk or protective alleles for immune mediated complications or individualizing drug dosing by screening for genetic mutations within drug metabolizing genes. Analysis of donor genetics could be used to identify organs more susceptible to ischemic and reperfusion damage, and this information might minimize the risk of delayed graft function (DGF).

The completion of the Human Genome Project, as well as the HapMap Project (www.hapmap.org), identified regions of genetic variability between individuals due to single nucleotide polymorphisms (SNPs) or microsatellite polymorphisms. Multiple studies have shown associations between polymorphisms in candidate genes and transplantation outcomes such as acute rejection, DGF, chronic allograft nephropathy (CAN), cardiovascular mortality, and pharmacokinetics. We will summarize the associations of candidate genes, with a particular emphasis on the functional variants and renal transplant outcomes, as well as discuss the limitations of these studies and future directions. A selection of influencing studies is summarized in Table 1.

2. Cytokine and chemokine system

Cytokines and chemokines comprise a family of secreted proteins that mediate and regulate immunity, inflammation,

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and hematopoiesis. Based on their functions, it is not surprising that these molecules have been the most studied in genetic association studies. Genetically affected differences in their production, binding, or signaling may significantly affect the immune responses of these individuals [4].

2.1. Cytokine and cytokine receptors

The influence of genetic variations in cytokine genes in organ transplantation, in particular TNF-α, IL-10, and IL-6, IFN-γ, has been examined in a number of studies with varying results. TNF-α stimulates neutrophil and macrophage function and participates in augmenting the delayed type hypersensitivity response. Polymorphisms within TNF-α have been shown to have functional effects. The SNP at position -308 (-308G/A) within the promoter region was associated with a 6- to 7-fold increase in transcription and protein expression in vitro [5,6]. The functional results seem convincing; however, the effects on the outcome in transplantation are controversial. Although several studies in kidney [7-11] and liver transplants [12] have found an association between this SNP and rejection, other studies have failed to find an effect on this outcome [13-18].

Within the IL-10 gene, 3 polymorphisms in the promoter region at position -1082 (A to G substitution), -819 (C to T), and -592 (C to A) correlated with different levels of IL-10 production in stimulated lymphocytes. The -1082G, -819C, and -592C alleles were 'high IL-10 producers' [19]. It may be anticipated that these SNPs might have beneficial effects due to the ability of IL-10 to down-regulate T-cell activation and MHC expression on antigen-presenting cells [20]. However, IL-10 appears to have paradoxical effects in kidney transplant recipients with high IL-10 producer SNPs being associated with increased acute rejection [8,10,13]. Consistent with this observation, functional tests found that the IL-10 G-allele decreased CD80 expression on peripheral blood cells [13].

There is now substantial evidence showing a cooperative effect between mutations in a specific gene or between genes that are inherited together, referred to as a haplotype or haplotype blocks. Renal transplant recipients with the combination of the TNF-α -308 G allele and the IL-10 -1082 G allele ('high producers') had a higher rate of acute rejection from HLA-DR–mismatched donors, whereas patients exhibiting the TNF-α -308 G allele alone had more steroid-resistant acute rejection episodes [10]. Conversely, recipients with TNF-α -308 G ('high')/IL-10 -1082A ('low') recipients showed more acute rejection after cardiac transplantation [19]. Other studies, however, have failed to find an association between these genes and clinical outcomes; this finding may be accounted for by differences in power variation in genetic backgrounds, and poorly defined outcomes. For example, rejection was not biopsy proven in all of the studies [13,14,16].

IL-6 is produced by many cell types that participate in both the innate and adaptive immune response. There are 3 polymorphisms within the promoter at positions -597 (G to C), -572 (G to A), and -174 (G to C). Functional analyses have indicated a cooperative effect between these SNPs on IL-6 transcriptional regulation [21]. The G allele at position -174 was independently associated with increased IL-6 plasma levels [22]. Despite the functional effect of the combination of the 3 promoter SNPs, no association with the incidence of acute kidney rejection rate has been found to date [14-16]. However, contrary to what would be expected, the high IL-6 status (-174 G-allele) was associated with improved long-term kidney graft survival [15,16]. Consistent with these observations was that the expression of the high producer IL-6 phenotype (-174 GG-allele) was associated with a delayed onset and a lower incidence of posttransplant coronary vasculopathy after 5 years [23].

The incidence of posttransplant diabetes mellitus (PTDM) is continuously increasing, resulting in a significant impact on long-term patient and graft survival. The incidence ranges from 2% to 53% and is influenced by genetic background and immunosuppressive protocols [24]. In one study, a striking increase in the risk for PTDM correlated with the presence of the high IL-6 production (-174 GG-allele) [25], emphasizing the importance of cytokine levels and chronic inflammation in the development of insulin resistance and diabetes. If confirmed, immunosuppressive regimens, which are less diabetogenic may be specifically selected to minimize the individual risk of PTDM.

2.2. Chemokine and chemokine receptors

Chemokines are a large and growing family of structurally related chemotactic cytokines, with 50 human chemokines and 19 related receptors [4]. They are involved in many diverse functions including cell migration, leukocyte activation, hematopoiesis, and angiogenesis, and they act as a link between innate and adaptive immunity [4]. The strongest data for the importance of chemokines in human kidney transplantation were published by Fischereder et al [26]. They examined the effect of a 32 base pair deletion in the CCR5 gene (CCR5Δ32), encoding a nonfunctional CCR5, on kidney allograft survival in 1227 transplant recipients. Twenty-one patients with the homozygous CCR5Δ32 mutation were identified, and of these, only 1 lost the kidney graft during follow-up, resulting in a significant prolongation in allograft half-life compared with the heterozygous or wild-type alleles (60 vs 17 years) [26]. Of note, the acute rejection rate was not influenced by the CCR5Δ32 mutation.

Our group has investigated the role of several other chemokines on transplant outcomes. The promoter region SNP of MCP-1 (-2518G/G) was associated with increased MCP-1 production in vitro ('high producers') in peripheral blood mononuclear cells from kidney transplant recipients carrying the G allele. The G/G genotype for MCP-1 -2518 behaved as a determinant for long-term allograft survival and resulted in reduction of the mean graft survival, as compared

Table 1
A summary of the important association for described genetic variation on outcome post kidney transplantation

Polymorphism	Outcomes	n	Reference
Chemokine/Cytokines			
MCP-1-2518A/G	This is the first study showing an association of an individual chemokine with outcomes in kidney transplantation. Clinical findings were supported by functional experiments.	232	[27]
CCR5Δ32	This study genotyped a large cohort of kidney transplant recipients for the rare CCR5Δ32 mutation. The presence of this loss of function mutation resulted in a significantly prolonged allograft half-life as compared with the heterozygous or wild-type allele.	1227	[26]
TNF-α-308 A/G [a] TGF-β codon 10 T/G [a] codon 25 C/G [a] IFN-γ 875 T/A [a] CCR5 59029 G/A [a]	This study examined the influence of several donor cytokine/chemokine SNPs on acute rejection and CAN in kidney transplants. Of these SNPs, the TGF-β high producer genotype and the CCR5 59029 low producer genotype were associated with a higher incidence of acute rejection. Furthermore, the latter mentioned genotype, as well as the IFN-γ T allele, which is associated with increased production, showed a significant higher frequency of CAN.	244	[30]
Innate immunity			
C3 [a]	C3F/F or C3F/S (donor) and C3S/S allotype (recipients) had a better graft survival. These effects were only seen when the recipient had the C3S/S allele, suggesting that allotypes of the donor and recipients interact.	513	[56]
TLR4 [a] D299G T399I	Mutations in the donor TLR4 correlated with decreased rate of acute rejection.	122	[46]
NOD2/CARD15 R708W R908G 1007fs	Carrying any mutated haplotype of the NOD2/CARD15 receptor was associated with a higher rate of cardiovascular mortality in a well-defined transplant cohort.	352	[52]
MBL codon 52 G/A codon 54 G/A codon 57 C/T	Lower levels of pretransplant MBL levels, which are coupled with a mutated sequence, were associated with a better graft and patient survival.	99	[58]
Fibrosis			
VEGF 936 C/T	In this case control study (early graft loss vs long-term graft survival), the CT and TT alleles were associated with a better graft outcome.	555	[85]
VEGF -1154A/G -2578C/A	This study described the significance of 2 polymorphisms in the VEGF gene for risk of acute rejection in kidney transplant recipients (-1154 G- and -2578 C-allele). The study provides also good functional data supporting the clinical findings.	173	[86]
Pharmacogenetic			
MDR1 129 C/T 1236 C/T 2677 G/T 3435 C/T CYP3A4 (∗1B) CYP3A5 (∗1/∗3)	The authors showed for a couple of important SNPs in the MDR1 gene their influence on CNI metabolism.	174 81	[94,96]
Coagulation			
Factor V Leiden Factor II 20210 A/G MTHFR C677T	In this multicenter study the authors found no association of factor V Leiden or the MTHFR C677T gene polymorphisms with kidney allograft survival. For the factor II 20210 A-allele, there was a significant benefit in graft survival after 3 and 5 years; however, the significance was lost after Bonfferoni correction for multiple testing.	671 + 651 [b]	[81]
Metabolism			
IL-6 -174 G/C	The risk of PTDM was significantly increased in patients carrying the GG-allele.	217 + 132 [c]	[25]

[a] Donor genotype. All other studies refer to recipient genotype.
[b] 671 first transplant and 651 retransplant patients.
[c] 217 retrospectively and 132 prospectively enrolled patients.

with the heterozygous (A/G) or wild-type (A/A) allele [27]. Interestingly, this effect was organ specific because this variant had no influence on the outcome in liver transplant recipients [28]. The chemokine RANTES has 3 functional SNPs, 2 in the promoter (-403 G/A, -28 G/C) and 1 in the intron (In 1.1 T/C), that are associated in different models with higher mRNA or protein levels (-403A, -28G, and In 1.1 T allele). However, in the largely redundant system of chemokines and chemokine receptors, the effect of polymorphisms on local regulation of protein on the immune response may prove difficult to predict. The RANTES-403 GA/AA and In 1.1 TC/TT have been associated with a higher rate of recurrent rejections in a study of 261 kidney transplant recipients [29]. Several chemokine receptor

antagonists are currently being tested in clinical trials. Polymorphisms within these genes may help predict outcomes and provide insight into the individual responsiveness to the antagonists currently under development.

In addition to the influences of recipient genetics on transplant outcome, donor organs may vary in their response to injury and immune stimuli in a genetically determined manner. However, until now, few studies have been published that address this particular question. In one of the largest cohort, 244 renal allograft donors were genotyped for different cyto/chemokines: IL-2 (-330 G/T), IL6 (-174 G/C), IL-10 (-1082 G/C, -819 C/T, -592 C/A), TNF-α (-308 A/G), TGF-β (codon 10 T/G, codon 25 C/G), IFN-γ (875 T/A), CCR2 (V64I), and CCR5 (Δ32, 59029 G/A). In this cohort, the TGF-β high producer phenotype, as well as the CCR5 59029 A-allele, with lower receptor expression, were significantly associated with higher rates of acute rejection. Furthermore, the CCR5 59029 A allele and, more strikingly, the IFN-γ T allele (higher production) were also associated with the occurrence of biopsy-proven CAN [30].

3. Adhesion molecules and cell migration

Adhesion molecules play a key role in the recruitment of inflammatory cells to the site of inflammation. Selectins, single-chain transmembrane glycoproteins, such as E-selectin (CD62E), L-selectin (CD62L), and P-selectin (CD62P), are involved in the process of leukocyte adherence and rolling on endothelium, whereas intracellular adhesion molecules-1 (ICAM-1) and platelet/endothelial cell adhesion molecule-1 (PECAM-1) are necessary for migration across the endothelium. Several polymorphisms in these molecules, lacking defined functional effects, have been analyzed with respect to their effects on transplant outcomes. Overall, the results of these studies have been diverse. Within the ICAM-1 gene, 2 SNPs, 469E/K and 241 G/R, were tested in both kidney and heart transplant recipients for their effect on the rate of acute rejection and graft survival [31,32]. In kidney transplants, the 469E/K SNP was associated with a decreased graft survival, whereas in heart transplantation, this same allele appeared to have a protective effect, whether it was expressed by the recipient or donor. The 241R allele was more common in a kidney transplant cohort with impaired kidney function compared with long-term survivors (defined >10 years) [31], an effect that again was not confirmed in heart transplant recipients. The same study tested genetic variations in E-selectin (128S/R) and L-selectin (206F/L) and found no effect on transplant outcomes either in heart or kidney transplant recipients [31].

4. Costimulatory molecules

Therapeutic agents targeting costimulatory pathways are currently under investigation in clinical trials in transplantation and autoimmunity [33]. The best-characterized interaction is the CD28/B7-1/B7-2 axis, which provides the stimulation required for full T-cell activation. CTLA4, a CD28 homologue, is a negative regulator of the signals for T-cell proliferation and plays a critical role in down-regulation of the immune response [33]. Polymorphisms in the CTLA4 gene have been associated with several autoimmune diseases including diabetes and thyroiditis [34-37]. We have examined the association of 2 polymorphisms in the CTLA-4 gene (microsatellite $(AT)_n$-repeat in the 3′ UTR region and SNP 49 A/G in exon 1) with acute rejection and graft survival in kidney and liver allograft recipients [38,39]. An increased incidence of acute rejection was found in association with the $(AT)_{92}$ and $(AT)_{94}$ microsatelitte polymorphisms in both liver and kidney transplant recipients. In addition, the $(AT)_{100}$ allele was associated with acute rejection in liver transplant recipients independent of ethnicity. Consistent with these findings, these alleles were associated with decreased graft survival in liver recipients.

The +49A/G SNP in exon 1 of the CTLA-4 gene results in a switch from alanine to threonine in the amino acid sequence. Lymphocytes from individuals with the GG genotype had an increased baseline proliferation and a decreased response to blockade of CTLA4 [40]. Liver transplant recipients homozygous for the G allele had a 48% and 85% decrease in 5- and 10-year graft survival, respectively [39,41]. The importance of this SNP in liver transplantation was further strengthened by 2 recent studies that found that the CTLA-4 +49 G/G genotype was associated with reduction in the incidence of acute rejection [42,43].

5. Innate immune system

5.1. Toll-like receptors

Much attention has been focused on the innate immune response since the discovery of special recognition molecules called toll-like receptors (TLRs). To date, 13 human proteins related to the *Drosophila* Toll gene have been characterized. These receptors specifically recognize pathogen-associated molecular patterns that are expressed in infectious agents, and their activation is crucial to both innate immunity and for an effective presentation of antigens to the adaptive system [44]. Upon ligand binding, TLR-mediated signaling activates signal transduction pathways that induce cytokines, chemokines, and increased expression of cellular membrane proteins related to inflammatory responses. The most studied polymorphisms in this group are the 299 A/G (aspartate to glycine) and 399 C/T (isoleucine to threonine) in the TLR4 gene, which have been shown to attenuate receptor signaling and diminish the inflammatory response to lipopolysaccharide. In renal transplantation, patients carrying at least one of these polymorphisms (G299 or T399) have a lower rate of posttransplant atherosclerotic events and acute allograft rejection, with more severe infectious complications [45]. In a study examining the

effect of these polymorphisms in donors, transplanted kidneys carrying at least one mutation in the TLR4 gene were found to have a lower rate of acute rejections [46]. These human genetic studies are consistent with experimental transplant models and suggest an important role for donor and recipient TLR activation in vascular disease and alloimmunity [47,48].

5.2. NOD2/CARD15-gene

The NOD2/CARD15 gene (nucleotide oligomerization domain-2/caspase-recruiting activating domain-15), which is a general (intracellular) receptor for both Gram-positive and Gram-negative bacteria and a member of the innate immune defense, is expressed in macrophages and has been shown to be expressed in atherosclerotic lesions and on endothelial cells [49]. Its main ligand is muramyl dipeptide, the minimal motif of all peptidoglycans. These ligands are potent inducers of cytokine secretion in both monocytes and dendritic cells. Furthermore, these ligands are also known to induce the maturation of dendritic cells, as measured by increased expression of CD80, CD86, and MHC class II. Since the discovery of a striking association of specific polymorphisms in the NOD2/CARD15 gene and the occurrence of Crohn's disease [50,51], several groups have tried to link these SNPs (SNP8 [R708W], SNP9 [R908G], and SNP13 [1007fs]) with outcomes in transplantation. All of these polymorphisms are thought to lead to loss of function; however, gain of function has also been reported in some cases, a difference which may be attributable to alternate stimulation pathways in different cell types [52]. In our study, we demonstrated a greater occurrence of the mutated NOD2/CARD15 haplotype in conjunction with "death with a functional graft" or cardiovascular death in renal transplant recipients, with no difference in rejection rates or graft survival [52]. These results do not clarify the issue of loss vs gain of function because there are differences in susceptibility between atherosclerotic disease vs CAN, which would have anticipated similar associations. However, this difference in the findings may also be attributable to the multifactorial nature of CAN.

5.3. Complement genes

The complement system is a link between innate and adaptive immunity [53]. Complement genes are activated during ischemia reperfusion injury and are involved in antigen-dependent responses directed toward the allograft [54,55]. The central player is complement factor 3 (C3), in which an SNP (R102G) is identified by the differences in mobility shift assays with fast (F) and slow (S) allotypic variants [56]. A recent study in caucasian kidney transplant recipients identified donor C3 allotypes as a factor that was associated with a better long-term outcome for cadaver allografts. Five hundred donor/recipient pairs had better graft survival with a C3F/F or C3F/S donor allotype in comparison to the C3S/S allotype. Of note, these effects were only seen when the recipient had the C3S/S allotype suggesting that allotypes of the donor and recipients interact. The exact mechanism of the protective effect of the C3F allotype is unclear because the functional effect is not well described; thus, the effect may be due to linkage disequilibrium with another SNP in the C3 gene or a neighboring gene. It will be important to confirm these results in another cohort of renal transplant patients.

Mannose-binding lectin (MBL) is the major recognition molecule in the lectin pathway of complement activation utilizing the same C3 convertase as the classical pathway. In host defense, MBL binds to carbohydrate moieties, leading to complement deposition, opsonization, and elimination of pathogens [57]. The SNPs at codon 52 (G/A), 54 (G/A), and 57 (C/T) are associated with low serum MBL levels and decreased host defense against various infectious agents. In simultaneous pancreas-kidney transplantation and kidney transplantation alone, pretransplant levels of MBL were shown to be associated with graft and patient survival [58,59]. Furthermore, a relation between MBL level and the occurrence of any mutation (ie, at codon 52, 54, 57) was established in these simultaneous pancreas-kidney transplantation recipients. Both studies lack detailed adjustment for confounders, and prospective controlled studies are needed to confirm these results.

6. Renin-angiotensin aldosterone system

Hypertension is a frequent posttransplant complication contributing to morbidity and mortality and reducing long-term graft survival [60]. Given the important role of the renin-angiotensin aldosterone system in regulating blood pressure, gene polymorphisms at several levels of this axis have been extensively investigated in renal and cardiovascular disease and also in transplantation. The most extensively studied genetic variant is the angiotensin-I-converting enzyme (ACE) gene, which contains an insertion (I) or deletion (D) of a 289 base pair Alu-repetitive sequence in intron 16. Circulating and tissue levels of ACE are higher in the presence of the D/D allele, and this has been associated with an increased risk for coronary artery disease and left ventricular hypertrophy [61,62] or progression of chronic kidney disease in glomerulonephritis [63]. Furthermore, it has correlated with worsening of outcomes in kidney transplantation, such as the development of CAN or graft survival, as well as a higher rate of transplant vasculopathy in heart transplantation [64-67]. However, a number of other investigations have been unable to confirm these findings and in some cases have found conflicting results [68-72].

Polymorphisms in the angiotensinogen gene and the angiotension type I and II receptors have also been studied; however, most of these studies revealed conflicting results likely due to the low number of patients enrolled. The T allele of a mutation in the steroidogenic factor-1 site of the aldosterone synthase gene (CYP11B2 -344C/T) has been

associated with increased secretion of aldosterone and the prevalence of hypertension [73,74]. In a study of 223 kidney transplant recipients, the TT allele showed a significant diminished graft survival in comparison to recipients with the CC or CT allele. However, in this study, only patients with a graft survival of at least 1 year were included [71].

Chronic renal failure is a major cause of morbidity and mortality after orthotopic liver transplantation, and the activation of the renin-angiotensin aldosterone system has been implicated in the pathogenesis. We retrospectively investigated genetic variants of the ACE gene, the angiotensin II receptor 1 gene, and the angiotensinogen gene and found that liver transplant patients with ACE gene D/D genotype are at a significant higher risk of developing chronic renal failure [75].

7. Coagulation and aggregation

The coagulation system not only plays a crucial role in transplantation through antigen-independent processes such as hypercoaguability, but it also contributes to the immune response directed toward the kidney by the formation of microthrombi on activated endothelium after injury in ischemia reperfusion and acute humoral rejection [76,77]. The most frequent cause of inherited thrombophilia exhibits the factor V 506 G/A (arginine to glutamine) mutation (protein C resistance). In numerous studies, the factor V mutation has several negative effects on kidney transplantation, for example, graft perfusion defects or acute graft losses due to “vascular rejection” [78-80]. However, in a recently published multicenter study, the factor V Leiden mutation had no influence on graft survival [81]. Interestingly, the factor V mutation of either recipient or donor origin has no detrimental effect in liver transplant recipients in terms of vascular thrombosis and subsequent graft loss again pointing to the potential tissue specific effects of certain genes and therefore SNPs within those genes [82]. In the case of liver transplantation, the lack of influence of the recipient genotype is not surprising because factor V is produced by the donor liver.

Another inherited thrombophilia arises because of the prothrombin 20210 G/A polymorphism, which has been associated with decline in long-term kidney graft survival [83]. However, in a recent study with over 1300 patients, this finding could not be confirmed after correction for multiple testing [81]. In addition to these polymorphisms in coagulation factors, a polymorphism in the platelet glycoprotein IIb/IIIa receptor (GPIIb/IIIa 33L/P) has been described in association with higher acute rejection rates (58.1% vs 35.5%) and decreased 2-year graft survival (87.7% vs 97.2%) in kidney transplant. However, one must take into account that these results were performed in only 119 patients [84]. The clinical impact of these polymorphisms is of particular interest because they are highly amenable to therapeutic intervention.

8. Fibrosis and other molecules

Vascular endothelial growth factor (VEGF) promotes endothelial cell proliferation, monocyte chemotaxis, and expression of adhesion molecules and is a major mediator of vascular permeability. Through its receptor VEGFR-1, it increases the inflammatory reactions that lead to migration of leukocytes into the graft. Various polymorphisms of the VEGF gene, associated with either lower or higher VEGF protein production, have been identified, and a recent publication reported an additional polymorphism at position 936 C/T, which is associated with low VEGF plasma levels [85]. In a recent case control study was conducted in which recipients with early immunological graft loss (<1 year) were compared with recipients with long-term functioning grafts. In this study, the C/T or the T/T genotypes were significantly more frequent in the long-term functioning group [85]. Furthermore, Shahbazi et al [86] demonstrated an association between 2 polymorphisms in VEGF (-1154A/G and -2578C/A) and increased in vitro VEGF production, which correlated with an increased rate of acute rejection in kidney transplants.

A SNP in the methylenetetrahydrofolate reductase gene, MTHFR 677C/T, results in reduced enzymatic activity and an increase in homocysteine levels [87]. Despite the potential contribution of hyperhomocysteinemia to coronary atherosclerosis and fibromuscular thickening of small arteries [88,89], no association was found between the MTHFR 677C/T mutation and graft survival in large cohorts of kidney transplant recipients [81,90]; however, contrary to studies in other patient populations, a recent study found a protective effect of homocysteine in renal transplant recipients [91].

9. Pharmacogenetics

Pharmacogenetics aims to identify the inherited basis for interindividual differences in drug response. There are numerous drug metabolizing enzymes, drug transport proteins, and drug targets, all of which may be affected by genetic variants with potential functional effects on the pharmacokinetics of specific drugs. Because cyclosporine, tacrolimus, and sirolimus are metabolized via the MDR1 gene, there has been much interest in the pharmacogenetic effects of SNPs within this gene on calcineurin inhibitors (CNI) and mTor dose requirements and bioavailability in transplant recipients [92-100,115]. The basis of the variation in pharmacokinetics is related to the cytochrome P450 (CYP450) system and to P-glycoprotein (PGP) encoded by the multidrug resistance gene (MDR1), a fact that complicates the dissection of the role of each gene variant in the metabolism of these drugs [101,102]. Intestinal mRNA expression of MDR1 and CYP3A4 has been examined in living-donor liver transplant recipients for a correlation with concentration/dose ratios of tacrolimus and survival. MDR1,

but not CYP3A4, was inversely related to the concentration/dose ratio of tacrolimus. Furthermore, high levels of MDR1, but not of CYP3A4, were also associated with survival rates [103]. Approximately, 32 SNPs in the MDR1 have been identified [104], but the most frequently studied are 3 SNPs located in exons 12 (C1236T), 21 (G2677T), and 26 (C3435T). These SNPs are in linkage disequilibrium, and their frequency differs widely between ethnicities; for example, the CGC haplotype is expressed in 61% of African Americans as compared with 36% of whites, whereas the TTT haplotype has a 6.5% to 27% distribution [105]. This family of enzymes, consisting of cytochromes P450 3A4, 3A5, 3A7, and 3A43, are prominent in the liver and gastrointestinal tract and have a 5- to 20-fold variability in its ability to clear a drug between individuals [106,107]. Both cyclosporine and tacrolimus are a substrate for CYP3A, and numerous polymorphisms have been described as having effects on pharmacokinetics. The CYP3A5∗3 and CYP3A5∗6 variants induce splice site variants that substantially decrease the amount of CYP3A hepatic content [108]. Debate exists about the functional significance of CYP3A4∗1B and its disease association [109-112]. The association of cytochrome P450 SNPs with CNI dose requirements predominantly in kidney transplant recipients was evaluated in various studies [94,95,97,100,113,114]. There are striking differences between the influences of the cytochrome P450 SNPs in patients with different CNIs. For example, the CYP3A4 ∗1B allele leads to decreased tacrolimus levels, whereas cyclosporine levels are unaffected. Other alleles like the CYP3A5 (∗1/∗3) increased daily dose, again only in patients using tacrolimus. The immunosuppressants, cyclosporine, rapamycin, and tacrolimus, are all substrates for the PGP, and overall, there is still considerable controversy about the correlation between genotypes and PGP expression and function in humans [92-95,99,102].

10. Limitations and future directions

There are several problems in the reporting of genetic associations with complex diseases, and guidelines have been proposed to increase the quality and reproducibility of genetic association studies [116-118]. The outcomes of interest in transplantation, DGF, acute rejection, and graft loss are referred to as complex disease traits, influenced by genetic factors in multiple immune and nonimmune genes, in both recipient and donor, and contrast dramatically with the well-defined monogenetic diseases determined by a single gene locus. The increase in relative risk for a specific gene may be masked or alternatively be a false-positive finding because of poorly designed studies. The conflicting results between genetic associations in studies discussed in this review may be accounted for by some of the following explanations: (1) small patient numbers with inadequate power to detect a difference; (2) failure to allow for linkage disequilibrium; (3) inadequately defined end points; (4) failure to control for confounding variables (eg, immunosuppressive protocol, baseline and concomitant diseases, donor factors); (5) inherent bias of retrospective studies (enrolled on a cross-sectional basis), for cohort studies an enrollment rate of at least 95% of the population within the study period is considered to be necessary to be reflective; (6) differences in genetic backgrounds.

Ethnicity clearly influences transplantation outcomes, with African American recipients traditionally being stratified as high immunological risk. In addition, differences in the allelic distribution between certain ethnicities have been well described [119]. Therefore, precise description and stratification of the study population is required to avoid misidentification of a SNP as associated with the end point as opposed to having increased expression in a particular ethnicity. Furthermore, association may occur by virtue of coinheritance or linkage disequilibrium. For example, the -308G/A in the TNF-α promoter is associated with a 6-fold increase in transcription and has been shown to be associated with an increase in acute rejection in renal, cardiac, and liver transplantation [5-8,11,12,19]. There are at least 10 other TNF-α SNPs that have never been studied or taken into consideration. Furthermore, this gene is located in the highly polymorphic immune region of the MHC class II genes and is in tandem with TGF-α. These circumstances make linkage studies essential to truly define the role of each SNP. Although the identified association may still be important irrespective of linkage, clearly identifying the risk gene furthers our understanding of the disease mechanisms and the design of therapeutic targets.

Another shortcoming has been the failure to clearly define end points, such as the time point and the method of diagnosis of acute rejection (clinical vs biopsy proven, clinically indicated vs protocol biopsies), and identification of the causes of graft loss in studies examining long-term graft survival. This is further complicated by the multiple factors that contribute to the development of CAN and the failure of the pathological diagnosis to differentiate between these. The revised Banff Schema for grading biopsies now attempts to take this into consideration and may aid in differentiating immune from nonimmune processes [120].

Although significant limitations exist, the impact of genetic variations on organ transplantation is an exciting and promising area of research. The dissection of the complex genetic determinants that control transplant end points offer us the opportunity to individually tailor our approach to each patient. Further advances in haplotype description and new technologies for high-throughput genotyping offer exciting possibilities in candidate gene or genome-wide association studies and further emphasize the need for large, well-controlled, prospective studies. The NIH/NIAID Genomics Consortium is currently funding 3 large multicenter grants with the aim of achieving this goal. The results of these studies should provide important clinically relevant data that will influence our approach to patient care in the future.

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