Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 7 July 2024

Research Article

Genes Govern the Acceptance or Rejection of a Transplant!

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Received: May 14, 2024

Published: June 27, 2024

Abstract

The current strategies of immunosuppression and prophylactic antimicrobial medication in a transplantation are exemplary. Still the desirable outcome of relief from rejection and infection risk aren't a guarantee! Graft survival relies not just on human leukocyte antigen matching, other crucial factors include the infection risk, extent of immunosuppression, and management of comorbidities. The transplant outcome can be improved through risk stratification of genetic predispositions and application of precision pharmacotherapy.

Keywords: Transplant Outcome; Transplant Donor; Transplant Recipient; Immunosuppression; Graft Survival; Graft Rejection; Genetic Predictors; Single Nucleotide Polymorphisms/SNPs; Precision Medicine

Introduction

Technology advancements in molecular genetics have paved way for increased accessibility to economical genetic tests with wider application in clinical care. Precision pharmacotherapy has evidence-based guidelines from varied organizations including the US Food and Drug Administration, the Clinical Pharmacogenetics Implementation Consortium, and the Pharmacogenomics Knowledge Base National Institute of General Medical Sciences (National Institutes of Health). A polygenic risk score or a single score predicting the overall genetic risk based on variants of allograft rejection should be clinically validated and integrated into practice for improved transplant outcomes [1-4].

Factors that decide the fate of a transplant

- The most important genes deciding the fate of a transplanted organ, cell or tissue, belong to the major histocompatibility complex or MHC.
- Prime role of MHC antigens is peptide presentation to the immune system to help distinguish self from non-self. These antigens are called HLA (human leukocyte antigens), and comprise three regions namely, class I (HLA-A,B,Cw), class II (HLA-DR,DQ,DP) and class III (no HLA genes).
- Located on the short arm of chromosome 6 (6p21.3), the MHC system or HLA has polymorphic genes. The structure and function of their gene products determine their classification as class I, class II or class III. The prime role of HLA class I gene products (HLA-A, -B, and -C) is presenting endogenous peptides to responding $CD8^+$ T Cells. The HLA class II gene encoded molecules HLA-DR, -DP, and –DQ show restricted expression, processing the exogenous peptides for presentation to $CD4^+$ helper T Cells. The gene products of HLA class III region encode immune regulatory molecules, such as tumour necrosis factor (TNF), factors C3, C4, and C5 of complement and heat shock proteins.

- Polymorphisms of genes in HLA class I and class II impact the amino acids in their peptide-binding groove thus altering their binding specificity. An open structure of the HLA class II peptide-binding groove with a greater length of the peptides bound in it favour greater flexibility in peptide binding. Polymorphisms of HLA class I and II loci have nucleotide substitutions mostly concentrated in the exons encoding the peptide-binding groove and the site of interaction with the T Cell receptor/TCR. 'T' cell activation is crucial in immune response; and during this process, the CD4 and CD8 molecules bind T cell receptor with major histocompatibility complex (class I and II molecules). T cell activation necessitates three definitive signals for optimally generating an immune response, and these include, signal 1 or T cell receptor engagement, signal 2 or costimulation, and signal 3 or cytokine stimulation.
- Transplantation with an allogeneic (non-self) tissue can stimulate a vigorous immune response directing towards graft rejection. Thus a majority of recipients with allogeneic organ transplants might require the use of immune suppressive agents for a lifetime. As 'T' cell activation directly mediates rejection and graft-versus-host disease (GVHD), usage of costimulation (signal 2) blockade can induce immune tolerance precisely to allogeneic antigens. This could assuredly be a potent alternative to immunosuppression, with substantial research evidence [5,6].
- The HLA matching contributes to the success rate of a majority of solid organ transplantations (such as kidney, liver, heart, lung, pancreas, and intestine).
- In transplantation, linking genetic insights with demographic profile and clinical outcomes can potentially pave way for personalized medicine. Precision medicine in transplantation can be achieved through individualized risk stratification and immunosuppression based on genetic variants encoding immune-mediated complications, post-transplant disease or alterations in drug-metabolism [1-4].

Discussion

Need for recognising Inter-individual differences in transplant acceptance

Recognizing the inter-individual difference in transplant outcome is becoming a possibility through integration of pharmacogenetics, pharmacoproteomics, epigenetics, and noncoding RNAs data into clinical practice [1]. The following figure, Figure 1 (as adapted from Nobakt., *et al*. 2021) illustrates the significance of recognising Inter-individual differences in transplant acceptance.

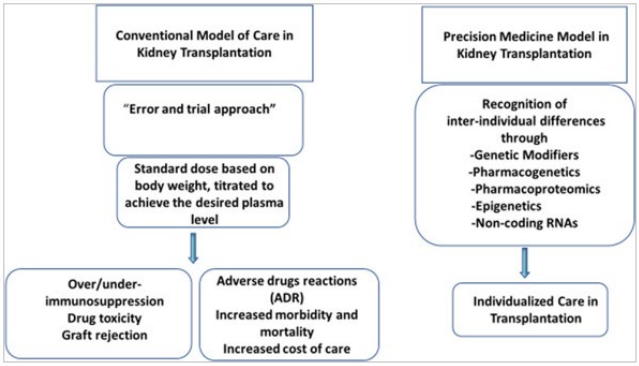

Figure 1: Need for recognising Inter-individual differences in transplant acceptance.

Precision medicine is vital in transplantation, WHY?

The polygenic risk score (PRS) is a single score which provides the overall genetic risk of an individual, including variants encoding allograft rejection in conjuncture with pharmacogenetics. This individualized approach may be integrated into practice for better outcomes after an appropriate clinical validation[1]. The following figure, Figure 2 (as adapted from Nobakt., *et al*. 2021) elucidates the vitality of precision medicine in transplantation.

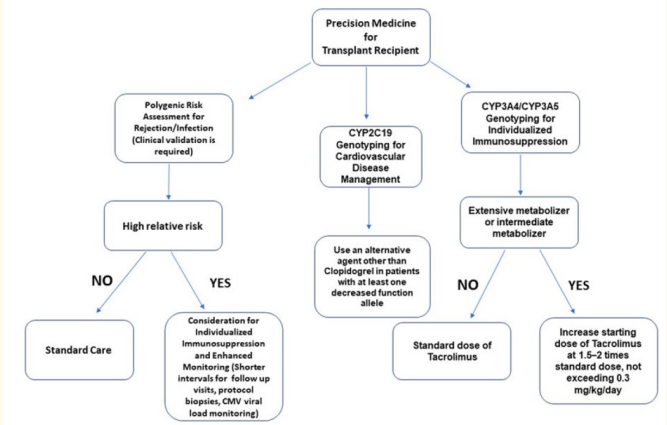

Figure 2: Vitality of precision medicine in transplantation.

A panel of genetic variants for transplant recipients and donors

Individualized care in transplantation is achievable through a gene panel that would aid the physician in managing the transplant outcome through an insight of donor as well as recipient's innate compatibility profile [1]. The following figure, figure 3 (as adapted from Nobakt., *et al*. 2021) explains the elements of such a gene panel.

The following table, Table 1 (as adapted from Nobakt., *et al*. 2021) provides a list of genetic predictors that decide the transplant outcome.

Significant role of genes and their variants in kidney transplantation

- The genetic interplay between a donor and a recipient strongly determines the kidney transplantation outcome. Nevertheless, our knowledge of these complex interactions is limited. Development of risk predication models that precisely assess the post-transplant risk may lead to a more personalized approach to kidney transplant care [7,8].
- Probability of severe rejection was strongly associated with the TLR-3 (rs3775296) polymorphism in the recipient, and donor carrying polymorphisms namely ficolin-2 (rs7851696; Ala258Ser) and C1qR1 (rs7492).

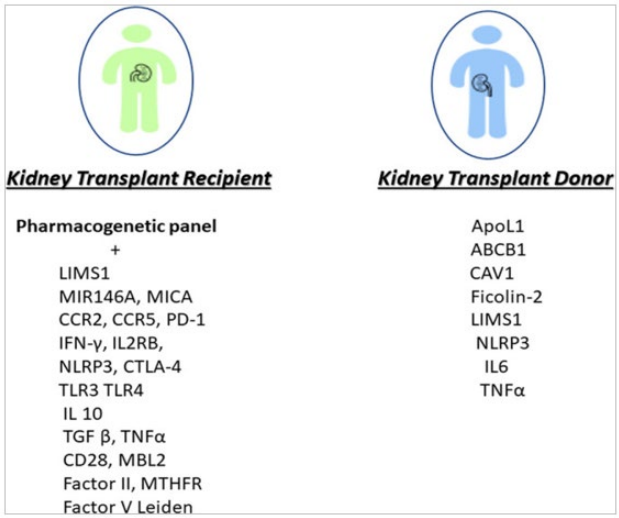

Figure 3: Essential elements of a donor-recipient transplant genetics' panel.

ABCB1, ATP binding cassette subfamily B member 1; ApoL1, Apolipoprotein L1; CAV1, caveolin-1; CCR, chemokine receptor; CTLA, Cytotoxic T-Lymphocyte Antigen; IFN-γ, interferon-gamma; IL2RB, IL2, Receptor Beta; MBL, mannose-binding lectin; MICA, MHC class I-related chain A; MiR, microRNA; MTHFR, methylenetetrahydrofolate reductase; NLRP3, NOD-like receptor family, pyrin domain containing 3; TGF-β, transforming growth factor; TLR, Toll-Like Receptor; TNF-α, tumor necrosis factor-alpha.

S. No.	Gene	Physiologic function	SNP identified	Associations with clinical outcomes
1	ApoL1 (Apolipoprotein 1)	Trypanosome killing function	rs71785313, rs60910145, rs73885319	Reduced kidney allograft survival
2	MICA (MHC class I-related chain A)	Stress-induced protein regulated at the cell surface	rs2596538, rs67841474	Anti-MICA sensitization and increased proteinuria in kidney transplant recipients and is a predictor of susceptibility to CMV infection
3	TLR3 (Toll-Like Receptor)	Cell-bound receptor involved in innate immune system	rs3775296	Increased acute kidney allograft rejection
4	TLR4 (Toll-Like Receptor)	Binds to endogenous ligands released from damaged tissues and exogenous ligands such as lipopolysaccharide	rs10759932	Increased rejection-free survival rate

5	FCN2	Soluble recognition molecule that can engage apoptotic and necrotic cells	rs7851696	Reduced incidence of severe kidney allograft rejection and graft loss
6	LIMS1	A minor histocompatibility antigen	rs893403	Increased kidney allograft rejection
7	MIR146A (Micro RNA)	Modulated Treg (regulatory T cells) and suppression of inflammatory responses	rs2910164	Increased kidney allograft rejection
8	CAV1 (caveolin-1)	Involved in cholesterol transport and transmembrane signaling	rs4730751	Increased kidney allograft failure
9	PD-1	Involved in the dysfunction of HIV-specific T cell response and CMV-specific CD8 T cells	rs11568821	Improved kidney allograft survival in recipients from cytomegalovirus /CMV-positive donors
10	IFN-γ (interferon-gamma)	Involved in immune response to viral and bacterial infections	rs2430561	Increased risk for the CMV (cytomegalovirus) infection
11	ABCB1 (ATP binding cassette subfamily B member 1)	An efflux pump for intestinal transport of medications including tacrolimus	rs1045642, rs2229109	Increased risk of renal allograft loss
12	NLRP3 (NOD-like receptor family, pyrin domain containing 3)	NOD-like receptor family, pyrin domain containing 3 is a member of inflammasome family with a causal role in several inflammatory disorders	rs35829419, rs6672995	Increased acute kidney allograft rejection with rs35829419 and Reduced acute kidney allograft rejection with rs6672995
13	CCR5 (chemokine receptor)	Chemokine receptor specific for the proinflammatory chemokines	rs1799987	Increased acute kidney allograft rejection
14	CCR2 (chemokine receptor)	Involved in immune response including monocyte recruitment and T cell proliferation	rs1799864	Increased acute kidney allograft rejection
15	IL2RB (Interleukin receptor)	Stimulating T-cell proliferation through complex of IL2RA-IL2RB-IL2	rs228942, rs228953	Increased acute kidney allograft rejection episodes
16	IL6 (Interleukin)	A pleiotropic cytokine with proinflammatory and anti-inflammatory properties	rs1800795	Increased acute kidney allograft rejection
17	IL8 (Interleukin)		rs16944	AA genotype was associated with 2.7-folds increased risk for allograft rejection in recipients experiencing rejection episodes as compared to non-rejecters. Lower mean time to first rejection episode for AA recipients (23 months) as compared to TT recipients (30 months)
18	IL10 (Interleukin)	An immunomodulatory cytokine with anti-inflammatory effects	rs1800896	Increased acute kidney allograft rejection

19	TNF-α (Tumor Necrosis Factor alpha)	Proinflammatory cytokine	rs1800629	(rs1800629 in Donor and Recipient) Increased acute kidney allograft rejection episodes(rs1800629 in Recipient)Modulates the effect of ATG/ antithymocyte globulin; treatment
20	TGF-β (transforming growth factor)	Anti-inflammatory but profibrotic cytokine	rs1982073, rs1800471	Reduced risk of late acute kidney allograft rejections with rs1800471 and increased kidney allograft subclinical rejection with rs1982073
21	CD 28	A costimulatory molecule involved in T cell-mediated immune response	rs3116496	Increased acute kidney allograft rejection
22	MBL2 (mannose-binding lectin)	Complement-activating MBL, a soluble pattern recognition receptor	rs7096206 rs5030737 rs1800450 rs1800451	Increased acute kidney allograft rejection
23	CTLA4 (cytotoxic T-lymphocyte antigen)	CTLA4 transduces signals that inhibit lymphocyte activation	rs231775 rs3087243	Reduced acute kidney allograft rejection with rs231775 and increased acute kidney allograft rejection with rs3087243
			rs231775	CTLA4 G allele/GG genotype is associated with the acute rejection risk in renal transplantation. However, the AA genotype was not associated with acute rejection risk in renal transplantation
24	Factor II	Prothrombotic factor	rs1799963	Increased acute kidney allograft rejection, especially vascular rejections, and early allograft failure
25	Factor V Leiden	Prothrombotic factor	rs6025	Increased acute kidney allograft rejection especially vascular rejections
26	MTHFR (Methylenetetrahydrofolate reductase)	Prothrombotic factor	rs1801133	Increased acute kidney allograft rejection, especially vascular rejections
27	FCGR3A	Encodes the IgG Fc receptor	rs396991	Increased risk of infection following Rituximab in recipients of liver transplant
28	ficolin-2	L-ficolin (P35, ficolin-2) is synthesised in the liver and secreted into the bloodstream where it is one of the major pattern recognition molecules of plasma/serum.	rs7851696	Presence of the ficolin-2 Ala258Ser variant in the donor predicted lower incidence of severe rejection and of graft loss independently of clinical risk factors.
29	FOXP3 (forkhead box P3)	FOXP3 is important for regulation and development of T cells, which are mediators of kidney allograft rejection	rs3761548	AA genotype carriers were associated with about a fourfold greater risk for rejection compared with CC genotype
			rs3761548	The AA genotype was associatied with a higher risk of rejection compared to the C/A genotype. The C/A genotype was also associated with a better response to treatment for rejection and better posttransplant graft function

			rs3761548	Patients with FOXP3 rs3761548 AA and AC genotypes had a 10-fold higher risk for Tacrolimus/TAC-induced acute nephrotoxicity than those with CC genotype
			rs3761549	Patients (taking Cyclosporine (CsA) as an immunosuppressant) with rs3761549 T/TT genotype showed a more rapid decline in the estimated glomerular filtration rate (eGFR) level during the 5years following transplantation than those with the C/CC genotype
30	NFATC1	Nuclear Factor of activated T-cells (NFATs)	rs3894049	GC was a risk factor for acute rejection compared with CC carriers.
			rs2280055	TT carriers had a more stable estimated glomerular filtration rate level than CC

Table 1: A panel of genetic predictors for transplant outcomes.

- Donors carrying ficolin-2 Ala258Ser prompted towards improved graft outcome, alongside predicting a lower incidence of severe rejection (odds ratio/OR = 0.3; 95% confidence interval, 0.1-0.9 and P = 0.024) and graft loss (hazard ratio/HR = 0.5; 95% confidence interval, 0.2-1.0; P = 0.046), independent of clinical risk factors. Such a functional polymorphism efficiently handles injured cells by phagocytozing in order to decrease the intragraft exposure to danger signals and dampened alloimmune responses [9]. Monocytes, monocyte-derived macrophages, and peripheral blood mononuclear cells express ficolin-2. Presence of ficolin-2 Ala258Ser variant in the donor graft significantly elevated the expression of interleukin 6, having ascribed cytoprotective effects. Presence of Ala258Ser variant may increase the binding capacity of ficolin-2 to N-acetylglucosamine. In this research study, Ficolin-2 expression was assessed with digitonin-conjugated monoclonal antibody to Ficolin-2 (GN4; Hycult Biotechnology, Uden, the Netherlands), followed by HRP-conjugated sheep anti-DIG (Roche Diagnostics, Mannheim, Germany).
- The forkhead box P3 or the FOXP3 gene is essential in the regulation and development of T cells. As regulatory T cells are crucial mediators of kidney allograft rejection, the polymorphisms of FOXP3 gene may potentially relate with kidney transplant rejection. FOXP3 rs3761548 polymorphism is associated with allograft rejection in renal transplantation. The 'AA' genotype of rs3761548 showed nearly fourfold greater risk for rejection compared to the CC genotype (5 years post-transplant: odds ratio 3.95, 95% confidence interval 1.27-12.29, P = 0.018). Analysis through 'Multivariate Cox regression' revealed that the AA genotype of rs3761548 had a twofold higher risk for rejection compared to CC genotype (hazard ratio 2.37, 95% confidence interval 1.17-4.80, P = 0.017). The average (mean) time of first rejection as revealed by the Kaplan-Meier analysis was lower in AA genotype of rs3761548 compared to the CC genotype (Log rank = 4.303, P = 0.038) [10].
- FOXP3 rs3761548 might serve as a biomarker to prevent Tacrolimus (TAC) toxicity and help progression toward individualized therapy of TAC. In a research study amongst Chinese population, 114 renal transplant patients underwent TAC-based maintenance immunosuppression with a follow-up of at least 2 years. The AA and AC genotype carriers at rs3761548 of FOXP3 gene showed a 10-fold higher risk for TAC-induced acute nephrotoxicity compared to CC genotype [11].
- Tacrolimus, a calcineurin inhibitor, is considered a cornerstone in immunosuppression regimen of renal transplantation for more than a couple of decades. With tacrolimus, there is a significant improvement in the renal transplant outcome, especially seen as reduced episodes of acute rejection, enhanced graft survival, betterment in renal function, and

diminished adverse effects of cyclosporine. Conversely, tacrolimus also has undesirable effects like nephrotoxicity, neurotoxicity, post-transplant diabetes, and disturbances in electrolyte balance. To lessen such adverse effects, numerous strategies are adopted with minimal or nil tacrolimus in maintenance regimens of immunosuppression, with some success [12].

- In FOXP3 gene, the polymorphism rs3761549 significantly correlates with the renal allograft function and hence could predict the renal transplant outcome in patients undergoing immunosuppression with Cyclosporine (CsA). In a research study involving 166 renal transplant patients with a minimum of five year follow-up, T/TT genotype at rs3761549 of FOXP3 gene exhibited a rapid decline in the estimated glomerular filtration rate or eGFR compared to the C/CC genotype (24.0% vs. 6.3%, P = 0.004) during the follow-up period of transplantation [13].
- In a study involving 118 kidney transplant patients, identification of FOXP3 gene polymorphism (rs 3761648, C/A genotype) was hypothesized to be helpful in categorizing recipients with a lower risk of rejection and better graft survival. The AA genotype correlated with a higher risk of rejection compared to the C/A genotype (odds ratio, 2.329; 95% confidence interval, 1.041 to 5.210). Carriers of C/A genotype showed a better response to treatment against rejection (odds ratio, 6.667; 95% confidence interval, 1.319 to 33.707) and exhibited improved post-transplant graft function (odd ratio, 5.833; 95% confidence interval, 1.727 to 19.704) [14].
- Amongst 155 renal transplant recipients with at least a 5-year follow-up, the efficacy of cyclosporine (CsA) was studied by assessing the polymorphisms of its target genes namely, PPIA, PPP3CB, PPP3R1, NFATC1 and NFATC2. These pathway genes belong to the NFATs or nuclear factor of activated T-cells, also known as cyclophilin A/calcineurin. A single nucleotide polymorphism or SNP (rs3894049) in the gene NFATC1 showed its GC genotype carriers to be at a higher risk for acute rejection compared to the CC carriers (p = 0.0005). Another SNP in the NFATC1 gene, namely rs2280055 showed that its TT genotype carriers had a more stable level of estimated glomerular filtration rate than the CC genotype (p = 0.0004). In this research study, genotyping was carried out with PCR and single base extension following standard protocols for iPLEX chemistry (Sequenom). The reaction products being cleaned and spotted on a 384-well spectro-CHIP using a MassARRAY Nanodispenser. Following this, sample detection was done using MALDI-TOF-MS (MassARRAY System, Sequenom) [15].
- In a study sample of 146 Chinese renal transplant patients, who were on a 2-year follow-up with tacrolimus/TAC-based maintenance immunosuppression, the efficacy and safety of tacrolimus was studied by assessing polymorphisms in its target pathway (namely, the FK506-binding protein/ FKBP-calcineurin/CaN-nuclear factor of activated T cells/NFAT signaling pathway). Two years post-transplantation, TT genotype carriers at rs6041749 of FKBP1A gene showed a more stable eGFR level compared to the CC and CT genotypes ($P = 2.08 \times 10^{-8}$) during the follow-up. Presence of 'C' allele at rs6041749 of FKBP1A gene may increase the transcription, posing a higher risk for eGFR deterioration in its carriers. The increased gene transcription with 'C' allele presence was confirmed through 'Dual-luciferase reporter assay', showing a relatively enhanced luciferase activity compared to the T variant. Thus assessment of FKBP1A gene polymorphism at rs6041749 can be considered as a potential biomarker for predicting allograft function in renal transplant patients [16].
- In the immunosuppression regimen of renal transplantation, tacrolimus is considered 'the cornerstone'. The hepatic and intestinal enzymes belonging to the subfamily of cytochrome P 450 3A/CYP3A, metabolize tacrolimus. The expression of this enzyme is influenced by a variation in the intron 3 of the CYP3A5 gene, thus affecting tacrolimus trough blood levels. The influence of CYP3A5 (A6986G) polymorphism on tacrolimus was studied in 25 renal transplant adults receiving a tacrolimus dose of 0.1 mg/kg/body weight, in 2 divided doses. Tacrolimus trough blood levels were assessed on post-operative, day 6. Patients homozygous for CYP3A5*1/*1 (40%) showed significantly higher frequencies of acute rejection episodes compared to carriers of CYP3A5*1/*3 (20%) or CYP3A5*3/*3 (13%) genotypes. Drug dosage of Tacrolimus showed significant correlation with CYP3A5 (A6986G) polymorphism; as evident from frequent acute rejection episodes in expressors who demand a higher drug dosage. While non-expressors exhibited a higher frequency in tacrolimus-induced nephrotoxicity. Thus insights on CYP3A5

variation prior to a renal transplantation might guide in optimizing tacrolimus dosage, besides preventing episodes of acute rejection and tacrolimus toxicity [17].

- In Japanese renal transplant recipients, the impact of CYP3A5 genetic variation, 6986A>G (*3) SNP, on the dose-adjusted tacrolimus trough concentrations (C_{0h}/D) and rejection incidence was studied. For genotyping, polymerase chain reaction-restriction fragment length polymorphism (PCRRFLP) was used and its results were confirmed using a fully automated SNP detection system (Prototype i-densy; Arkray Inc., Kyoto, Japan). The recipients taking tacrolimus formulations for a year post- transplantation as either Tac-BID twice a day (n = 140) or Tac-QD, a modified-release once-daily (n = 80). Recipients carrying even one CYP3A5*1 wild-type allele (EMs) and those showing homozygous expression of the variant allele CYP3A5*3 (PMs) were significantly identified using the tacrolimus C_{0h}/D cut-off values of 2.77 and 0.85 ng/mL/mg, respectively with a discrimination rate of 75.3 (Tac-BID group) and 85.4% (Tac-QD group). The coefficients of variation or %CVs) of tacrolimus C_{0h}/D in CYP3A5 EMs taking Tac-QD was significantly lower compared to those taking Tac-BID (20.4 versus 23.3%, P = 0.003). The %CV of the tacrolimus C_{0h}/D was an independently predicts rejection risk (OR = 1.028, P = 0.033). The stability of the C_{0h}/D achieved using Tac-QD might prevent the development of rejection, and this is undeniably influenced by the genetic variation in CYP3A5 [18].
- Episodes of acute rejection represent an important risk factor for the development of chronic allograft nephropathy. The interleukin 8 (IL8) -251AA genotype potentially predicted allograft outcome in renal transplant recipients of North-Indian ethnicity. The 'AA' genotype of IL8 T251A, increased the risk of allograft rejection by 2.7-folds in recipients suffering rejection episodes as compared to non-rejecters (OR = 2.70, P = 0.032). This was supported by the Cox proportional analysis which showed more than two fold increase in the susceptibility of allograft rejection (HR = 2.38, P = 0.010) in IL8 -251AA recipients. Kaplan-Meier analysis revealed a lower mean time to first rejection episode in AA genotype recipients (23 months) as against TT recipients (30 months) (log rank P = 0.022) [19].
- Cytotoxic T-lymphocyte-associated antigen 4 or CTLA4 might transmit an inhibitory signal to T cells, and might serve as a therapeutic target for acute rejection following a renal transplantation. Variations in CTLA4 gene such as +49A/G polymorphism, may be a possible genetic susceptibility locus for acute rejection, as supported by a meta-analysis showing significant correlation (for GG vs. AG + AA: OR = 1.35, 95% CI = 1.05-1.73, p = 0.02; for G vs. A: OR = 1.21, 95% CI = 1.03-1.42, p = 0.02), especially in the Asian subgroup (for GG vs. AG + AA: OR = 1.79, 95% CI = 1.15-2.78, p = 0.009; for G vs. A: OR = 1.47, 95% CI = 1.04-2.07, p = 0.03) [20]. Amongst the influencers, ratios of GG to GA+AA (p = 0.046) and G to A (p = 0.017) were significant factors [21].
- In a meta-analysis, CTLA4 G allele/GG genotype is associated with the acute rejection risk in renal transplantation (G allele: OR = 1.21, 95% CI: 1.03-1.44, P = .02; GG genotype: OR = 1.37, 95% CI: 1.10-1.69, P = .004). The AA genotype did not show any association with acute rejection risk in renal transplantation [22].
- In a transplanted kidney, the CC chemokine receptors (CCR2 and CCR5), influence the trafficking of leucocytes into the immune response sites. Eventually, inter-individual differences in the gene expression of CCR2 and CCR5 owing to their polymorphisms potentially alter several immune responses within the graft, ultimately deciding the allograft outcome. In a case-control study involving North Indians, 296 renal transplant recipients and 277 healthy controls were genotyped for CCR2V64I and CCR5-Delta32 polymorphisms. In CCR2V64I, at the 190 nucleotide position, substituting 'A' at amino acid position 64 encodes isoleucine and yields restriction fragments of 145 and 18 bp. On the contrary, if 'G' encoding a valine is present then the 163-bp amplicon remains uncut. The incidence of genotypes namely, CCR2+/64I (heterozygous) and CCR2-64I/64I (homozygous mutant) were relatively higher amongst non-rejecters in comparison with transplant recipients experiencing one or more rejection episodes (20.4% versus 8.2%), thereby resulting in a significantly reduced risk of allograft rejection (OR = 0.331, P = 0.026). The Kaplan-Meier curve also suggested higher mean time for the first rejection episode in CCR2-64I allele carriers (32.83 +/- 1.36 months) when compared with CCR2+/+ recipients (28.09 +/- 0.93 months, log P = 0.027).

The variant, CCR5-Delta32, did not show any profound effect on allograft outcome. This study supports the association of CCR2-64I allele with reduced risk for allograft rejection in North Indian transplant recipients, also influencing allograft outcome (statistical software package -SPSS 11.5; SPSS Inc., Chicago, IL, USA) [23].

- Ongoing advancements in the medical care of renal transplantation is evidenced through improvements in allograft survival. Still, episodes of renal allograft rejection in recipients is posing a major obstacle to successful organ transplantation. Efficacy in the development of antirejection strategies can be achieved with an insight on underlying molecular mechanisms of allograft rejection. For instance, the effect of genetic variations in chemokine receptors (namely, CCR5- 59029 A/G and CCR2-V64I) on allograft survival was studied in 84 renal transplant recipients by genotyping using polymerase chain reaction/PCR in a thermal cycler (ABI 9700). In CCR2V64I polymorphism, 173 bp region was amplified and the primer sequences were 5′TTGGTTTTGTGGGCAACATGATGG-3′ and 5′-CATTGCATTCCCAAAGACCCACTC-3′. For CCR5-59029G>A polymorphism, a region of 268 bp was amplified, and with sense primer as 5′-CCCGTGAGCCCATAGTTAA AACTC-3′ and the antisense primer as 5′-TCACAGGGCTTTTCAACAG TAAGG-3′. Renal transplant recipients were categorized as either 'Rejector group' rejection episode within a year of transplantation, or 'Non-rejector group' with stable graft function for at least 5 years. Rejection risk was significantly reduced in recipients possessing the CCR2-64I (A) allele (p = 0.03) or 59029-A allele (p = 0.03), implying the impact of genetic variations renal allograft survival (for statistically analysing the clinical and genotyping data the 'Statistical Package for Social Sciences software was employed - SPSS version 17; SPSS Inc., Chicago, IL, USA) [24].
- Therapeutic interventions such as blocking the CCR2 receptor, specifically its 'G' polymorphism might render better survival rates of renal allograft in this patient group. Chemokine receptors can potentially grab a place in the spectrum of immunogenetic factors (known association with renal allograft rejection). The influence of genetic variations in chemokine receptors (CCR2V64I, CCR5-59029G>A and CCR5Δ32) on the renal allograft rejection was studied in 606 renal transplant recipients along with an equal number of their donors. The GG genotype in CCR2V64I related with a high frequency of allograft rejection (p = 0.009; OR = 2.14; 95% CI = 1.2-3.7). Rejection episode(s) were significantly lower in the GA+AA genotypes were as compared to the GG genotype (p = 0.009; OR = 0.4; 95% CI = 0.2-0.8). The Kaplan-Meier curve also indicated a reduced overall allograft survival for carriers of GG genotype in CCR2V64I (59.2 ± 1.4 weeks, log p = 0.008). Associations with rejection were significant in female donors possessing the CCR2 GG genotype (p = 0.02; OR = 2.6; CI = 1.1-6.3) and male donors carrying the CCR5-59029 GG genotype (p = 0.004; OR = 1.7; CI = 1.03-3.01). CCR2V64I (G/G) genotype is associated with renal allograft rejection [25].
- Acute rejection (AR) contributes to the development of chronic allograft nephropathy that is the major cause of graft failure. Presence of the A allele, i.e. recipients carrying 'A' allele (+) grafts exhibited poor graft survival (P = 0.008 by a log-rank test). To add on, graft survival was affected by the number of 'A' alleles; recipients carrying more number of 'A' alleles showed poor graft survival ('A' allele number 0 and 1 versus 2 versus 3 and 4, P = 0.011; 0 and 1 versus 3 and 4, P = 0.08; 0 and 1 versus 2, P = 0.002; by a log-rank test using SPSS for Windows package 16.0; SPSS Inc., Chicago, IL, USA) [26].
- A meta-analysis provided evidence for the association of recipient's TNF-A -308G/A polymorphism with acute rejection. The presence of TNF2 allele positive genotype in donor or recipient probably increased the incidence of acute rejection of renal allograft. The TNF2 allele positive genotype in recipient associates with an increased risk of recurring acute rejection episodes in renal allograft. A pooled OR of 1.44 (95% CI = 1.05-1.99, p = 0.03) was obtained based on 460 cases (whose recipient developed Acute Rejection/AR) and 623 controls (whose recipient did not develop AR) [27].
- Association of the genetic variation IL-6 -174G/C (rs1800795) in donor/recipient with acute rejection (AR) of renal allograft was analysed in a case-control study (with 341 cases, whose recipient developed acute rejection and 702 controls, whose recipient didn't develop acute rejection). The IL-6 -174G/C polymorphism's high producer genotype (G/G and G/C) in donor showed lowered risk tendency for acute rejection, although it was not statistically significant [28].
- A study involved 199 subsequent kidney graft recipients from deceased donors (without induction therapy). The aim was to associate genetic variations of TGF-β1/Transforming growth

factor-β1 at 10 (þ869) T/C (leucine/proline) and codon 25 (þ915) G/C (arginine/proline) codons with the incidence of delayed graft function (DGF), acute rejection (AR) and 5-year kidney graft loss (STATISTICA 8.0 PL for Windows software package StatSoft Polska, Krakow, Poland and MEDCALC 11.3.8., Mariakerke, Belgium used for statistical analysis). The TGF-β1 genotype showed significant associations with frequent early AR episodes. Genetic variation of IL-6 gene (-174G/C) showed association with the death-censored kidney graft survival. The risk of graft loss during 5-year follow-up period was greater by 57% for GG or GC (higher IL-6 production) than for CC carriers. None of the other analysed polymorphisms significantly influenced both patients and kidney graft survival, also in the analysis of the subgroup with human leucocyte antigen-DR mismatch. The IL-6 gene variant (-174G/C) in a kidney graft recipient can modulate the rate of graft excretory function deterioration and the risk of graft loss by influencing their constitutional expression. Elaborating on the methodology, for DNA amplification, thermal cycler Biometra UNO II (Biometra GmbH, Gottingen, Germany) was used [29].

- In solid organ transplantation, acute rejection episodes are implicated by the cytokines. Evidence form a meta-analysis suggests an association of IL-4 -590C/T polymorphism in the recipient with acute rejection of liver transplantation (not observed for heart or renal transplantation). Additionally, a combined genotype of IL-4 -590*T-negative in recipient/T-positive in donor may probably suffer a lower risk for acute rejection of solid allograft [30].
- In solid organ transplant recipients, the 'Transforming growth factor beta-1' or TGFB1 is involved in episodes of acute rejection (AR). Scientific data from a meta-analysis and a systematic review reveal that the TGFB1 gene polymorphism (+869T/C) in donor shows significant association with acute rejection of solid organ in transplant recipients (especially amongst those in CsA/FK 506 group compared to the CsA group). This meta-analysis considered PUBMED, EMBASE, China National Knowledge Infrastructure (CNKI) and Wanfang Database until 10th October, 2013 for identifying eligible studies on association of TGFB1 +869 T/C polymorphism with AR after solid organ transplantation. The STATA software (version 10.0; Stata Corporation, College Station, Texas, USA) was used for statistical analysis [31].
- Association of genetic polymorphisms in TNF-alpha/tumour necrosis factor-alpha and TGF-beta1/transforming growth factor-beta1 with renal allograft rejection was studied in Koreans. The patients (100 controls and 164 patients) underwent renal transplantation, having one or more Human leukocyte antigen (HLA)-A, HLA-B and HLA-DR antigens mismatched with their donors. Frequencies of variants namely, high-producer genotype (-308GA) in TNF-alpha gene, and lower (intermediate)-producer genotype (codon 10 CC and codon 25 GG) of TGF-beta1 gene were significantly higher in patients with recurrent acute rejection episodes (REs), compared to those with no or one RE. Analysis of chronic renal allograft dysfunction (CRAD) revealed that the highest risk group for developing CRAD showed the combination of recipient's TNF-alpha high- and donor's TGF-beta1 high-producer genotypes. In this research study, the SNP sites analysed in the TGF-b1 gene were at the position of codon 10 (þ869) T/C (leucine/proline) and codon 25 (þ915) G/C (arginine/proline). For associating genotype groups with the occurrence of acute REs and CRAD, linear-by-linear association analysis (using 2 x k tables) was employed. While statistical analysis through SPSS for Windows version 11.5 (SPSS Inc, Chicago, IL, USA) with a significance level of $P<0.05$ was considered [32].
- Fibrinogen and von Willebrand factor have a significant role in platelet aggregation; both have a membrane receptor called 'Glycoprotein IIIa/IIb'. This receptor's beta integrin chain known as GPIIIa is polymorphic, with an allele (PlA2) linked with coronary thrombosis. The PlA2 polymorphism independently predicts the risk of acute renal graft rejection by affecting the graft survival (short-term). In a cohort of 119 consecutive renal allograft recipients (46.3 +/- 13 yr; 85 M/34 F; 24.4% diabetic patients), the GPIIIa genotype was determined by PCR-restriction fragment length polymorphism. After a year of follow-up, those who suffered an acute rejection (n = 52) showed a lower proportion of HLA-DR beta1 identity with the donor (7.7% versus 23.9%; P = 0.03), a higher proportion of cytomegalovirus-positive (CMV+) donors/CMV- recipients (21% versus 7.5%; P = 0.05). And the PlA2 allele was more frequent in 52 acute rejection sufferers compared to the 67 patients who were free from acute rejection (48.1% versus 26.9%; P = 0.02). Incidence of PlA2 allele gave an odds ratio

of 2.75 (1.01 to 7.93, with 95% confidence interval), and a HLA-DR beta1 identity of 0.2 (95% confidence interval, 0.06 to 0.99) for suffering an acute rejection episode. At discharge, the serum level of creatinine was relatively higher in PlA2-positive patients compared to the PlA2-negatives (2.2 +/- 1.6 versus 1.5 +/- 0.6 mg/dl, respectively; P = 0.01). A year after transplantation, the prevalence of proteinuria (>1.5 g/day) was significantly higher among patients showing the PlA2 allele (16% versus 3%; P = 0.02). On the whole, a two-year graft survival rate was significantly reduced in PlA2-positive patients (n = 43) compared to PlA2-negatives (n = 76) patients (85.7% versus 97.2%; P = 0.015) [33].

- The antibody-mediated rejection or AMR is defined by the presence of a donor-specific antibody (DSA) directed towards the human leukocyte antigen (HLA).The AMA emerges as a foremost reason for limiting the long-term survival of a graft. The paucity in overcoming the clinical treatment of AMR, insists the urgency to identify potential biomarkers for AMR. During inflammatory circumstances, B cells may differentiate into antibody-secreting plasmablasts and regulatory B cells (Bregs). Plasmablasts generate alloantibodies which result in AMR, while the Bregs are a special B cell subset capable of secreting the immunosuppressive cytokines, especially interleukin (IL)-10. Thus, Bregs have immunoregulatory functions inducing immune homeostasis, and resistance to AMR after kidney transplantation [34].

- Immune response in a transplantation is modulated by the regulatory B cells /Breg which serve as a transcription factor or specific phenotypic marker for prolonging experimental allograft survival. This attribute of Breg potentially gives them a scope for clinical use as an immune monitoring tool with exciting prospects for cell therapy. The most widely used marker of Breg is Interleukin-10/ IL-10. The ratio of B-cell IL-10 and tumor necrosis factor α can predict immunologic reactivity and clinical outcome of liver and kidney transplantation. Identification of patients who require more immunosuppression can be achieved through the assessment of Breg:B effector balance using their IL-10/tumor necrosis factor α ratio which might hike the success rate of potential therapies [35].

Conclusion

The best choice of treating an end-stage, chronic organ disease is transplantation. In transplantation, a functionally compromised organ of an individual gets replaced with a functionally efficient organ, and it all starts with a donor. Donor and recipient genetic interplay influences transplant outcome, but these complex interactions are at a nascent stage of exploration in a clinical setting. A more personalized approach to transplant care can be achieved through the development of genetic risk prediction models, which consider the profile of both donor and the recipient in precisely assessing the post-transplant outcomes.

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