

# Influence of Genetic Polymorphisms on Renal Allografts: Role of Immunological and Non-Immunological Genetic Variants

Gaurav Tripathi<sup>1</sup>, Manuel Arias<sup>2</sup>, Minal Borkar<sup>1</sup>, Gema Fernandez-Fresnedo<sup>2</sup> and Marcos López-Hoyos<sup>1</sup>

<sup>1</sup>Immunology and <sup>2</sup>Nephrology Services, Hospital Universitario Marqués de Valdecilla-IFIMAV, Santander, Spain

## Abstract

**Renal failure arises as a complication of many diseases such as diabetes and vascular disease, and is affected by several factors including age and ethnic origin of the patient. Dialysis or renal transplantation remains the only treatment modality for end-stage renal disease. There are several single nucleotide polymorphisms reported to be associated with different degrees of severity or of success of allografts after renal transplantation. These single nucleotide polymorphisms are located in or around those genes which directly or indirectly result in renal allograft failure or affect survival.**

**However, the field of organ transplantation has generated many fundamental principles of the mechanisms of acute rejection based on the analysis of selected genes, with characterization of their specific fundamental role in the immunological cascade leading to organ rejection. Nevertheless, the redundancy of the immune system suggests that numerous other molecular pathways with their resident genes interplay in the effector response and contribute to the heterogeneity of the graft outcome. Therapeutic and prognostic heterogeneity of organ injury is currently impossible to predict, resulting in a “one treatment fits all” approach. Finally, the targets would be towards patient-specific prognosis and therapy. There are several single nucleotide polymorphisms present in different genetic determinants of donor and recipient, which alters the success or failure of grafts after kidney transplantation. The list includes several gene polymorphisms of both immunological and non-immunological factors.**

**The present review examines recent data on the inheritable genetic variations in the genes of different immunological (other than human leukocyte antigen) and non-immunological factors and their effect on renal allograft graft survival or rejection. We have mainly focused on relevant studies that have shown an association with particular genetic variants, where the findings have also been replicated in different groups of patients at different transplant centers. (Trends in Transplant. 2011;5:83-112)**

Corresponding author: Marcos López Hoyos, mlopezhoyos@humv.es

## Key words

**Acute rejection. Allograft. Polymorphism. SNP. Th1 and Th2 system.**

### Correspondence to:

Marcos López Hoyos  
Servicio de Inmunología  
Hospital Universitario Marqués de Valdecilla  
39008 Santander, España  
E-mail: mlopezhoyos@humv.es

## Introduction

The recent emergence of the concept of a “genetic predisposition” associated with renal allograft failure highlights the presence of inheritable allelic differences in the genes encoding various immunological and non-immunological factors, which are associated with different rates of graft survival. Depending upon their effects on the transcription level of the concerned genes, such allelic variants can result in high, moderate, and low producing phenotypes<sup>1-6</sup>, suggesting that to some extent the expression level of these factors is predetermined in each individual. Hence, a genotypic profile of the relevant genes might provide a clinical guideline for monitoring a transplanted patient.

In this context, human leukocyte antigen (HLA) matching both at class I and II loci is well established and is generally undertaken for specific donor-recipient pairs that are known to contribute to significantly better transplant outcomes worldwide. However, the variability in clinical outcome observed with HLA-matched renal transplants highlights the redundancy of the immune system, and suggests that numerous other molecular genetic pathways are involved in maintaining or diminishing graft function. Furthermore, the issues of genetic predisposition and the success of renal transplantation become more complicated when the different genetic associations observed in different ethnic groups are considered<sup>7,8</sup>.

This review examines recent data on the inheritable genetic variations in the genes of different immunological (other than HLA) and non-immunological factors and their effect on renal allograft graft survival (Tables 1 and 2). We have mainly focused on relevant studies that have shown an association with particular genetic variants, where the findings have also been replicated in different groups of patients at different transplant centers. Furthermore, we have tried to incorporate those studies that

have utilized such novel approaches as dual analysis of both donor and recipient; studies dealing with other important aspects such as the type of HLA mismatch or immunosuppressive therapy in addition to the non-HLA genetic variants were also included.

## Immunological factors leading to renal failure

### *Immunobiology of transplantation*

The knowledge of molecular immunology, with a better understanding of the cellular and molecular mechanisms that underline the immunological response to transplanted organs, led to the discovery of new immunosuppressive agents such as tacrolimus, rapamycin, interleukin-2 receptor monoclonal antibodies, and mycophenolate mofetil. All these drugs show selective mechanisms for T- and B-cell alloimmune responses<sup>9</sup>. Presently combinations of various drugs have reduced the rejection rates tremendously. However, vigorous and prolonged immunosuppression results in infections and malignancies. If immune tolerance can be developed, then the side effects of immunosuppression can be reduced. The trend is to develop agents that are capable of blocking the co-stimulatory pathway of allo-recognition, which can result in tolerance<sup>9</sup>.

### **Non-classical HLA and non-HLA genes in the HLA class I/II regions**

The application of molecular techniques like cloning, sequencing, and gene mapping have also revealed a number of additional HLA and non-HLA genes in the class I/II regions. In the class I region, there are known to be 17 ‘non-classical’ genes or gene fragments, although only three of these (HLA E, F, and G) are known to be transcribed<sup>10,11</sup>. Little is yet known of the possible function of HLA E and F. More is known of HLA G, which is

Table 1. Role of immunological genetic variants and their association with renal allograft

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Region	Association with allograft rejection (Reference)	No. association with allograft rejection (Reference)
Immunological biomarkers/polymorphisms								
Proinflammatory								
1.	TNF- $\alpha$	G(-308)A, Chr-6p21	rs1800629	Inflammation, proinflammatory	Proinflammatory, local inflammation, endothelial activation	USA, Iran, Bulgaria, UK, Poland, Belgium, Mexico, India, Czech Republic, Finland, Greece, Germany, France	2, 28, 30, 33, 36, 37, 40, 41, 42, 43, 45, 46, 47, 48, 64	4, 28, 30, 47
2.	TNF- $\alpha$	G(-238)A, Chr-6	rs361525	Inflammation, proinflammatory	Proinflammatory, local inflammation, endothelial activation	USA, UK, Iran	2, 28, 33	NA
3.	IFN- $\gamma$	T(+874)A, Chr-12	rs2430561	Inflammation, proinflammatory	Proinflammatory, pro-cellular, play a pivotal role in both innate and adaptive immune responses	USA, Iran, Bulgaria, Italy, Belgium, UK	6, 8, 26, 30, 32, 33, 34, 35	2, 32, 33, 36, 37, 39
4.	INF- $\gamma$	CA repeat, Chr-12	PubMed ID: 10068907	Inflammation, proinflammatory	Proinflammatory, pro-cellular	UK, USA, Iran, Mexico, Finland	4, 33, 35	2, 6, 43
5.	IL-6	-174G/C, Chr-7	rs1800795	Inflammation, proinflammatory	Pro-cellular and humoral, acute phase protein production, fever	USA, Japan, UK, Bulgaria, Poland, India, Germany	2, 6, 22, 23, 46	26, 27, 28, 29, 30
6.	IL-6	+572 G/A, Chr-7	Not found	Inflammation, proinflammatory	Pro-cellular and humoral, acute phase protein production, fever	USA, UK, Germany	2	8, 29
7.	IL-4	C590T, Chr-1	rs2243250	Inflammation, proinflammatory	IL-4 inhibits the release of inflammatory mediators (such as TNF- $\alpha$ , IL-6, and IL-1 $\alpha$ ) from activated monocytes, thus counteracting their damaging effects in the graft	UK, Finland, Poland, Korea, USA	NA	38, 43, 46, 48, 62
8.	IL-4	+1902 G/A, Chr-1	rs1801275	Inflammation, proinflammatory	Inhibits the release of inflammatory mediators	UK	NA	46
9.	IL-2	-330, Chr-4	PubMed ID: 15895884	Inflammation, proinflammatory	Control the immune response, regulate and coordinate acute renal allograft rejection	USA, USA, Finland	26, 43	2

(Continue).

Table 1. Role of immunological genetic variants and their association with renal allograft (continued)

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Region	Association with allograft rejection (Reference)	No. association with allograft rejection (Reference)
10.	CCR5	d32, Chr-3	PubMed ID: 9508061 I	inflammation, proinflammatory	Regulate the trafficking of leukocytes in immunity and inflammation	Iran, Germany, USA	74, 76, 78, 81	77, 79
11.	CX3CR	-V249I, Chr-16	rs3732379	Inflammation, proinflammatory	Regulate the trafficking of leukocytes in immunity and inflammation	Germany, USA	76, 80, 81	77
12.	CX3CR	-T280M, Chr-16	rs3732378	Inflammation, proinflammatory	Regulate the trafficking of leukocytes in immunity and inflammation	Germany, USA	76, 81	77
13.	CCR2	-V64I, Chr-3	rs1799864	Inflammation, proinflammatory	Regulate the trafficking of leukocytes in immunity and inflammation	Iran, USA	74, 77, 80, 81	NA
14.	CCR5	(-59029) A/G, Chr-3	rs1799987	Inflammation, proinflammatory	Regulate the trafficking of leukocytes in immunity and inflammation	Iran, USA, Germany	74, 77, 78, 80	NA
15.	Lymphotoxin	+249A/G, Chr-6	rs909253	Inflammation, proinflammatory	A protein that is produced by "killer" CD8+ T-cells that kill virally infected cells by producing holes in the cell's cell membrane	Tunisia, UK, USA	97	7, 98
16.	Lymphotoxin	+365 C/G, Chr-6	Not found	Inflammation, proinflammatory	Associated with increased production of the TNF- $\beta$ lymphotoxin following PHA mitogenic stimulation <i>in vitro</i>	Tunisia, UK, USA	97	7, 98
17.	Lymphotoxin	+720 C/A, Chr-6	Not found	Inflammation, proinflammatory	Associated with increased production of the TNF- $\beta$ lymphotoxin following PHA mitogenic stimulation <i>in vitro</i>	Tunisia, UK, USA	97	7, 98
18.	MIF	-G173C, Chr-22	rs755622	Inflammation, proinflammatory	Macrophage migration inhibitory factor induces the expression of proinflammatory mediators by macrophages and activates T-cells	Iran, Sweden, China, Italy	33, 53, 55, 94, 95	NA
19.	IL-1 $\alpha$	-C889T, Chr-2	rs1800587	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland, Sweden, UK	54, 55	7, 46
20.	IL-1 $\beta$	-C5887T, Chr-2	rs1143633	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland, Sweden UK, UK	54, 55	7, 46

(Continue).

Table 1. Role of immunological genetic variants and their association with renal allograft (continued)

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Region	Association with allograft rejection (Reference)	No. association with allograft rejection (Reference)
21.	<i>IL-1<math>\beta</math></i>	-T1903C, Chr-2	rs1143627	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Sweden	55, 56	NA
22.	<i>IL-1<math>\beta</math></i>	-C3953T, Chr-2	rs1143634	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Sweden, India	55, 56	NA
23.	<i>IL-1<math>\beta</math></i>	T(+3962)C, Chr-2	rs16944	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland, Sweden, India	55, 56	54
24.	<i>IL-1<math>\beta</math></i>	-C511T, Chr-2	rs16944	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland, Sweden, India	55, 56	54
25.	<i>IL-1RN</i>	+A9589T, Chr-2	rs454078	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	India	NA	56
26.	<i>IL-1RN</i>	+C8061T, Chr-2	rs423904	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland	NA	54
27.	<i>IL-1RN</i>	+C11100T, Chr-2	rs315952	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland	NA	54
28.	<i>IL-1Ra</i>	86bpVNTR, Chr-2	PubMed ID: 14563376	Inflammation, proinflammatory	IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells	Poland, India	54, 56	NA
29.	<i>ICAM-1</i>	E469 exon 6, Chr-19	rs5498	Inflammation, proinflammatory	Involved at distinct stages in the host response	USA, UK, Tunisia, Mexico	85, 86, 87	87, 88
30.	<i>ICAM-2</i>	R241 exon 4, Chr-19	PubMed ID: 16432463	Inflammation, proinflammatory	Upregulation of adhesion molecules in CAN	Mexico, Tunisia	88	87

(Continue).

Table 1. Role of immunological genetic variants and their association with renal allograft (continued)

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Region	Association with allograft rejection (Reference)	No. association with allograft rejection (Reference)
31.	<i>L-selectin</i>	+206 F/L, Chr-1	PubMed ID: 10330415	Inflammation, proinflammatory	Involved at the stage of leukocyte rolling and tethering to the vascular endothelium	UK, Tunisia	17	87
32.	<i>E-selectin</i>	+128 S/R, Chr-1	PubMed ID: 11764211	Inflammation, proinflammatory	Involved at the stage of leukocyte rolling and tethering to the vascular endothelium	UK, Tunisia	17	87
33.	<i>E-selectin</i>	+554 L/F, Chr-1	PubMed ID: 19420919	Inflammation, proinflammatory	Involved at the stage of leukocyte rolling and tethering to the vascular endothelium	UK, Tunisia	17	87
34.	<i>CTLA4</i>	-318 G/C, Chr-2	rs5742909	Inflammation, proinflammatory	CTLA-4 is T lymphocyte receptors involved in the regulation of T-cell activation	Poland, USA, Tunisia, Tunis	20, 96, 97	98
35.	<i>CTLA4</i>	+49 (A/G), Chr-2	rs231775	Inflammation, proinflammatory	Involved in the regulation of T-cell activation	Poland, USA, Tunis, Tunisia	20, 96, 97	98
36.	<i>TAP1</i>	1A to 1C, Chr-6	PubMed ID: 12827444	Inflammation, proinflammatory	Involved in antigen presentation by MHC-I, in the transport of endogenous peptides	Japan, Turkey	16	164
37.	<i>TAP2</i>	2A to 2E, Chr-6	PubMed ID: 8894702	Inflammation, proinflammatory	Involved in antigen presentation by MHC-I, in the transport of endogenous peptides	Japan, Turkey	16	164
38.	<i>LMP2</i>	Chr-6	PubMed ID: 8089211	Inflammation, proinflammatory	Takes part in cytosolic proteolysis	Japan	16	NA
39.	<i>VEGF</i>	-1154A/G	rs 1570360	Proinflammatory	Expressed widely in renal tissues and a potent regulator of normal and abnormal angiogenesis	UK, The Netherlands	72	73
40.	<i>VEGF</i>	-2578C/A	rs 699947	Proinflammatory	Potent regulator of normal and abnormal angiogenesis	The Netherlands	NA	73
41.	<i>FC receptor</i>	FcγRIIA	Not found	Proinflammatory	Susceptibility to autoimmune diseases and link between the humoral branch and the effector cells of the immune system	China, Australia, Poland	89, 90	91

(Continue)

Table 1. Role of immunological genetic variants and their association with renal allograft (continued)

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Region	Association with allograft rejection (Reference)	No. association with allograft rejection (Reference)
42.	TLR	TLR4, Asp299Gly and Thr399Ile	rs4986790 and rs4986791	Proinflammatory	Toll-like receptors have a key role in innate immunity	Poland, Germany, Brazil, France, Hungary	106, 110, 112	111, 118
43.	TGFB	T(+869)C, Chr-19q13.2	rs1982073	Inflammation, anti-inflammatory	Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth	Iran, Bulgaria, Mexico, Germany, Korea, China	4, 28, 30, 31, 33, 62, 64, 68	21, 69
44.	TGFB	C(+915)G, Chr-19q13.2	rs1800471	Inflammation, anti-inflammatory	Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth	USA, Iran, Bulgaria, Germany, Korea, China	30, 47, 62, 68	21, 69
45.	TGFB	aa25 R/P, Chr-19q13.2	Not found	Inflammation, anti-inflammatory	Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth	USA, Iran, UK, India	30, 33, 68	8, 30
46.	TGFB	-509 C/T, Chr-19q13.2	rs1800469	Inflammation, anti-inflammatory	Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth	USA, UK, India	68	8, 30
47.	TGFB	-880 G/A, Chr-19q13.2	rs7067496	Inflammation, anti-inflammatory	Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth	USA, UK	NA	8, 30
48.	IL-10	G(-1082)A, Chr6q22-q23	rs1800896	Inflammation, anti-inflammatory	Play an important role in modulating inflammatory response in acute kidney injury	Iran, Bulgaria, Mexico, UK, Germany, India	4, 30, 43	20, 33, 35, 43, 48
49.	IL-10	C(-819)T, Chr-6q22q23	rs1800871	Inflammation, anti-inflammatory	Play an important role in modulating inflammatory response in acute kidney injury	Iran, Bulgaria, Mexico, India, Germany	30, 33, 38, 43	33, 35, 43, 62
50.	IL-10	C(-592)A, Chr-6q22q23	rs1800872	Inflammation, anti-inflammatory	Pro-humoral, anti-inflammatory, macrophage suppressant	UK, Bulgaria, Iran USA, Germany	30, 38, 43	28, 35, 43, 62
51.	DAFC	Arg89Cys	rs3027020	Anti-inflammatory	Duffy receptor for chemokines (DARC) show increased expression on post-capillary venules following transplantation	USA	80, 83	84

Table 2. Role of non-immunological genetic variants and their association with renal allograft

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Ethnicity	Association (Reference)	No. association (Reference)
Non-immunological biomarkers/polymorphisms								
1.	ACE	287 bp I/D intron 16, Chr-17q23	ID: 2554286	Hypertension	Increased p-ACE levels, association with CIN and ATN	Iran, USA, UK, China, Iran, India	33, 120, 121, 122, 149	123
2.	AGT	M235T, Chr-1 at 1p36-p34	rs4762	Hypertension	Progression with chronic Interstitial nephritis	Iran, USA, Turkey	33, 119, 120, 124	164
3.	ApoE	e2, e4, Chr-19q13.2.5	ID: 10662539	Atherosclerosis	Increases risk of cardiovascular disease after transplantation, elevated plasma Lp (a) levels	Spain, Spain, USA	145, 146, 147	NA
4.	eNOS	Intron 4 STR, 27 bp STR, Chr-7q36	ID: 18390539	Atherosclerosis	Increase severity of urinary protein excretion	China, India, Turkey, Italy	126, 129, 163	127, 128, 149
5.	Factor V Leiden	FVL G1691A, at Chr1q23	rs2232708	Factor Xa activation	Venous thromboembolism and deep venous thrombosis, increased coagulation	Germany, Australia, Turkey, India	134, 135, 137	143
6.	G protein	GNB3 C825T, Chr12p13, exon 10	rs5443	Hypertension	Higher CRP levels and mortality	India, Germany, Turkey, Czech Republic	131, 132, 148	149
7.	GSTT1	(±)480 bp, Chr-11q13	ID: 17000715	Phase II metabolizing enzymes	Activating and inactivating oxidative metabolites of carcinogenic compounds may associated with renal failure	Italy, Israel, India, Brazil	152, 153	154, 155
8.	GSTM1	(±)215 bp, Chr-11q13	ID: 12117698	Involved in detoxification	Activating and inactivating oxidative metabolites of carcinogenic compounds may associated with renal failure	Israel, India, Brazil	153, 154	155
9.	GSTP1	-313 A/G, Chr-11q13	rs1695	Involved in detoxification	Activating and inactivating oxidative metabolites of carcinogenic compounds may associated with renal failure	Israel, India	153, 154	NA
10.	Leptin	G2548A, Chr-7q22-35	rs7799039	Energy expenditure	Fat accumulation during dialysis	India	149	NA
11.	MTHFR	A1298C, 1 at Chr1p36.3	rs2066462	Pro-coagulation	Increases plasma homocysteine level, thrombotic risk	Bosnia and Herzegovina-Europe, India, Italy, Iran	134, 143, 144, 145	129

(Continue)



Table 2. Role of non-immunological genetic variants and their association with renal allograft (continued)

Sr No.	SNP	Position at chromosome	dbSNP ID/ PubMed ID	Effect on	Role	Ethnicity	Association (Reference)	No. association (Reference)
12.	MTHFR	C677T, Chr-1 at Chr 1p36.3	rs1801133	Pro-coagulation	Increases plasma homocysteine level, thrombotic risk	Iran, Italy, Bosnia and Herzegovina-Europe, India	33, 134, 143, 144, 145	129
13.	Prothrombin	PT G20210A, Chr-11	rs1799963	Factor XIII and fibrinogen activation	Thrombotic complications of the vascular access, increased F2 production	Australia, Turkey, Sweden, India	135, 137, 138	137, 143
14.	UCP2	45 bp I/D exon 8, Chr11q13	ID: 18839467	Energy expenditure	Fat accumulation during dialysis	Turkey, India, Sweden	148, 149, 150	NA
15.	VDR	Taq1, Chr-12q Apa1, Chr-12q Fok1, Chr-12q Bsm1 Chr-12q	rs731236 rs7975232 rs10735810 rs15444	Immune response	Involved in calcium absorption, excretion and modulation of cellular proliferation and differentiation	Iran, Ireland	157	33, 157

SNP: single nucleotide polymorphism; dbSNP ID: data base single nucleotide polymorphism; NA: not available; rs: reference sequence.

closely homologous to other class I gene sequences and was thought to show little polymorphism<sup>12</sup>.

Taken together, these collected discoveries have overthrown earlier concepts of the major histocompatibility complex (MHC) class I and II regions as solely containing genes encoding for molecules which present antigenic peptides to T-cells. Rather, the current view is of a genetic region encoding many different types of molecules collectively involved in pathways of antigen processing and presentation to helper and cytotoxic T-cells. These gene products may have a role in immunologically mediated rejection<sup>13,14</sup>.

### Role of cytokines in allograft rejection

Cytokines are potent immunomodulatory molecules that act as mediators of inflammation and the immune response. Transplant patients reveal inflammation and also show impaired immune response. There are two types of cytokines: (i) proinflammatory cytokines like interleukin-1 (IL-1), interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF- $\alpha$ ), and interferon gamma (IFN- $\gamma$ ) etc. that are involved in cell mediated immunity and cause allograft rejection; and (ii) anti-inflammatory cytokines like IL-4, IL-6, IL-10 etc., which preferentially control humoral responses and are correlated with allograft protection<sup>15</sup> (Table 1). Polymorphisms of cytokine genes may modulate gene expression at transcriptional level. As these polymorphisms segregate independently, each individual is a mosaic of high, moderate, and low cytokine-producing phenotype. The majority of the polymorphisms observed in cytokine genes are single nucleotide polymorphisms (SNP) or length polymorphisms (short tandem repeat, STR).

The vast majority of polymorphisms are found in the cytokine genes and their receptors

that are located in their regulatory promoter regions, while various others are found in the intronic, exonic, and untranslated regions. The promoter gene polymorphisms may disrupt or abolish transcription regulatory elements such as those involved for nuclear factor kappa-B (NF $\kappa$ B) and signal transducers and activators of transcription (STAT)<sup>16,17</sup>. All these regulatory elements regulate RNA polymerase binding, influencing the rate at which the gene is transcribed into mRNA. Intronic variation may affect enhancer/silencer sequences and certain polymorphisms may alter architectural transcription factor binding elements<sup>18,19</sup>. Several studies have addressed the extent to which specific polymorphisms directly or indirectly influence the level of cytokine production and expression<sup>20,21</sup>. Some of these cytokine gene variants are discussed below (Table 1).

## Interleukin-6

Interleukin-6 (IL-6) regulates many aspects of the immune response. The concentration of IL-6 increases in the cases of inflammation, tissue damage, hypoxia, or infection. Circulating IL-6 can be detected in healthy individuals in the 1 pg/ml range and is significantly elevated in some end-stage renal disease (ESRD) patients but not all<sup>22,23</sup>. It has a specific IL-6 receptor and a signal-transducing subunit (gp130). Circulating gp130 acts as an antagonist of IL-6 biological functions<sup>24</sup>.

The potential causes of elevated plasma IL-6 levels in renal transplant patients<sup>22</sup> may be due to (i) the loss of kidney function, (ii) uremia *per se* (and its sequelae, such as fluid overload, oxidative stress and susceptibility to infections), and (iii) dialysis-related factors. There is an association between plasma levels<sup>23</sup> of specific IL-6R and the progression rate of renal function in the pre-dialysis phase, as well as an association between changes in glomerular filtration rate and changes in IL-6 during peritoneal dialysis treatment<sup>23</sup>. Elevated circulating

IL-6 levels were independently associated with progressive carotid atherosclerosis during the first 12 months of dialysis treatment<sup>25</sup>.

Within the promoter region of the *IL-6* gene, a G/C polymorphism at position -174 affects the rate of transcription, producing high- or low-IL-6 producing phenotypes. The high-IL-6 genotypes (GG or GC) are found more frequently in Caucasians with type-1 diabetes mellitus and in African Americans with hypertension<sup>25,26</sup>. This *IL-6* genetic variant shows a direct correlation with graft failure, where -174C allele has a 3.7-fold relative risk of graft loss compared with G allele<sup>27</sup>. Furthermore, Marshall, et al.<sup>34</sup> have demonstrated the protective effect of the presence of the -174G allele from the donor on graft survival. Muller-Steinhardt, et al.<sup>28</sup> have shown that two more polymorphisms (572G/C and -597G/A) are present in *IL-6* gene, where -597 and -174 SNP are in tight linkage disequilibrium, and carriers of GGG/GGG genotype of the three loci have a superior three-year graft survival rate. Despite studies supporting its role as a determinant of renal allograft survival<sup>28-30</sup>, IL-6 is still grouped with the controversial cytokines<sup>20,27</sup> (Table 1).

## Interferon-gamma

Activated T-cells produce IFN- $\gamma$ , which has several properties: activation of macrophages, lytic effect, potentiation of the actions of other interferons, and inhibition of intracellular microorganisms other than viruses. The *IFN- $\gamma$*  gene lies in 12q15, is 4974 bp long, and encodes a protein of 166 amino acids long<sup>31</sup>. The *IFN- $\gamma$*  acts both as an antirejection and pro-rejection cytokine, e.g. by induction of microvascularization in the grafted organ and increasing the expression of MHC. Whether the main effect will be anti- or pro-rejection depends mainly on the secretion time after kidney transplantation, being protective early on and then antagonistic later<sup>32,33</sup>.

Further SNP in *IFN- $\gamma$*  may affect the production of *IFN- $\gamma$* . A polymorphic dinucleotide (CA)<sub>n</sub> microsatellite marker in the first intron of *IFN- $\gamma$*  at position -874 from the translation start site contains a SNP, T/A at the 59 end of the repeat, which produces high- and low-producer phenotypes. The high-producer recipient genotype is associated with acute rejection of kidney and heart, occurrence of infections, and development of fibrosis after lung transplantation<sup>6,34,35</sup>. A study has shown that early accumulation of donor's *IFN- $\gamma$*  in the graft causes immune tolerance induction, resulting in better graft survival<sup>36</sup>. In contrast, another study<sup>37</sup> reported that the presence of the high-producing 874T allele in donors is strongly associated with biopsy proven chronic allograft nephropathy. Apart from the 874T/A polymorphism, a 12 CA repeat allele (*IFNG\*2*) of intron first has been reported to be associated with high *IFN- $\gamma$*  production<sup>35</sup>. The 12 CA repeat was found in 54.3% of patients with acute rejection episodes in that study<sup>35</sup>. Furthermore, Asderakis, et al.<sup>35</sup> in their study of 88 renal transplant patients reported that the frequency of high *IFN- $\gamma$* -producing genotypes was significantly higher in patients with rejection episodes who were HLA-DR-mismatch (OR: 2.87). Additionally, they reported that immunosuppressant monotherapy (cyclosporine) in graft recipients with high *IFN- $\gamma$* -producing genotypes resulted in more rejection episodes (61%; OR: 3.06) than triple therapy. However, there are also reports available<sup>6,38</sup> that suggest that high *IFN- $\gamma$* -producing genotypes have no significant association with the rejection episodes.

The 'low-producer' *IFN- $\gamma$*  +874 A/A genotype was associated with a preventive effect on long-term C-reactive protein (CRP) elevation in hemodialysis patients, possibly mediated by decreased gene expression of *IFN- $\gamma$* <sup>39</sup>. On the contrary, another report<sup>33</sup> has shown an association of high-producing 874 T allele donors with biopsy proven chronic allograft nephropathy (Table 1).

## Tumor necrosis factor-alpha

The most studied cytokine is TNF, mainly known for its devastating effects on sepsis, leading to massive inflammatory reactions. It causes proinflammatory responses (fever, hypotension, shock, etc.) to several immunological challenges<sup>40</sup>. The *TNF* gene lies on 6p21.3 in the region of MHC class III and is in linkage disequilibrium with classical *HLA* genes. The *TNF* gene is 2778 bp long and encodes a protein of 233 amino acids<sup>40</sup>.

According to the classic proinflammatory scenario, failure to regulate the production of *TNF* at a site of immunological injury may lead to chronic activation of innate immune cells and to chronic inflammatory responses, which may consequently lead to organ-specific inflammatory pathology and tissue damage. Several studies have reported that *TNF* may also directly promote or downregulate the adaptive immune response<sup>41</sup>.

The most widely studied polymorphism among cytokine genes is within the *TNF- $\alpha$*  gene, involving a G→A substitution at position 308 in the promoter region encoding for a high responder genotype (GA or AA) and is associated with acute rejection of heart, kidney, and liver transplants<sup>2,42-45</sup>. The "A" allele results in a six- to sevenfold increase in the *in vitro* transcription of the *TNF- $\alpha$*  gene. Other important polymorphisms studied are -238 and +484<sup>28,33</sup>. It is reported that 14 repeats of (AC) di-nucleotide microsatellite in *TNF- $\alpha$*  is associated with renal allograft failure<sup>3</sup>. The *TNF- $\alpha$*  gene is one of those cytokine genes whose variants accompanied most of the contradictory reports. In the general population, the 308 G→A transition is associated with a state of high *TNF- $\alpha$*  production and susceptibility to several diseases<sup>33,40,46</sup>. Akalin and Murpy<sup>47</sup>, Hutchinson, et al.<sup>4</sup> and Marshal, et al.<sup>28</sup> have shown that high producing *TNF- $\alpha$* -308A allele is mainly affective in HLA-DR-mismatched, steroid-resistant, acute rejection

episodes. The two groups have reported that there was no significant association of *TNF- $\alpha$ -308* with *IL-101082* and *TGF- $\beta$*  codon 10 and 25 variants. However, both Poole, et al.<sup>48</sup> and McDaniel, et al.<sup>2</sup>, on the basis of their studies on 120 and 77 renal transplant recipients, have shown that *TNF- $\alpha$ -308* with *IL-10-1082* and *TGF- $\beta$*  codon 10 and 25 variants are highly associated with each other, and mainly have a cumulative effect on graft rejection episodes. In our view, this controversy could be because of the different immunotherapy regimes (triple versus single) used in these studies, as all the immune suppressors have significant effects on the expression profiles of cytokines. Furthermore, Hutchinson, et al.<sup>4</sup> have used the posttransplantation data at five years while Marshall, et al.<sup>28</sup> have used data at 30 days posttransplantation. Such discrepancies can only be ruled out if expression analysis of all associated genes can be carried out and experimental designs are of a similar nature (Table 1).

### Interleukin-1 gene cluster

Interleukin-1 is a pleiotropic cytokine, which is actively involved in the inflammatory response. The *IL-1* gene cluster is a 430 kb region of chromosome 2 (2q12-21). Vandembroeck, et al.<sup>49</sup> have reported that its three well-studied members, i.e. two agonists, *IL-1 $\alpha$*  and *IL-1 $\beta$* , and the antagonist *IL-1Ra*, *IL-1 $\alpha$*  and  $\beta$ , are proinflammatory cytokines that bind to the *IL-1* receptor, while the receptor antagonist, a competitive inhibitor at the receptor site of both molecules, downregulates the immune response<sup>49</sup>. The *IL-1* gene polymorphisms have been associated with a variety of diseases in which inflammation has been suspected to play a role<sup>50</sup>, including systemic lupus erythematosus<sup>51</sup> and cardiovascular disease<sup>52</sup>. Among them, some polymorphisms are located within the regulatory regions of the genes. Their localization in the regulatory region suggests that they may modulate IL-1 protein

production by directly affecting transcription, leading to their association with altered levels of *IL-1*. The *IL-1Ra* is expressed from the *IL-1RN* gene, which has a length variation within intron 2 caused by 86 bp variable number of tandem repeats (VNTR)<sup>53</sup>. According to the number of 86 bp repeats, there are five alleles corresponding to allele I (410 bp), II (240 bp), III (500 bp), IV (325 bp) and V (595 bp)<sup>53</sup>. Allele I and II mainly play a role in the production of *IL-1Ra*. Thus, the effect of this gene is to control inflammatory and host defense response as it causes vasorelaxation, increase adherence of lymphocytes and neutrophils to endothelial cells, and might be implicated in the immunobiology of renal allografts. Buraczynska, et al.<sup>54</sup> have reported that *IL-1Ra* gene polymorphism affects the progression of chronic renal failure. They further demonstrated that the *IL-1Ra* allele II is associated with a faster progression to renal failure.

Interleukin-1 beta (*IL-1 $\beta$* ) may further amplify inflammation and lead to malnutrition, by inducing anorexia, and muscle wasting due to increased protein breakdown. Several clinical studies have shown that the circulating level of *IL-1 $\beta$*  may affect the nutritional status, especially the body composition. Several *IL-1* gene cluster polymorphisms were reported and they may affect the prevalence of cytokine-mediated diseases. Although a number of factors are related to malnutrition and wasting in ESRD, proinflammatory cytokines, such as *IL-1 $\beta$* , may play an important role<sup>55</sup>. Manchanda, et al. studied the genetic association of *IL-1 $\beta$*  and *IL-1Ra* gene polymorphism with allograft function in 136 renal transplant patients<sup>56</sup>. They demonstrated that genetically determined low production of IL-1Ra may be a risk factor for rejection episodes and delayed graft function and that IL-1 $\beta$ /IL-1R $\alpha$  haplotype influences the impact of allograft outcome (Table 1). This could in part be due to genetic factors. Further research, especially regarding the *IL-1* gene cluster polymorphisms, is necessary to validate this hypothesis.

## Interleukin-2

Interleukin-2 (IL-2) is a lymphokine secreted by activated lymphocytes, which acts as a cofactor in the replication and differentiation of T-cells, B-cells, and natural killer (NK) cells during inflammation. The *IL-2* has a T→G substitution at position 330 relative to the transcription start site that has a variable frequency within the population<sup>57</sup>. The substitution is expected to have an influence on the level of *IL-2* production. However, to date no such correlation has been reported. It appears to interact with *IL-2* to stimulate the proliferation of T lymphocytes.

Pawlik, et al.<sup>58</sup> evaluated the role of promoter polymorphism of *IL-2* gene (−330), along with, *TNF-α* gene (−308 promoter polymorphism), *IL-4* gene (−590 promoter polymorphism), and *IL-6* gene (−174 promoter polymorphism) in 197 renal transplant recipients with well-functioning grafts for 2-18 years. They reported that there was no difference in relation to IL-2 and IL-4 promoter polymorphism. However, *TNF-α* −308 and *IL-6* −174 may be the genetic factors influencing renal allograft survival.

## Interleukin-4

Interleukin-4 (*IL-4*) is a T helper cell type 2 (Th2) cytokine produced by activated Th cells that inhibits macrophage migration<sup>59</sup>. The most commonly studied genetic variant of *IL-4* is 590C/T while that of *IL-4* receptor is 1902A/G. Lee, et al.<sup>46</sup> worked on IL-4 590C/T on the incidence of acute renal allograft rejection in HLA-DR mismatched transplants. They reported no association between the cytokine gene polymorphisms tested and the incidence of posttransplant acute rejection. Marshall, et al.<sup>60</sup>, along with IL-4 (−590T/C) polymorphism, studied 21 more polymorphisms in 11 cytokine and cytokine-receptor genes in 209 cadaveric renal transplant recipients. They did not find any association between

IL-4 polymorphism and the incidence or severity of acute renal allograft rejection.

Poole, et al.<sup>48</sup> have indicated that 96% of a total of 25 rejection episodes carries 590T allele. Similarly, 1902GG genotype of *IL-4R* results in high producer phenotype and increases the IL-4 binding several fold<sup>42</sup>, but no correlation with renal allograft failure has been reported so far.

## Interleukin-10

Interleukin-10 (*IL-10*) promotes the Th2-type immune response, leading to antibody production. The effects of *IL-10* are generally thought to be anti-inflammatory and to suppress the Th1-type immune response. It is also involved in the regulation and secreted by Th1 cells<sup>61</sup>. The *IL-10* gene lies in 1q32.2, is 4892 bp long and encodes a protein of 178 amino acids. There are several polymorphisms in the promoter region of *IL-10*. However, the effects of these on *IL-10* production remain unclear<sup>61</sup>.

Polymorphisms in *IL-10* regulatory sites was found at positions 1082G/A, 819C/T and 592C/A<sup>4</sup>. Corresponding to these polymorphisms, GCC haplotype is associated with high production and ATA haplotype with low production of *IL-10*<sup>35</sup>. The high-producer *IL-10* genotype is associated with allograft survival in heart transplants and has a long-term protective effect in renal transplants, but is less effective in acute rejections<sup>30</sup>. However, Alkaluppi, et al.<sup>43</sup> have found that genotype ACC/ACC and ATA/ATA (for 1082, 819 and 392 SNP) are predisposed to acute kidney rejection.

In *IL-10*, three polymorphisms (1082G/A, 819C/T and 592C/A) are the most important polymorphisms associated with graft survival. Park, et al.<sup>62</sup> and Kocierz, et al.<sup>20</sup> have reported that none of these SNP except 1082G/A have a significant effect on graft survival rate, but their combination with various low-producing polymorphisms of Th1 cytokine genes, like

*IFN- $\gamma$* , *TGF- $\beta$*  and *TNF- $\alpha$* , have shown strong protective effects.

## Growth factors

### *Transforming growth factor-beta 1*

Transforming growth factor-beta 1 (*TGF- $\beta$ 1*) regulates proliferation and differentiation of many cell types. In the immune system, *TGF- $\beta$ 1* affects many cell types, e.g. T-cell survival, proliferation, Th differentiation, and effector functions<sup>63</sup>. The *TGF- $\beta$ 1* gene lies in 19q13.2, is 23402 bp long and encodes a protein of 390 amino acids<sup>63</sup>. In kidney transplantation, *TGF- $\beta$ 1* is known as a fibrogenetic factor, whose increased expression has been associated with chronic allograft rejection<sup>62,64</sup>. The *TGF- $\beta$ 1* is probably the second most important fibrogenic cytokine after *IL-1* in renal interstitial fibrogenesis and is mainly involved in tissue differentiation and repair<sup>65</sup>.

The *TGF- $\beta$ 1* gene polymorphisms, located in the positions 869T/C and 915G/C at codons 10 and 25, respectively, in the signal sequence, have variable effects on the production of *TGF- $\beta$* <sup>166,67</sup>. The TT and the GG genotypes of the 869T/C and 915G/C are high producers, whereas the CC is a low-producer type. A T/C variant at position 869 on codon 10 replaces leucine to proline, causing modification of charge and distortion of  $\alpha$ -helix involved in transport of *TGF- $\beta$*  protein through the cell. In renal transplants, the exact role of this polymorphism is not yet elucidated. However, Cho, et al.<sup>68</sup> and Li, et al.<sup>69</sup> have shown its role in chronic allograft nephropathy. Melk, et al.<sup>21</sup> studied 75 renal allograft dysfunction patients and 125 controls and found that codon 10 low-producing genotype showed a low expression of *TGF- $\beta$*  gene, but their expression was still higher than the controls. They have deduced that the SNP may not play a significant role once the immune system is activated as no significant correlation was found between the

codon 10-869C allele and graft rejection<sup>21</sup>. Some studies<sup>30,42,62,70</sup> have clearly illustrated that high-producing *TGF- $\beta$*  genotypes (GG at codon 25) in recipients have high acute rejection episodes, while those with genotypes TT/TC at codon 10 in donors are associated with chronic renal allograft dysfunction (Table 1).

### *Vascular endothelial growth factor*

Vascular endothelial growth factor (VEGF), expressed widely in renal tissues, is a potent regulator of normal and abnormal angiogenesis<sup>71</sup>. Shahbazi, et al.<sup>72</sup> have studied two SNP in the promoter region, 1154A/G and 2578C/A, and have reported that 1154G and 2758C alleles encode higher VEGF production and increase the rejection risk several fold. Lemos, et al.<sup>73</sup> investigated the SNP at 1154, 1157, and 2578 positions of VEGF gene among 305 donors and 387 recipients of renal transplants. No effect was seen in cases of acute rejection, but -2578C/A, AA homozygote, associated with low production of VEGF, had worse graft survival ( $p = 0.03$ ) in comparison to the high-producing CC homozygotes or CA heterozygotes (Table 1).

### ***Role of chemokines and their receptors***

Chemokine biology underlies the diverse pathologies that take place during transplant rejection<sup>74</sup>. Sequential waves of chemokine expression seen in the graft shortly after transplantation play a central role in graft injury through the orchestration of recruitment of chemokine receptor-expressing leukocytes in the weeks, months, and years following transplantation. Events that occur during or shortly after transplantation induce early alloantigen-independent organ injury. The initiators of such an injury subsequent to transplantation are multifactorial and include ischemic damage and surgical stress leading to intra-graft recruitment of phagocytes and monocytes through specific

chemokine expression<sup>75</sup>. Chemokine receptor polymorphisms may have diverse effects on the rejection process<sup>74</sup>. The common genetic variants described in renal transplant patients<sup>76</sup> are *CCR5-D32*, *CCR5-59029-A/G*, *CCR2-V64I*, *CX3CR1-V249I*, and *CX3CR1T280M* (Table 1). With the exception of *CCR2-V64I*, each of these genetic variants has been shown to affect chemokine receptor function and/or expression. Data suggest that relatively few chemokine receptors play central roles in allograft rejection, and chemokine blockade, either nonselective or specific, has shown promising results in experimental transplantation<sup>76,77</sup>.

### **Chemokine receptor-5 deletion of 32 base pairs**

The most important and common gene polymorphism in the chemokine receptor CCR5 is the deletion of 32 bp from its coding gene (*CCR5-D32*), which causes a functional blockage of CCR5. Homozygosity for the *CCR5-D32* allele in transplant recipients was associated with a highly significant survival rate. Nelson, et al.<sup>78</sup> have reported that absence of *CCR5* results in a significantly prolonged renal allograft half-life (60 vs. 17 years) as compared to the heterozygous or wild-type allele.

The functional importance of *CCR5-D32* in human allograft dysfunction was dramatically demonstrated in a large cohort of patients. Approximately 1% of individuals of northern European heritage are homozygous for *CCR5-D32*, a null allele of *CCR5* resulting from a 32 bp deletion within the coding region. These individuals show no obvious phenotype, but are highly resistant to productive infection with HIV. The prevalence of the *CCR5-D32* genotype was studied in 1,227 renal transplant recipients<sup>79</sup>. Twenty-one patients were identified as homozygous for *CCR5-D32*. Of the 22 renal transplants performed in these 21 patients, only one transplant showed a loss of function during follow-up<sup>79</sup>.

### **Chemokine receptor-5 –59029-A mutation**

Nelson, et al.<sup>78</sup> analyzed data from 163 renal allograft recipients for chemokine receptor polymorphisms and the incidence of acute renal allograft rejection. Patients homozygous for *CCR5-59029-A* mutation, showed a twofold reduction ( $p < 0.05$ ) in the incidence of acute rejection, whereas patients with the *CCR5-59029-G* allele showed an increased rate of acute rejection ( $p < 0.05$ ). The *CCR5-59029-A* homozygosity is potentially associated with increased promoter activity and enhanced *CCR5* expression by CD4<sup>+</sup> T-cells<sup>78</sup>. In another study, Abdi, et al.<sup>77</sup> analyzed data from 163 renal allograft recipients with regards to chemokine receptor polymorphisms and the incidence of acute renal allograft rejection. Patients homozygous for *CCR5-59029-A*, showed a twofold reduction ( $p < 0.05$ ) in the incidence of acute rejection, whereas patients with the *CCR5-59029-G* allele showed an increased rate of acute rejection ( $p < 0.05$ ).

### **Chemokine receptor-8 and CX3CR1**

Chemokine receptors play pivotal roles for leukocyte recruitment in acute and chronic inflammatory processes. In the promoters of human *CCR8* and *CX3CR1* as well as *CX3CR1* coding polymorphisms, SNP are associated with arteriosclerosis susceptibility and this may have a deleterious effect on the allograft<sup>80</sup>.

Chemokine receptor *CX3CR1* plays a role in transplant rejection based on animal models of heart transplantation. A study was recently performed by Hoffmann, et al.<sup>81</sup> to analyze the expression, distribution, and cellular localization of *CX3CR1* in human renal transplant biopsies. The *CX3CR1* was prospectively analyzed in 174 renal graft biopsies from patients with normal morphology ( $n = 76$ ), antibody-mediated acute rejection ( $n = 6$ ), acute tubulointerstitial rejection ( $n = 27$ ), acute

vascular rejection (n = 31), and with acute tubulus necrosis (n = 34). They reported that CX3CR1 was associated with acute renal allograft rejection in human renal transplantation.

### Duffy antigen/receptor for chemokines

The Duffy antigen/receptor for chemokines (DARC) show increased expression on postcapillary venules following transplantation<sup>82</sup>. Expression of DARC on red blood cells has been suggested to have an anti-inflammatory role by acting as a “chemokine sink”. Targeted disruption of DARC leads to impaired neutrophil infiltration, and thus this receptor may be important in the initial injury response in organ transplantation<sup>80</sup>. Genetic differences in DARC are well characterized. Reduced Duffy expression results from mutations affecting transcription (mutated GATA box in one allele) or instability of the translated protein (Arg89Cys). The frequency of these mutations varies among populations. Delayed graft function was strongly associated with graft failure for Duffy (a<sup>-</sup>b<sup>-</sup>) African American patients (p = 0.003)<sup>83</sup>. Duffy (a<sup>-</sup>b) patients were found to have a lower allograft survival in the presence of delayed graft function. However, a prospective, multicenter cohort study reported by Mange, et al. enrolled 222 African American recipients of cadaveric renal allografts from eight adult transplant centers<sup>84</sup>. Subjects were typed for the polymorphism of DARC at position 535 that determines the level of transcription. They confirmed that rejection was not found to be associated with DARC alleles or genotype.

### Adhesion molecule expression in allograft rejection

Intercellular adhesion molecule-1 (ICAM-1) is involved in leukocyte adhesion and enhancement of vascular permeability, causing infiltration of Th1 cytokines into the graft<sup>85</sup>. The genetic variant *ICAM1* Gly241Arg,

resulting from a G/A SNP in exon 4, is reported to increase the expression profile of ICAM-1 and is significantly associated with graft loss<sup>86-88</sup>. However, another SNP (C/A in exon 6, resulting in the amino acid substitution Glu469Lys) has shown no such association<sup>86</sup> (Table 1).

### Fc receptor

Several studies have identified FcγRIIIb (Fcgr3b) polymorphisms that determine susceptibility to autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. The human Fc gamma receptor IIA (FcγRIIA) forms an essential link between the humoral branch and the effector cells of the immune system. Yuan, et al found an association between FcγRIIA polymorphism and acute renal-allograft rejection<sup>89</sup>. The frequency of homozygosity for FcγRIIA-R/R131 in the rejectors was significantly higher than that in the non-rejector recipients. In another study, Xu, et al. worked on FcγRIIIb allele polymorphisms in 171 renal recipients. They reported that FcγRIIIb polymorphisms did not affect the acute rejection in a Chinese population<sup>90</sup>. Pawlik, et al.<sup>91</sup> have shown that individuals are homozygous for either arginine 131 (RR131) or histidine 131 (HH131) or are heterozygous for these two alleles (RH131). The HH131 genotype binds human IgG2 with high RR131 with low, and RH131 with intermediate affinity. They studied 121 renal transplant recipients. They reported that the FcγRIIA polymorphism is not an important genetic risk factor for chronic rejection of kidney allografts (Table 1).

### Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is an important cytokine in the innate immune system, which plays an important role in the control of inflammatory responses<sup>92</sup>. The MIF has a unique role as the physiologic



counter regulator of the immunosuppressive effects of glucocorticoids. It has been implicated in the pathogenesis of glomerular inflammation and its role has been already reported in end-stage kidney failure<sup>93</sup> and data are available for its role in graft rejection<sup>94</sup>. The MIF promoter contains a single nucleotide G→C polymorphism at position -173 (SNP rs755622). Marina, et al.<sup>95</sup> studied this polymorphism in nephritic syndrome patients and reported a positive association of the MIF-173\*C allele with childhood nephrotic syndrome. No studies of the association of this SNP and renal transplantation outcome have been published to our knowledge.

### **Cytotoxic T-lymphocyte antigen-4**

Binding of homologous cytotoxic T-lymphocyte antigen-4 (CTLA4) to the same ligand has an inhibitory effect on T-cells. Two polymorphism-(AT)*n* repeat in exon 3 and 49A/G SNP in exon 1 of *CTLA4* gene have been studied by Slavecheva, et al.<sup>96</sup>, where allele 1 of (AT)*n* STR showed a potential protective effect, but no such correlation was found for the SNP. Gorgi, et al.<sup>97</sup> investigated the association between kidney transplant rejection and the polymorphisms of *CTLA4* gene exon 1(+49) and promoter (-318), genomic DNA of 70 renal transplant recipients and compared this with 110 healthy blood donors and reported that the *CTLA4* polymorphism gene was associated with susceptibility to chronic allograft dysfunction. Recently, a study published by Krichen, et al.<sup>98</sup> reported no association with acute renal allograft rejection; however, they suggested that (+49) A and CT60 (G) alleles may confer protection against renal allograft loss.

### **Natural killer cell receptor gene polymorphisms**

Studies in haplo-identical bone marrow transplantation have shown that mismatching

for killer immunoglobulin receptor (KIR) ligands leads to a reduced risk of relapse in acute myeloid leukemia<sup>99,100</sup>. Recent studies have also shown that *KIR* genotype differences between donors and recipients, in terms of numbers of *KIR* genes, influence levels of graft rejection in kidney transplantation<sup>101</sup>. When the recipient and donor genotype is same, there was a significant increase in graft rejection and graft-versus-host disease. These results show that compatibility between *KIR* genotypes may influence outcome in transplantation. However, there are no further available data on renal transplantation and more work is needed to confirm these findings. Matching for *KIR* and related receptors between donors and recipients may prove at least as difficult as HLA matching, given the polymorphic and polygenic nature of NK receptors<sup>102</sup>.

### **Toll-like receptors**

Toll-like receptors (TLR) belong to the family of signaling pattern-recognition receptors of the innate immunity system. They recognize various common pathogenic components, such as lipopolysaccharides (LPS), peptidoglycans, RNA from viruses, and bacterial oligodeoxynucleotides<sup>103</sup>. Their tasks include phagocytosis and activation of the complement pathway and of numerous cytokines, such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$ <sup>104</sup>. Toll-like receptors are also able to recognize endogenous molecules, which are released upon cell damage and necrosis and have been shown to be present in numerous autoimmune diseases. Therefore, the release of endogenous TLR ligands during inflammation and consequently the activation of TLR signaling pathways may be one mechanism initiating and driving autoimmune diseases. An increasing body of circumstantial evidence implicates a role of TLR signaling in systemic lupus erythematosus, atherosclerosis, asthma, type-1 diabetes, multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis<sup>105</sup>.

*TLR4* is probably the most widely studied receptor in the TLR family. In humans, *TLR4* polymorphisms have been documented to be associated with an increased susceptibility to inflammatory diseases including renal diseases<sup>106</sup>, systemic lupus erythematosus<sup>107</sup>, giant cell arteritis and polymyalgia rheumatica<sup>108</sup>, rheumatoid arthritis<sup>109</sup>, etc.

Toll-like receptors might have an important role in the pathogenesis of renal diseases: their exaggerated activation is associated with ischemic kidney damage, acute kidney injury, end-stage renal failure, acute tubulointerstitial nephritis, acute renal transplant rejection, and delayed allograft function. To date, the impact of the TLR system on early and late kidney transplantation outcome, such as acute rejection episodes or cardiovascular morbidity and mortality, has still not been elucidated conclusively. However, recently Krüger, et al.<sup>110</sup> studied 11 SNP in TLR2, TLR3, TLR4, TLR5, TLR9 and within a coreceptor CD14 in 265 patients receiving their first kidney transplant, and the association of these with the occurrence of delayed graft function and acute rejection episodes. They reported that patients carrying the TLR3 TT/CT allele were more prone to delayed graft function and acute rejection episodes. Another study by Nogueira, et al. worked on only TLR4 polymorphisms at Asp299Gly and Thr399Ile codons in 201 kidney transplant patients<sup>111</sup>. They observed that neither polymorphism was associated with renal allograft outcomes. However, Ducloux, et al.<sup>112</sup> reported that renal transplant recipients with TLR4 polymorphism presented a lower risk of posttransplant atherosclerotic events and acute allograft rejection.

### ***Minor histocompatibility antigens***

Minor histocompatibility antigens (miHAg) may play an important role in graft rejection and are defined as cell surface antigens other than the MHC antigens. These antigens may not

be universally present on all the cells and they do not interact functionally with MHC antigens. However, the role of these antigens is not well defined in humans. Disparities in miHAg between HLA-matched organ and hematopoietic stem cell donors and recipients create the risks of graft failure and graft-versus-host disease, respectively<sup>113</sup>. A decade ago, technical advances combined with genomic information resulted in the identification of the chemical nature of the first series of miHAg, facilitating their molecular typing. A new era of research had begun in exploring the role of miHAg in physiological and non-physiological settings. The miHAg HY is associated with acute rejection, and male grafts in female recipients have reduced graft survival; both cellular and humoral responses are observed<sup>113</sup>. Studies on autosomal miHAg on graft rejection are less conclusive; their role in transplant tolerance, however, offers perspective. Information on the clinical relevance of miHAg alloimmune responses in solid organ allografting is still scarce. Experimental data obtained from studies of congenic strains of mice suggests that polymorphism of minor HLA antigens may be similar to that of the MHC. Important differences between them are that miHAg are less potent and immunogenic and they do not initiate the immune response independently, while MHC antigens are more immunogenic and can trigger antibody production against incompatible alloantigens. These miHAg account for comparatively slower and more chronic rejection<sup>113,114</sup>.

### ***Heat shock proteins in transplantation***

Heat shock proteins (HSP) may be involved in the pathogenesis of chronic rejection. The cellular stress response decreases cellular injury, either via primary induction of cytoresistance or by secondary enhancement of cellular repair mechanisms. The most frequently studied and best understood effectors of cellular stress response are the HSP. These

are among the oldest tools in the cellular protein machinery, demonstrating extremely high conservation of the genetic code since bacteria. Molecular chaperons, with HSP-70 being the prototype, cooperate in the transport and folding of proteins, preventing aggregation, and even making injured proteins soluble again. Increasing evidence supports a role for HSP during the recovery from renal ischemia, in particular in cellular salvage from apoptotic cell death and cytoskeletal restoration<sup>115</sup>. Some studies also report the potential for biomolecular profiling of newborns for the risk of acute renal failure<sup>116</sup> and the risk of nephropathy<sup>117</sup>.

The most studied gene polymorphisms in HSP-70 are HSPA1A G(190)C, HSPA1B A(1267)G in human renal transplant. Fekete, et al.<sup>118</sup> evaluated the association between HSPA1A G(190)C, HSPA1B A(1267)G and TLR4 A(299)G polymorphisms and allograft survival in patients with acute rejection and healthy controls. They reported that the increased frequency of HSPA1B (1267)AA and TLR4 (299)AG indicated that better cytoprotective functions in HSPA1B (1267)AA and reduced proinflammatory response in TLR4 (299)AG carriers might improve the renal allograft survival.

### **Non-immunological factors leading to renal failure**

A number of genetically determined factors without any known immunological function also complement their immunological counterparts in causing renal graft failures. All these factors are governed by/associated to different genes and are mainly involved in causing loss of graft function as most of them are well known for their role in causation of renal dysfunction. They either intensify immunological injuries like thrombosis or cause unresponsiveness to immunosuppressors like hypertension (Table 2).

### **Genetic variants associated with hypertension and hemodynamics**

Hypertension is one of the most important hemodynamics that leads to chronic allograft failure or loss of graft function, mainly by causing posttransplant atherosclerosis or erythrocytosis. Three important genetic systems (renin-angiotensin system or RAS, *eNOS* and *G-proteins*), associated either with the risk or severity of hypertension have been analyzed by different researchers in the recent past for their role in the success of renal transplantation<sup>119</sup>.

#### **Renin-angiotensin system**

The renin-angiotensin aldosterone system (RAAS) has a key role in both cardiovascular and renal pathophysiology<sup>120</sup>. Angiotensin II (angII), the most important biological active product, is synthesized via a pathway that involves several precursor peptides and enzymes, some of them regulated by separate genes. The main known action of angII is its potency to constrict vascular smooth muscle cells and to stimulate fluid and sodium retention by directly acting on tubular cells and through stimulating aldosterone release. Therefore, it is clear that genetic polymorphisms of RAAS have gained interest in the search for genetic factors that might influence the progression of chronic renal failure and the response to treatment to renoprotective regimens.

Decreased renal allograft function has also been associated with increased activity of RAAS, which may be genetically determined. The human *ACE* gene is located on chromosome 17q23 and includes 26 exons. The polymorphism involves the presence (insertion; II) or absence (deletion; DD) of a 287 bp sequence of DNA in intron 16 of the gene coding for ACE. Recent studies have suggested a high prevalence of DD genotype among individuals with hypertension and irregular renal

dysfunction<sup>33,121,122</sup>. Zhang, et al.<sup>122</sup> studied 206 renal transplant recipients with steroid refractory acute rejections (SRAR). They reported that renal allograft recipients with SRAR had significantly higher occurrences of the DD genotype of ACE than recipients without SRAR. Their study provides evidence that determination before transplantation of the polymorphism of the gene encoding components of RAS may help identify patients who are at risk for SRAR. A similar effect was also reported by Azarpira, et al.<sup>33</sup>. They reported that the transplant recipients with chronic allograft dysfunction had significantly higher frequencies of the DD genotype than those without it ( $p < 0.05$ ). Still, there are contradictory reports available<sup>123</sup>. This inconsistency could be in part due to the genetic and environmental heterogeneity among different ethnic groups.

### Angiotensinogen gene

The M235T polymorphism within the angiotensinogen (*AGT*) gene was associated with interstitial nephritis, where the 235t allele was transmitted significantly more frequently than expected and may thus play a significant role in progression of disease in these patients<sup>119,120,124</sup>. Azarpira, et al.<sup>33</sup> investigated the possible links between angiotensinogen (*AGT* M235T) genotypes with 77 chronic allograft dysfunctions. Kidney recipients with chronic allograft dysfunction had significantly higher frequencies of the TT than the recipients without it ( $p < 0.05$ ). They propose that determination of *AGT* M235T and ACE genotypes prior to transplantation may be useful to identify patients who are at risk for chronic renal transplant dysfunction.

### Endothelial nitric oxide synthetase gene

Changes in hemodynamics are thought to be the important factor in the deterioration of the renal functions<sup>125</sup>. Nitric oxide (NO), a relaxing factor expressed in endothelium, is

reported to be a potent regulator of intrarenal hemodynamics. The endothelial nitric oxide synthase (*eNOS*) gene comprises 26 exons that span 21 kilobases (kb) and is located on chromosome 7q35-3b<sup>125</sup>. Production of NO may be influenced by *eNOS* gene polymorphism. The three-dimensional structure of the enzyme may be altered by the polymorphism in exons, whereas polymorphism in introns may change the transcriptional activity, which may be the reason for a decrease in NO production and subsequently an increase in arterial pressure or intra-glomerular hypertension. Highly polymorphic (CA)<sub>n</sub> repeat in intron 13 and two biallelic markers in intron 18 have been proved to be not associated with hypertension<sup>125</sup>.

Wang, et al.<sup>126</sup> showed that *eNOS*-a allele in a homozygous condition is a risk factor for coronary artery diseases among smokers. In *eNOS* intron 4 they identified two alleles 'a' and 'b'; b allele is larger and consists of five tandem 27 bp repeats. The first three repeats had A and last two had G at the 19th base of 27 bp repeats, whereas the smaller 'a' allele has only four tandem 27 bp of which the first two repeats had A and last two had G at the 19th base of 27 bp repeats, respectively. The 'a' allele is 393 bp long whereas the 'b' allele is 420 bp long.

Shenker, et al.<sup>127</sup> worked on a patient cohort comprised of 140 renal transplant recipients, including 61 patients with biopsy-proven chronic allograft failure and 79 with stable graft function for at least 10 years. They conclude that recipient *eNOS* gene polymorphisms do not alter the risk of chronic allograft failure after renal transplantation<sup>127</sup>. Likewise, Akcay, et al. also showed that graft function was not affected by *eNOS* gene polymorphisms<sup>128</sup>.

Artifoni, et al.<sup>129</sup> carried out a genetic analysis of *eNOS* 894G>T, methionine synthase (*MTR*) 2756A>G and methylenetetrahydrofolate reductase (*MTHFR*) 677C>T/1298A>C in 268 renal allograft recipients. They found *eNOS* -894 genotype and clinically acute rejection

episodes as independent risk factors for graft loss, suggesting a role for this gene in chronic allograft injury.

## G-protein gene

A single nucleotide polymorphism (C→T at position 825) in the gene encoding the  $\beta 3$  subunit of heterotrimeric G-proteins (*GN $\beta$ 3*) mapping to chromosome 12p has been identified and has demonstrated to be significantly associated with essential hypertension<sup>130</sup>. The *GN $\beta$ 3* 825 T allele is also associated with enhanced G-protein activation, resulting in increased cell proliferation and hypertension and significant outcome in pediatric transplantation<sup>131</sup>. The  $\beta 3$  subunit of G-protein is known to be significantly associated with the risk and not severity of hypertension in people of different ethnic descent. The *G $\beta$ 3-825C/T* polymorphism has been shown to be associated with twofold elevated risk of kidney allograft loss. Beige, et al.<sup>132</sup> have shown that *G $\beta$ 3-TT* genotype contributes significantly in accelerated loss of graft function. They also suggested that high graft loss is associated with *G $\beta$ 3-825TT* genotype. The *G $\beta$ 3-825C/T* genetic system offers more chances of assessing reasons of chronic allograft nephropathy than the genes of RAS genetic system mainly because of the known functional relevance of the *G $\beta$ 3-825* polymorphism<sup>131</sup>.

### **Genetic variants associated with thrombosis and homeostasis**

Venous or arterial thrombosis is responsible for 1-5% early graft loss in adult renal transplants and up to 12% in pediatric renal transplants<sup>133</sup>, mainly due to the immunological injury upon the vascular wall exacerbated or induced by the prothrombotic state. Genetic prothrombotic risk factors like factor V Leiden mutation -1691G/A, and prothrombin gene mutation -20210G/A are a major determinant for the occurrence of various thromboembolic

diseases and are also studied at some transplant centers as a genetic predisposition factor for graft loss<sup>134</sup>.

## Factor V Leiden mutation

The *FV* gene lies in 1q24.2, is 72,422 bases long, and encodes a protein of 2,224 amino acids long. In the context of kidney transplantation, the factor V Leiden mutation (*FVL*, *F5 R506Q*, *G1691A*) is currently the most thoroughly investigated polymorphism of the genes of the coagulation cascade. The *FVL* mutation associates with thrombotic events, rejection episodes, and infarction. Patients with the *FVL* mutation are often given antithrombotic prophylaxis. However, in some studies investigators have been unable to demonstrate any association between the *FVL* mutation and outcome of renal transplantation<sup>135</sup> (Table 2).

The *FVL* mutation has been recognized as the most prevalent genetic risk factor for venous thromboembolic disease. Single nucleotide substitution (G1691A) in factor V gene leads to an amino acid substitution, Arg-506Gln, disrupting the cleavage site recognized by activated protein C<sup>136</sup>. Activated protein C is a natural anticoagulation molecule, which acts by cleaving the molecule of activated factor V, thus limiting its pro-coagulation activity. In 2004, an Oxford transplant group<sup>135</sup> studied 300 kidney allograft recipients and found 45% of graft failures have A allele (1691A), either in homozygous AA or heterozygous AG state. Significant association of prothrombotic mutations with acute rejection signifies the rationale of screening the hypercoagulable state before transplantation to intensify the anticoagulatory treatment posttransplantation<sup>137</sup>.

## Prothrombin (*PT G20210A*)

The prothrombin *G20210A* mutation leads to production of increased amount of prothrombin, and is associated with increased

risk of venous thrombosis. The prothrombin (*PT* G20210A) gene polymorphism has been associated with thrombotic complications of the vascular access; hence it may influence the risk of renal failure<sup>138</sup>.

## Methylenetetrahydrofolate reductase gene

Folate and homocysteine (Hcy) metabolisms are partly under genetic control. The gene for methylenetetrahydrofolate reductase (MTHFR) is located on chromosome 1 at 1p36.3 and consists of 11 exons spanning 2.2 kb<sup>139</sup>. Most of the allelic variants are rare and induce severe MTHFR deficiency, and may be one of the genetic factors of homocystinuria. Human MTHFR catalyses the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine re-methylation to methionine homocysteine, which occupies an important position in the metabolism of the essential amino acid, methionine. Elevated Hcy levels have been identified as a risk factor for diabetic nephropathy in ESRD patients<sup>140</sup>. In addition, increased plasma Hcy is an independent risk factor for several vasculopathies including arteriosclerosis, acute myocardial infarction, and cerebrovascular disease, arterial and venous thrombosis<sup>140,141</sup>. Hyperhomocysteinemia is common in patients with ESRD and may partially explain the high prevalence of vascular diseases observed in these patients. A common C677T transition was identified in *MTHFR* gene, which leads to the synthesis of a thermolabile enzyme with reduced catalytic activity particularly in carriers of TT alleles<sup>142</sup>. This mutation causes an alanine to valine amino acid substitution. The result is a thermolabile *MTHFR* variant that has reduced catalytic activity and is stabilized by folate. Homozygosity for this variant predisposes individuals to the development of hyperhomocysteinemia, especially during folate insufficiency<sup>151</sup>. In particular, the *MTHFR* C677T

polymorphism in the gene was shown to influence total Hcy and/or folate plasma levels in healthy adults, but also in patients on hemodialysis treatment, peritoneal dialysis treatment, and in ESRD<sup>143</sup>. Azarpira, et al.<sup>33</sup> reported that *MTHFR* T677 allele is associated with the presence of chronic allograft nephropathy. Another polymorphic site (A→C change at position 1298) has also been reported to be associated with renal allograft rejection. It is shown that this lead to diminished enzyme activity and has also been reported with kidney allograft rejection<sup>129,144</sup>.

## Genetic variants associated with atherosclerosis

### Apo E gene

Lipid abnormalities are found frequently in transplant recipients, and abnormal metabolism may contribute to the progression of renal failure as well as accelerated atherosclerosis. It has been reported that *ApoE* may play an important role in lipoprotein metabolism, and variations at the *ApoE* locus have been shown to affect low-density lipoprotein (LDL) cholesterol levels in continuous ambulatory peritoneal dialysis (CAPD) subjects, suggesting that ApoE4 carries more susceptibility to accelerated development of atherosclerosis in this condition<sup>145</sup>. In another study, Hernández, et al.<sup>146</sup> reported that ApoE4 allele was associated with chronic allograft nephropathy after renal transplantation. Moreover, in chronic kidney disease patients, Chu, et al.<sup>145</sup> found a high frequency of the e2 allele, suggesting that ApoE2 is associated with a possible predisposition to ESRD. They demonstrated that the impact of ApoE2 and ApoE4 on the lipid profile of renal patients was unique and different from the normal population. However, a study including 269 patients indicated that *ApoE* polymorphisms significantly affected serum levels of total cholesterol and LDL cholesterol; it was not associated with the presence of atherosclerosis<sup>147</sup>. Thus, the association

between LDL *ApoE* gene polymorphisms and atherosclerosis still remains unclear in renal transplant patients.

### **Genetic variants associated with obesity**

#### **Leptin gene CODON 25 (CAA/CAG)**

Leptin is an adipocyte-derived hormone that regulates food intake and energy expenditure. Recent functional studies have suggested a direct effect of leptin on blood pressure<sup>148</sup>. In addition to its effect on appetite and energy expenditure, a direct effect of leptin on blood pressure has recently been reported<sup>148</sup>. The mechanism by which leptin increases blood pressure is thought to be through sympathetic activation. Because patients with essential hypertension were reported to be hyperleptinemic, and plasma immunoreactive, leptin was reported to correlate with blood pressure. Another study reported by Tripathi, et al. in a north-Indian population reported that GG genotype was associated with ESRD<sup>149</sup>. Up to now there is no study on the association of this polymorphism in post-transplant patients.

#### **Uncoupling proteins**

In mammals, it is believed that a portion of tissue metabolic rate is driven by counteraction of uncoupling, in which the energetically inefficient process of proton leak acts to diminish the mitochondrial electrochemical membrane potential. It is proposed that specific proteins associated with the mitochondrion catalyze uncoupling, and the biology of such putative uncoupling proteins (UCP) is the subject of active research efforts. Chronic treatment with peritoneal dialysis is a unique long-term metabolic procedure entailing a continuous 24-hour supply of glucose absorbed from the dialysis fluid. One common

and important side effect of this treatment is weight gain and accumulation of body fat stores. However, not all patients accumulate body fat mass during peritoneal dialysis, and the reason for this is not clear<sup>150</sup>. Recently, two new mitochondrial uncoupling proteins (UCP2 and UCP3) have been found to have thermogenic properties that suggest involvement in the control of metabolic efficiency in humans. Both UCP2 and UCP3 are ubiquitously expressed throughout the body. Moreover, recent results suggest that a polymorphism in the *UCP2* gene may contribute to adipose tissue accumulation through its effects on energy metabolism. It could therefore be speculated that genetic differences might contribute to renal allograft functioning. The *UCP2* maps to regions of human chromosome 11q13. The deletion/deletion (D/D) polymorphism of UCP2 was associated with decreased energy expenditure in diabetic and obese patients. An I/D in the *UCP2* gene has been shown to be associated with lower metabolic rate, and it has been demonstrated that this polymorphism influences changes in body composition during 12 months of peritoneal dialysis<sup>150</sup>. A common and important side effect of dialysis is accumulation of body fat stores and that could be a determinant for renal allograft rejection in transplant recipients. There is currently no information regarding the expression and distribution of the different UCP isoforms in the kidney.

### **Genetic variants affecting free radicals**

Protection from the deleterious effects of free radicals is essential to the successful function of the transplanted organ. Enzymes including glutathione S-transferase (*GST*) and manganese superoxide dismutase (MnSOD) are important factors and their genetic variants could influence the graft outcome<sup>151</sup>. A member of the *GST* superfamily of genes provides protection to the cells against reactive oxygen species and plays a vital role in phase II of

biotransformation of many substances. Overexpression of *GST* has been documented in the erythrocytes of patients with chronic renal failure and this may be of clinical relevance.

Soluble GST represents a superfamily of inducible enzymes, comprising at least seven classes of cytoplasmic proteins ( $\alpha$ ,  $\mu$ ,  $\pi$ ,  $\sigma$ ,  $\theta$ ,  $\kappa$ ,  $\xi$ )<sup>152</sup>, which catalyze the conjugation of glutathione (GSH) with different species of electrophilic compound. The human *GST* genes are divided into four major subfamilies designated as *GST*  $\alpha$  or A, *GST*  $\mu$  or m, *GST*  $\theta$  or T and *GST*  $\pi$  or P. The class  $\pi$  *GST* genes exist as a single functional gene in humans, whereas class  $\alpha$ ,  $\mu$  and  $\theta$  families contain multiple distinct genes, sharing ~55, 65, and 50% homology, respectively<sup>152</sup>. For both *GSTM1* and *GSTT1*, the variant allele is a deletion of the gene, and individuals who are homozygous for the deleted allele are said to possess the “null” genotype and do not express the enzyme and *GSTP1* gene show polymorphism within its coding region, of which well-known are an A→G transition at nucleotide position 1578, causing an isoleucine to valine substitution at codon 105 in exon 5. The C→T base change occurs at position 2293, giving rise to the replacement of alanine to valine at the amino acid position 114 in exon 6. This results in decreased enzyme activity<sup>152</sup>.

Recently, Elhasid, et al.<sup>153</sup> found in hematopoietic stem cell transplanted children that when *GSTT1*-null recipients were transplanted with a *GSTT1*-positive graft, rejection due to an antibody-mediated immune response against *GSTT1* displayed on transplanted stem cells may take place. Thus, it seems that detection of anti-*GSTT1* antibodies in patients with a *GSTT1*-null genotype before transplantation may be predictive of graft rejection in the event of a *GSTT1*-positive donor. Singh, et al.<sup>154</sup> studied the role of genetic polymorphisms in *GSTM1*, *GSTM3*, *GSTT1* and *GSTP1* on allograft outcome in renal transplant recipients and reported that *GST* genes may

influence the graft outcome in renal transplant patients. However, contradictory reports are available stating no association of either *GSTT1*-null or *GSTM1*-null genotypes with chronic allograft dysfunction<sup>155</sup>.

### **Vitamin D receptor genetic variants in renal transplant recipients**

Vitamin D receptor (VDR) [1,25(OH)<sub>2</sub>D<sub>3</sub>] agonists have been shown to reduce short- and long-term allograft rejection in animal models<sup>156</sup>. The *VDR* gene is reported to be involved in calcium absorption, excretion, and modulation of cellular proliferation and differentiation. Several common polymorphisms in the *VDR* gene have been reported, with functional consequences in immune regulation. The functional significance and potential effect on disease susceptibility have been investigated in different diseases like prostate cancer, urolithiasis, and osteoporosis, etc. However, there are very few reports related to *Apa1*, *Taq1*, *Fok1* and *Bsm1* polymorphisms of *VDR* gene among renal transplant recipients. Lavin, et al.<sup>157</sup> performed these gene polymorphisms in 379 renal transplant recipients and were genotyped for *VDR* (*Fok1* and *Apa1*). There was significantly improved allograft survival for patients who were homozygous or heterozygous for the *VDR Fok1* T allele. This finding shows the prevention of chronic allograft rejection with the use of vitamin D receptor agonists. However, Azarpira, et al. reported no association of *VDR* polymorphisms in incidence of acute rejection or graft survival after renal transplantation<sup>33</sup>.

### **Genetic variants affecting pharmacokinetics of immunosuppressors**

Immunosuppressor drugs carry a narrow therapeutic range and a wide interindividual variation in their pharmacokinetics<sup>158</sup>.



Tacrolimus has a narrow therapeutic window and shows significant interindividual difference in dose requirement. Wang, et al.<sup>159</sup> worked to identify the genetic factors that impact tacrolimus dose using a candidate gene association approach, and then generating a personalized algorithm combining identified genetic and clinical factors to predict an individualized tacrolimus dose. They screened 768 SNP in 15 candidate genes in metabolism, transport and calcineurin inhibition pathways of tacrolimus, for association with tacrolimus dose in a discovery cohort of 96 patients. They reported that the *CYP3A5* genotype was the most significant genetic factor that impacted tacrolimus dose among the genes studied. This study generated the first pharmacogenomics model that predicted tacrolimus stable dose based on age, ethnicity, genotype, and co-medication use.

The most important factor that affects interpatient variability is P-glycoprotein (p-GP), a product of multidrug resistant gene-1 (*MDR1*) that uses these immunosuppressors as its substrate<sup>160</sup>. Four important SNP have been studied in *MDR1* gene, namely 129T/C in exon 1b, 1236C/T in exon 12, 2677G/T in exon 21, and 3435C/T in exon 26, where the latter three SNP are more frequently found in the general population<sup>160</sup>. Exon 21-2677G/T SNP was found highly associated with tacrolimus dose as TT homozygous showed 40% higher dose requirement of tacrolimus but not of cyclosporin<sup>161</sup>. Exon 26 SNP-3435C/T is reported to be associated with increased expression of *MDR1* gene in CD56<sup>+</sup> NK cells. However, the report regarding its role in drug kinetics is contradictory. More focused and targeted studies on such genes will lead to individualization of drug treatment and enhance drug safety and efficacy. The studies to evaluate these polymorphisms may be helpful to individualize immunosuppressive protocols to inhibit or retard the progression of both chronic allograft nephropathies as well as graft rejection.

## Personalized medicine and pharmacogenomics

Advances in a number of molecular profiling technologies, including proteomic profiling, metabolomics analysis, and genetic testing, may allow for a greater degree of personalized medicine than is currently available. Information about a patient's proteinaceous, genetic, and metabolic profile could be used to tailor medical care to that individual's needs. As renal transplant treatment protocols have evolved to overcome the frequent rejection episodes of the early days of transplantation, the aim of improving therapies currently emphasizes individualized immunotherapy to lengthen the graft and patient survival as well as to improve the recipient's quality of life. Excessively potent immunosuppression increases the risk of infections, cancer, and nephrotoxicity, while insufficient immunosuppression results in rejection. Immunosuppression is monitored also by pharmacokinetics measuring concentration of drugs in blood. In addition, functional assays can be performed; a new pharmacodynamic method detects cell-mediated immunity by measuring the concentration of ATP from whole blood CD4 cells following stimulation<sup>162</sup>. Constant search and development continues to find other noninvasive tools needed. Two utilized strategies are to monitor alloimmune responses in the graft or global changes in immune responses of the host. Alloimmune monitoring takes place before and after transplantation by measuring the reactivity of recipient HLA antibodies to donor cells or HLA antigens in beads. These antibody-based tools have been more successful than T-cell-based tests for predicting rejection. However, the two strategies are successful only when an alloimmune response takes place also in circulation and not only in the graft. The second strategy, to monitor global changes in immune response, faces the problem of separating rejection and infection-mediated immune responses from each other. Routinely used creatinine measurement

detects mainly glomerular injury but also tubulointerstitial injury to a lesser extent.

Tubular markers have been found from urine that may separate ischemia from acute rejection, chronic rejection, infection, and nephrotoxicity, although not separating the latter from each other.

This leads to the conclusion that whereas a single biomarker for diagnosing any of the entities may never be found, a combination of several biomarkers may distinguish compartments and reveal the reason for injury. A new emerging field is global analysis of different biomolecule classes that can be studied by proteomics, glycomics, lipidomics, or metabolomics. However, these scientific disciplines are much more complex than genomics. Various genetic typing methods are now available both to identify these non-HLA genetic risk factors and to type them in the future. Other SNP, such as those that effect the metabolism of immunosuppressive drugs, are also likely to be of importance as efforts are made to individualize patient care based on genetic profiles. Further studies to identify such markers will broaden the options that can be brought into practice, such as more accurate timing and administration of immunosuppressive therapy, supplementation with folic acid, or type of antihypertensive and anticoagulation therapy to increase the still poor long-term outcome in renal transplantation.

## Conclusions

Despite many studies in this area, there are still no clear indications of which non-HLA genetic polymorphisms are likely to have the greatest clinical use in the future. The discovery of an increasing number of gene polymorphisms provides us with a possible explanation for the marked variability in outcomes in transplant recipients. Although recent studies indicated the importance of some gene polymorphisms in transplantation, the

results are not yet conclusive. This area of investigation is in its early stages. Categorical reviewing of the studies carried out on different genetic variants indicated that for success or failure of renal transplantation, the genetic predisposition of a patient involves more factors than just *HLA* genes.

At present it is premature to use recipient or donor genotyping to predict the transplant outcome or to individualize the immunosuppressive therapy. It is likely that, as with diseases such as hypertension and diabetes, an individual's risk status is determined by a complex genetic trait resulting from the interplay of several genes rather than a single gene. Advances in technology, such as the identification of SNP by oligonucleotide microarrays, suggest the typing of patients on a large-scale basis for multiple polymorphisms thereby making it clinically applicable. This approach is aimed at tailoring drug therapy at a dosage that is most appropriate for an individual patient, with the potential benefits of increasing the efficacy and safety of medications.

## Sources of support

This work was partially supported by grants from the Fondo de Investigaciones Sanitarias-ISCIII (PI070683, PI080157, RED-INREN 06/16 ISCIII) and from the Fundación Mutua-Madrileña (ACI 07/01). Gaurav Tripathi is a recipient of a grant from the Fundación Marqués de Valdecilla-IFIMAV.

## References

1. Tinckam K, Rush D, Hutchinson I, et al. The relative importance of cytokine gene polymorphisms in the development of early and late acute rejection and six-month renal allograft pathology. *Transplantation*. 2005;79:836-41.
2. McDaniel DO, Barber WH, Nguyen C, et al. Combined analysis of cytokine genotype polymorphism and the level of expression with allograft function in African-American renal transplant patients. *Transpl Immunol*. 2003;11:107-19.
3. George S, Turner D, Reynard M, et al. Significance of cytokine gene polymorphism in renal transplantation. *Transplant Proc*. 2001;33:483-4.
4. Hutchinson IV, Pravica V, Perrey C, et al. Cytokine gene polymorphisms and relevance to forms of rejection. *Transplant Proc*. 1999;31:734-6.

5. Hutchinson IV, Turner D, Sankaran D, et al. Cytokine genotypes in allograft rejection: guidelines for immunosuppression. *Transplant Proc.* 1998;30:3991-2.
6. Hahn AB, Kasten-Jolly JC, Constantino DM, et al. TNF-alpha, IL-6, IFN-gamma, and IL-10 gene expression polymorphisms and the IL-4 receptor alpha-chain variant Q576R: effects on renal allograft outcome. *Transplantation.* 2001;72:660-5.
7. Hoffmann SC, Stanley EM, Cox ED, et al. Ethnicity greatly influences cytokine gene polymorphism distribution. *Am J Transplant.* 2002;2:560-7. ***\*\*This is an interesting paper, which demonstrates an association between ethnicity and gene polymorphisms.***
8. Delaney NL, Esquenazi V, Lucas DP, et al. TNF-alpha, TGF-beta, IL-10, IL-6, and INFgamma alleles among African Americans and Cuban Americans. Report of the ASHI Minority Workshops: Part IV. *Hum Immunol.* 2004;65:1413-9.
9. Noris N, Casiraghi F, Todeschini M, et al. Regulatory T Cells and T Cell depletion: role of immunosuppressive drugs. *J Am Soc Nephrol.* 2007;18:1007-18.
10. Pyo CW, Williams LM, Moore Y, et al. HLA-E, HLA-F, and HLA-G polymorphism: genomic sequence defines haplotype structure and variation spanning the nonclassical class I genes. *Immunogenetics.* 2006;58:241-51.
11. Lepourcelet M, Andrieux N, Giffon T, et al. Systematic sequencing of the human HLA-A/HLA-F region: establishment of a cosmid contig and identification of a new gene cluster within 37 kb of sequence. *Genomics.* 1996;37:316-26.
12. Apps R, Gardner L, Moffett A. A critical look at HLA-G. *Trends Immunol.* 2008; 29:313-21.
13. Li C, Houser BL, Nicotra ML, Strominger JL. HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. *Proc Natl Acad Sci USA.* 2009;106:5767-72.
14. Singh N, Pirsch J, Samaniego M. Antibody-mediated rejection: treatment alternatives and outcomes. *Transplant Rev (Orlando).* 2009;23:34-46.
15. Bonafe M, Olivieri F, Cavallone L. A gender-dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. *Eur J Immunol.* 2001;31:2357-61.
16. Kobayashi T, Yokoyama I, Inoko H, et al. Significance of transporter associated with antigen processing gene polymorphism in living related renal transplantation. *Hum Immunol.* 2000;61:670-4.
17. Pigott R, Dillon LP, Hemingway IH, Gearing AJ. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun* 1992;187:584-9.
18. Kim BC, Kim WY, Park D, Chung WH, Shin KS, Bhak J. SNP@Promoter: a database of human SNPs (single nucleotide polymorphisms) within the putative promoter regions. *BMC Bioinformatics.* 2008;9(Suppl 1):S2.
19. Andersen MC, Engström PG, Lithwick S, et al. In silico detection of sequence variations modifying transcriptional regulation. *PLoS Comput Biol.* 2008;4:e5
20. Kocierz M, Kujawa-Szewiczek A, Kolonko A, Chudek J, Wiecek A. The influence of selected cytokine gene polymorphisms on the occurrence of acute and chronic rejection and on kidney graft survival. *Postepy Hig Med Dosw.* 2009;63:613-26.
21. Melk A, Henne T, Kollmar T, et al. Cytokine single nucleotide polymorphisms and intrarenal gene expression in chronic allograft nephropathy in children. *Kidney Int.* 2003;64:314-20.
22. Liu Y, Berthier-Schaad Y, Fallin MD, et al. IL-6 haplotypes, inflammation, and risk for cardiovascular disease in a multiethnic dialysis cohort. *J Am Soc Nephrol.* 2006;17:863-70.
23. Hasuike Y, Nonoguchi H, Ito K, et al. Interleukin-6 is a predictor of mortality in stable hemodialysis patients. *Am J Nephrol.* 2009;30:389-98.
24. Nairn J, Hodge G, Henning P. Intracellular cytokines in peripheral blood leucocytes in children with chronic renal failure. *Pediatr Nephrol.* 2006;21:251-6.
25. Shevchenko AO, Shevchenko OP, Orlova OV, Tulikov MV. Neoangiogenesis and coronary atherosclerosis: diagnostic value of a novel biochemical marker placenta growth factor in patients with ischemic heart disease. *Kardiologiya.* 2006;46:9-15.
26. Suthanthiran M. The importance of genetic polymorphisms in renal transplantation. *Curr Opin Urol.* 2000;10:71-5.
27. Muller-Steinhardt M, Hartel C, Muller B, et al. The interleukin-6 -174 promoter polymorphism is associated with long-term kidney allograft survival. *Kidney Int.* 2002;62:1824-7.
28. Marshall SE, McLaren AJ, McKinney EF, et al. Donor cytokine genotype influences the development of acute rejection after renal transplantation. *Transplantation.* 2001;71:469-76. ***\*\*They identify donor IL-6 genotype as a major genetic risk factor for the development of acute rejection after renal transplantation. Thus, not only recipient but donor genotype has importance on kidney acute rejection.***
29. Muller-Steinhardt M, Fricke L, Muller B, et al. Cooperative influence of the interleukin-6 promoter polymorphisms -597, -572 and -174 on long-term kidney allograft survival. *Am J Transplant.* 2004;4:402-6.
30. Nikolova PN, Ivanova MI, Mihailova SM, et al. Cytokine gene polymorphism in kidney transplantation-impact of TGF-beta 1, IL10, TNF-alpha and IL-6 on graft outcome. *Transpl Immunol.* 2008;18:3-8. ***\*Pro and anti-inflammatory cytokine gene polymorphisms on development of Chronic Allograft Nephropathy in a group of renal transplant patients and donors is analyzed. The high producer genotype of profibrogenetic TGF-beta 1, pro-inflammatory TNF-alpha and genetically determined low production of immunoregulatory IL-6 cytokine are determined as risk factors for CAN development.***
31. Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. *Annu Rev Immunol.* 1993;11:571-611.
32. Omrani MD, Mokhtari MR, Bagheri M, Ahmadpoor P. Association of interleukin-10, interferon-gamma, transforming growth factor-beta, and tumor necrosis factor-alpha gene polymorphisms with long-term kidney allograft survival. *Iran J Kidney Dis.* 2010;4:141-6.
33. Azarpira N, Aghdai MH, Raisjalali GA, Darai M, Tarahi MJ. Influence of recipient and donor IL-10, TNFA and INFg genotypes on the incidence of acute renal allograft rejection. *Mol Biol Rep.* 2009;36:1621-6.
34. Pravica V, Asderakis A, Perrey C, et al. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. *Eur J Immunogenet.* 1999;26:1-3.
35. Asderakis A, Sankaran D, Dyer P, et al. Association of polymorphisms in the human interferon-gamma and interleukin-10 gene with acute and chronic kidney transplant outcome: the cytokine effect on transplantation. *Transplantation.* 2001;71:674-7.
36. Pirenne J, Kitade H, Kawai M, et al. Regulatory cells, TH1/TH2 imbalance, and antibody-induced chronic rejection in operational tolerance induced by donor specific blood transfusion. *Transplantation.* 2005;79:S25-7.
37. Hoffmann S, Park J, Jacobson LM, et al. Donor genomics influence graft events: the effect of donor polymorphisms on acute rejection and chronic allograft nephropathy. *Kidney Int.* 2004;66:1686-93.
38. Pelletier R, Pravica V, Perrey C, et al. Evidence for a genetic predisposition towards acute rejection after kidney and simultaneous kidney-pancreas transplantation. *Transplantation.* 2000;70:674-80.
39. Amoroso A, Savoldi S, Bosutti A, et al. Association of interferon-gamma +874A polymorphism with reduced long-term inflammatory response in haemodialysis patients. *Nephrol Dial Transplant.* 2006;5:1317-22.
40. Bradley JR. TNF-mediated inflammatory disease. *J Pathol.* 2008;214:149-60.
41. Kollias G, Douni E, Kassiotis G, Kontoyiannis D. The function of tumour necrosis factor and receptors in models of multi-organ inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. *Ann Rheum Dis.* 1999; 58(Suppl 1):I32-9.
42. Mytilineos J, Laux G, Opelz G. Relevance of IL10, TGFbeta1, TNFalpha, and IL4Ralpha gene polymorphisms in kidney transplantation: a collaborative transplant study report. *Am J Transplant.* 2004;4:1684-90.
43. Alakulppi NS, Kyllonen LE, Jantti VT, et al. Cytokine gene polymorphisms and risks of acute rejection and delayed graft function after kidney transplantation. *Transplantation.* 2004;78:1422-8. ***\*It shows that cytokine gene polymorphisms influence the outcome of kidney transplantation, especially identify the TNF-alpha-308A-genotype and IL10 haplotypes as genetic factor predisposing for acute rejection episodes.***
44. Bathgate AJ, Pravica V, Perrey C, et al. The effect of polymorphisms in tumor necrosis factor-alpha, interleukin-10, and transforming growth factor-beta1 genes in acute hepatic allograft rejection. *Transplantation.* 2000;56:890-6.
45. Abdallah AN, Cucchi-Mouillot P, Biteau N, et al. Analysis of the polymorphism of the tumour necrosis factor (TNF) gene

- and promoter and of circulating TNF alpha levels in heart-transplant patients suffering or not suffering from severe rejection. *Eur J Immunogenet.* 1999;26:249-55.
46. Lee H, Clark B, Gooi HC, Stoves J, Newstead CG. Influence of recipient and donor IL1alpha, IL-4, and TNFalpha genotypes on the incidence of acute renal allograft rejection. *J Clin Pathol.* 2004;57:101-3.
  47. Akalin E, Murphy B. Gene polymorphisms and transplantation. *Curr Op Immunol.* 2001;13:572-6.
  48. Poole KL, Gibbs PJ, Evans PR, et al. Influence of patient and donor cytokine genotypes on renal allograft rejection: evidence from a single centre study. *Transpl Immunol.* 2001;8:259-65.
  49. Vandenbroeck K, Goris A. Cytokine gene polymorphisms in multifactorial diseases: gateways to novel targets for immunotherapy? *Trends Pharmacol Sci.* 2003;24:284-9.
  50. Geismar K, Enevold C, Sørensen LK, Gyntelberg F, Bendtzen K, Sigurd B. Involvement of interleukin-1 genotypes in the association of coronary heart disease with periodontitis. *J Periodontol.* 2008;79:2322-30.
  51. Sánchez E, Sabio JM, Callejas JL, et al. Association study of genetic variants of proinflammatory chemokine and cytokine genes in systemic lupus erythematosus. *BMC Med Genet.* 2006;7:48.
  52. Arman A, Soylu O, Yildirim A, Furman A, Ercelen N, Aydogan H. Interleukin-1 receptor antagonist gene VNTR polymorphism is associated with coronary artery disease. *Arq Bras Cardiol.* 2008;91:293-8.
  53. Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB. The human IL-1 receptor antagonist gene (IL1RN) maps to chromosome 2q14-q21, in the region of the IL-1 alpha and IL-1 beta loci. *Genomics.* 1992;13:654-7.
  54. Buraczynska M, Ksiazek P, Kubit P, Zaluska W. Interleukin-1 receptor antagonist gene polymorphism affects the progression of chronic renal failure. *Cytokine.* 2006;36:167-72.
  55. Maruyama Y, Stenvinkel F, Lindholm B. Role of interleukin-1beta in the development of malnutrition in chronic renal failure patients. *Blood Purif.* 2005;23:275-81.
  56. Manchanda PK, Bid HK, Kumar A, Mittal RD. Genetic association of interleukin-1beta and receptor antagonist (IL-1Ra) gene polymorphism with allograft function in renal transplant patients. *Transpl Immunol.* 2006;15:289-96.
  57. Zachwieja J, Zaniew M, Runowski D, Lewandowska-Stachowiak M, Stefaniak E, Sipińska A. Abnormal cytokine synthesis as a consequence of increased intracellular oxidative stress in children treated with dialysis. *Nephron Clin Pract.* 2005;101:c100-8.
  58. Pawlik A, Domanski L, Rozanski J, et al. The association between cytokine gene polymorphisms and kidney allograft survival. *Ann Transplant.* 2008;13:54-8.
  59. Varin A, Gordon S. Alternative activation of macrophages: immune function and cellular biology. *Immunobiology.* 2009;214:630-41.
  60. Marshall SE, McLaren AJ, Haldar NA, Bunce M, Morris PJ, Welsh KI. The impact of recipient cytokine genotype on acute rejection after renal transplantation. *Transplantation.* 2000;70:1485-91. *\*\*In this paper the cytokine gene association and renal allograft failure in a large cohort of renal transplant recipients was evaluated in a large number of cytokines. They found no association.*
  61. Sabat R, Grütz G, Warszawska K, et al. Biology of interleukin-10. *Cytokine Growth Factor Rev.* 2010;21:331-44.
  62. Park JY, Park MH, Park H, et al. TNF-alpha and TGF-beta1 gene polymorphisms and renal allograft rejection in Koreans. *Tissue Antigens.* 2004;64:660-6.
  63. Rubtsov YP, Rudensky AY. TGFbeta signalling in control of T-cell mediated self-reactivity. *Nat Rev Immunol.* 2007;7:443-53.
  64. Pribylova-Hribova P, Kotsch K, Lodererova A, et al. TGF-beta1 mRNA upregulation influences chronic renal allograft dysfunction. *Kidney Int.* 2006;69:1872-9.
  65. Govinden R, Bhoola KD. Genealogy, expression, and cellular function of transforming growth factor-beta. *Pharmacol Ther.* 2003;98:257-65.
  66. Schirmer MA, Hoffmann AO, Campean R, et al. Bioinformatic and functional analysis of TGFBR1 polymorphisms. *J Pharmacogenet Genomics.* 2009;19:249-59.
  67. Buck MB, Knabbe C. TGF-beta signaling in breast cancer. *Ann N Y Acad Sci.* 2006;1089:119-26.
  68. Cho JH, Huh S, Kwon TG, et al. Association of C-509T and T869C polymorphisms of transforming growth factor-beta1 gene with chronic allograft nephropathy and graft survival in Korean renal transplant recipients. *Transplant Proc.* 2008;40:2355-60.
  69. Li CJ, Yu LX, Xu J, et al. Association between transforming growth factor beta-1 gene polymorphism and chronic allograft nephropathy. *Nan Fang Yi Ke Da Xue Xue Bao.* 2007;27:535-7.
  70. Manchanda PK, Mittal RD. Analysis of cytokine gene polymorphisms in recipient's matched with living donors on acute rejection after renal transplantation. *Mol Cell Biochem.* 2008;311:57-65.
  71. Watabe T. Roles of old players in the suppression of a new player: Networks for the transcriptional control of angiogenesis. *J Biochem.* 2011;149:117-9.
  72. Shahbazi M, Fryer AA, Pravica V, et al. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol.* 2002;13:260-4.
  73. Lemos FB, Mol WM, Roodnat JI, et al. The beneficial effects of recipient-derived vascular endothelial growth factor on graft survival after kidney transplantation. *Transplantation.* 2005;79:1221-5.
  74. Omrani MD, Mokhtari MR, Tagizadeh A, Bagheri M, Ahmad-Poor P. Association of CCR5-59029 A/G and CCR2-V64I variants with renal allograft survival. *Iran J Immunol.* 2008;5:201-6.
  75. Womer KL, Vella JP, Sayegh MH. Chronic allograft dysfunction: mechanisms and new approaches to therapy. *Semin Nephrol.* 2000;20:126-47.
  76. Fischereder M, Schroppel B. The role of chemokines in acute renal allograft rejection and chronic allograft injury. *Front Biosci.* 2009;14:1807-14.
  77. Abdi R, Huong TTB, Sahagun-Ruiz A, et al. Chemokine receptor polymorphism and risk of acute rejection in human renal transplantation. *J Am Soc Nephrol.* 2002;13:754-8. *\*This paper demonstrates an association with genetic variation in the chemokine receptors CCR2 and CCR5 for the risk of acute rejection in renal transplantation.*
  78. Nelson PJ, Krensky AM. Chemokines, chemokine receptors, and allograft rejection. *Immunology.* 2001;14:377-86.
  79. Fischereder M, Luckow B, Hoher B, et al. CC chemokine receptor 5 and renaltransplant survival. *Lancet.* 2001;357:1758-61.
  80. Hancock WW, Gao W, Faia KL, Csizmadia V. Chemokines and their receptors in allograft rejection. *Curr Opin Immunol.* 2000;12:511-16.
  81. Hoffmann U, Bergler T, Segerer S, et al. Impact of chemokine receptor CX3CR1 in human renal allograft rejection. *Transpl Immunol.* 2010;23:204-8.
  82. Segerer S, Bohmig GA, Exner M, et al. When renal allografts turn DARC. *Transplantation.* 2003;75:1030-4.
  83. Akalin E, Neylan JF. The influence of Duffy blood group on renal allograft outcome in African Americans. *Transplantation.* 2003;75:1496-500.
  84. Mange KC, Prak EL, Kamoun M, et al. Duffy antigen receptor and genetic susceptibility of African Americans to acute rejection and delayed function. *Kidney Int.* 2004;66:1187-92.
  85. Kamoun M. Cellular and molecular parameters in human renal allograft rejection. *Clin Biochem.* 2001;34:29-34.
  86. McLaren AJ, Marshall SE, Haldar NA, et al. Adhesion molecule polymorphisms in chronic renal allograft failure. *Kidney Int.* 1999;55:1977-82.
  87. Khazen D, Jendoubi-Ayed S, Gorgi Y, et al. Adhesion molecule polymorphisms in acute renal allograft rejection. *Transplant Proc.* 2007;39:2563-4. *\*Authors genotype a total of 120 renal transplant recipients and 100 controls for seven nucleotide polymorphisms in ICAM-1, PECAM-1, L-selectin, and E-selectin and do not find an association of any polymorphism with acute rejection.*
  88. Mendoza-Carrera F, Ojeda-Durán S, Angulo E, et al. Influence of cytokine and intercellular adhesion molecule-1 gene polymorphisms on acute rejection in pediatric renal transplantation. *Pediatr Transplant.* 2008;12:755-61.
  89. Yuan FF, Watson N, Sullivan JS, et al. Association of Fc gamma receptor IIA polymorphisms with acute renal-allograft rejection. *Transplantation.* 2004;78:766-9.
  90. Xu G, He Q, Shou Z, et al. Association of Fc gamma receptor IIIB polymorphism with renal-allograft in Chinese. *Transpl Immunol.* 2007;18:28-31.

91. Pawlik A, Florczyk M, Bak L, et al. The FcγRIIIa polymorphism in patients with chronic kidney graft rejection. *Transplant Proc.* 2004;36:1311-13.
92. Bach JP, Deuster O, Balzer-Geldsetzer M, Meyer B, Dodel R, Bacher M. The role of macrophage inhibitory factor in tumorigenesis and central nervous system tumors. *Cancer.* 2009;115:2031-40.
93. Tripathi G, Borkar M, Akhter A, Sankhwar SN, Sharma RK, Agrawal S. Association of proinflammatory cytokines with end stage renal disease. *Cytokine.* 2010;50:278-83.
94. Javeed A, Zhao Y, Zhao Y. Macrophage-migration inhibitory factor: role in inflammatory diseases and graft rejection. *Inflamm Res.* 2008;57:45-50.
95. Vivarelli M, D'Urbano LE, Stringini G, et al. Association of the macrophage migration inhibitory factor -173°C allele with childhood nephrotic syndrome. *Pediatr Nephrol.* 2008; 23:743-8.
96. Slavcheva E, Albanis E, Jiao Q, et al. Cytotoxic T-lymphocyte antigen 4 gene polymorphisms and susceptibility to acute allograft rejection. *Transplantation.* 2001;72:935-40. *\*\*This was the first study reported the association between polymorphisms in costimulatory molecules and susceptibility to acute rejection.*
97. Gorgi Y, Sfar I, Abdallah TB, et al. Ctlα-4 exon 1 (+49) and promoter (-318) gene polymorphisms in kidney transplantation. *Transplant Proc.* 2006;38:2303-5.
98. Krichen H, Sfar I, Jendoubi-Ayed S, et al. Genetic polymorphisms of immunoregulatory proteins in acute renal allograft rejection. *Transplant Proc.* 2009;41:3305-7.
99. Stringaris K, Adams S, Uribe M, et al. Donor KIR Genes 2DL5A, 2DS1 and 3DS1 are associated with a reduced rate of leukemia relapse after HLA-identical sibling stem cell transplantation for acute myeloid leukemia but not other hematologic malignancies. *Biol Blood Marrow Transplant.* 2010;16:1257-64.
100. Ruggeri L, Mancusi A, Capanni M, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood.* 2007;110:433-40.
101. Yu LX, Xiao F, Xiao LL, et al. KIR/HLA ligand matching and acute rejection after kidney transplantation. *Nan Fang Yi Ke Da Xue Xue Bao.* 2010;30:288-91. *\*This paper stress the importance of KIR/HLA class I ligand combinations between the donor and recipient may reduce the incidence of AR. A good KIR/HLA class I ligand matching will benefit the survival of the renal allograft.*
102. Ruggeri L, Mancusi A, Burchielli E, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. *Blood Cells Mol Dis.* 2008;40:84-90.
103. Zuany-Amorim C, Hastewell J, Walker C. Toll-like receptors as potential therapeutic targets for multiple diseases. *Nat Rev Drug Discov.* 2002;1:797-807.
104. Pasare C, Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. *Microbes Infect.* 2004; 6:1382-7.
105. Drexler SK, Foxwell BM. The role of toll-like receptors in chronic inflammation. *Int J Biochem Cell Biol.* 2010;42:506-18.
106. Gluba A, Banach M, Hannam S, Mikhailidis DP, Sakowicz A, Rysz J. The role of Toll-like receptors in renal diseases. *Nat Rev Nephrol.* 2010;6:224-35.
107. Richez C, Blanco P, Rifkin I, Moreau JF, Schaefferbeke T. Role for toll-like receptors in autoimmune disease: The example of systemic lupus erythematosus. *Joint Bone Spine.* 2011;78:124-30.
108. Boiardi L, Casali B, Farnetti E, et al. Toll-like receptor 4 (TLR4) gene polymorphisms in giant cell arteritis. *Clin Exp Rheumatol.* 2009;27(Suppl 52):S40-4.
109. Enevold C, Radstake TR, Coenen MJ, et al. Multiplex screening of 22 single nucleotide polymorphisms in 7 Toll-like receptors: an association study in rheumatoid arthritis. *J Rheumatol.* 2010;37:905-10.
110. Krüger B, Banas MC, Walberer A, et al. A comprehensive genotype-phenotype interaction of different Toll-like receptor variations in a renal transplant cohort. *Clin Sci (Lond).* 2010;119:535-44.
111. Nogueira E, Ozaki KS, Macusso GD, Quarim RF, Câmara NO, Pacheco-Silva A. Incidence of donor and recipient toll-like receptor-4 polymorphisms in kidney transplantation. *Transplant Proc.* 2007;39:412-4.
112. Ducloux D, Deschamps M, Yannarakis M, et al. Relevance of Toll-like receptor-4 polymorphisms in renal transplantation. *Kidney Int.* 2005;67:2454-61.
113. Dierselhuys M, Goulmy E. The relevance of minor histocompatibility antigens in solid organ transplantation. *Curr Opin Organ Transplant.* 2009;14:419-25.
114. Goulmy E, Schipper R, Pool J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med.* 1996; 334:281-5.
115. Kelly KJ. Heat shock (stress response) proteins and renal ischemia/reperfusion injury. *Contrib Nephrol.* 2005;148:86-106.
116. Aufricht C. Heat-shock protein 70: molecular supertool? *Pediatr Nephrol.* 2005;20:707-13.
117. Buraczynska M, Swatowski A, Buraczynska K, Dragan M, Ksiazek A. Heat-shock protein gene polymorphisms and the risk of nephropathy in patients with Type 2 diabetes. *Clin Sci (Lond).* 2009;116:81-6.
118. Fekete A, Viklick O, Hubáček JA, et al. Association between heat shock protein 70s and toll-like receptor polymorphisms with long-term renal allograft survival. *Transpl Int.* 2006; 19:190-6.
119. Israni AK, Li N, Sidhwani S, et al. Association of hypertension genotypes and decline in renal function after kidney transplantation. *Transplantation.* 2007;84:1240-7.
120. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev.* 2007;59:251-87.
121. Tripathi G, Dharmani P, Khan F, Sharma RK, Pandirikkal V, Agrawal S. High prevalence of ACE DD genotype among north Indian end stage renal disease patients. *BMC Nephrol.* 2006;17:7-15.
122. Zhang G, Wang H, Wang F, et al. Gene polymorphisms of the renin-angiotensin-aldosterone system and angiotensin II type 1-receptor activating antibodies in renal rejection. *Tohoku J Exp Med.* 2007;213:203-14.
123. Saggari-Malik AK, Afzal AR, Swisaman JS, Bland M, Sagnella GA, Eastwood JB. Lack of association of ACE/angiotensinogen association genotype with renal function in autosomal dominant polycystic kidney disease. *Genet Test.* 2000;4:299-303.
124. Cooper Worobey C, Fisher ND, Cox D, Forman JP, Curhan GC. Genetic polymorphisms and the risk of accelerated renal function decline in women. *PLoS One.* 2009;4:e4787.
125. Kone BC, Baylis C. Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. *Am J Physiol.* 1997;41:F561-78.
126. Wang Y, Kikuchi S, Suzuki H, Nagase S, Koyama A. Endothelial nitric oxide synthase gene polymorphism in intron 4 affects the progression of renal failure in non-diabetic renal diseases. *Nephrol Dial Transplant.* 1999;14:2898-902.
127. Shenker NS, Haldar NA, Reilly JJ, Bunce M, Welsh KI, Marshall SE. The impact of endothelial nitric oxide synthase polymorphisms on long-term renal allograft outcome. *Transpl Int.* 2003;16:391-5.
128. Akcay A, Sezer S, Ozdemir FN, et al. Association of the genetic polymorphisms of the renin-angiotensin system and endothelial nitric oxide synthase with chronic renal transplant dysfunction. *Transplantation.* 2004;78:892-8.
129. Artifoni L, Benetti E, Centi S, et al. The impact of eNOS, MTR and MTHFR polymorphisms on renal graft survival in children and young adults. *Nephrol Dial Transplant.* 2009;24:2931-7.
130. Pitrucc F, Moritz A, Montemurro M, et al. Selectively enhanced cellular signaling by Gi proteins in essential hypertension: Gαi2, Gαi3, Gβ1 & Gβ2 are not mutated. *Circ Res.* 1996;79:974-83.
131. Hocher B, Pleschka A, Yang F, Kraus R, Neumayer HH, Filler G. G protein beta3 subunit 825T genotype is not associated with differing outcome in pediatric renal transplant recipients. *Pediatr Transplant.* 2002;6:141-6.
132. Beige J, Kreutz R, Tscherkaschka I. Matrix analysis for the dissection of interactions of G-protein beta3 subunit C825T genotype, allograft function, and posttransplant hypertension in kidney transplantation. *Am J Kidney Dis.* 2002;40:319-24.
133. Cossu M, Carru C, Pes GM. Plasma homocysteine levels and C677T MTHFR gene polymorphism in stable renal graft recipients. *Transplant Proc.* 2001;33:1156-8.
134. Heidenreich S, Nowak-Gottl U, August C. Hypercoagulable state and graft rejection: is there a link? *Nephrol Dial Transplant.* 1999;14: 2293-6.

135. Irish A. Hypercoagulability in renal transplant recipients: identifying patients at risk of renal allograft thrombosis and evaluating strategies for prevention. *Am J Cardiovasc Drugs*. 2004;4:139-49.
136. Guermazi S, Znazen R. [Activated protein C resistance and factor V Leiden: Clinical interest.] *Pathol Biol (Paris)*. 2009. [Epub ahead of print].
137. Ekmekci Y, Keven K, Akar N, et al. Thrombophilia and avascular necrosis of femoral head in kidney allograft recipients. *Nephrol Dial Transplant*. 2006;21:3555-8.
138. Biglarnia A, Bergqvist D, Johansson M, Wadström J. Venous thromboembolism in live kidney donors—a prospective study. *Transplantation*. 2008;86:659-61.
139. Trifonova EA, Spiridonova MG, Stepanov VA. Genetic diversity and the structure of linkage disequilibrium in the methylenetetrahydrofolate reductase locus. *Genetika*. 2008;44:1410-9.
140. Mtiraoui N, Ezzidi I, Chaieb M, et al. MTHFR C677T and A1298C gene polymorphisms and hyperhomocysteinemia as risk factors of diabetic nephropathy in type 2 diabetes patients. *Diabetes Res Clin Pract*. 2007;75:99-106.
141. Ebbesen LS. Hyperhomocysteinemia, thrombosis and vascular biology. *Cell Mol Biol*. 2004;50:917-30.
142. Frosst P, Bloom HJ, Milos R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genet*. 1995;10:111-13.
143. Tripathi G, Sankhwar SN, Sharma RK, Baburaj VP, Agrawal S. Role of thrombotic risk factors in end-stage renal disease. *Clin Appl Thromb Hemost*. 2010;16:132-40.
144. Jamison RL, Shih MC, Humphries DE, et al. Effect of the MTHFR C677T and A1298C polymorphisms on survival in patients with advanced CKD and ESRD: a prospective study. *Am J Kidney Dis*. 2009;53:779-89.
145. Chu AY, Parekh RS, Astor BC, et al. Association of APOE polymorphism with chronic kidney disease in a nationally representative sample: a Third National Health and Nutrition Examination Survey (NHANES III) Genetic Study. *BMC Med Genet*. 2009;10:108.
146. Hernández D, Salido E, Linares J, et al. Role of apolipoprotein E epsilon 4 allele on chronic allograft nephropathy after renal transplantation. *Transplant Proc*. 2004;36:2982-4.
147. Cofán F, Cofan M, Rosich E, et al. Effect of apolipoprotein E polymorphism on renal transplantation. *Transplant Proc*. 2007;39:2217-8.
148. Kartal O, Inal V, Baysan O, Saqlam K. Relationship between serum leptin levels and left ventricular hypertrophy in obese hypertensive patients. *Anadolu Kardiyol Derg*. 2008;8:342-6.
149. Tripathi G, Sharma RK, Baburaj VP, Sankhwar SN, Jafar T, Agrawal S. Genetic risk factors for renal failure among north Indian ESRD patients. *Clin Biochem*. 2008;41:525-31.
150. Wang X, Axelsson J, Nordfors L, et al. Changes in fat mass after initiation of maintenance dialysis is influenced by the uncoupling protein 2 exon 8 insertion/deletion polymorphism. *Nephrol Dial Transplant*. 2007;22:196-202.
151. McLeod HL, Siva C. The thiopurine S-methyltransferase gene locus: implications for clinical pharmacogenomics. *Pharmacogenomics*. 2002;3:89-98.
152. Buzio L, De Palma G, Mazzoni P, et al. Glutathione S-transferase M1-1 and T1-1 as risk modifiers for renal cell cancer associated with occupational exposure to chemicals. *Occup Environ Med*. 2003;60:789-93.
153. Elhasid R, Krivoy N, Rowe JM, Sprecher E, Efrati E. Glutathione S-transferase T1-null seems to be associated with graft failure in hematopoietic SCT. *Bone Marrow Transplant*. 2010;45:1728-31.
154. Singh R, Manchanda PK, Kesarwani P, Srivastava A, Mittal RD. Influence of genetic polymorphisms in GSTM1, GSTM3, GSTT1 and GSTP1 on allograft outcome in renal transplant recipients. *Clin Transplant*. 2009;23:490-8.
155. Pagliuso RG, Abbud-Filho M, Alvarenga MP, et al. Role of glutathione s-transferase polymorphisms and chronic allograft dysfunction. *Transplant Proc*. 2008;40:743-5.
156. Amuchastegui S, Daniel KC, Adorini L. Inhibition of acute and chronic allograft rejection in mouse models by BXL-628, a nonhypercalcemic vitamin D receptor agonist. *Transplantation*. 2005;80:81-7.
157. Lavin PJ, Laing ME, O'Kelly P, et al. Improved renal allograft survival with vitamin D receptor polymorphism. *Ren Fail*. 2007;29:785-9.
158. Kahan BD. Current approaches to the use of sirolimus in renal transplantation. *Transplant Proc*. 2009;41:3011-5.
159. Wang P, Mao Y, Razo J, et al. Using genetic and clinical factors to predict tacrolimus dose in renal transplant recipients. *Pharmacogenomics*. 2010;11:1389-402.
160. Spiewak M, Malek LA, Kostrzewa G, et al. Influence of C3435T multidrug resistance gene-1 (MDR-1) polymorphism on platelet reactivity and prognosis in patients with acute coronary syndromes. *Kardiol Pol*. 2009;67:827-34.
161. Loh PT, Lou HX, Zhao Y, Chin YM, Vathsala A. Significant impact of gene polymorphisms on tacrolimus but not cyclosporine dosing in Asian renal transplant recipients. *Transplant Proc*. 2008;40:1690-5.
162. Sánchez-Velasco P, Rodrigo E, Valero R, et al. Intracellular ATP concentrations of CD4 cells in kidney transplant patients with and without infection. *Clin Transplant*. 2008;22:55-60.
163. Tang FY, Liu FY, Xie XW. Association of angiotensin-converting enzyme and endothelial Nitric Oxide synthase gene polymorphisms with vascular disease in ESRD patients in a Chinese population. *Mol Cell Biochem*. 2008;319:33-9.
164. Akman B, Tarhan C, Arat Z, Sezer S, Ozdemir FN. Renin-angiotensin system polymorphisms: a risk factor for progression to end-stage renal disease in vesicoureteral reflux patients. *Ren Fail*. 2009;31:196-200.