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

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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)

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Key words

Acute rejection. Allograft. Polymorphism. SNP. Th1 and Th2 system.
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 phenotypes1-6, 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 considered7,8.

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 underlie 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 responses9. 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 tolerance9.

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 transcribed10,11. 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 rejection

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>SNP</th>
<th>Position at chromosome</th>
<th>dbSNP ID/ PubMed ID</th>
<th>Effect on</th>
<th>Role</th>
<th>Region</th>
<th>Association with allograft rejection (Reference)</th>
<th>No. association with allograft rejection (Reference)</th>
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<tbody>
<tr>
<td>1.</td>
<td>TNF-α</td>
<td>G(−308)A, Chr-6p21</td>
<td>rs1800629</td>
<td>Inflammation, proinflammatory</td>
<td>Proinflammatory, local inflammation, endothelial activation</td>
<td>USA, Iran, Bulgaria, UK, Poland, Belgium, Mexico, India, Czech Republic, Finland, Greece, Germany, France</td>
<td>2, 28, 30, 33, 36, 37, 40, 41, 42, 43, 45, 46, 47, 48, 64</td>
<td>4, 28, 30, 47</td>
</tr>
<tr>
<td>2.</td>
<td>TNF-α</td>
<td>G(−238)A, Chr-6</td>
<td>rs361525</td>
<td>Inflammation, proinflammatory</td>
<td>Proinflammatory, local inflammation, endothelial activation</td>
<td>USA, UK, Iran</td>
<td>2, 28, 33</td>
<td>NA</td>
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<tr>
<td>3.</td>
<td>IFN-γ</td>
<td>T(+874)A, Chr-12</td>
<td>rs2430561</td>
<td>Inflammation, proinflammatory</td>
<td>Proinflammatory, pro-cellular, play a pivotal role in both innate and adaptive immune responses</td>
<td>USA, Iran, Bulgaria, Italy, Belgium, UK</td>
<td>6, 8, 26, 30, 32, 33, 34, 35</td>
<td>2, 32, 33, 36, 37, 39</td>
</tr>
<tr>
<td>4.</td>
<td>INF-γ</td>
<td>CA repeat, Chr-12</td>
<td>PubMed ID: 10068907</td>
<td>Inflammation, proinflammatory</td>
<td>Proinflammatory, pro-cellular</td>
<td>UK, USA, Iran, Mexico, Finland</td>
<td>4, 33, 35</td>
<td>2, 6, 43</td>
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<td>5.</td>
<td>IL-6</td>
<td>−174G/C, Chr-7</td>
<td>rs1800795</td>
<td>Inflammation, proinflammatory</td>
<td>Pro-cellular and humoral, acute phase protein production, fever</td>
<td>USA, Japan, UK, Bulgaria, Poland, India, Germany</td>
<td>2, 6, 22, 23, 26, 27, 28, 29, 30</td>
<td>46</td>
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<tr>
<td>6.</td>
<td>IL-6</td>
<td>+572 G/A, Chr-7</td>
<td>Not found</td>
<td>Inflammation, proinflammatory</td>
<td>Pro-cellular and humoral, acute phase protein production, fever</td>
<td>USA, UK, Germany</td>
<td>2</td>
<td>8, 29</td>
</tr>
<tr>
<td>7.</td>
<td>IL-4</td>
<td>C590T, Chr-1</td>
<td>rs2243250</td>
<td>Inflammation, proinflammatory</td>
<td>IL-4 inhibits the release of inflammatory mediators (such as TNF-α, IL-6, and IL-1α) from activated monocytes, thus counteracting their damaging effects in the graft</td>
<td>UK, Finland, Poland, Korea, USA</td>
<td>NA</td>
<td>38, 43, 46, 48, 62</td>
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<td>8.</td>
<td>IL-4</td>
<td>+1902 G/A, Chr-1</td>
<td>rs1801275</td>
<td>Inflammation, proinflammatory</td>
<td>Inhibits the release of inflammatory mediators</td>
<td>UK</td>
<td>NA</td>
<td>46</td>
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<tr>
<td>9.</td>
<td>IL-2</td>
<td>−330, Chr-4</td>
<td>PubMed ID: 15895884</td>
<td>Inflammation, proinflammatory</td>
<td>Control the immune response, regulate and coordinate acute renal allograft rejection</td>
<td>USA, USA, Finland</td>
<td>26, 43</td>
<td>2</td>
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<tr>
<td>Sr No.</td>
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<tr>
<td>10.</td>
<td>CCR5</td>
<td>d32, Chr-3</td>
<td>PubMed ID: 95080611</td>
<td>Inflammation, proinflammatory</td>
<td>Regulate the trafficking of leukocytes in immunity and inflammation</td>
<td>Iran, Germany, USA</td>
<td>74, 76, 78, 81</td>
<td>77, 79</td>
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<tr>
<td>11.</td>
<td>CX3CR</td>
<td>−V249I, Chr-16</td>
<td>rs3732379</td>
<td>Inflammation, proinflammatory</td>
<td>Regulate the trafficking of leukocytes in immunity and inflammation</td>
<td>Germany, USA</td>
<td>76, 80, 81</td>
<td>77</td>
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<tr>
<td>12.</td>
<td>CX3CR</td>
<td>−T280M, Chr-16</td>
<td>rs3732378</td>
<td>Inflammation, proinflammatory</td>
<td>Regulate the trafficking of leukocytes in immunity and inflammation</td>
<td>Germany, USA</td>
<td>76, 81</td>
<td>77</td>
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<tr>
<td>13.</td>
<td>CCR2</td>
<td>−V64I, Chr-3</td>
<td>rs1799864</td>
<td>Inflammation, proinflammatory</td>
<td>Regulate the trafficking of leukocytes in immunity and inflammation</td>
<td>Iran, USA</td>
<td>74, 77, 80, 81</td>
<td>NA</td>
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<tr>
<td>14.</td>
<td>CCR5</td>
<td>(−590) A/G, Chr-3</td>
<td>rs1799987</td>
<td>Inflammation, proinflammatory</td>
<td>Regulate the trafficking of leukocytes in immunity and inflammation</td>
<td>Iran, USA, Germany</td>
<td>74, 77, 78, 80</td>
<td>NA</td>
</tr>
<tr>
<td>15.</td>
<td>Lymphotoxin</td>
<td>+249 A/G, Chr-6</td>
<td>rs909253</td>
<td>Inflammation, proinflammatory</td>
<td>A protein that is produced by “killer” CD8+ T-cells that kill virally infected cells by producing holes in the cell’s cell membrane</td>
<td>Tunisia, UK, USA</td>
<td>97</td>
<td>7, 98</td>
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<tr>
<td>16.</td>
<td>Lymphotoxin</td>
<td>+365 C/G, Chr-6</td>
<td>Not found</td>
<td>Inflammation, proinflammatory</td>
<td>Associated with increased production of the TNF-β lymphotoxin following PHA mitogenic stimulation in vitro</td>
<td>Tunisia, UK, USA</td>
<td>97</td>
<td>7, 98</td>
</tr>
<tr>
<td>17.</td>
<td>Lymphotoxin</td>
<td>+720 C/A, Chr-6</td>
<td>Not found</td>
<td>Inflammation, proinflammatory</td>
<td>Associated with increased production of the TNF-β lymphotoxin following PHA mitogenic stimulation in vitro</td>
<td>Tunisia, UK, USA</td>
<td>97</td>
<td>7, 98</td>
</tr>
<tr>
<td>18.</td>
<td>MIF</td>
<td>−G173C, Chr-22</td>
<td>rs755622</td>
<td>Inflammation, proinflammatory</td>
<td>Macrophage migration inhibitory factor induces the expression of proinflammatory mediators by macrophages and activates T-cells</td>
<td>Iran, Sweden, China, Italy</td>
<td>33, 53, 55, 94, 95</td>
<td>NA</td>
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<tr>
<td>19.</td>
<td>IL-1α</td>
<td>−C889T, Chr-2</td>
<td>rs1800587</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland, Sweden, UK</td>
<td>54, 55</td>
<td>7, 46</td>
</tr>
<tr>
<td>20.</td>
<td>IL-1β</td>
<td>−C5887T, Chr-2</td>
<td>rs1143633</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland, Sweden UK, UK</td>
<td>54, 55</td>
<td>7, 46</td>
</tr>
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<td>Sr No.</td>
<td>SNP</td>
<td>Position at chromosome</td>
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<tr>
<td>21.</td>
<td><em>IL-1</em> β</td>
<td>-T1903C, Chr-2</td>
<td>rs1143627</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Sweden</td>
<td>55, 56</td>
<td>NA</td>
</tr>
<tr>
<td>22.</td>
<td><em>IL-1</em> β</td>
<td>-C3953T, Chr-2</td>
<td>rs1143634</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Sweden, India</td>
<td>55, 56</td>
<td>NA</td>
</tr>
<tr>
<td>23.</td>
<td><em>IL-1</em> β</td>
<td>T(3962)C, Chr-2</td>
<td>rs16944</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland, Sweden, India</td>
<td>55, 56</td>
<td>54</td>
</tr>
<tr>
<td>24.</td>
<td><em>IL-1</em> β</td>
<td>-C511T, Chr-2</td>
<td>rs16944</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland, Sweden, India</td>
<td>55, 56</td>
<td>54</td>
</tr>
<tr>
<td>25.</td>
<td><em>IL-1RN</em></td>
<td>+A9589T, Chr-2</td>
<td>rs454078</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>India</td>
<td>NA</td>
<td>56</td>
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<td>26.</td>
<td><em>IL-1RN</em></td>
<td>+C8061T, Chr-2</td>
<td>rs423904</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland</td>
<td>NA</td>
<td>54</td>
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<tr>
<td>27.</td>
<td><em>IL-1RN</em></td>
<td>+C11100T, Chr-2</td>
<td>rs315952</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland</td>
<td>NA</td>
<td>54</td>
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<td>28.</td>
<td><em>IL-1Ra</em></td>
<td>86bpVNTR, Chr-2</td>
<td>PubMed ID: 14563376</td>
<td>Inflammation, proinflammatory</td>
<td>IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells</td>
<td>Poland, India</td>
<td>54, 56</td>
<td>NA</td>
</tr>
<tr>
<td>29.</td>
<td><em>ICAM-1</em></td>
<td>E469 exon 6, Chr-19</td>
<td>rs5498</td>
<td>Inflammation, proinflammatory</td>
<td>Involved at distinct stages in the host response</td>
<td>USA, UK, Tunisia, Mexico</td>
<td>85, 86, 87</td>
<td>87, 88</td>
</tr>
<tr>
<td>30.</td>
<td><em>ICAM-2</em></td>
<td>R241 exon 4, Chr-19</td>
<td>PubMed ID: 16432463</td>
<td>Inflammation, proinflammatory</td>
<td>Uregulation of adhesion molecules in CAN</td>
<td>Mexico, Tunisia</td>
<td>88</td>
<td>87</td>
</tr>
</tbody>
</table>
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 and Inflammation | Region       | Association with allograft rejection (Reference) | No. association with allograft rejection (Reference) |
|--------|-------|------------------------|--------------------|----------------------|------------------------|--------------|------------------------------------------------|
| 31     | L-selectin | +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     | E-selectin | +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     | E-selectin | +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     | CTLA4  | −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     | CTLA4  | +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     | TAP1   | 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     | TAP2   | 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     | LMP2   | Chr-6                  | PubMed ID: 8089211  | Inflammation, proinflammatory | Takes part in cytosolic proteolysis | Japan       | 16 | NA |
| 39     | VEGF   | −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     | VEGF   | −2578C/A               | rs 699947        | Proinflammatory        | Potent regulator of normal and abnormal angiogenesis | The Netherlands | NA | 73 |
| 41     | FC receptor | 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>
<thead>
<tr>
<th>Sr No.</th>
<th>SNP</th>
<th>Position at chromosome</th>
<th>dbSNP ID/ PubMed ID</th>
<th>Effect on</th>
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<th>No. association with allograft rejection (Reference)</th>
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</thead>
<tbody>
<tr>
<td>42.</td>
<td>TLR</td>
<td>TLR4, Asp299Gly and Thr399Ile</td>
<td>rs4986790 and rs4986791</td>
<td>Proinflammatory</td>
<td>Toll-like receptors have a key role in innate immunity</td>
<td>Poland, Germany, Brazil, France, Hungary</td>
<td>106, 110, 112</td>
<td>111, 118</td>
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<tr>
<td>43.</td>
<td>TGFB</td>
<td>T(+869)C, Chr-19q13.2</td>
<td>rs1982073</td>
<td>Inflammation, anti-inflammatory</td>
<td>Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth</td>
<td>Iran, Bulgaria, Mexico, Germany, Korea, China</td>
<td>4, 28, 30, 31, 33, 62, 64, 68</td>
<td>21, 69</td>
</tr>
<tr>
<td>44.</td>
<td>TGFB</td>
<td>C(+915)G, Chr-19q13.2</td>
<td>rs1800471</td>
<td>Inflammation, anti-inflammatory</td>
<td>Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth</td>
<td>USA, Iran, Bulgaria, Germany, Korea, China</td>
<td>30, 47, 62, 68</td>
<td>21, 69</td>
</tr>
<tr>
<td>45.</td>
<td>TGFB</td>
<td>aa25 R/P, Chr-19q13.2</td>
<td>Not found</td>
<td>Inflammation, anti-inflammatory</td>
<td>Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth</td>
<td>USA, Iran, UK, India</td>
<td>30, 33, 68</td>
<td>8, 30</td>
</tr>
<tr>
<td>46.</td>
<td>TGFB</td>
<td>–509 C/T, Chr-19q13.2</td>
<td>rs1800469</td>
<td>Inflammation, anti-inflammatory</td>
<td>Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth</td>
<td>USA, UK, India</td>
<td>68</td>
<td>8, 30</td>
</tr>
<tr>
<td>47.</td>
<td>TGFB</td>
<td>–880 G/A, Chr-19q13.2</td>
<td>rs7067496</td>
<td>Inflammation, anti-inflammatory</td>
<td>Anti-inflammatory, immunosuppressive, profibrogenic, inhibits cell growth</td>
<td>USA, UK</td>
<td>NA</td>
<td>8, 30</td>
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<tr>
<td>48.</td>
<td>IL-10</td>
<td>G(-1082)A, Chr6q22-q23</td>
<td>rs1800896</td>
<td>Inflammation, anti-inflammatory</td>
<td>Play an important role in modulating inflammatory response in acute kidney injury</td>
<td>Iran, Bulgaria, Mexico, UK, Germany, India</td>
<td>4, 30, 43</td>
<td>20, 33, 35, 43, 48</td>
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<tr>
<td>49.</td>
<td>IL-10</td>
<td>C(-819)T, Chr-6q22q23</td>
<td>rs1800871</td>
<td>Inflammation, anti-inflammatory</td>
<td>Play an important role in modulating inflammatory response in acute kidney injury</td>
<td>Iran, Bulgaria, Mexico, India, Germany</td>
<td>30, 33, 38, 43</td>
<td>33, 35, 43, 62</td>
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<tr>
<td>50.</td>
<td>IL-10</td>
<td>C(-592)A, Chr-6q22q23</td>
<td>rs1800872</td>
<td>Inflammation, anti-inflammatory</td>
<td>Pro-humoral, anti-inflammatory, macrophage suppressant</td>
<td>UK, Bulgaria, Iran USA, Germany</td>
<td>30, 38, 43</td>
<td>28, 35, 43, 62</td>
</tr>
<tr>
<td>51.</td>
<td>DARC</td>
<td>Arg89Cys</td>
<td>rs3027020</td>
<td>Anti-inflammatory</td>
<td>Duffy receptor for chemokines (DARC) show increased expression on post-capillary venules following transplantation</td>
<td>USA</td>
<td>80, 83</td>
<td>84</td>
</tr>
</tbody>
</table>
Table 2. Role of non-immunological genetic variants and their association with renal allograft

<table>
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<tr>
<th>Sr No.</th>
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<th>Position at chromosome</th>
<th>dbSNP ID/ PubMed ID</th>
<th>Effect on</th>
<th>Role</th>
<th>Ethnicity</th>
<th>Association (Reference)</th>
<th>No. association (Reference)</th>
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<td>ACE</td>
<td>287 bp ID intron 16, Chr-17q23</td>
<td>ID: 2554286</td>
<td>Hypertension</td>
<td>Increased p-ACE levels, association with CIN and ATN</td>
<td>Iran, USA, UK, China, Iran, India</td>
<td>33, 120, 121, 122, 149</td>
<td>123</td>
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<tr>
<td>2</td>
<td>AGT</td>
<td>M235T, Chr-1 at 1p36-p34</td>
<td>rs4762</td>
<td>Hypertension</td>
<td>Progression with chronic interstitial nephritis</td>
<td>Iran, USA, Turkey</td>
<td>33, 119, 120, 124</td>
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<tr>
<td>3</td>
<td>ApoE</td>
<td>e2, e4, Chr-19q13.2.5</td>
<td>ID: 10662539</td>
<td>Atherosclerosis</td>
<td>Increases risk of cardiovascular disease after transplantation, elevated plasma Lp (a) levels</td>
<td>Spain, Spain, USA</td>
<td>145, 146, 147</td>
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<tr>
<td>4</td>
<td>eNOS</td>
<td>Intron 4 STR, 27 bp STR, Chr-7q36</td>
<td>ID: 18390539</td>
<td>Atherosclerosis</td>
<td>Increase severity of urinary protein excretion</td>
<td>China, India, Turkey, Italy</td>
<td>126, 129, 163</td>
<td>127, 128, 149</td>
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<td>5</td>
<td>Factor V Leiden</td>
<td>FVL G1691A, at Chr1q23</td>
<td>rs2232708</td>
<td>Factor Xa activation</td>
<td>Venous thromboembolism and deep venous thrombosis, increased coagulation</td>
<td>Germany, Australia, Turkey, India</td>
<td>134, 135, 137</td>
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<td>6</td>
<td>G protein</td>
<td>GNB3 C825T, Chr-1p36, exon 10</td>
<td>rs5443</td>
<td>Hypertension</td>
<td>Higher CRP levels and mortality</td>
<td>India, Germany, Turkey, Czech Republic</td>
<td>131, 132, 148</td>
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<td>GSTT1</td>
<td>(±)480 bp, Chr-11q13</td>
<td>ID: 17000715</td>
<td>Phase II metabolizing enzymes</td>
<td>Activating and inactivating oxidative metabolites of carcinogenic compounds may associated with renal failure</td>
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<td>8</td>
<td>GSTM1</td>
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<td>ID: 12117698</td>
<td>Involved in detoxification</td>
<td>Activating and inactivating oxidative metabolites of carcinogenic compounds may associated with renal failure</td>
<td>Israel, India, Brazil</td>
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<td>9</td>
<td>GSTP1</td>
<td>–313 A/G, Chr-11q13</td>
<td>rs1695</td>
<td>Involved in detoxification</td>
<td>Activating and inactivating oxidative metabolites of carcinogenic compounds may associated with renal failure</td>
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<td>Leptin</td>
<td>G2548A, Chr-7q22-35</td>
<td>rs7799039</td>
<td>Energy expenditure</td>
<td>Fat accumulation during dialysis</td>
<td>India</td>
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<td>11</td>
<td>MTHFR</td>
<td>A1298C, at Chr1p36.3</td>
<td>rs2066462</td>
<td>Pro-coagulation</td>
<td>Increases plasma homocysteine level, thrombotic risk</td>
<td>Bosnia and Herzegovina-Europe, India, Italy, Iran</td>
<td>134, 143, 144, 145</td>
<td>129</td>
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closely homologous to other class I gene sequences and was thought to show little polymorphism\textsuperscript{12}.

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\textsuperscript{13,14}.

**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\textsuperscript{15} (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 (NFκB) and signal transducers and activators of transcription (STAT)\textsuperscript{16,17}. 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\textsuperscript{18,19}. Several studies have addressed the extent to which specific polymorphisms directly or indirectly influence the level of cytokine production and expression\textsuperscript{20,21}. 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\textsuperscript{22,23}. It has a specific IL-6 receptor and a signal-transducing subunit (gp130). Circulating gp130 acts as an antagonist of IL-6 biological functions\textsuperscript{24}.

The potential causes of elevated plasma IL-6 levels in renal transplant patients\textsuperscript{22} may be due to (i) the loss of kidney function, (ii) uremia \textit{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\textsuperscript{23} 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\textsuperscript{23}. Elevated circulating IL-6 levels were independently associated with progressive carotid atherosclerosis during the first 12 months of dialysis treatment\textsuperscript{25}.

Within the promoter region of the \textit{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\textsuperscript{25,26}. This \textit{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\textsuperscript{27}. Furthermore, Marshall, et al.\textsuperscript{34} have demonstrated the protective effect of the presence of the –174G allele from the donor on graft survival. Muller-Steinhardt, et al.\textsuperscript{28} have shown that two more polymorphisms (572G/C and -597G/A) are present in \textit{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\textsuperscript{28-30}, IL-6 is still grouped with the controversial cytokines\textsuperscript{20,27} (Table 1).

**Interferon-gamma**

Activated T-cells produce IFN-\(\gamma\), which has several properties: activation of macrophages, lytic effect, potentiating the actions of other interferons, and inhibition of intracellular microorganisms other than viruses. The \textit{IFN-\(\gamma\)} gene lies in 12q15, is 4974 bp long, and encodes a protein of 166 amino acids long\textsuperscript{31}. The \textit{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\textsuperscript{32,33}.  

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\(\text{IFN-\(\gamma\)}\)
Further SNP in \( IFN-\gamma \) may affect the production of \( IFN-\gamma \). A polymorphic dinucleotide (CA)n 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\(^6,^{34,35}\). A study has shown that early accumulation of donor's \( IFN-\gamma \) in the graft causes immune tolerance induction, resulting in better graft survival\(^36\). In contrast, another study\(^37\) 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\(^35\). The 12 CA repeat was found in 54.3% of patients with acute rejection episodes in that study\(^35\). Furthermore, Asderakis, et al.\(^35\) 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\(^6,^{38}\) 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 \( IFN-\gamma \)\(^39\). On the contrary, another report\(^33\) 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\(^40\). 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\(^40\).

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\(^41\).

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\(^2,^{42-45}\). 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\(^28,^{33}\). It is reported that 14 repeats of (AC) di-nucleotide microsatellite in \( TNF-\alpha \) is associated with renal allograft failure\(^3\). 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\(^33,^{40,46}\). Akalin and Murpy\(^47\), Hutchinson, et al.\(^4\) and Marshal, et al.\(^28\) 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-α-308 with IL-10-1082 and TGF-β codon 10 and 25 variants. However, both Poole, et al.48 and McDaniel, et al.2, on the basis of their studies on 120 and 77 renal transplant recipients, have shown that TNF-α-308 with IL-10-1082 and TGF-β 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.4 have used the posttransplantation data at five years while Marshall, et al.28 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). Vandebroek, et al.49 have reported that its three well-studied members, i.e. two agonists, IL-1α and IL-1β, and the antagonist IL-1Ra, IL-1α and β, 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 response49. The IL-1 gene polymorphisms have been associated with a variety of diseases in which inflammation has been suspected to play a role50, including systemic lupus erythematosus51 and cardiovascular disease52. 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)53. 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)53. 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.54 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-1β) 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-1β 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-1β, may play an important role55. Manchanda, et al. studied the genetic association of IL-1β and IL-1Ra gene polymorphism with allograft function in 136 renal transplant patients56. 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-1β/IL-1Ra 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. 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. 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. The most commonly studied genetic variant of IL-4 is 590C/T while that of IL-4 receptor is 1902A/G. Lee, et al. 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., 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. 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, 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. 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.

Polymorphisms in IL-10 regulatory sites was found at positions 1082G/A, 819C/T and 592C/A. Corresponding to these polymorphisms, GCC haplotype is associated with high production and ATA haplotype with low production of IL-10. 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. However, Alkaluppi, et al. 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. and Kocierz, et al. 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-γ, TGF-β and TNF-α, have shown strong protective effects.

**Growth factors**

*Transforming growth factor-beta 1*

Transforming growth factor-beta 1 (TGF-β1) regulates proliferation and differentiation of many cell types. In the immune system, TGF-β1 affects many cell types, e.g. T-cell survival, proliferation, Th differentiation, and effector functions. The TGF-β1 gene lies in 19q13.2, is 23402 bp long and encodes a protein of 390 amino acids. In kidney transplantation, TGF-β1 is known as a fibrogenetic factor, whose increased expression has been associated with chronic allograft rejection. The TGF-β1 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-β1. 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 α-helix involved in transport of TGF-β protein through the cell. In renal transplants, the exact role of this polymorphism is not yet elucidated. However, Cho, et al. and Li, et al. have shown its role in chronic allograft nephropathy. Melk, et al. studied 75 renal allograft dysfunction patients and 125 controls and found that codon 10 low-producing genotype showed a low expression of TGF-β 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. Some studies have clearly illustrated that high-producing TGF-β 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. Shahbazi, et al. 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. 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. 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\textsuperscript{75}. Chemokine receptor polymorphisms may have diverse effects on the rejection process\textsuperscript{74}. The common genetic variants described in renal transplant patients\textsuperscript{76} 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\textsuperscript{76,77}.

### 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.\textsuperscript{78} 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\textsuperscript{79}. 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\textsuperscript{79}.

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

Nelson, et al.\textsuperscript{78} 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\textsuperscript{+} T-cells\textsuperscript{78}. In another study, Abdi, et al.\textsuperscript{77} 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\textsuperscript{80}.

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.\textsuperscript{81} 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\(^8\). 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\(^8\). 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–b–) African American patients (p = 0.003)\(^8\). Duffy (a–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\(^8\). 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\(^8\). 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\(^8\). However, another SNP (C/A in exon 6, resulting in the amino acid substitution Glu469Lys) has shown no such association\(^8\) (Table 1).

**Fc receptor**

Several studies have identified FcγRIIa 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\(^8\). 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 FcγRIIb allele polymorphisms in 171 renal recipients. They reported that FcγRIIb polymorphisms did not affect the acute rejection in a Chinese population\(^8\). Pawlik, et al. 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\(^8\). 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\textsuperscript{93} and data are available for its role in graft rejection\textsuperscript{94}. The MIF promoter contains a single nucleotide G→C polymorphism at position −173 (SNP rs755622). Marina, et al.\textsuperscript{95} 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.\textsuperscript{96}, where allele 1 of (AT)n STR showed a potential protective effect, but no such correlation was found for the SNP. Gorgi, et al.\textsuperscript{97} 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.\textsuperscript{98} 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\textsuperscript{99,100}. 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\textsuperscript{101}. 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\textsuperscript{102}.

**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\textsuperscript{103}. Their tasks include phagocytosis and activation of the complement pathway and of numerous cytokines, such as IL-1\textsubscript{β}, IL-6, and TNF-α\textsuperscript{104}. 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\textsuperscript{105}. 


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\textsuperscript{106}, systemic lupus erythematosus\textsuperscript{107}, giant cell arteritis and polymyalgia rheumatica\textsuperscript{108}, rheumatoid arthritis\textsuperscript{109}, 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.\textsuperscript{110} 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\textsuperscript{111}. They observed that neither polymorphism was associated with renal allograft outcomes. However, Ducloux, et al.\textsuperscript{112} 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\textsuperscript{113}. 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\textsuperscript{113}. 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\textsuperscript{113,114}.

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\(^\text{115}\). Some studies also report the potential for biomolecular profiling of newborns for the risk of acute renal failure\(^\text{116}\) and the risk of nephropathy\(^\text{117}\).

The most studied gene polymorphisms in HSP-70 are HSPA1A G(190)C, HSPA1B A(1267)G in human renal transplant. Fekete, et al.\(^\text{118}\) 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\(^\text{119}\).

### Renin-angiotensin system

The renin-angiotensin aldosterone system (RAAS) has a key role in both cardiovascular and renal pathophysiology\(^\text{120}\). 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. Zhang, et al. 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. 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. 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 235 allele was transmitted significantly more frequently than expected and may thus play a significant role in progression of disease in these patients. Azarpira, et al. investigated the possible links between angiotensinogen (AGT M235T) genotypes and 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. Nitric oxide (NO), a relaxing factor expressed in endothelium, is reported to be a potent regulator of intrarenal homodynamics. The endothelial nitric oxide synthase (eNOS) gene comprises 26 exons that span 21 kilobases (kb) and is located on chromosome 7q35-3b. 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)n repeat in intron 13 and two biallelic markers in intron 18 have been proved to be not associated with hypertension.

Wang, et al. 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. 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. Likewise, Akcay, et al. also showed that graft function was not affected by eNOS gene polymorphisms.

Artifoni, et al. 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 β3 subunit of heterotrimeric G-proteins (GNβ3) mapping to chromosome 12p has been identified and has demonstrated to be significantly associated with essential hypertension. The GNβ3 825 T allele is also associated with enhanced G-protein activation, resulting in increased cell proliferation and hypertension and significant outcome in pediatric transplantation. The β3 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 GNβ3-825C/T polymorphism has been shown to be associated with twofold elevated risk of kidney allograft loss. Beige, et al. have shown that GNβ3-TT genotype contributes significantly in accelerated loss of graft function. They also suggested that high graft loss is associated with GNβ3-825TT genotype. The GNβ3-825C/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 GNβ3-825 polymorphism.

**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, 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.

**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, F5R506Q, 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 (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, Arg506Gln, disrupting the cleavage site recognized by activated protein C. 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 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.

**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\(^{138}\).

**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\(^{139}\). 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\(^{140}\). 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\(^{140,141}\). 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\(^{142}\). 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\(^{151}\). 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\(^{143}\). Azarpira, et al.\(^{33}\) 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\(^{129,144}\).

**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\(^{145}\). In another study, Hernández, et al.\(^{146}\) reported that ApoE4 allele was associated with chronic allograft nephropathy after renal transplantation. Moreover, in chronic kidney disease patients, Chu, et al.\(^{145}\) 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\(^{147}\). 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. In addition to its effect on appetite and energy expenditure, a direct effect of leptin on blood pressure has recently been reported. 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. 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. 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. 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. 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 (α, μ, π, σ, θ, κ, ζ)\(^{152}\), 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 α or A, GST μ or m, GST θ or T and GST π or P. The class π GST genes exist as a single functional gene in humans, whereas class α, μ and θ families contain multiple distinct genes, sharing ~55, 65, and 50% homology, respectively\(^{152}\). 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\(^{152}\).

Recently, Elhasid, et al.\(^{153}\) 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.\(^{154}\) 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\(^{155}\).

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

Vitamin D receptor (VDR) [1,25(OH)2D3] agonists have been shown to reduce short-and long-term allograft rejection in animal models\(^{156}\). 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.\(^{157}\) performed these gene polymorphisms in 379 renal transplant recipients and were genotyped for VDR (FokI and ApaI). There was significantly improved allograft survival for patients who were homozygous or heterozygous for the VDR FokI 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\(^{33}\).

**Genetic variants affecting pharmacokinetics of immunosuppressors**

Immunosuppressor drugs carry a narrow therapeutic range and a wide interindividual variation in their pharmacokinetics\(^{158}\).
Tacrolimus has a narrow therapeutic window and shows significant interindividual difference in dose requirement. Wang, et al.\textsuperscript{159} 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 \textit{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 (\textit{MDR1}) that uses these immunosuppressors as its substrate\textsuperscript{160}. Four important SNP have been studied in \textit{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\textsuperscript{160}. 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\textsuperscript{161}. Exon 26 SNP-3435C/T is reported to be associated with increased expression of \textit{MDR1} gene in CD56\textsuperscript{+} 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\textsuperscript{162}. 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 affect 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.

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