The Transcriptome of the Implant Biopsy Identifies Donor Kidneys at Increased Risk of Delayed Graft Function


Abbreviations: DD, deceased donor; LD, living donor; DGF, delayed graft function; IGF, immediate graft function; GFR, glomerular filtration rate; IQR, interquartile range; PCA, principal components analysis; PC1, principal component 1; RMA, robust multi-chip averaging.

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Introduction

The quality of the donor organ is a key determinant of early and late allograft function. Evaluation of organ quality at the time of transplantation, as a predictor of performance, is a serious challenge in terms of acceptance of an organ as well as individualization of posttransplant management. Screening and scoring systems utilizing biopsies and/or established clinical parameters have been proposed to assess acceptability of a deceased donor (DD) kidney for transplantation and risk of delayed graft function (DGF) (1–11). The existing scoring systems, however, have low predictive value for individual transplants (2,4). Improved assessment of organ quality and risk of DGF would therefore have important clinical significance (12–16).

Donor factors have been estimated to account for 35–45% of the variability in early allograft function, demonstrating the interaction of donor and recipient factors in determining the posttransplant course (17). Factors intrinsic to the transplanted kidney include donor age, size, gender and diseases such as hypertension. Acute injury includes stresses of brain death, donor instability, organ recovering and preservation. Recipient factors include hypotension, poor cardiac output and age. The interplay between intrinsic, acute and recipient features is reflected by less DGF and better outcomes in living donor (LD) compared to DD kidneys, as LD kidneys have fewer peri-transplant stresses and more selection against unfavorable intrinsic donor factors (18,19). The relatively poor performance of current screening and scoring systems when applied to individual kidneys likely reflects their inability to capture the combined impact of all these participating factors on the kidney itself.

The assessment of gene expression in the donor organ itself is an appealing strategy to determine organ quality...
and predict performance. The transcriptome may provide a comprehensive measurement of the individual kidney’s response to intrinsic donor and acute injury factors. Previous microarray analyses in small numbers of patients examined the transcriptome prior to revascularization in human kidney transplant biopsies. In one study, induction of pneumoperitoneum, prior to laparoscopic donor nephrectomy, was associated with altered gene expression compared to open donor nephrectomy (20). Another study identified 132 transcripts including coagulation and complement genes that separated DD and LD kidneys (21). No study thus far has convincingly identified transcriptome changes predictive of early graft function.

The current study analyzes the transcriptome of postperfusion implant biopsies, thus encompassing all intrinsic and acute features. Unsupervised analyses in a large group of renal transplant recipients identified gene sets that discriminate DD from LD kidneys, and among DD kidneys a group at increased risk of DGF.

Materials and Methods

Patients and clinical data collected

Implant biopsies taken just before the end of the transplant surgery have been standard of care in our center. Consent was obtained from renal transplant recipients, as approved by the Health Research Ethics Board of the University of Alberta, for an additional 18-gauge core sample for transcriptome analysis. Donor data were collected retrospectively and recipient data were collected prospectively. Renal allografts were biopsied intra-operatively within 1 h of revascularization. One core was sent for histology. The additional core sample was immediately placed into “RNA-later” for subsequent RNA extraction. All biopsies were read using conventional renal histopathologic techniques by nephropathologists. Biopsies with cortical tissue and at least 20 glomeruli per sample were considered adequate. Individual donor kidney histologic scores were calculated based on the global kidney score described by Remuzzi et al. (1,9), the Banff classification (22) and the percentage of sclerosed glomeruli (5,10).

DGF was defined as the need for dialysis within the first week after transplantation. The decision to initiate dialysis was at the discretion of the attending physicians, without involvement of study investigators. Known risk factors for poor posttransplant function were defined based on extended donor criteria and other factors predisposing to acute kidney injury (2–7,11,13). Glomerular filtration rate (GFR) was estimated using Cockcroft Gault equation: \( \text{GFR} = \frac{140 \times \text{age} \times \text{weight}}{72 \times \text{serum creatinine} \times 0.0113} \). Clinical risk scores were calculated according to the weighted nomogram for predicting DGF by Irish, and variables identified by Nyberg and Schold for assessing quality and early function of DD kidneys (2,3,7).

The maintenance immunosuppressive regimen consisted of corticosteroids (n = 82 patients), mycophenolate mofetil (n = 85) and either tacrolimus (n = 65) or cyclosporine (n = 15). Altogether six patients received sirolimus and two azathioprine. As induction therapy 62 received anti-CD25 monoclonal antibodies (Basiliximab or Daclizumab), 8 received polyclonal rabbit ATG antibodies. All rejection episodes were treated with steroid bolus therapies, patients with steroid-resistant T-cell-mediated rejections received polyclonal (n = 2 cases) or monoclonal (n = 1 case) antibodies, patients with antibody-mediated rejections received in addition polyclonal (n = 3 cases) and/or i.v. immunoglobulins (n = 1) and/or plasmapheresis (n = 1).

RNA preparation and amplification

Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, Valencia, CA), and amplified according to the Affymetrix® protocol (Santa Clara, CA) protocol. If the starting input of cRNA was below 2.5 μg an additional round of linear amplification was conducted. RNA yields were measured by UV absorbance and RNA fixed and acute features assessed by Agilent Bioanalyzer.

Microarray processing

RNA labeling and hybridization to the Affymetrix® GeneChip microarrays (human Hu133 plus 2.0) was carried out according to the protocols included in the Affymetrix® GeneChip Expression Analysis Technical Manual (www.affymetrix.com). The RNA of each individual sample was run on an individual chip, no samples were pooled.

Analysis of the transcriptome and clinical data

As a first step the raw data of all individual sample chips were normalized in one batch and preprocessed using robust multi-chip averaging (RMA), implemented in Bioconductor version 1.7 and R version 2.2. An inter-quartile range (IQR) cutoff of 0.5 log2 units was then used to filter out probe sets with low variability across the entire dataset. Unsupervised clustering methods, DNA intelligent analysis (DIANA) and K-means and principal component analysis (PCA) were then used to visualize patterns in the dataset without any a priori sample classification. Among the different patient groups, dichotomous variables were compared using the chi-square test or Fisher’s exact test. Continuous variables were compared using the t-test for those variables that were approximately normally distributed, and the nonparametric Mann-Whitney U-test for those that were not. Standard class comparison methods were used to compare known classes in search of differentially expressed genes. All adjusted p-values reported refer to false discovery rates, e.g. an adjusted p-value of 0.01 signifies that 1% of the probe sets identified as significant at the 0.01 level will, on average, be false positives. Biological pathways were identified using the KEGG-library (www.genome.ad.jp/kegg). A logistic regression model was developed with DGF as the dependent variable and principal component 1 loadings (PC1), clinical and histopathological scores as independent variables.

Results

Patient demographics

Eighty-seven consecutive implant biopsies from patients undergoing renal transplantation in our center between August 2004 and September 2006 were included: 42 from 31 DDs, including 11 pairs and 45 from LDs (Table 1). The early function was better in LD kidneys. Incidence of DGF was significantly greater in DD kidneys: of 42 DD kidneys, 10 manifested DGF, whereas 1 of 45 LD recipients had DGF (p = 0.003). LD kidneys had a lower serum creatinine level at day 7 posttransplant, but there was no difference in renal function at 1 year between DD and LD. The mean duration of follow-up was 381 ± 187 days. During follow-up, two patients died with functioning grafts, no further grafts were lost, giving a graft and patient survival rate of 98%. Demographics and clinical characteristics of all LD and DD implants are outlined in Table 1. Differences
Mueller et al.

Table 1: Patient demographics and general characteristics of the implant biopsies

<table>
<thead>
<tr>
<th>Variables</th>
<th>LD (n = 45 kidneys)</th>
<th>DD (n = 42 kidneys)</th>
<th>p</th>
<th>DD1 (n = 21 kidneys)</th>
<th>DD2 (n = 21 kidneys)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.5 ± 13.5</td>
<td>53.0 ± 12.4</td>
<td>0.387</td>
<td>50.6 ± 13.0</td>
<td>55.4 ± 11.5</td>
<td>0.213</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.3 ± 20.6</td>
<td>76.8 ± 16.0</td>
<td>0.107</td>
<td>75.0 ± 13.7</td>
<td>78.5 ± 18.2</td>
<td>0.494</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (69%)</td>
<td>27 (64%)</td>
<td>0.649</td>
<td>13 (62%)</td>
<td>14 (67%)</td>
<td>0.747</td>
</tr>
<tr>
<td>Female</td>
<td>14 (31%)</td>
<td>15 (36%)</td>
<td>8 (38%)</td>
<td>7 (33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.0 ± 11.3</td>
<td>43.7 ± 18.3</td>
<td>0.605</td>
<td>42.8 ± 19.2</td>
<td>45.2 ± 18.4</td>
<td>0.677</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (31%)</td>
<td>261 (62%)</td>
<td>0.004</td>
<td>17 (81%)</td>
<td>9 (43%)</td>
<td>0.011</td>
</tr>
<tr>
<td>Female</td>
<td>31 (69%)</td>
<td>161 (38%)</td>
<td>4 (19%)</td>
<td>12 (57%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>72 ± 13</td>
<td>69 ± 26</td>
<td>0.577</td>
<td>66 ± 19</td>
<td>73 ± 21</td>
<td>0.386</td>
</tr>
<tr>
<td>Cold ischemia time (h)</td>
<td>3.7 ± 2.0</td>
<td>16.2 ± 5.7</td>
<td>&lt;0.001</td>
<td>17.2 ± 5.5</td>
<td>15.2 ± 5.8</td>
<td>0.249</td>
</tr>
<tr>
<td>Revascularization time (min)</td>
<td>36 ± 6</td>
<td>40 ± 9</td>
<td>0.058</td>
<td>39 ± 8</td>
<td>40 ± 9</td>
<td>0.492</td>
</tr>
<tr>
<td>Intraoperative MAP2 (mmHg)</td>
<td>77 ± 8</td>
<td>81 ± 8</td>
<td>0.044</td>
<td>80 ± 8</td>
<td>81 ± 8</td>
<td>0.775</td>
</tr>
<tr>
<td>Banff score of the kidneys ≥1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulitis ’g’</td>
<td>0 (0%)</td>
<td>1 (2.6%)</td>
<td>0.464</td>
<td>0 (0%)</td>
<td>1 (4.8%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Glomerulopathy ’cg’</td>
<td>1 (2.2%)</td>
<td>0 (0%)</td>
<td>1.000</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Interstitial fibrosis ’ci’</td>
<td>9 (20.0%)</td>
<td>19 (48.7%)</td>
<td>0.010</td>
<td>10 (55.6%)</td>
<td>9 (42.9%)</td>
<td>0.527</td>
</tr>
<tr>
<td>Tubulitis ‘t’</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>N/A</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Tubular atrophy ’ct’</td>
<td>15 (33.3%)</td>
<td>21 (53.8%)</td>
<td>0.078</td>
<td>9 (50.0%)</td>
<td>12 (67.1%)</td>
<td>0.752</td>
</tr>
<tr>
<td>Intimal arteritis ’v’</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>N/A</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Fibrous intimal thickening ’cv’</td>
<td>30 (66.7%)</td>
<td>30 (78.9%)</td>
<td>0.231</td>
<td>15 (88.2%)</td>
<td>15 (71.4%)</td>
<td>0.257</td>
</tr>
<tr>
<td>Arteriolar hyalnosis ’ah’</td>
<td>19 (42.2%)</td>
<td>21 (55.3%)</td>
<td>0.381</td>
<td>7 (38.9%)</td>
<td>14 (66.7%)</td>
<td>0.113</td>
</tr>
<tr>
<td>Mesangial matrix increase ’mm’</td>
<td>1 (2.2%)</td>
<td>5 (12.8%)</td>
<td>0.092</td>
<td>3 (16.7%)</td>
<td>2 (9.5%)</td>
<td>0.647</td>
</tr>
<tr>
<td>Banff score total</td>
<td>2.0 ± 1.7</td>
<td>3.0 ± 2.0</td>
<td>0.011</td>
<td>2.8 ± 1.7</td>
<td>3.2 ± 2.2</td>
<td>0.468</td>
</tr>
<tr>
<td>Glomerulosclerosis (≥20%)</td>
<td>0 (0%)</td>
<td>2 (4.8%)</td>
<td>0.23</td>
<td>0 (0%)</td>
<td>2 (9.5%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Global kidney score3</td>
<td>2.4 ± 1.6 (12)</td>
<td>3.4 ± 1.9 (12)</td>
<td>0.177</td>
<td>3.4 ± 1.9 (12)</td>
<td>0.177</td>
<td></td>
</tr>
<tr>
<td>'Irish' score4 (%)</td>
<td>35 ± 14</td>
<td>38 ± 13</td>
<td>0.418</td>
<td>35 ± 14</td>
<td>38 ± 13</td>
<td>0.418</td>
</tr>
<tr>
<td>'Schold' score5</td>
<td>0.69 ± 0.30</td>
<td>0.79 ± 0.29</td>
<td>0.305</td>
<td>0.69 ± 0.30</td>
<td>0.79 ± 0.29</td>
<td>0.305</td>
</tr>
<tr>
<td>'Nyberg' score5</td>
<td>6.6 ± 5.6</td>
<td>7.0 ± 5.6</td>
<td>0.716</td>
<td>6.6 ± 5.6</td>
<td>7.0 ± 5.6</td>
<td>0.716</td>
</tr>
<tr>
<td>Principal component score (PC1)</td>
<td>22.1 ± 10.4</td>
<td>-22.4 ± 18.1</td>
<td>&lt;0.001</td>
<td>-10.0 ± 10.6</td>
<td>-34.7 ± 15.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patients with delayed graft function</td>
<td>1 (2%)</td>
<td>10 (24%)</td>
<td>0.003</td>
<td>2 (10%)</td>
<td>8 (38%)</td>
<td>0.030</td>
</tr>
<tr>
<td>U-Vol first h (mL)</td>
<td>370 ± 445</td>
<td>248 ± 243</td>
<td>0.033</td>
<td>302 ± 245</td>
<td>198 ± 238</td>
<td>0.129</td>
</tr>
<tr>
<td>U-Vol first 8 h (mL)</td>
<td>3352 ± 2105</td>
<td>2419 ± 2026</td>
<td>0.005</td>
<td>2617 ± 2029</td>
<td>2230 ± 2056</td>
<td>0.606</td>
</tr>
<tr>
<td>Creatinine reduction ratio on day 28 (%)</td>
<td>40 ± 19 (45)7</td>
<td>27 ± 29 (38)7</td>
<td>0.026</td>
<td>30 ± 26 (20)7</td>
<td>24 ± 31 (18)7</td>
<td>0.493</td>
</tr>
<tr>
<td>Serum creatinine on day 7 (μmol/L)</td>
<td>140 ± 49 (44)</td>
<td>167 ± 96 (34)</td>
<td>0.338</td>
<td>139 ± 53 (19)</td>
<td>202 ± 125 (15)</td>
<td>0.103</td>
</tr>
<tr>
<td>Serum creatinine on day 7 (all values) (μmol/L)</td>
<td>153 ± 99 (45)</td>
<td>234 ± 185 (42)</td>
<td>0.028</td>
<td>162 ± 123 (21)</td>
<td>307 ± 210 (21)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

52 kidneys from 31 deceased donors.
22 kidneys from 18 male donors and 16 from 13 female donors.
2Intraoperative mean arterial pressure.
3Calculated for samples with ≥20 glomeruli (1.9).
4'Schold' score (2).
5'Nyberg' score (7).
6Number of measurements.
7Fall of serum creatinine from day 1 to day 2, not calculated in case of renal replacement therapy on day 1.
8Excluding creatinine measurements during ongoing renal replacement therapy.
9Cockroft-Gault formula: ([140 – recipient age] × recipient lean body weight at time of transplant × 0.85 [if female])/[72 × recipient crea × 0.0113].
10Cytomegalovirus (CMV) disease was diagnosed in case of viremia plus clinical symptoms/signs plus treatment.

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between LD versus DD groups included more female LD ($p = 0.004$), longer cold ischemia time in DD ($p < 0.001$) and greater HLA-mismatches in DD ($p < 0.001$). There were no differences in panel reactive antibody and cytomegalovirus status between DD and LD (data not shown).

**Unsupervised transcriptome analysis**

Unsupervised methods were used to reveal the intrinsic heterogeneity in the kidney population. Of the 54675 probe sets on the microarray, 7376 probe sets passed the IQR filter. The results of the hierarchical DIANA cluster analysis are shown in Figure 1. The dendrogram demonstrates three major groups (plus one solitary outlier), distinguished primarily by donor origin: LD, and two clusters of DD kidneys, designated DD1 and DD2. K-means clustering with $K = 3$ confirmed the robustness of the clustering— independent runs of the algorithm produced clusters highly consistent with those obtained from the DIANA method.

**Clinical characteristics and outcomes in the groups clustered by the transcriptome**

Table 1 outlines the clinical and functional data for the 42 samples within the DD group as clustered by DIANA (the single LD kidney within the DD2 cluster was omitted, to focus exclusively on DD samples). The clusters defined in this analysis proved to be associated with the risk of DGF within the DD kidneys: 2 of 21 patients in cluster DD1 (9.5%) and 8 of 21 in cluster DD2 (38.1%) ($p = 0.03$). Only one LD kidney (2.3%) experienced DGF. The incidence of DGF was significantly different between LD and DD2 ($p < 0.001$) and DD1 and DD2 ($p < 0.05$), but not between clusters LD and DD1. The serum creatinine on day 7 was higher in DD2 than DD1 kidneys ($p = 0.008$ including all values). When the patients with DGF were excluded, creatinine levels in the remaining DD2 patients were still higher than DD1, although this did not reach statistical significance. Thus the analysis distinguished both heterogeneity of LD versus DD and among the DD (DD1 ‘good’ vs. DD2 ‘bad’), and similarity in the transcriptome of the ‘good’ DD1 to the LD kidneys.

Unlike the transcriptome findings, the clinical parameters identified no major differences in donor and recipient characteristics between the DD1 and DD2 groups, with the exception of more female donors in the DD2 group ($p = 0.011$). The probability of DGF calculated using the weighted nomogram by Irish et al. was not different between the patients in DD1 and DD2 ($p = 0.418$, Table 1).
or among patients that developed DGF compared to those who did not (38 ± 13% vs. 36 ± 14%, p = 0.585) (3). In addition classifications recommended in publications by Schold et al. and Nyberg et al. were not different between the patients in DD1 and DD2 (Table 1) (2,7).

Histopathology features based on the Banff scoring system, extent of glomerulosclerosis, as well as the global kidney score recommended by Remuzzi et al. were not different between DD1 and DD2 kidneys (Table 1) or between kidneys that did or did not develop DGF (data not shown) (1,5,9,10,22).

**Differentially expressed transcripts**

In comparison between DD versus LD kidneys, 3718 probe sets were found to be differentially expressed at an adjusted p-value <0.01 (DD vs. LD set, Suppl Table 1). A total of 1789 probe sets showed a significantly lower and 1929 probe sets a significantly higher expression in DD than in LD samples. Transcripts most significantly increased in DD versus LD included acute phase proteins, complement components and chemokines. Transcripts reduced in DD versus LD kidneys included many related to metabolism (e.g. fatty acids) and amino acid and solute transporters and members of the albumin gene family.

Between DD1 and DD2 kidneys, 1051 probe sets were differentially expressed at an adjusted p-value <0.01: 404 probe sets were increased and 647 decreased in the DD2 kidneys compared to the DD1 kidneys (DD2 vs. DD1 set, Suppl Table 2). Transcripts demonstrating higher expression in DD2 than DD1 included genes associated with inflammation (immunoglobulins), collagens, integrins, chemokines, Toll-like receptor signaling, antigen processing and presentation and renal injury. Transcripts with lower expression in DD2 than DD1 included genes related to transport, glucose, fatty acid and amino acid metabolism.

In contrast, when kidneys with immediate graft function (IGF) versus DGF were compared using the same adjusted p-value <0.01, no transcripts were differentially expressed. Using less stringent criteria (unadjusted p-value <0.05), a total of 1109 transcripts differed between DGF and IGF (DGF vs. IGF set, Suppl Table 3). Transcript levels of 838 probe sets were higher in DGF including genes associated with inflammation and immune responses and to a lesser degree genes related to injury and repair. Transcripts with lower expression in kidneys developing DGF were predominantly associated with metabolism. The supplement tables show the KEGG pathway annotations for the individual genes.

**Principal components analysis**

PCA is a visualization method designed to reduce the dimensionality of multi-variable (i.e. multigene) systems. The lowest PCs (PC1, PC2) typically capture the most important patterns of covariation in the data. PCA confirmed the separation of LD from DD kidneys and the extensive heterogeneity among DD organs (Figure 2). The three previously defined clusters operated as a continuum across the space of the first two principal components. Supplement table 4 provides the PC1 scores of the individual genes. The single outlier identified in Figure 1 lies to the most extreme left in Figure 2. This patient had the worst outcome of all 87 patients, requiring dialysis for 2 months posttransplantation. The one LD kidney clustered in DD2 had unexpected arteriosclerosis on implant histopathology. This transplant had the poorest immediate posttransplant function among the LD kidneys, although not requiring dialysis. Thus PCA demonstrates the greater heterogeneity in transplant function among the LD kidneys and an overall continuous distribution of the 87 kidney samples from the worst (on the left) to the best (on the right) performing kidneys.

**Probability of DGF**

The PC1 scores, in contrast to the scores derived from clinical or histopathological data, were significantly different between DD1 (–10.0 ± 10.6) and DD2 (–34.7 ± 15.6) kidneys (p < 0.001) as shown in Table 1. Subjects with lower PC1 values are more likely to develop DGF (OR = 1.06, 95% CI 1.01–1.12, p = 0.022). Neither the clinical nor the histopathological scores predicted DGF (Table 2). Recipient age was the only demographic factor strongly correlated with DGF.

![Figure 2: Principal component analysis of all 87 kidney biopsy samples based on the expression of 7376 probe sets that passed the IQR filter shows a continuum of the transcriptome changes across PC1 from the LD kidneys (Δ) to the DD1 (○) and then to the DD2 (□) kidneys. Filled symbols represent kidneys with delayed graft function (DGF). x-axis = principal component 1 (PC1), y-axis = principal component 2 (PC2).](image-url)
higher creatinines again likely re
in DD2 did develop DGF, but there was a trend toward
major factor. It is important to recognize that not all patients
posttransplantation suggesting poor kidney quality as the
DD2 had stable blood pressures and positive
inconsistent that these kidneys clustered in the low risk
group. Conversely all eight patients who developed DGF in
patients with DGF both had signi
the predetermined susceptibility plus the environmental
factors operating before the 1 h biopsy (intrinsic and acute
inflammation, apoptosis, and adhesion, as shown by other stud-
ings to be characteristic for DGF (20,21,23,24). However,
this DGF signature was not predictive in our study. The
factors operating before the 1 h biopsy (intrinsic and acute
injury can only be expected to account for a portion of the
risk of developing DGF: the actual DGF phenotype reflects
the predetermined susceptibility plus the environmental
pressures posttransplant. Indeed, in the DD1 group, the two
patients with DGF both had significant donor-independent
posttransplant hemodynamic instability. It is therefore not
inconsistent that these kidneys clustered in the low risk
group. Conversely all eight patients who developed DGF in
DD2 had stable blood pressures and positive fluid balances
posttransplantation suggesting poor kidney quality as the
major factor. It is important to recognize that not all patients
in DD2 did develop DGF; but there was a trend toward
higher creatinines again likely reflecting poorer kidney qual-
ity. The implant transcriptome is therefore analogous to an
endophenotype in a genetic association study—it provides
a quantifiable link between the observable disease pheno-
type and the underlying but difficult to measure biological
processes that are the true causative agents of disease
(25). Recipient age was found to be a significant predictor
of DGF. This finding, however, is likely confounded by the
practice that older patients tend to receive less optimal kid-
neys. Neither the DD versus LD nor the DD2 versus DD1
gene sets were associated with functional differences at
3, 6 and 12 months posttransplant. The limited sample size
and short follow-up time invite caution.

PCA confirmed the grouping of kidneys as identified by
cluster analysis. In addition PCA demonstrated a contin-
uum across all kidneys from LD to DD1 to DD2, again
confirming the functional relevance of the transcriptome. The
variation in gene expression captured by the PC1 scores
defines the risk of poor initial function among DD kid-
neys. Thus DD1 kidneys having PC1 scores more similar
to those of LD kidneys performed better than DD2 kid-
neys with more disturbed transcriptomes. In contrast, the
clinical and histopathologic risk scores did not correlate
with risk of DGF in our cohort. The global kidney score
could be calculated retrospectively in 24 DD kidney biop-
sies having more than 20 glomeruli per sample (1,9,10).
The score was ≥4 in 12 of the 24 samples and 7 in one
sample, in which dual kidney transplantation or discarding
respectively would have been recommended. All of these
kidneys are functioning well in our cohort thus far. These
observations suggest limitations of histopathology in as-
essment of organ quality and underline that much of the
variability in donor organs is not captured by traditional risk
factors. Ultimately, interpretation of the transcriptome, in
conjunction with clinical data, may offer the best predictive
value (26).

Major limitations of this study include relatively small sam-
ple size and short duration of follow-up. However, our study
is the largest microarray-based analysis of implant biopsies
thus far. A further limitation is the use of DGF as a clini-
cal outcome. Although this is an established outcome in

### Table 2: Scoring systems for early graft function in deceased donor kidneys based on clinical, histopathological and transcriptome data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate OR (95% CI)</th>
<th>p</th>
<th>Multivariate OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age &gt;55 yrs</td>
<td>6.67 (1.21–36.74)</td>
<td>0.029</td>
<td>9.14 (1.33–62.63)</td>
<td>0.024</td>
</tr>
<tr>
<td>Principal component score PC1</td>
<td>1.05 (1.01–1.10)</td>
<td>0.025</td>
<td>1.06 (1.01–1.12)</td>
<td>0.022</td>
</tr>
<tr>
<td>‘Irish’ score</td>
<td>1.02 (0.96–1.07)</td>
<td>0.576</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Schold’ score</td>
<td>2.21 (0.19–25.83)</td>
<td>0.527</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Nyberg’ score</td>
<td>1.04 (0.91–1.19)</td>
<td>0.605</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global kidney score</td>
<td>1.18 (0.82–1.70)</td>
<td>0.371</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banff score total</td>
<td>1.23 (0.84–1.78)</td>
<td>0.284</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 OR = odds ratio for DGF.
2 95% CI = 95% confidence interval.
3 ‘Irish’ score = probability of DGF (3).
4 ‘Schold’ score = short-term function, 6 months (2).
5 ‘Nyberg’ score = short-term function, 30 days (7).
6 Global kidney score calculated for samples with ≥20 glomeruli (1,9).

Discussion

The present study analyzed whether variability in the tran-
scriptome of kidneys at the time of transplantation would
identify DD kidneys at increased risk of poor initial function.
By including LD and DD kidneys, we were able to compare
a wide range of kidney stress with unsupervised analyses.
The cluster analysis revealed differences between kidneys
from DD and LD and differences among DD kidneys—
DD1 versus DD2—that corresponded with increased risk
of poor initial function.

At an adjusted p-value <0.01 many transcripts differen-
tiated these kidney groups—3718 between DD versus LD,
and 1051 between DD1 versus DD2, but none separated
DGF from IGF in a direct analysis. However, using a less
stringent cutoff (unadjusted p-value of <0.05), a total of
1110 transcripts were differentially expressed, represent-
ing predominantly pathways and genes involved in inflam-
ation, apoptosis, and adhesion, as shown by other stud-
ies to be characteristic for DGF (20,21,23,24). However,
this DGF signature was not predictive in our study. The
factors operating before the 1 h biopsy (intrinsic and acute
injury can only be expected to account for a portion of the
risk of developing DGF: the actual DGF phenotype reflects
the predetermined susceptibility plus the environmental
pressures posttransplant. Indeed, in the DD1 group, the two
patients with DGF both had significant donor-independent
posttransplant hemodynamic instability. It is therefore not
inconsistent that these kidneys clustered in the low risk
group. Conversely all eight patients who developed DGF in
DD2 had stable blood pressures and positive fluid balances
posttransplantation suggesting poor kidney quality as the
major factor. It is important to recognize that not all patients
in DD2 did develop DGF; but there was a trend toward
higher creatinines again likely reflecting poorer kidney qual-
ity. The implant transcriptome is therefore analogous to an
endophenotype in a genetic association study—it provides
a quantifiable link between the observable disease pheno-
transplant studies, the decision to dialyze may vary from
one physician to another, and the absence of need for dial-
ysis does not necessarily imply excellent graft function.
Categorical outcomes, however, are necessary in studies
such as ours. While discovery-phase studies like this re-
quire validation, there are many aspects of the study that
engender confidence in the conclusions. The unsupervised
approach generated findings with biological/clinical signif-
icance. This study is prospective and inclusive of all biop-
sies and has extensive conventional phenotyping to enrich
the interpretation of microarray findings. The emerging pre-
dictive strategy may have clinical significance in that the
transcriptome performs better than existing risk scores.
Further validation and a more refined understanding of the
information contained in the transcriptome could guide re-
source allocation and clinical management in individual pa-
ients. Our findings would have greater implications if they
could be validated in predonation biopsies, to better iden-
tify kidneys with favorable transcriptomes that may other-
wise have been discarded. This could potentially result in a
significant increase in numbers of kidneys transplanted per
year.

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Supplementary Material

The following supplementary material is available for this article:

Table S1.
Table S2.
Table S3.
Table S4.

This material is available as part of the online article from: http://www.blackwellpublishing.com/doi/abs/10.1111/j.1600-6143.2007.02032.x

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