A porcine model to study the effect of brain death on kidney genomic responses

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Introduction. A majority of transplanted organs come from donors after brain death (BD). Renal grafts from these donors have higher delayed graft function and lower long-term survival rates compared to living donors. We designed a novel porcine BD model to better delineate the incompletely understood inflammatory response to BD, hypothesizing that adhesion molecule pathways would be upregulated in BD.

Methods. Animals were anesthetized and instrumented with monitors and a balloon catheter, then randomized to control and BD groups. BD was induced by inflating the balloon catheter and animals were maintained for 6 hours. RNA was extracted from kidneys, and gene expression pattern was determined.

Results. In total, 902 gene pairs were differently expressed between groups. Eleven selected pathways were upregulated after BD, including cell adhesion molecules.

Conclusions. These results should be confirmed in human organ donors. Treatment strategies should target involved pathways and lessen the negative effects of BD on transplantable organs.

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observed at increased levels in humans and animals after receiving an organ from a DBD [8–10]. Increases in these circulating and tissue mediators in the recipient have been shown to be associated with poor graft outcomes in heart, liver, and renal transplants [11–16]. These studies have looked at small animal models, examined circulating or tissue protein levels in the recipient after transplantation, and/or focused on donor tissue after prolonged periods of warm or cold ischemia. None have focused on both a larger, more translationally relevant animal model and gene expression levels in the DBD before organ recovery. It is essential to better understand the state of potentially transplantable organs at the time of organ recovery, as this is when decisions are being made by transplant programs about the suitability of organs for transplantation. Combining these 2 approaches is necessary to inform potential human organ donor interventions aimed at increasing the availability of organs suitable for transplantation, as there may be targets for intervention identified in the donor.

Kidney transplantation is a common research target, since kidneys are the most frequently donated organs, and because obtaining biopsy data is a common practice. Further, delayed graft DGF, defined as a drop in blood flow to <15% of baseline, MAP persistently greater than mean arterial pressure (MAP), and fixed and dilated pupils [18]. BD was also verified in the first two animals in the BD group using conventional apnea testing in order to validate the previous model upon which our study was based [18]. Apnea testing was performed after preoxygenation with 100% oxygen for 5 minutes, followed by disconnecting the ventilator. Base-line levels of pCO₂ were measured. After 10 minutes of being disconnected from the ventilator, another level of pCO₂ was measured before placing the animal back on mechanical ventilation. If there was an increase of 20 mmHg pCO₂ compared with baseline, BD was confirmed, consistent with national guidelines.

In total, 20 animals were randomized into 2 groups: a control group (burr hole, catheters, and anesthesia only) and a BD group (instrumentation plus inflation of subdural balloon catheter and confirmation of BD). Animals were supported on mechanical ventilation for 6 hours after randomization and 2 cc/kg boluses of 0.9% NaCl were given as needed to maintain a MAP above 35 mmHg to prevent cardiovascular collapse. Vasopressors were not utilized, consistent with the validated model upon which our study was based. Both groups were maintained with isoflurane for the duration of the case. A laparotomy was then performed and kidney specimens were obtained and snap frozen.

Vital signs were recorded every minute during the balloon inflation phase and at regular intervals during the 6-hour maintenance phase. Repeated measures ANOVA statistics were used to analyze mean physiologic variables and determine difference between groups during the balloon inflation phase and an independent-samples t-tests was used to analyze terminal values for ICP, MAP, and HR.

**Materials and Methods**

**Porcine Model of Brain Death**

The animal protocols used in this study were approved by the UCI institutional animal care and use committee (UCI-IACUC). In total, 20 domestic female Chesterwhite or Yorkshire swine at 6 weeks of age were purchased from S&S Farms in San Diego County, CA. Animals (20–30 kg) were acclimated for 72 hours, fasted for 12 hours, and induced with an intramuscular combination of ketamine (21 mg/kg), xylazine (2.2 mg/kg), and atropine (0.04 mg/kg). Pentothal (10 mg/kg) was then given intravenously for induction via an ear vein catheter and endotracheal intubation was performed. Ventilation and anesthetic were maintained with isoflurane (0.75%–4%). Electrocardiogram leads, pulse oximetry, and end-tidal carbon dioxide (CO₂) were monitored and adequate anesthesia was confirmed by assessing jaw tone, movement, and vital signs. Femoral artery and external jugular catheters were placed for hemodynamic monitoring, blood collection, and fluid administration. Based on a previously described model [18], a left-sided parietal burr hole was created, and a 15 cc epidural balloon catheter and subdural intracranial pressure (ICP) monitor were placed through this access point. A subdural laser Doppler flow probe was placed in the right frontal area (Fig. 1). After a 30-minute rest period, a physiologic baseline was established and brainstem herniation was induced by inflating the 15-cc balloon catheter over 1 min, mimicking the effects of a space-occupying lesion and eventual herniation. The balloon remained inflated for 20 minutes, then was deflated. Herniation was evaluated with a contralateral laser Doppler flow probe and an ipsilateral ICP catheter. BD was defined as a drop in blood flow to <15% of baseline, MAP persistently greater than mean arterial pressure (MAP), and fixed and dilated pupils [18]. BD was also verified in the first two animals in the BD group using conventional apnea testing in order to validate the previous model upon which our study was based [18]. Apnea testing was performed after preoxygenation with 100% oxygen for 5 minutes, followed by disconnecting the ventilator. Base-line levels of pCO₂ were measured. After 10 minutes of being disconnected from the ventilator, another level of pCO₂ was measured before placing the animal back on mechanical ventilation. If there was an increase of 20 mmHg pCO₂ compared with baseline, BD was confirmed, consistent with national guidelines.

**RNA Extraction**

Total RNA for gene expression analysis was extracted from the porcine kidney tissue using TRIzol® (Gibco BRL Life Technologies, Rockville, MD, USA). RNA was purified using Qiagen-RNeasy Mini Kit.
RNA pellets were resuspended in diethyl pyrocarbonate-treated water. RNA integrity was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies, Inc., Palo Alto, CA, USA). All samples had RNA Integrity Number (RIN) ≥9.1.

**Gene Expression Microarrays**

Microarray processing was performed as recommended by the manufacturer and is available in the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA, USA). In brief, first-strand cDNA was synthesized from 250 ng of total RNA. After making the complementary second strand, the double-stranded cDNA is used to generate biotin-tagged cRNA from an in-vitro transcription using T7 RNA polymerase. Ten μg of fragmented target cRNA was hybridized on an Affymetrix GeneChip Porcine Genome Array. Arrays were scanned using GeneChip® Scanner 3000 7G and Com- by ANOVA testing.

**Gene Expression Data Analysis**

The results were analyzed using GeneSpring GX 12.1 Software (Agilent Technologies, Inc.). Raw data were normalized using GC-RMA. Only probe sets that reached a signal value ≥50 in at least 50% of the values in anyone out of the 2 conditions were included in the analysis. Overall, 17,566 of 54,675 probe sets represented on the microarray cell met these criteria. The microarray cell data have been deposited in the GEO database (series accession number https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94709). Traditional Student’s paired t-test was first applied to each probe set and fold change (increase or decrease) >1.5 and Multiple Testing Correction (false discovery rate [FDR] <0.05) (Benjamini–Hochberg) procedure was carried out for statistical analysis.

The final list of significantly different expression probe sets between the 2 groups was then additionally analyzed using the functional annotation tools provided by DAVID 6.8, the Database for Annotation, Visualization and Integrated Discovery to classify the genes into pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Only pathways with Expression Analysis Systematic Explorer (EASE) score ≤0.05 are presented in this analysis. The EASE score is a modified Fisher exact p value in the DAVID system used for gene-enrichment analysis. An EASE score p value = 0 represents perfect enrichment. p value ≤0.05 is considered as gene enrichment in a specific annotation category (http://david.abcc.ncifcrf.gov/helps/functional_annotation.html#summary).

**Microarray Results Corroboration Using RT-PCR**

For confirmation of gene expression microarray findings, reverse transcriptase polymerase-chain reaction (RT-PCR) assays were carried out on 6 genes from the KEGG cell adhesion pathway (SLA-DRA, SLA-1, SLA-DQA, ITGB2, ITGB8, and VCAM1). Analysis was performed with the Applied Biosystems 7900HT PCR System by using TaqMan Universal PCR Master Mix and Assays-on-Demand Gene Expression probes (Applied Biosystems) (SLA-DRA: assay ID, Ss03389945_m1; ITGB2: assay ID, Ss03392626_u1; ITGB8: assay ID, Ss03385280_g1; and SLA-1: Ss03395429_s1; SLA-DQA: Ss03889541_g1; VCAM1: assay ID, Ss03390914_m1). Actin β was used as an endogenous control.

**Results**

**Animal Model**

In total, 10 animals were assigned to the BD group and 10 animals were assigned to the control group. One animal in the BD group and 2 animals in the control group were unable to be maintained for 6 hours under general anesthesia and suffered cardiac arrest; upon autopsy, it was discovered that these animals had pre-existing illnesses.

Hemodynamic and intracranial physiologic results during the balloon inflation phase are displayed in Fig. 2. All animals in the experimental group and none in the control group met criteria for BD. Animals in BD and control groups had similar physiologic variables at initiation of general anesthesia: control animals had a mean heart rate (HR) of 80.3 ± 18.4 beats per minute (BPM) and experimental animals had a mean HR of 85.1 ± 9.0 BPM (p = 0.49); MAP at induction was 64.6 ± 11.6 mm of mercury (mmHg) for control animals and 68.7 ± 11.0 mmHg for experimental animals (p = 0.47); and ICP at induction was 17.3 ± 5.5 mmHg for control animals and 16.8 ± 7.3 mmHg for experimental animals (p = 0.88).

The expected catecholamine surge associated with herniation was demonstrated in the animals in the BD group during the time of balloon inflation. Initially, HR and MAP increased dramatically to a highest mean HR of 125.3 ± 30.8 BPM at 4 minutes after balloon inflation and a highest MAP of 106.1 ± 16.9 at 3 minutes postinflation. Means were compared using repeated measures ANOVA, and significant differences were found for MAP at minutes 2–6 and for HR at minutes 3–5 (Fig. 2) during balloon inflation are displayed in Fig. 2. At the conclusion of the balloon inflation phase, there were no significant differences between the groups with regard to HR or MAP (Fig. 2).

After maintenance of 6 hours of general anesthesia, the terminal ICP values were significantly different between control and experimental groups (19.3 ± 4.5 vs. 59.3 ± 18.8, p <0.001), however mean MAP (59.7 ± 17.1 vs. 56.9 ± 13.8, p =0.73) and HR (85 ± 19.3 vs. 122.3 ± 43.8, p =0.06) between groups were not significant.

**The Effects of BD on Kidney Gene Expression**

Using FDR <0.05 with 95% confidence, a total of 902 probe sets were differentially expressed between BD and control group which
represent 233 annotated genes. In total, 139 genes had higher expression in the BD compared with control, and 94 genes had lower expression in the BD compared to control (online Supplementary Table 1).

We classified the final list of genes with significantly different expression between the 2 groups into pathways using the KEGG database. In total, 40 pathways (EASE score ≤ 0.01, FDR < 0.05) were enriched with genes that expressed significantly different between the 2 groups. Table 1 presents 11 selected pathways linked to the immune response, cell communication, allograft rejection, and graft-versus-host disease. Table 2 presents the individual upregulated genes within our primary pathway of interest, cell adhesion molecules, and the degree of fold change associated with the upregulation. Fig. 3 is a diagrammatic representation of the KEGG pathway for the cell adhesion molecules.

In this diagram, the various genes and their interactions are displayed and those upregulated after BD in our animal model are noted.

RT-PCR was performed to corroborate the microarray results in our primary outcome of interest, the cell adhesion molecule pathway. A total of 6 representative genes were upregulated, 5 of which were noted to be significant (p < 0.05) (Table 3).

### DISCUSSION

Efforts are needed to improve the quantity and quality of organs available for transplantation, and a better understanding of the inflammatory response to brainstem herniation may help to identify targets for future therapeutic interventions. Toward that end, we examined the genomic response to herniation in the porcine kidney and found significant differences in the expression levels of 233 genes, which were classified into gene pathways. These affected pathways play a central role in inflammation, allograft rejection, antigen processing and presentation, Toll-like receptor signaling, tumor necrosis factor (TNF) signaling, graft-versus-host disease, cell adhesion molecules, natural killer cell-mediated toxicity, Janus kinase/signal transducers and activators of transcription (Jak-STAT) signaling, peroxisome proliferator-activated receptor signaling, and chemokine signaling. Additionally, the results confirmed our hypothesis that adhesion molecule pathways are significantly upregulated after brainstem herniation and BD.

It has been established that a cascade of events result from severe neurologic injuries and brainstem herniation. This cascade usually begins with activation of the parasympathetic pathway, leading to hypothermia, hypotension, and electrolyte disturbances. This derangement is then followed by sympathetic stimulation, resulting in high catecholamine release, tachycardia, and hypertension [10]. Though this response to BD is well described in the literature, there is a dearth of information concerning the inflammatory response to brainstem herniation at the genetic level.

In reviewing the genetic contribution to organ transplantation after BD, both existing genotypes and regulation of these genotypes, or epigenetic events, should be examined. Several groups have examined the idea that certain genetic makeups are predictive of success in transplantation. One early study, limited by sample size, was unable to show any association between donor single-nucleotide polymorphisms and DGF [28]. However, subsequent studies have shown that differences in TNF-α and TGF-β gene polymorphisms between donor and recipient were associated with increased acute rejection [29, 30]. The fact that genetic polymorphisms are associated with differential rejection rates suggests that examining downstream gene expression patterns may increase our understanding of BD and how it affects transplantation outcomes.

In addition to polymorphisms that may contribute to graft function, gene pathways that are affected during the process of BD have been described in the literature. However, these previous genomic studies have been performed on tissue immediately prior to implantation, after prolonged periods of both warm and cold ischemia. In our model, we examine the effects of BD on the genomic pathways in the donor, prior to any period of ischemia, cold storage, or interaction with the recipient immune response. With respect to the pre-implantation literature, several groups have examined apoptotic pathway, and DGF has been shown to correlate with increased expression in biopsies obtained after cold storage [31]. Confounding these results is that fact that longer durations of CIT have been shown to increase apoptosis expression in specimens from DBDs [7]. In another study, higher pre-implant lipocalin 2 (LCN-2) expression correlated with increased DGF rates and acute rejection episodes [32]. In an effort to avoid alterations in gene expression patterns that cold ischemia may induce, our animal model focuses on tissue specimens obtained at the time of organ

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**Table 1.** Selected Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in porcine model significantly upregulated 6 hours following brainstem herniation when compared with control animals

<table>
<thead>
<tr>
<th>KEGG pathway</th>
<th>Number of genes</th>
<th>EASE score</th>
<th>FDR</th>
<th>Role in inflammation and transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen processing and presentation</td>
<td>7</td>
<td>7.20E-05</td>
<td>7.60E-04</td>
<td>Associated with macrophage presence in rejected renal allograft specimens [19]</td>
</tr>
<tr>
<td>Toll-like receptor signaling pathway</td>
<td>8</td>
<td>1.00E-04</td>
<td>1.00E-03</td>
<td>Initiate allograft inflammation and promote development of acute and chronic rejection [20]</td>
</tr>
<tr>
<td>TNF-signaling pathway</td>
<td>8</td>
<td>1.50E-04</td>
<td>1.40E-03</td>
<td>Inhibition protects kidney from ischemia-reperfusion injury by reducing accumulation of macrophages/monocytes [21]</td>
</tr>
<tr>
<td>Graft-versus-host disease</td>
<td>5</td>
<td>4.60E-04</td>
<td>4.00E-03</td>
<td>Associated with immune function, chronic inflammation [22]</td>
</tr>
<tr>
<td>Allograft rejection</td>
<td>5</td>
<td>6.40E-04</td>
<td>4.40E-03</td>
<td>Upregulated renal dysfunction in transplant recipients [23]</td>
</tr>
<tr>
<td>Cell adhesion molecules (CAMs)</td>
<td>8</td>
<td>8.50E-04</td>
<td>5.40E-03</td>
<td>Mediate infiltration to graft, disseminate antigenic message to host lymphoid tissue [24]</td>
</tr>
<tr>
<td>Natural killer cell-mediated cytotoxicity</td>
<td>7</td>
<td>1.00E-03</td>
<td>5.90E-03</td>
<td>Play a role in renal transplant loss [25]</td>
</tr>
<tr>
<td>Cytokine-cytokine receptor interaction</td>
<td>9</td>
<td>2.60E-03</td>
<td>1.20E-02</td>
<td>Upregulated in renal dysfunction in transplant recipient [23]</td>
</tr>
<tr>
<td>Jak-STAT signaling pathway</td>
<td>7</td>
<td>4.00E-03</td>
<td>1.80E-02</td>
<td>Upregulated in renal dysfunction in transplant recipients [23]</td>
</tr>
<tr>
<td>PPAR signaling pathway</td>
<td>5</td>
<td>5.80E-03</td>
<td>2.50E-02</td>
<td>Postulated involvement in UC after renal transplant [26]</td>
</tr>
<tr>
<td>Chemokine signaling pathway</td>
<td>7</td>
<td>1.00E-02</td>
<td>4.20E-02</td>
<td>Upregulated in inflammatory states like SCI [27]</td>
</tr>
</tbody>
</table>

EASE, expression analysis systematic explorer; FDR, false discovery rate; Jak-STAT, Janus kinase/signal transducers and activators of transcription; PPAR, peroxisome proliferator-activated receptor; UC, ulcerative colitis; SCI, spinal cord injury; TNF, tumor necrosis factor.
<table>
<thead>
<tr>
<th>Gene name (gene symbol)</th>
<th>FC</th>
<th>Directional change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen processing and presentation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC class II DR-α (SLA-DRA)</td>
<td>1.6</td>
<td>Up</td>
</tr>
<tr>
<td>MHC class II histocompatibility antigen SLA-DQA (SLA-DQA1)</td>
<td>1.8</td>
<td>Up</td>
</tr>
<tr>
<td>SLA-DQ J1 domain (SLA-DQB1)</td>
<td>1.6</td>
<td>Up</td>
</tr>
<tr>
<td>Major histocompatibility complex, class II, DO α (SLA-DOA)</td>
<td>1.8</td>
<td>Up</td>
</tr>
<tr>
<td>Proteasome activator subunit 2 (PSME2)</td>
<td>1.7</td>
<td>Up</td>
</tr>
<tr>
<td>Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) (TAP1)</td>
<td>2.2</td>
<td>Up</td>
</tr>
<tr>
<td><strong>Toll-like receptor signaling pathway</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBJ murine osteosarcoma viral oncogene homolog (FOS)</td>
<td>3.3</td>
<td>Up</td>
</tr>
<tr>
<td>Caspase 8, apoptosis-related cysteine peptidase (CASP8)</td>
<td>1.8</td>
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</tr>
<tr>
<td>Chemokine (C-C motif) ligand 4 (CCL4)</td>
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<tr>
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<tr>
<td>Interferon (α, β, and ω) receptor 1 (IFNAR1)</td>
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<tr>
<td>Interferon regulatory factor 7 (IRF7)</td>
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</tr>
<tr>
<td>Signal transducer and activator of transcription 1, 91 kDa (STAT1)</td>
<td>1.9</td>
<td>Up</td>
</tr>
<tr>
<td>Toll-like receptor-4 (TLR-4)</td>
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</tr>
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<td><strong>TNF signaling pathway</strong></td>
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<td></td>
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<td>FBJ murine osteosarcoma viral oncogene homolog (FOS)</td>
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<td>Up</td>
</tr>
<tr>
<td>Activating transcription factor 4 (ATF4)</td>
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<td>Caspase 3, apoptosis-related cysteine peptidase (CASP3)</td>
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<td>Ribosomal protein S6 kinase α-5 (LOC100152046)</td>
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<tr>
<td><strong>Vascular cell adhesion molecule 1 (VCAM1)</strong></td>
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<td>Integrin, β 2 (complement component 3 receptor 3 and 4 subunit) (ITGB2)</td>
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<td>FYN proto-oncogene, Src family tyrosine kinase (FYN)</td>
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</tr>
<tr>
<td>Growth hormone receptor (GHR)</td>
<td>2.2</td>
<td>Down</td>
</tr>
<tr>
<td>Interferon (α, β, and ω) receptor 1 (IFNAR1)</td>
<td>1.8</td>
<td>Up</td>
</tr>
<tr>
<td>Interleukin 10 receptor, β 1 (IL10RB)</td>
<td>2.7</td>
<td>Up</td>
</tr>
<tr>
<td><strong>Transforming growth factor, β 1 (TGFβ1)</strong></td>
<td>2.0</td>
<td>Up</td>
</tr>
</tbody>
</table>
recovery, prior to cold preservation, to isolate those pathways strictly related to the process of brainstem herniation that could be targets for intervention during organ donor management.

Many of the described studies have been performed in mice or rats, which have poor genetic correlation with human pathways [33]. Due to both greater genetic and anatomic similarities with humans, swine are one of the most commonly used species in biomedical translational research models. In 2012, Hume et al. published the first porcine genome-wide transcriptional analysis that included 62 tissues and cell types, providing an important resource for understanding the relationship between porcine and human gene expression [34]. In one of the first porcine models of brainstem herniation, Mclean et al. examined the effect of glucocorticoid administration on circulating levels of

Table 2. Continued

<table>
<thead>
<tr>
<th>Gene name (gene symbol)</th>
<th>FC</th>
<th>Directional change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jak-STAT signaling pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Janus kinase 2 (JAK2)</td>
<td>2.0</td>
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</tr>
<tr>
<td>Growth hormone receptor (GHR)</td>
<td>2.2</td>
<td>Down</td>
</tr>
<tr>
<td>Interferon (α, β, and ω) receptor (IFNAR1)</td>
<td>1.8</td>
<td>Up</td>
</tr>
<tr>
<td>Interleukin 10 receptor, β (IL10RB)</td>
<td>2.7</td>
<td>Up</td>
</tr>
<tr>
<td>Protein tyrosine phosphatase, nonreceptor type 6 (PTPN6)</td>
<td>1.7</td>
<td>Up</td>
</tr>
<tr>
<td>Signal transducer and activator of transcription I, 91 kDa (STAT1)</td>
<td>1.9</td>
<td>Up</td>
</tr>
<tr>
<td>Signal transducer and activator of transcription 5A (STAT5A)</td>
<td>1.6</td>
<td>Up</td>
</tr>
<tr>
<td>PPAR signaling pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiopoietin-like 4 (ANGPTL4)</td>
<td>6.9</td>
<td>Down</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase 1 A (liver) (CPT1A)</td>
<td>1.8</td>
<td>Down</td>
</tr>
<tr>
<td>Fatty acid binding protein 4, adipocyte (FABP4)</td>
<td>1.8</td>
<td>Down</td>
</tr>
<tr>
<td>Fatty acid binding protein 5 (psoriasis-associated) (FABP5)</td>
<td>1.7</td>
<td>Up</td>
</tr>
<tr>
<td>Glycerol kinase (GK)</td>
<td>2.1</td>
<td>Up</td>
</tr>
<tr>
<td>Chemokine signaling pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell division cycle 42 (CDC42)</td>
<td>1.5901718</td>
<td>Up</td>
</tr>
</tbody>
</table>

FC, fold change; TNF, tumor necrosis factor.

Fig. 3. Diagrammatic representation of the interactions among genes within the cell adhesion molecule Kyoto Encyclopedia of Genes and Genomes pathway, with significantly upregulated genes in our experiments annotated. *Upregulated in porcine brain death model.
models [21]. Additionally, we identi
promise with regards to being protective against I:R injury in animal
mediated toxicity [45], Jak-STAT signaling [46], peroxisome
rejection, such as graft-versus-host disease [44], natural killer cell-
common immunologic processes affecting renal transplantation and
antibody-mediated rejection has been a target of anti-rejection medi-
presence in rejected human renal allograft specimens [19], and
signi
further investigation is warranted.

treated with the drug [41]. These results were obtained in rats, and
treating donors to mitigate the I:R injury, recently proposing that
mortality in transplant recipients [40]. Several publications have examined
in ischemia-reperfusion (I:R) injuries in kidney tissue [39], and
hemostasis, the immune response, in
molecules (CAMs) are glycoproteins expressed on the cell surface and
upregulation was hypothesized to be particularly affected. Cell adhesion
chronic [38] rejection of organs, and cell adhesion molecule upregu-
were classi
herniation, when compared with those in a control group. These genes
were included into KEGG gene pathways that are related to immune
modulation. The immune system is responsible for the acute [13] and
rejection of organs, and cell adhesion molecule upregu-
was hypothesized to be particularly affected. Cell adhesion
molecules (CAMs) are glycoproteins expressed on the cell surface and
play a critical role in a wide array of biologic processes that include
hemostasis, the immune response, inflammation, embryogenesis, and
development of neuronal tissue. They have been shown to be integral
in ischemia-reperfusion (I:R) injuries in kidney tissue [39], and
increased levels of CAMs have been associated with increased mor-
tality in transplant recipients [40]. Several publications have examined
treating donors to mitigate the I:R injury, recently proposing that
CAM-mediated I:R injury can be treated with N-octanoyl dopamine. In
this study, the researchers showed better early graft function and
reduced acute rejection in renal allograft recipients after donors were
wrested with the drug [41]. These results were obtained in rats, and
further investigation is warranted.

In our porcine model of BD, we found that 233 unique genes were
noted to be significantly altered in those animals subject to brainstem
herniation, when compared with those in a control group. These genes
were classified into KEGG gene pathways that are related to immune
modulation. The immune system is responsible for the acute [13] and
pro-inflammatory cytokines, and found that glucocorticoids
shifted toward a more anti-inflammatory state [18]. Additional studies
have examined mRNA and protein levels in heart, liver, and kidney
 tissues, looking at TNF-α, IL-6, and IL-10 in one study [35] and TNF-α, IL-6, IL-1β, IL-6R, ICAM-1, MCP-1, and TGF-β in another [36]. These
studies have helped to delineate the inflammatory response to BD, and
may explain organ-specific differences in transplantation outcomes
after BD. However, variable results were obtained, with the former
study did not show increased levels of cytokine mRNA between organ
tissues [35], whereas the latter study showed a mixed picture of
cytokine upregulation in the studied organs [36]. Whereas these stud-
ies have addressed targeted genes, we have sought to take a more
global approach and use microarrays to identify a large numbers of
genes simultaneously, identify their molecular interaction and classify
them to biological gene pathways. Perhaps most similar to our study is
a comparison between BD and living donor swine that examined dif-
fferences in apoptotic and protective gene expression patterns in
multiple tissues, albeit after cold storage [37].

In our porcine model of BD, we found that 233 unique genes were
classified into KEGG gene pathways that are related to immune
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further investigation is warranted.

Ten additional gene pathways related to the immune system were
significantly affected by the process of herniation in our study. Antigen
processing and presentation has been associated with macrophage
presence in rejected human renal allograft specimens [19], and
antibody-mediated rejection has been a target of anti-rejection medi-
cations for years. Toll-like receptor-4 (TLR-4) has been increasingly
recognized as playing a critical role in the pathogenesis of I:R injury of
renal grafts [42, 43], and research into anti-TNF agents have shown
promise with regards to being protective against I:R injury in animal
models [21]. Additionally, we identified pathways associated with
common immunologic processes affecting renal transplantation and
rejection, such as graft-versus-host disease [44], natural killer cell-
mediated toxicity [45], Jak-STAT signaling [46], peroxisome
proliferator-activated receptor signaling [47], chemokine signaling, and
allograft rejection.

The limitations of this study include correlative data only. Though
unable to prove causation, these data are a first step in identifying gene
pathways that are altered in kidney tissue after BD. Additionally, there
are no internal controls performed prior to herniation in each speci-
men, which potentially would aid in the delineation of the inflamma-
tory response. Isoflurane anesthesia also may be considered to alter
the inflammatory response. However, we attempted to control for
this by administering this anesthetic to both the experimental and
control animals, even though it was not needed for sedation or com-
fort in the BD group. It is also unclear as to the ultimate source of the
inflammatory response to BD. Some investigators have postulated that
neutrophils, platelets [48], and the nervous system [49] may be sour-
ces of the inflammatory response, but we were unable to address
these concepts in the current study. Our intent was to address only
the kidney, and future work would involve analysis of all transplantable
organs, protein levels within organs, and circulating markers. In addi-
tion, expanding the animal model to include allotransplantation of
organs into another subject would allow correlations between donor
gene expression/biomarker patterns and recipient graft outcomes, the
ultimate consequence of interest. This expansion would facilitate
investigation of whether interventions in the donor can abrogate the
inflammatory response and improve transplantation outcomes.

In this study, we addressed only the 6-hour time point after herniation. This
time point was chosen for this initial study for several reasons. This
time point is a practical surrogate for when actual interventions could begin to be
carried out in a donor. In general, declaration of BD occurs 1–2 hours after
the actual BD event, and then it requires several additional hours of
obtaining authorization for donation and examining the donor before the
organ procurement organization can begin to make interventions. A 6-hour
time point marks the approximate start of donor management by the organ
procurement organization, when interventions in the donor possibly could
begin. Future studies would examine later time points, further character-
izing the gene expression and gene pathways affected by BD and potentially
guiding therapeutic interventions at these times. The raw data of the
6-hour time point can be found in GEO database (series accession number
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94709) and is
available for additional analyses and comparisons with current and future
genomic datasets related to kidney injury or other kidney donor studies.
Many studies have shown conflicting results in examination of the impact
duration of donor management [7, 50–52] and the best strategy for optimal
duration of management is still under investigation. Later time points in a
porcine model may help in determining kidney-specific genomic changes in
examining duration of donor management.

Using a porcine model of brainstem herniation, we showed for the first
time that multiple gene pathways associated with inflammation and
organ rejection are altered in the donor after BD when compared with
control animals. Building on this approach, new studies will generate
data on additional time points with a goal to characterize potential
biomarkers and gene pathways and identify the window of opportunity
for organ management/procurement and. Animal models that corre-
late gene expression patterns in organs at the time of recovery with
post-transplant outcomes would be essential in identifying appropriate
targets in the DBD for future interventions. With the development of

| Table 3. Reverse transcriptase polymerase-chain reaction verification of specific genes in the cell adhesion pathway |
|-------------------------------------------------|----------------|----------|
| Gene name (gene symbol)                        | Fold change  | Directional change | p-Value |
| MHC class I antigen 1 (SLA-I)                  | 1.27         | Up        | 0.06    |
| MHC class II DR-α (SLA-DRA)                    | 1.31         | Up        | 0.018   |
| MHC class II histocompatibility antigen SLA-DQA (SLA-DQA1) | 1.34     | Up        | 0.05    |
| Integrin, β 2 (complement component 3 receptor 3 and 4 subunit) (ITGB2) | 1.63  | Up        | 0.003   |
| Integrin, β 8 (ITGB8)                          | 1.83         | Up        | 4.12E-06|
| Vascular cell adhesion molecule 1 (VCAM1)      | 4.03         | Up        | 7.93E-05|

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valid animal models, possible interventions could be tested with little risk to transplantable human organs. Donor management after BD is one step in the process of organ donation in which these interventions could occur. This study begins to identify the underlying molecular mechanisms associated with this event, and aims to act as a first step towards targeting specific pathways that could optimize organs for successful transplantation.

Supplementary materials

To view supplementary material for this article, please visit https://doi.org/10.1017/cts.2018.312

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Disclosures

The authors have no conflicts of interest to declare. The contents of this article do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

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