Differences in Gene Expression Profiles of Diabetic and Nondiabetic Patients Undergoing Cardiopulmonary Bypass and Cardioplegic Arrest

Pierre Voisine, MD; Marc Ruel, MD, MPH; Tanveer A. Khan, MD; Cesario Bianchi, MD, PhD; Shu-Hua Xu, PhD; Isaac Kohane, MD, PhD; Towia A. Libermann, PhD; Hasan Otu, PhD; Alan R. Saltiel, PhD; Frank W. Sellke, MD

Background—Diabetes mellitus is an independent risk factor for early postoperative mortality and complications after coronary artery bypass grafting (CABG). We sought to compare the cardiac gene expression responses to cardiopulmonary bypass (CPB) and cardioplegic arrest (C) in patients with and without diabetes.

Methods and Results—Twenty atrial myocardium samples were harvested from 5 type II insulin-dependent diabetic and 5 matched nondiabetic patients undergoing CABG, before and after CPB/C. Oligonucleotide microarray analyses of 12625 genes were performed on the 10 sample pairs using matched pre-CPB tissues as controls. Array results were validated with Northern blotting and immunoblotting. Compared with pre-CPB/C, post-CPB/C myocardial tissues revealed 851 upregulated and 480 downregulated genes with a threshold $P < 0.025$ (signal-to-noise ratio, 4.04) in the diabetic group, compared with 480 upregulated and 626 downregulated genes (signal-to-noise ratio, 3.04) in the nondiabetic group ($P < 0.001$). There were 18 genes that were upregulated >4-fold in diabetic and nondiabetic patients (including inflammatory/transcription activators FOS, CYR 61, and IL-6, apoptotic gene NR4A1, stress gene DUSP1, and glucose-transporter gene SLC2A3). However, 28 genes showed such marked upregulation in the diabetic group exclusively (including inflammatory/transcription activators MYC, IL8, IL-1β, growth factor vascular endothelial growth factor, amphiregulin, and glucose metabolism-involved gene insulin receptor substrate 1), and 27 genes in the nondiabetic group only, including glycogen-binding subunit PPP1R3C.

Conclusions—Gene expression profile after CPB/C is quantitatively and qualitatively different in patients with diabetes. These results have important implications for the design of tailored myocardial protection and operative strategies for diabetic patients undergoing CPB/C. (Circulation. 2004;110[Suppl II]:II-280–II-286.)

Key Words: cardioplegia ■ cardiopulmonary bypass ■ complications ■ diabetes mellitus ■ genes
able fuel for adenosine triphosphate production via the glycolytic pathway during ischemia, whereas the latter favors oxidation of glucose and lactate during reperfusion and suppresses oxidation of fatty acids, which can be detrimental in the postischemic heart. Despite promising results with the optimization of perioperative insulin management in diabetic patients, more work is needed to elucidate the complex mechanisms governing glucose and fatty acid oxidation in the ischemic-reperfused myocardium and better-understand their association with postoperative complications.

Transcriptional profiling using high-density microarrays provides unique data about disease mechanisms, drug responses, regulatory pathways, and gene function by comparing the level of mRNA transcribed in cells in a given pathologic state versus a control. In this study, we used this technology to bring insight into the pathophysiologic processes involved in the diabetic myocardial response to CPB and cardioplegic arrest (C) directly at the gene expression level, by comparing the cardiac gene expression responses to CPB and C of patients with and without diabetes. These findings could in turn provide a reference framework for the evaluation of prognostic gene markers and lead to the development of tailored cardioprotective strategies for diabetic patients.

Methods

Tissue Samples
The study protocol was approved by the Research Ethics Committee of the Beth Israel Deaconess Medical Center and informed consent was obtained from the patients. Atrial muscle samples were collected immediately before and after CPB in 5 insulin-dependent type II diabetic and 5 matched nondiabetic patients undergoing primary elective CABG. Samples were harvested with cold sharp dissection and handled in a nontraumatic fashion, and consisted of intact tissue that was not involved in a purse string suture. Pre-CPB tissues were taken from the right atrial appendage before venous cannulation, and post-CPB samples were harvested after the construction of a second purse string suture on the right atrial appendage and decannulation. The samples (~10×10×2 mm in size) were immediately snap-frozen in liquid nitrogen and stored at ~80°C. Phenotypically similar patients undergoing identical procedures were selected to minimize biological heterogeneity. Patient characteristics and CPB/C durations are shown in Table 1. For all patients, mild hypothermic CPB with intermittent cold-blood hyperkalemic (25 mmol/L) cardioplegia was used. Serum glucose levels were kept <130 mg/dL by intermittent intravenous insulin injections.

RNA Isolation
Total RNA was isolated from samples of ~200 mg with a Trizol-based method, following the manufacturer’s protocol (Gibco BRL). Further purification and concentration determinations were performed as previously described.

Microarray Processing
cDNA was prepared according to protocols provided with the Affymetrix U95 GeneChip system (Affymetrix), as previously described. Using the BioArray High-Yield RNA Transcript Labeling Kit (Enzo Diagnostics), the purified cDNA was incubated at 37°C for 5 hours in an in vitro transcription reaction to produce cRNA labeled with biotin.

Microarray Hybridization
cRNA was processed as described before. Briefly, cRNA was fragmented and then mixed with eukaryotic hybridization controls (containing control cRNA and oligonucleotide B2) and hybridized with a pre-equilibrated human U95Av2 Affymetrix chip at 45°C for 16 hours. The chips were washed and stained with streptavidin phycoerythrin (SAPE). This process was followed by incubation with normal goat immunoglobin G and biotinylated mouse anti-streptavidin antibody, then re-staining with streptavidin phycoerythrin.

Statistical Analysis of Microarray Data
The chips were scanned in an HP ChipScanner (Affymetrix Inc) to detect hybridization signals. Scanned image output files were visually examined for major chip defects and hybridization artifacts. Positive hybridization was defined as hybridization signal intensity of 1000 or greater. Two statistical methods were combined to assess differential gene expression between the pre-CPB and post-CPB/C and cardioplegic arrest tissues. To ensure a minimal number of false-positives, only the probe sets commonly yielded by the 2 methods were included in the list of genes differentially expressed in post-CPB/C versus pre-CPB/C samples.

Method 1 identified probe sets with significant intensity differences between pre-CPB/C and post-CPB/C tissues. For each gene, a Wilcoxon signed-rank test was applied to the absolute signal intensities in the pre-CPB/C versus post-CPB/C data set. The use of a nonparametric paired Wilcoxon test was selected over that of a standard t-test to avoid a distributional assumption; the threshold was set at P=0.0253 or less, which corresponds to the lowest possible probability value in the particular experimental setting of the study (observed when 5 out of 5 patients from 1 group show the same pattern of differential expression). By using this probability value, the number of genes that could have reached significance by chance alone was determined and compared with the number of observed significant genes, and a signal-to-noise-ratio was computed.

Method 2 determined the differentiability of a probe set by its signal intensity fold change. Nonparametric determination of genes with significantly different fold changes between the pre-CPB/C and post-CPB/C states was performed by computing and ordering by increasing size the median relative signal intensities of each probe set. A particular gene was considered to be upregulated after CPB/C if a median fold change of 4 (4:1) or more was observed in
post-CPB/C versus pre-CPB/C samples. Conversely, a gene was considered downregulated if the median fold change of post-CPB/C versus pre-CPB/C samples was 0.25 (1:4) or lower. Only genes identified by both methods were ultimately considered to be differentially expressed and are reported.

**Microarray Validation**

For validation of mRNA expression yielded by the microarray technique, both a significantly altered gene (DUSP1) and a nonaltered gene (H9252-actin) were chosen to validate positive and negative chip signals by use of Northern blotting. In addition, immunoblotting was performed to determine whether protein expression also correlated with the mRNA regulation of DUSP1, and that of the 2 most upregulated genes in the diabetic (amphiregulin) and the nondiabetic (PPP1R3C) exclusive groups.

**Northern Blotting**

cDNA probes of DUSP1 and H9252-actin were labeled with 32P-dCTP (New England Nuclear, Boston, Mass) using a random-priming labeling kit (Boehringer) and purified from unincorporated nucleotides using G-50 Quick Spin Columns (Boehringer). As previously described, the blots were hybridized and washed, autoradiography was performed, and the blots were analyzed after digitization and quantification, on x-ray films, of the gene mRNA to loading band density ratio. Results are presented as a median (minimum, maximum) ratio of post-CPB/C to pre-CPB/C mRNA expression in both groups.

**Immunoblotting**

Protein extraction was performed from total atrial muscle tissue lysates, then membranes prepared as described before. Membranes were incubated with rabbit polyclonal antibodies (anti-DUSP1 1:1000 [v/v] dilution, anti-PPP1R3C 1:1000 [v/v] dilution, and anti-amphiregulin [BD Pharmigen]) in 2.5% nonfat dry milk in TBST (50 mmol/L Tris-HCl, pH 8.0, 100 mmol/L NaCl, and 0.1% Tween 20) for 2 hours. After washing with TBST, the membranes were incubated for 1 hour in 2.5% nonfat dry milk in TBST diluted with antirabbit immunoglobulin G (Jackson Immunolabs), both at 1:3000 [v/v] dilution conjugated to horseradish peroxidase. Peroxidase activity was visualized using enhanced chemiluminescence and exposed to x-ray films (Amersham). Post-CPB/C to pre-CPB/C ratios of protein expression are presented as median (minimum, maximum) ratios.

**Reporting and Functional Classification**

Genes identified to be significant by microarray analysis with both statistical methods were the object of a literature search and reported, along with their GenBank accession number, according to the current

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**TABLE 2. Genes Exhibiting a 4-Fold or Greater Increase in Expression in Post-CPB Versus pre-CPB/C Arrest Atrial Samples of Diabetic and Nondiabetic Patients**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Symbol</th>
<th>GenBank Accession Number</th>
<th>Expression Ratio in Diabetic Subjects*</th>
<th>Expression Ratio in Controls*</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 6</td>
<td>IL6</td>
<td>X04430</td>
<td>41:1</td>
<td>25:1</td>
<td>Mediates leukocytosis, thrombosis, and lymphocyte activation</td>
</tr>
<tr>
<td>Proto-oncogene JUN-B</td>
<td>JUNB</td>
<td>M29039</td>
<td>27:1</td>
<td>25:1</td>
<td>Pro-apoptotic transcription factor, acting with FOS</td>
</tr>
<tr>
<td>Nuclear receptor subfamily 4, group A, member 1</td>
<td>NR4A1</td>
<td>L13740</td>
<td>21:1</td>
<td>16:1</td>
<td>Steroid receptor; regulates apoptosis</td>
</tr>
<tr>
<td>Nuclear receptor subfamily 4, group A, member 2</td>
<td>NR4A2</td>
<td>X75918</td>
<td>19:1</td>
<td>23:1</td>
<td>Coactivator of transcription; stimulated by PGE2, IL1B, and TNF</td>
</tr>
<tr>
<td>Dual-specificity phosphatase 1</td>
<td>DUSP1</td>
<td>X68277</td>
<td>14:1</td>
<td>12:1</td>
<td>Regulator of the cellular response to stress</td>
</tr>
<tr>
<td>Proto-oncogene C-FOS</td>
<td>FOS</td>
<td>K00650</td>
<td>11:1</td>
<td>10:1</td>
<td>Pro-apoptotic transcription factor, acting with JUN</td>
</tr>
<tr>
<td>Zinc finger transcriptional regulator</td>
<td>ZFP36</td>
<td>M92843</td>
<td>9:1</td>
<td>13:1</td>
<td>Component of a negative feedback loop that interferes with TNF production</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif), ligand 2, solute carrier family 2, member 3</td>
<td>CXCL2</td>
<td>M57731</td>
<td>9:1</td>
<td>6:1</td>
<td>Chemotactic factor for monocytes</td>
</tr>
<tr>
<td>Early growth response 3</td>
<td>EGR3</td>
<td>X63741</td>
<td>6:1</td>
<td>10:1</td>
<td>Immediate–early transcription activator; induced by stress or injury</td>
</tr>
<tr>
<td>Cysteine-rich angiogenic inducer 61</td>
<td>CYR61</td>
<td>Y11107</td>
<td>6:1</td>
<td>4:1</td>
<td>Immediate–early transcription factor; promotes adhesion of endothelial cells, chemotaxis, angiogenesis</td>
</tr>
<tr>
<td>Early growth response 2</td>
<td>EGR2</td>
<td>J04076</td>
<td>6:1</td>
<td>6:1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>E-selectin</td>
<td>SELE</td>
<td>M24736</td>
<td>5:1</td>
<td>7:1</td>
<td>Adhesion of leukocytes to cytokine-stimulated endothelial cells</td>
</tr>
<tr>
<td>Oncogene FOS-B</td>
<td>FOSB</td>
<td>L49169</td>
<td>5:1</td>
<td>7:1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Chemokine (C-C motif), ligand 2</td>
<td>CCL2</td>
<td>BC00976</td>
<td>5:1</td>
<td>6:1</td>
<td>Chemotactic factor for monocytes</td>
</tr>
<tr>
<td>Kruppel-like factor 4</td>
<td>KLF4</td>
<td>BC030811</td>
<td>5:1</td>
<td>6:1</td>
<td>Zinc-finger–containing transcription factor</td>
</tr>
<tr>
<td>PI3 kinase-related kinase</td>
<td>SMG1</td>
<td>AY014957</td>
<td>5:1</td>
<td>4:1</td>
<td>Controls numerous cellular functions (growth, differentiation, migration, survival, metabolism)</td>
</tr>
</tbody>
</table>

*Median expression ratio of post-CPB/C versus pre-CPB/C samples (paired for each patient exposed to CPB).

**II-282 Circulation September 14, 2004**
Table 3. Genes Exhibiting a 4-Fold or Greater Increase in Expression in Post-CPB versus pre-CPB/C Arrest Atrial Samples of Diabetic Patients, Exclusively

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Symbol</th>
<th>GenBank Accession Number</th>
<th>Expression Ratio*</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphiregulin</td>
<td>AREG</td>
<td>M30704</td>
<td>16:1</td>
<td>Autocrine growth factor</td>
</tr>
<tr>
<td>Interleukin 1β</td>
<td>IL1B</td>
<td>M15330</td>
<td>9:1</td>
<td>Cytokine involved in inflammation, cell–cell signaling, signal transduction, and antimicrobial response</td>
</tr>
<tr>
<td>Nuclear receptor subfamily 4, group A, member 3</td>
<td>NR4A3</td>
<td>D78579</td>
<td>8:1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Regulator of G protein signaling 1</td>
<td>RGS1</td>
<td>S59049</td>
<td>8:1</td>
<td>Immediate–early response gene</td>
</tr>
<tr>
<td>Activating transcription factor 3</td>
<td>ATF3</td>
<td>L19871</td>
<td>8:1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Insulin receptor substrate 1</td>
<td>IRS1</td>
<td>S62539</td>
<td>7:1</td>
<td>Substrate of the insulin receptor tyrosine kinase, participant in insulin signaling</td>
</tr>
<tr>
<td>V-maf musculoaponeurotic fibrosarcoma oncogene family, protein F (avian)</td>
<td>MAFF</td>
<td>BC015037</td>
<td>7:1</td>
<td>Transcription regulator</td>
</tr>
<tr>
<td>Ras homolog gene family, member B (Rho B)</td>
<td>ARHB</td>
<td>X06820</td>
<td>6:1</td>
<td>Growth factor-responsive early gene</td>
</tr>
<tr>
<td>Complement component 1, q subcomponent, receptor 1</td>
<td>C1QR1</td>
<td>U94333</td>
<td>6:1</td>
<td>Regulation of phagocytic activity</td>
</tr>
<tr>
<td>Nuclear antigen SP100</td>
<td>SP100</td>
<td>M60618</td>
<td>6:1</td>
<td>Component of nuclear domain, role in autoimmunity, infections, tumorigenesis</td>
</tr>
<tr>
<td>Chemokine (C-C motif), ligand 4</td>
<td>CCL4</td>
<td>J04130</td>
<td>6:1</td>
<td>Chemotactic factor for monocytes</td>
</tr>
<tr>
<td>Special AT-rich sequence-binding protein 2</td>
<td>SATB2</td>
<td>AB028957</td>
<td>6:1</td>
<td>Transcriptional repressor, maintains T cells in a quiescent state</td>
</tr>
<tr>
<td>Interleukin 8</td>
<td>IL8</td>
<td>M28130</td>
<td>5:1</td>
<td>Mediates neutrophil chemotaxis and migration</td>
</tr>
<tr>
<td>Interleukin 1 receptor antagonist</td>
<td>IL1RN</td>
<td>M63099</td>
<td>5:1</td>
<td>Inhibits interleukin 1α and interleukin 1β</td>
</tr>
<tr>
<td>Oncogene MYC</td>
<td>MYC</td>
<td>V00568</td>
<td>5:1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>VEGF</td>
<td>M32977</td>
<td>5:1</td>
<td>Growth factor, mitogen primarily for vascular endothelial cells</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif), ligand 3</td>
<td>CXCL3</td>
<td>X53800</td>
<td>5:1</td>
<td>Chemotactic factor for monocytes</td>
</tr>
<tr>
<td>B-cell translocation gene 2</td>
<td>BTG2</td>
<td>U72649</td>
<td>5:1</td>
<td>Cell cycle regulator in response to DNA damage</td>
</tr>
<tr>
<td>Cerebellar degeneration-related autoantigen 1</td>
<td>CDR1</td>
<td>M31423</td>
<td>4:1</td>
<td>Unclear</td>
</tr>
<tr>
<td>Barren (homolog of Drosophila) 1</td>
<td>BRNN1</td>
<td>D38553</td>
<td>4:1</td>
<td>Involved in cell division</td>
</tr>
<tr>
<td>Oncogene JUN</td>
<td>JUN</td>
<td>J04111</td>
<td>4:1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Sodium channel, voltage-gated, type IX, alpha subunit</td>
<td>SCN9A</td>
<td>X82835</td>
<td>4:1</td>
<td>Subunit of voltage-gated sodium channel</td>
</tr>
</tbody>
</table>

*Median expression ratio of post-CPB/C versus pre-CPB/C samples (paired for each patient exposed to CPB).

Results

Microarray Signal-to-Noise Characteristics

Of 12625 genes examined, 1294 and 1106 genes were identified by statistical method 1 to have $P=0.0253$ or less for differential expression in post-CPB/C versus pre-CPB/C atrial tissues from diabetic and nondiabetic patients, respectively (Figure 1). The projected number of genes that could have reached significance because of chance alone was 320 for each group, corresponding to a signal-to-noise ratio of 4.04 in diabetic and 3.46 in nondiabetic microarrays. There were more upregulated genes (851 versus 480, $P<0.001$) and, contrastingly, less downregulated genes (443 versus 626, $P<0.001$) in the diabetic than in the nondiabetic group.

Identification of Significantly Altered Genes

By using statistical method 2 alone, the numbers of genes upregulated >4-fold were, respectively, 66 for the diabetic group and 50 for the nondiabetic patients, including 22 genes that were shared by both groups. Genes whose expression was found to be significantly upregulated by using both statistical methods are reported in Tables 2 to 4. Of these, there were 18 shared by both groups, which included mostly transcription factors and mediators of the inflammatory response, as well as glucose transporter-3. As shown in Figure 2, respectively, 28 and 27 genes were exclusively upregulated in the diabetic and the nondiabetic groups, displaying a highly significantly different pattern of expression between these groups ($P=0.0001$). Genes from the diabetic group included mostly other transcription factors, mediators of inflammation such as IL8, as well as vascular endothelial growth factor, amphiregulin, and insulin-receptor substrate 1. Glycogen-targeting subunit PPP1R3C overexpression was very significant (16:1 ratio) and found only in nondiabetic patients (Figure 3 C).

DUSP1

The microarray results for DUSP1 revealed increased expression (median 12-fold; 3.2, 27.1; $P=0.025$) in the atrium from
Validation studies revealed the median mRNA expression of DUSP1 in post-CPB/C versus pre-CPB/C samples from nondiabetic patients to be increased 3-fold (1.7, 7.1; \( P = 0.05 \)) and 4-fold for diabetic patients (1.5, 13.3; \( P = 0.05 \)) by Northern blotting (Figure 3A), with changes in protein levels of 4.6-fold (1.3, 5.7; \( P = 0.05 \)) and 4.3-fold (1.5, 4.3, \( P = 0.05 \)), respectively, for nondiabetic and diabetic patients by immunoblotting.

**Amphiregulin**

Immunoblotting demonstrated a 3.2-fold increase (1.3, 5.7; \( P < 0.05 \)) in amphiregulin protein level in diabetic atrial tissue after CPB/C (Figure 3B), coherent with microarray data showing a 16-fold increase (3.7, 21.0; \( P = 0.025 \)) in mRNA expression, whereas no significant changes in expression were observed in the nondiabetic group by either assessment technique.

**β-actin**

Microarray results revealed no significant differential expression of that gene after CPB/C in atrial tissues. On Northern blotting, the median differential expression of post-CPB/C to pre-CPB/C in the nondiabetic group was 1.2, and 1.0 for the diabetic group (\( P = \) not significant).

**PPP1R3C**

Immunoblotting demonstrated a 5.2-fold increase (2.2, 6.1 \( P < 0.05 \)) in expression in the diabetic patients but no significant change in the nondiabetic group, corroborating the microarray results that showed a median expression ratio of
18:1 in the former group (2.4, 41.3; \(P=0.025\)) and no change in the latter (Figure 3 C).

**Discussion**

This study reports a significantly modified response to CPB and C in insulin-dependent type II diabetic patients at the gene expression level, both quantitatively and qualitatively.

A statistically larger number of upregulated genes with subthreshold probability values were identified in the diabetic myocardial samples after CPB/C compared with the nondiabetic group, whereas the inverse relationship was found for the downregulated genes. Sixty-six percent (851/1294) of the significantly altered genes in the diabetic group were upregulated, compared with only 43% (480/1106) in the nondiabetic group. Although these genes may not all have clinical relevance, this relationship emphasizes how strikingly different the pattern of expression is between these 2 groups of patients.

The selection of genes with a 4-fold (or greater) change in expression further outlines targets with potential clinical significance. It was observed that most of the genes found to be upregulated in both study groups were early transcription factors (Table 2), followed by mediators of the inflammatory response such as IL6, E-selectin, and CCL2. IL6 showed the most important change of all genes studied and was most upregulated in diabetic subjects (41:1 ratio). Of note is that high IL6 levels after CPB have been associated with hepatic and renal dysfunction. Glucose transporter-3 as well as phosphatidylinositol 3-kinase-related-kinase were also upregulated in both groups and are, respectively, an important regulator of insulin action and a downstream participant in cellular glucose uptake in response to insulin. These findings suggest that other messengers in the insulin signaling pathway may play a significant role in inducing resistance to insulin.

Twenty-eight genes were upregulated by a factor of 4-fold in the diabetic group exclusively. Among them are important transcription activators MYC and JUN and potent mediators of the inflammatory response IL8 and IL1β, in accordance with previous studies that have shown an exaggerated response to CPB in diabetic patients. The increase in expression of vascular endothelial growth factor may be explained by the increased oxidative stress in response to CPB and increased NO production in diabetic patients. Insulin receptor substrate 1 was also among that group of overexpressed genes in diabetic myocardium and is a key controlling element in insulin and insulin-like growth factor actions.

Contrastingly, genes that were exclusively upregulated in the nondiabetic group involved mostly cell-cycle regulators and mediators of apoptosis. Of particular interest is protein phosphatase regulatory subunit 3C, which was overexpressed by an 18:1 ratio in the nondiabetic patients but was not significantly altered in the diabetic group. It is a scaffold protein that plays an important role in activating protein phosphatase 1 by juxtaposing the enzyme with its substrates in a macromolecular complex. Protein phosphatase 1 is a regulator of insulin-dependent activation of glycogen synthase and inactivation of glycogen phosphorylase, both promoting glycogen deposition. Given the lower-than-normal glycogen levels in diabetic patients, the absence of studies on the effects of this mechanism of insulin-resistance after CPB and the capacity of adenoviral-mediated PPP1R3C overexpression to stimulate glucose disposal while still allowing substrate-mediated and hormone-mediated regulation of glycogen turnover in isolated hepatocytes, it is conceivable that this gene could be a target for specifically designed cardio-protective strategies aimed at insulin-resistant patients undergoing CPB/C. Other genes with potential clinical applications are presented in Tables 2 to 4.

**Limitations**

Microarray techniques, despite their immense potential to improve disease understanding, have inherent shortcomings related to the selection of tissues, the lack of standardized methods for the statistical analysis of chip data, the presentation of vast amounts of results, and the generalizability of findings. In this study, we have attempted to limit these shortcomings by selecting subjects who underwent the same anesthetic, surgical and perfusion techniques, and who were phenotypically similar on all respects with the exception of diabetic status. In addition, we have combined 2 independent
nonparametric statistical approaches to minimize false-positive results; however, it is possible as a consequence that not all genes may necessarily have been identified in the study (false-negatives). Expression patterns were validated with signal-to-noise ratios determinations and specifically by confirming mRNA expression patterns and protein levels of altered as well as nonaltered genes with conventional molecular techniques. Finally, we have attempted, based on current literature, to suggest a potential functional role for genes whose expression was markedly altered.

Another limitation of the study is that, for ethical reasons, we have used atraumatically harvested human atrial biopsy specimens rather than ventricular biopsy specimens to examine the cardiac gene expression changes that result from CPB and cardioplegic arrest. In contrast to ventricular biopsy specimens, atrial sampling involves little or no intrinsic risk of morbidity, provides full-thickness samples, and is clinically reproducible. Furthermore, Stirling et al have shown that CPB and antegrade cold blood cardioplegia distribute to the right atrium.25 Although atrial myocardium differs from ventricular myocardium with respect to the relative percentage of myocytic, endothelial, connective, and neural elements, these cell types are present in each tissue type and demonstrate ultrastructural changes in disease.26–27 Previous animal work from our laboratory has shown that genes of the critical MEK/ERK pathway show similar patterns of expression in both ventricular and atrial tissue before and after CPB.28

Despite the aforementioned shortcomings, the results from this analysis help better-understand the molecular mechanisms pertaining to the cardiac response to CPB/C in diabetic patients and their associated clinical implications. Further investigation based on these data could lead to the development of tailored cardioplegic approaches as well as alternative operative strategies, resulting in lower morbidity and mortality after cardiac surgery in these high-risk patients.

Acknowledgments

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References

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