Genomic expression pathways associated with brain injury after cardiopulmonary bypass

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Objectives: Neurologic injury after cardiac surgery, often manifested as neurocognitive decline, is a common postoperative complication without clear cause. We studied acute variations in gene-expression profiles of patients with neurocognitive decline (NCD group) compared with those without neurocognitive decline (NORM group) after cardiopulmonary bypass.

Methods: Forty-two patients undergoing coronary artery bypass grafting, valve procedures, or both by using cardiopulmonary bypass were administered a validated neurocognitive battery preoperatively and postoperatively at day 4. Neurocognitive decline was defined as 1 standard deviation from baseline on 25% or greater of tasks. Whole-blood mRNA was isolated preoperatively and at 6 hours after surgical intervention for fold-change calculation. Relative gene expression in the NCD versus the NORM group was assessed by using Affymetrix GeneChip U133 Plus 2.0 (>40,000 genes) from mRNA samples collected. Differential expression, clustering, gene ontology, and canonical pathway analysis were performed. Validation of microarray gene expression was performed with SYBR Green real-time polymerase chain reaction.

Results: Patients with neurocognitive decline (17/42 [40.5%] patients) were associated with a significantly different gene-expression response compared with that of healthy patients. Compared with preoperative samples, 6-hour samples had 531 upregulated and 670 downregulated genes uniquely in the NCD group compared with 2214 upregulated and 558 downregulated genes uniquely in the NORM group ($P < .001$; lower confidence bound, ≥ 1.2). Compared with patients in the NORM group, patients with neurocognitive decline had significantly different gene-expression pathways involving inflammation (including *FAS*, *IL2RB*, and *CD59*), antigen presentation (including *HLA-DQ1*, *TAP1*, and *TAP2*), and cellular adhesion (including *ICAM2*, *ICAM3*, and *CAD7*) among others.

Conclusions: Patients with neurocognitive decline have inherently different genetic responses to cardiopulmonary bypass compared with those of patients without neurocognitive decline. Genetic variations in inflammatory, cell adhesion, and apoptotic pathways might be important contributors to the pathophysiology of neurologic injury after cardiopulmonary bypass and could become a target for prevention and risk stratification.

Brain injury after cardiopulmonary bypass (CPB) remains a common and serious complication that is often misdiagnosed.^{1,2} Brain-protective strategies include improved operative approach selection (eg, “no-touch” technique for the calcified aorta), preoperative investigation (eg, carotid duplex scan-

Abbreviations and Acronyms

BP	= biologic process
CNS	= central neurologic system
CPB	= cardiopulmonary bypass
FC	= fold change
GO	= gene ontology
LCB	= lower confidence bound
NCD	= neurocognitive decline
NORM	= without neurocognitive decline
PRE	= preoperatively after induction of anesthesia and before skin incision
SD	= standard deviation
6h	= 6 hours postoperatively in the cardiovascular intensive care unit

ning), and intraoperative measures (eg, hypothermia and CPB filters). Despite these advances in surgical technique, CPB, and anesthesia, central neurologic system (CNS) injury remains an important complication for patients. The American College of Cardiology/American Heart Association guidelines for coronary artery bypass graft surgery divide postoperative neurologic deficits into 2 categories.³ Type 1 deficits (incidence of 1%-5%) include major focal neurologic events, stupor, and coma. Type 2 deficits, with an incidence as high as 65% in some studies,^{4,5} describe more global cognitive deficits, such as deterioration in intellectual function, memory, and confusion without evidence of focal injury. Type 1 deficits are usually caused by identifiable sources of cerebral hypoxia caused by intraoperative hypoperfusion or embolic phenomena. In contrast, the cause of type 2 deficits is unclear and likely multifactorial, where factors such as hypoxia, time on CPB, age, type of procedure, preoperative creatinine levels, and perioperative inflammatory response have been implicated in its pathophysiology.⁶

The remarkably high incidence of type 2 brain injury (measured at 1 week postoperatively), usually improves and decreases to around 10% to 30% at 1 year. Interestingly, Newman and colleagues⁷ have reported that the occurrence of early neurocognitive decline (NCD) in cardiac surgical patients is predictive of long-term decline. One of the reasons why the incidence of type 2 CNS injury remains so high in spite of recent advances might be a fundamental lack in the understanding of the pathophysiology of this type of injury.^{1,6}

The inflammatory response and associated oxidative stress have been implicated in the development of postoperative CPB-associated complications.⁸⁻¹² These are triggered by the activation of blood components on the artificial surface of the extracorporeal circuit, such as the activation of leukocytes, complement, expression of adhesion molecules, cytokine release, and an increase in reactive oxygen

species, such as peroxides, that mediate oxidative stress. It is increasingly recognized that there exists a certain amount of variability in the magnitude and duration of response to CPB between patients, which has been implicated in transient and permanent end-organ damage. Our group, among others, has reported on the strong association between NCD and the magnitude of the inflammatory response after CPB.^{13,14}

Transcriptional profiling with 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. This technology can potentially elucidate complex pathophysiologic association mechanisms directly at the gene-expression level. The present study was conducted to examine the differences in gene-expression profiles of patients who have NCD after cardiac surgery compared with those who do not have this complication in an effort to improve our understanding of type 2 brain injury and provide perioperative strategies aimed at prevention and treatment.

Materials and Methods**Patient Enrollment**

We carried out a single-institution, prospective cohort study that was approved by the Beth Israel Deaconess Medical Center Institutional Review Board/Committee on Clinical Investigations. Forty-three patients scheduled for elective or urgent primary coronary artery bypass grafting, valvular surgery (aortic or mitral), or a combination of the 2 with CPB provided informed written consent and were enrolled. Exclusion criteria included the following: patients undergoing aortic arch/root procedures and those with calcified aorta, recent stroke, severe preoperative neurologic deficits, known high-grade carotid stenosis, advanced hepatic disease (cirrhosis), and chronic renal failure (serum creatinine, >2.0 mg/dL). Patients who were unable to complete the baseline neuropsychological battery because of severe cognitive impairment, psychiatric disease, substance abuse, blindness, or poor English were also excluded. One patient was excluded from the analysis because of inability to complete the neuropsychological assessment before discharge. Thus 42 patients were included in the analysis.

Anesthetic and Surgical Techniques

The conventional operative approach at our institution was followed, including induction of general anesthesia, invasive monitoring, midline sternotomy, and systemic heparinization. CPB was initiated through cannulation of the right atrium and ascending aorta with a nonpulsatile system, membrane oxygenator, and 40- μ m arterial filter. Crystalloid pump prime was used. For all patients, mild hypothermic CPB (minimum temperature, 32°C-34°C) with intermittent cold blood hyperkalemic (25 mmol/L) cardioplegia was used. Serum glucose levels were monitored, and we attempted to maintain a value of less than 130 mg/dL by means of intermittent intravenous insulin injections or insulin infusion. During CPB, pump blood flow was maintained at 2 to 2.4 L $\cdot$ min⁻¹ $\cdot$ m⁻² body surface area. Arterial partial oxygen

pressure was maintained at between 150 and 250 mm Hg, and alpha-stat pH monitoring was used. Mean blood pressure was maintained between 50 and 90 mm Hg by using conventional vasoactive medications.

Neurocognitive Assessment

Patients underwent neurocognitive testing with a battery of evaluations preoperatively (1-10 days before surgical intervention) and postoperatively before discharge at postoperative day 4, as well as at 3 months. Before baseline neurocognitive assessment, all patients underwent a depression assessment by using the Geriatric Depression Scale (positive if score ≥ 9 of 15). All evaluations were carried out by trained and dedicated psychometricians who were blinded to serologic testing. The battery was chosen to be consistent with the "Statement of consensus on neurobehavioral outcomes after cardiac surgery"¹⁵ and consisted of 8 validated assessments covering memory, executive function, attention, language, and global cognition.

From the Hopkins Verbal Learning Test, a validated 12-item verbal learning, retention, and recall measure, we assessed the number of items learned, the number of items recalled after a 20-minute delay divided by the maximum number of items learned, and the number of items correctly identified from a list. Confrontational naming was measured with the Boston Naming Test.¹⁶ Time to complete Trailmaking A and B, a measure of shifting attention abilities, was recorded. Digit span forward and backward of the Revised Wechsler Adult Intelligence Scale is a measure of working memory and sustained attention requiring participants to hold and manipulate information. Fluency tasks are measures that assess language and knowledge storage patterns by requiring the patient to spontaneously generate words in a category (semantic) or beginning with a specific letter (phonemic). Performance on the Wechsler Test of Adult Reading, an irregular-word reading task, was used as a measure of premorbid intelligence. The Stroop Color-Word Interference test assesses executive function, and we recorded the number of correct responses. The visual search and acuity test requires visuospatial abilities and executive function.

Following the "Consensus statement on neurobehavioral changes following cardiac surgery" guidelines, we defined cognitive decline as a 1-standard-deviation (SD) decline from baseline on 25% of the tasks (2/8 measures).¹⁷ The SD was derived by performing the cognitive battery in a similar population undergoing cardiac surgery.¹⁸

Blood Sampling and Microarray Processing

For each of the 42 patients, blood samples were collected from the central venous line preoperatively after induction of anesthesia and before skin incision (PRE), as well as 6 hours postoperatively in the cardiovascular intensive care unit (6h). Blood samples were collected into PAXgene tubes (QIAGEN, Inc, Valencia, Calif) for immediate mRNA stabilization and extraction, as per the manufacturer's recommendation. Transcriptional profiles of samples were probed by using Affymetrix HG (Affymetrix Inc, Santa Clara, Calif) U133 Plus 2.0 chips for a total of 84 chips. Total RNA extraction and purification, cDNA synthesis, in vitro transcription reaction for production

of biotin-labeled cRNA, hybridization of cRNA with Affymetrix GeneChips, and scanning of arrays were done according to previously described protocols.¹⁹ All arrays went through stringent quality control assessment with regard to 3'/5' ratios (by using glyceraldehyde-3-phosphate dehydrogenase and β -actin probes), percentage of present calls, and array outlier call percentage within 2 SDs of the mean. All scanned array images passed the quality controls and were analyzed by using dChip,²⁰ which has been shown to be more robust than Affymetrix software Microarray Analysis Suite 5.0 in signal calculation for about 60% of genes.²¹ In the dChip analysis a smoothing spline normalization method was applied before obtaining model-based gene-expression indices, also known as signal values. There were no outliers identified by using dChip, and therefore all samples were carried on for subsequent analysis.

Unsupervised and Differential Expression Analysis

A hierarchic clustering technique was used to construct an unweighted pair group method with arithmetic-mean tree by using the Pearson correlation as the metric of similarity. When comparing 2 groups of samples to identify genes enriched in a given group, we used the lower confidence bound (LCB) of the fold change (FC) between the 2 groups (6H vs PRE) as the cutoff criteria. If 90% LCB of FC between the 2 groups was greater than 1.2, the corresponding gene was considered to be differentially expressed. LCB is a stringent estimate of the FC and has been shown to be the better ranking statistic.²⁰ Recently, dChip's LCB method for assessing differentially expressed genes has been shown to be superior to other commonly used approaches, such as Microarray Analysis Suite 5.0- and Robust Multiarray Average-based methods.^{22,23} By using custom arrays and quantitative reverse transcriptase real-time polymerase chain reaction, it has been suggested that Affymetrix chips might underestimate differences in gene expression.¹⁷ Based on this work and others,²⁴ a criterion of selecting genes that have an LCB of greater than 1.2 most likely corresponds to genes with an "actual" FC of at least 3 in gene expression.

Gene Ontology Analysis

Once gene lists that are differentially expressed between various groups have been calculated, we identified the gene ontology (GO) categories for each of these input gene sets.²⁵ This approach suggests biologic areas that warrant further study by discovering GO categories that exist in significant abundance in the input gene list by using hypergeometric distribution ($P < .05$). For each category, the actual number represents the number of upregulated genes in that category. The expected number is a calculated value obtained by determining the ratio of genes in the particular category to the total number of genes and multiplying by the number of genes found to be upregulated. The expected number is therefore the number of genes predicted to be upregulated if the genes were distributed randomly among categories.

Pathway Analysis

Pathway analyses of genes were performed by using the Ingenuity Pathways Knowledge Base (Redwood City, Calif), which is a manually curated database of previously published findings

TABLE 1. Baseline patient characteristics for the entire cohort, as well as those with (NCD group) and without (NORM group) neurocognitive deficits after cardiopulmonary bypass (mean $\pm$ standard deviation)

Characteristic	Cohort (42 patients)	NCD group (17/42)	NORM group (25/42)	P value
Preoperative data				
Age (y)	66.7 $\pm$ 10.5	68.2 $\pm$ 10.7	65.7 $\pm$ 10.5	.45
Sex (% male)	85.7 (36/42)	82.4 (14/17)	72.0 (22/25)	.71
Race (% white)	88.1 (37/42)	94.0 (16/17)	84.0 (21/25)	.33
Hypertension (% of cohort)	76.2 (32/42)	82.4 (14/17)	72.0 (18/25)	.45
Hypercholesterolemia (% of cohort)	61.9 (26/42)	58.8 (10/17)	64.0 (16/25)	.74
Diabetes mellitus (% of cohort)	76.2 (32/42)	82.4 (14/17)	72 (18/25)	.45
Creatinine (mg/dL)	40.5 (17/42)	35.3 (6/17)	44.0 (11/25)	.58
Leukocytes (10^3 cells/ μ L)	1.31 $\pm$ 1.4	1.68 $\pm$ 2.2	1.07 $\pm$ 0.4	.18
Hematocrit (% of cohort)	7.6 $\pm$ 2.2	7.5 $\pm$ 2.0	7.6 $\pm$ 2.3	.86
Hemoglobin (g/dL)	35.8 $\pm$ 4.3	36.1 $\pm$ 4.7	35.6 $\pm$ 4.2	.73
Operative data				
Procedure (% CABG)	76.2 (32/42)	82.4 (14/17)	72.0 (18/25)	.45
CPB time (min)	85.3 $\pm$ 31.2	91.2 $\pm$ 26.3	81.3 $\pm$ 33.7	.15
Crossclamp time (min)	62.3 $\pm$ 28.7	65.9 $\pm$ 23.8	59.8 $\pm$ 31.5	.24
Aprotinin (% of cohort)	33.3 (14/42)	35.3 (6/17)	32.0 (8/25)	.42
Aminocaproic acid (Amicar; % of cohort)	66.7 (28/42)	64.7 (11/17)	68.0 (17/25)	.42
Cardiotomy suction (% of cohort)	61.9 (26/42)	58.8 (10/17)	64.0 (16/25)	.37
Cell saver (% of cohort)	42.9 (18/42)	35.3 (6/17)	32.0 (8/25)	.37

NCD, Neurocognitive decline; NORM, without neurocognitive decline; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass.

on mammalian biology from the public literature. We used the network analysis, using the knowledge base to identify interactions of input genes within the context of known biologic pathways. To this end, we investigated the established pathways in the knowledge base.

Real-time Polymerase Chain Reaction

After total RNA extraction, concentrations were determined by means of spectrophotometry; each sample yielded a minimum of 10 μ g of total RNA with an A260/A280 ratio ranging between 1.7 and 1.9. Integrity of total RNA was confirmed by means of Agarose gel electrophoresis, and only samples with a ratio of 2:1 28S/18S were used for analysis.

The quantitative real-time SYBR Green reaction was performed in duplicate, according to recommended protocols provided by the TaqMan ABI 7700 (Applied Biosystems, Foster City, Calif). RT2 polymerase chain reaction primers for the human genes analyzed were obtained commercially available (Superarray Bioscience Corp, Frederick, Md). Primers for the 3' ends of genes were used for microarray validation, including the downregulated genes *HLA-DQA1*, *FBP2*, and *PYGB* and the upregulated genes *CYCL2*, *IFIH1*, and *TFAP2B*.

Clinical Data Analysis

Clinical data were expressed as means $\pm$ SD. Following the "Statement of consensus on assessment of neurobehavioral outcomes after cardiac surgery" guidelines,¹⁵ we defined cognitive decline through a dichotomous variable as a 1-SD decrease from baseline on 25% of the tasks (2/8 measures). The χ^2 test or Fisher exact test was used to compare proportions, and the Student *t* test was used to compare continuous variables.

Software packages used were GraphPad Prism 4 (GraphPad, San Diego, Calif) and SPSS (SPSS 11.5 for Windows; SPSS, Inc, Chicago, Ill).

Results

Patient Characteristics

From 68 patients who were eligible for the study and approached for enrollment/consent during the study period, 24 refused enrollment. One patient was approached but was subsequently not consented because of delirium. Forty-three patients provided informed written consent and were enrolled. One patient was subsequently excluded from the analysis because of refusal to complete neuropsychologic assessment before discharge (voluntary withdrawal). Thus 42 patients were included in the analysis.

Early NCD rate at postoperative day 4 was 40.5% (17/42). Almost all patients returned to baseline cognitive function at the 3-month time point because the NCD rate was 2.5% (1/42). Baseline patient characteristics and key perioperative data were similar between patients who had NCD (NCD group) at postoperative day 4 compared with those who did not have NCD (NORM group) and are summarized in Table 1. Patients underwent similar intraoperative courses with respect to CPB technique, temperature, anesthesia, and perioperative monitoring. No focal neurologic deficits or stroke occurred in enrolled patients. Similarly, no differences were observed in other postoperative complications for patients in the NCD group com-

Figure 1. Genes that were differentially expressed after cardiopulmonary bypass (CPB) in patients with (NCD group) and without (NORM group) neurocognitive decline: **A**, upregulated genes; **B**, downregulated genes. **6H**, Six hours postoperatively in the cardiovascular intensive care unit; **PRE**, preoperatively after induction of anesthesia and before skin incision.

pared with those in the NORM group. All patients had negative test results for depression preoperatively.

Differential Gene Expression in NCD Compared With That in NORM Patients

Using the described microarray GeneChip, we generated a nearly comprehensive database of gene expression in patients with and without NCD after CPB. A complete list of gene expression, upregulated and downregulated gene lists, and functional and correlation analyses are provided in the online supplementary data Web site (<http://www.bidmcgenomics.org/NCD/index.html>).

Cluster analysis of complete array expression values produced expression similarities across the samples. Hierarchic clustering of all samples demonstrated a clear distinction based on the sample collection time: PRE versus 6h. In the NCD group 6490 genes were upregu-

lated and 1147 genes were downregulated at 6h compared with PRE. In the NORM group 8173 genes were upregulated and 1035 genes were downregulated at 6h compared with PRE. When these gene lists were compared, we found that 5959 genes were commonly upregulated at 6h versus PRE regardless of the NCD status, 531 genes were uniquely upregulated in NCD group patients, and 2214 genes were uniquely upregulated in NORM patients. Among the genes that were downregulated at 6h versus PRE, 477 genes were commonly downregulated regardless of the NCD status, 670 genes were uniquely downregulated in patients with NCD, and 558 genes were uniquely downregulated in patients without NCD (Figure 1). Selected upregulated and downregulated genes are presented for NCD group patients only (Tables E1 and E2) and NORM patients only (Tables E3 and E4).

TABLE 2. Gene ontology biologic process pathways that are differentially expressed at 6 hours postoperatively versus preoperatively in patients with neurocognitive decline compared with patients without neurocognitive decline

Gene regulation (6h vs PRE)	Patient group	Biologic process no.	Gene ontology pathway	Genes observed	P value
Down	NCD present	6955	Immune response	32	.000008
		19884	Antigen presentation, exogenous	6	.000026
		19886	Antigen processing through MHC II	6	.00003
		6959	Humoral immune response	5	.002033
		7166	Cell-surface receptor transduction	14	.003332
		42110	T-cell activation	4	.004954
			Cell-cell adhesion	7	.006279
		6952	Defense response	6	.03404
		7218	Neuropeptide signaling pathway	4	.046216
Up	NCD present	7596	Blood coagulation	7	.001267
		6917	Induction of apoptosis	6	.048673
	NCD absent	45454	Cell redox homeostasis	5	.003162
		6950	Response to stress	19	.007486
		6916	Antiapoptosis	20	.010814
		6635	Fatty acid β -oxidation	4	.039869

6h, Six hours postoperatively; PRE, preoperatively; NCD, neurocognitive decline; MHC II, major histocompatibility complex II.

Gene Ontology of Pathways Differentially Expressed in NCD Patients

As expected, unsupervised analysis of gene-expression data did not reveal an overarching separation based on NCD status and implied that the expression difference because of NCD status is more subtle and requires supervised analysis of expression data. Genes that were uniquely upregulated or downregulated at 6h compared with PRE in either group (NCD or NORM) were classified into GO groups based on their biologic process (BP). For pathway analysis, we first obtained genes that are uniquely upregulated or downregulated between the 6h and PRE time points with respect to NCD status. To this end, we combined the 531 and 670 genes (from Figure 1) that were specifically changed in patients with NCD (up or down) to obtain genes that are differentially expressed uniquely in patients with NCD. Similarly, we combined the 2214 and 558 genes (from Figure 1) that were specifically changed in NORM patients to obtain genes that are differentially expressed uniquely in NORM patients. We then investigated the expression of these 2 gene sets on the established pathways in Ingenuity Pathways Knowledge Base, also known as “canonical pathways.”

Table 2 summarizes selected categories of genes that contained significantly more than expected numbers of genes for a particular pathway when comparing the NCD and NORM groups ($P < .05$). Among the genes that were downregulated uniquely in the NCD group compared with the NORM group, GO BP categories of pathways that were significant included immune response, antigen presentation, antigen processing, humoral immune response, T-cell activation, and cell-cell adhesion (Figure 2). Genetic pathways that were uniquely downregulated in NORM patients in-

cluded defense response and neuropeptide signaling pathway categories. When upregulated genes in patients with NCD were analyzed, we identified blood coagulation as an important GO BP category that was represented with a significantly greater number of genes than the NORM group. Similarly, cell redox homeostasis, response to stress, antiapoptosis, and fatty acid β -oxidation were identified as selected GO BP categories that were in significant abundance among the genes that were upregulated in NORM patients. A complete list of GO BP categories that are represented significantly in genes that were uniquely upregulated or downregulated depending on their NCD status can be found in online supplementary data (<http://www.bidmcgenomics.org/NCD>).

In Figure 3 we show the results of the aforementioned analysis on the antigen presentation pathway. Colored nodes represent genes that exist in the input gene lists, where red denotes upregulation at 6h compared with PRE and green denotes downregulation at 6h compared with PRE in the respective gene lists. We observe a partial activation of the pathway for both gene lists, where the direction of this activation is opposite in the NORM versus NCD groups. Genes that were changed specifically in patients with NCD show downregulation at 6h compared with PRE, as reflected on this pathway.

Microarray expression validation with RT2-polymerase chain reaction was consistent with GeneChip data for all 6 gene primers studied, as summarized in Figure 4.

Discussion

Extensive research has been focused on clinical and intraoperative parameters as the culprits of this most

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common cardiac surgical complication. Several groups have shown significant associations between factors such as microemboli, perioperative temperature, anesthesia, and other neuroprotective strategies that clearly affect the pathophysiology of NCD after cardiac surgery.²⁶⁻³⁰ As a result of this body of data, we have a clearer understanding of the possible mechanisms that contribute to the development of neurologic dysfunction after cardiac surgery. Most importantly, we have learned that it is likely a multifactorial process that is difficult to prevent or treat because this extensive research has failed to translate into improvements in NCD occurrence after cardiac surgery. In fact, the incidence of NCD after CPB is arguably as high today as it was a decade ago. Why is this?

We believe that this study sheds some light on this issue because it highlights the important inherent differences that exist at the genetic level between patients who have type 2 brain injury after CPB, as manifested by NCD, and those who do not have this complication. By using genome-wide microarray gene-expression methods, we identified several genetic pathways that were differentially regulated in patients with NCD with statistical significance. For example, patients in the NCD group had significant downregulation of pathways involving inflammation, antigen presentation, T-cell activation, and intercellular adhesion. Such pathways are critical components of the patients' response to the CPB insult. We cannot, however, determine exactly from this study how such downregulation affects the complex cascade of intracellular and extracellular events that eventually culminate in NCD because their clinical significance might not be entirely clear. It is possible that downregulation of the cell-cell adhesion pathway leads to increased vascular permeability and increased brain edema in these patients. Similarly, it might be that upregulation of the blood coagulation pathway in patient with NCD leads to increased microthrombotic events, which lead to decreased perfusion to the hippocampus and cerebrum. Also, upregulation of genes involved in the response-to-stress pathway in NORM patients might have had a protective effect against brain injury.

Our data point to the fact that CPB does not cause an indiscriminate variation in gene expression in all patients. Rather, depending on the patient, CPB induces a distinct pattern of changes in specific pathways that are highly associated with NCD postoperatively. These data and subsequent conclusions were based on patients with similar baseline clinical and perioperative characteristics and then subjected to stringent bioinformatics analysis and validation by using conventional techniques. Sample size is a clear limitation to this study because some of the baseline clinical parameters between the 2 groups might have reached significance if the sample size was larger. This study, however, demonstrates these strong associa-

tions despite the sample size, which attests to the strength of relationships between particular pathway regulation and NCD status.

Another important observation from this study is that inflammatory and immunologic responses to CPB are inherently different at the genetic level in patients, depending on NCD status. This corroborates previous work by our group that revealed proteomic differences in which patients with NCD after CPB had significantly higher serologic inflammatory indices compared with those of patients without NCD after CPB.¹⁴ This link to the patients' differential inflammatory response to brain injury is not isolated to the cardiac surgical sphere. During the inflammatory response, peripheral cytokines have been found to penetrate the blood-brain barrier directly through active transport mechanisms or indirectly through vagal nerve stimulation.³¹ It has been shown that inflammatory mechanisms within the CNS contribute to cognitive impairment through cytokine-mediated interactions between neurons and glial cells (eg, neurodegenerative diseases, such as Alzheimer's and vascular dementia).³¹ Also, this response promotes membrane permeability and edema, contributing to end-organ dysfunction and possible neurologic injury observed in many patients.

In a recent study it was found that long-term cognitive outcomes after cardiac surgery are no different in patients undergoing conventional coronary revascularization with CPB compared with those in medically treated cardiac patients as control subjects.³² Such important studies, as well as this one (NCD rate at 3 months decreases to 2.5%) underscore the increasing body of evidence that such type 2 neurologic deficits are transient rather than long term. Potentially, in genetically susceptible patients the cognitive deficits are manifested during times of perioperative inflammatory stress and subsequently resolve as the inflammatory response subsides.

Our data certainly point to an inherently different response to CPB at the genetic level in patients who have NCD postoperatively. Certainly, such findings also point to a bigger question: Do these patients, or other cohorts, react differently to different insults, and thus are they more prone to increased complications? Such studies might prove to be beneficial in improving preoperative risk assessment and elucidating the pathophysiology of other surgical complications. We hope that further study of differentially expressed genetic pathways, in addition to established knowledge on clinical risk factors and perioperative factors, will translate into better understanding of NCD pathophysiology after CPB. In the short-term, however, it might be that such data would form the basis for a database of genetic information that could prove beneficial gene-based risk-stratification strategies.

Figure 2. Heat maps with hierarchic clustering of genes in pathways showing differential expression in patients with neurocognitive decline (NCD) versus those without neurocognitive decline (NORM group). The cell-cell adhesion (A) and immune response (B) pathways were both downregulated postoperatively uniquely in the NCD group. The blood coagulation pathway (C) was upregulated uniquely in the NCD group. Also, the response-to-stress pathway (D) was uniquely upregulated in the NORM group.

Figure 3. Significantly different expression of genes involved in the antigen presentation pathway in patients with neurocognitive decline (NCD; A) in contrast to patients without neurocognitive decline (NORM; B). Red, Upregulation; green, downregulation.

Figure 4. Confirmation of microarray gene-expression data by means of SYBR Green real-time polymerase chain reaction in 6 differentially expressed genes. Human 18S ribosomal RNA (18SrRNA) was used to normalize mRNA levels between samples. Values are shown for each gene as fold change (FC) in mRNA expression at 6 hours postoperatively (6H) compared with preoperative expression (PRE). Mean fold change $\pm$ standard error of the mean is shown.

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TABLE E1. Selected genes exhibiting significant upregulation at 6 hours postoperatively compared with preoperatively uniquely in patients with neurocognitive deficit

Accession	Gene ID	Gene	FC	LCB
NM_030937	81669	Cyclin L2	5.06	1.44
NM_003221	7021	Transcription factor AP-2 β	4.31	1.75
AV658684	4616	Growth arrest and DNA damage-inducible, β	3.18	1.76
AI653117	966	CD59 antigen p18–20 (antigen identified by monoclonal antibodies)	2.96	1.6
BC029442	5797	Protein tyrosine phosphatase, receptor type, M	2.9	1.39
NM_000570	2214	Fc fragment of IgG, low-affinity IIIa, receptor (CD16a)	2.59	1.23
U25975	5062	p21 (CDKN1A)-activated kinase 2	2.55	1.66
AK074045	85302	Fas (TNFRSF6) binding factor 1	2.54	1.24
NM_004486	2801	Golgi autoantigen, golgin subfamily a, 2	2.52	1.42
U80802	2649	Nuclear receptor subfamily 6, group A, member 1	2.48	1.33
BF196874	64163	Interferon-responsive gene 15	2.37	1.38
NM_000250	4353	Myeloperoxidase	2.27	1.37
NM_002686	5409	Phenylethanolamine N-methyltransferase	2.24	1.57
BF311866	5738	Prostaglandin F ₂ receptor-negative regulator	2.16	1.22
NM_145046	125972	Calreticula 3	2.12	1.2
AB005043	8651	Suppressor of cytokine signaling 1	2.1	1.23
BF589679	9255	Inducible cytokine subfamily E, member 1 (endothelial monocyte activating)	2.08	1.39
BF221850	2181	Acyl-CoA synthetase long-chain family member 3	2.07	1.45
NM_016185	51155	Hematologic and neurologic expressed 1	2.05	1.4
U39657	5608	Mitogen-activated protein kinase kinase 6	2	1.36
NM_004620	7189	TNF receptor-associated factor 6	1.94	1.28
AL577322	6383	Ssyndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	1.9	1.35
AF251120	27178	Interleukin 1 family, member 7 (ζ)	1.88	1.27
AI126453	170712	Cytochrome c oxidase subunit VIIb2	1.87	1.37
AV649018	4179	Membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	1.75	1.2
D38169	80271	Inositol 1,4,5-trisphosphate 3-kinase C	1.74	1.32
AI821602	3480	Insulin-like growth factor 1 receptor	1.73	1.27
W72082	22918	Complement component 1, subcomponent, receptor 1	1.71	1.24
M81695	3687	Integrin α X (antigen CD11C (p150) α polypeptide)	1.7	1.24
NM_005391	5165	Pyruvate dehydrogenase kinase, isoenzyme 3	1.67	1.29

For a complete list, please refer to the Web site referenced in the text.

FC, Fold change; LCB, lower confidence bound; TNF, tumor necrosis factor; Acyl-CoA, acyl-coenzyme A.

TABLE E2. Selected genes exhibiting significant downregulation at 6 hours postoperatively compared with preoperatively uniquely in patients with neurocognitive deficit

Accession	Gene ID	Gene	FG	LCB
NM_002589	5099	BH-protocadherin (brain-heart)	-4.18	-2.23
NM_000954	5730	Prostaglandin D ₂ synthase 21 kd (brain)	-3.41	-1.47
NM_004936	1030	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	-2.62	-1.54
NM_002122	3117	Major histocompatibility complex, class II, DQ α 1	-2.58	-1.2
NM_000878	3560	Interleukin 2 receptor, β	-2.54	-1.48
NM_006137	924	CD7 antigen (p41)	-2.54	-1.43
NM_005816	10225	CD96 antigen	-2.21	-1.41
U47924	920	CD4 antigen (p55)	-2.2	-1.62
NM_002185	3575	Interleukin 7 receptor	-2.2	-1.58
AI797836	921	CD5 antigen (p56-62)	-2.17	-1.56
NM_000839	2912	Glutamate receptor, metabotropic 2	-2.13	-1.25
NM_014349	80833	Apolipoprotein L, 3	-2.06	-1.42
NM_022819	64600	Phospholipase A ₂ , group IIF	-2.06	-1.31
AA035414	9881	Lupus brain antigen 1	-2.05	-1.46
X76775	3108	Major histocompatibility complex, class II, DM α	-2	-1.39
M28590	972	CD74 antigen (invariant polypeptide of MHC-II antigen associated)	-1.98	-1.25
AF005487	3128	Major histocompatibility complex, class II, DR β 6	-1.93	-1.39
NM_016579	51293	CD320 antigen	-1.93	-1.29
AB002328	23523	Calcineurin-binding protein 1	-1.92	-1.45
BC015186	5156	Platelet-derived growth factor receptor, α polypeptide	-1.91	-1.53
NM_003790	8718	Tumor necrosis factor receptor superfamily, member 25	-1.91	-1.29
AI084226	9214	Fas apoptotic inhibitory molecule 3	-1.91	-1.2●
NM_172139	282616	Interleukin 28A (interferon, λ 2)	-1.91	-1.23
M27487	3113	Major histocompatibility complex, class II, DP α 1	-1.84	-1.3
NM_000206	3561	Interleukin 2 receptor, γ	-1.83	-1.42
AF269133	50615	Interleukin 21 receptor	-1.8	-1.32
AI829170	23015	Golgi autoantigen, golgin subfamily a, BA	-1.76	-1.24
BC001830	7041	Transforming growth factor β 1 induced transcript 1	-1.72	-1.28
AJ297586	3123	Major histocompatibility complex, class II, DR β 1	-1.72	-1.21
AA126728	3384	Intercellular adhesion molecule 2	-1.71	-1.36
NM_005601	4818	Natural killer cell group 7 sequence	-1.66	-1.21
NM_004361	1005	Cadherin 7, type 2	-1.65	-1.26
NM_002346	4061	Lymphocyte antigen 6 complex, locus E	-1.63	-1.28
AI358867	348	Apolipoprotein E	-1.51	-1.24
U36759	171558	Pre-T-cell antigen receptor α	-1.51	-1.23
NM_001571	3661	Interferon regulatory factor 3	-1.51	-1.2
BG236280	942	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	-1.43	-1.22
NM_031440	83597	Transmembrane protein 7	-1.39	-1.22

For a complete list, please refer to the Web site referenced in the text.

FC, Fold change; LCB, lower confidence bound; MHC-II, major histocompatibility complex II.

TABLE E3. Selected genes exhibiting significant upregulation at 6 hours postoperatively compared with preoperatively uniquely in patients without neurocognitive deficit

Accession	Gene ID	Gene	FC	LCB
NM_022168	64135	Interferon induced with helicase C domain 1	5.89	2.77
AF204231	23015	Golgi autoantigen, golgin subfamily a, 8A	3.62	2.44
W95035	948	CD36 antigen (thrombospondin receptor)	3.5	2.34
AW300598	160760	T-cell activation protein phosphatase 2C	2.98	1.81
NM_013374	10015	Programmed cell death 6-interacting protein	2.79	1.95
H05812	3480	Insulin-like growth factor 1 receptor	2.79	1.84
AI275597	2909	Glucocorticoid receptor DNA binding factor 1	2.36	1.59
AW207699	29767	Tropomodulin 2 (neuronal)	2.3	1.4
AI185160	27250	Programmed cell death 4	2.28	1.64
AI657064	387837	Macrophage antigen h	2.26	1.44
AA742237	7916	HLA-B-associated transcript 2	2.25	1.64
M16276	3119	Major histocompatibility complex, class II, DQ β 1	2.24	1.23
AW117229	22926	Activating transcription factor 6	2.23	1.58
BE217880	3575	Interleukin 7 receptor	2.21	1.39
AW411030	374868	ATPase, class II, type 9B	2.09	1.63
NM_004763	9270	Integrin β 1 binding protein 1	2.04	1.21
AU153405	835	Caspase 2, apoptosis-related cysteine peptidase	2.02	1.51
BG230614	961	CD47 antigen (Rh-related antigen, integrin)	2	1.35
NM_001558	3587	Interleukin 10 receptor, α	1.97	1.47
NM_002198	3659	Interferon regulatory factor 1	1.97	1.41
NM_000591	929	CD14 antigen	1.95	1.51
AU151801	708	Complement component 1, q binding protein	1.94	1.38
AA700015	965	CD58 antigen (lymphocyte function antigen 3)	1.93	1.37
NM_002610	5163	Pyruvate dehydrogenase kinase, isozyme 1	1.91	1.27
NM_021949	492	ATPase, Ca ⁺⁺ transporting, plasma membrane 3	1.9	1.21
U08839	5329	Plasminogen activator, urokinase receptor	1.87	1.38
M31933	2213	Fc fragment of IgG, low-affinity IIb, receptor (CD32)	1.87	1.37
AW242432	359948	Interferon regulatory factor 2 binding protein 2	1.87	1.25
BC005830	8416	Annexin A9	1.87	1.24
AI347128	3476	Immunoglobulin (CD79A) binding protein 1	1.72	1.3
AI870617	3732	CD82 antigen	1.68	1.25
NM_003798	8727	Catenin (cadherin-associated protein) α -like 1	1.68	1.25
AL552534	960	CD44 antigen (homing function)	1.65	1.23
AF455755	10018	BCL2-like 11 (apoptosis facilitator)	1.57	1.2
U36501	6672	Nuclear antigen Sp100	1.56	1.2
AF015452	8837	CASP8 and FADD-like apoptosis regulator	1.55	1.23
NM_005526	3297	Heat shock transcription factor 1	1.55	1.22
BC000251	2932	Glycogen synthase kinase 3 β	1.52	1.29
NM_001861	1327	Cytochrome c oxidase subunit IV isoform 1	1.48	1.2

For a complete list, please refer to the Web site referenced in the text.

FC, Fold change; LCB, lower confidence bound; ATPase, adenosine triphosphatase; BCL2, B-cell CLL/Lymphoma 2; CASP8, casapase-8; FADD, FAS-associated death domain.

TABLE E4. Selected genes exhibiting significant downregulation at 6 hours postoperatively compared with those preoperatively uniquely in patients without neurocognitive deficit

Accession	Gene ID	Gene	FC	LCB
BC038712	57194	ATPase, class V, type 10A	−15.23	−1.88
NM_145275	147700	Kinesin light chain 3	−5.4	−1.75
NM_003837	8789	Fructose-1,6-bisphosphatase 2	−4.73	−1.68
NM_002862	5834	Phosphorylase, glycogen; brain	−3.58	−1.71
BE221674	152404	Immunoglobulin superfamily, member 11	−2.72	−1.34
AI394679	43849	Kallikrein 12	−2.51	−1.34
M25915	1191	Clusterin (complement lysis inhibitor)	−2.46	−1.59
NM_016140	51673	Brain-specific protein	−2.37	−1.53
AW057540	1636	Angiotensin 1-converting enzyme (peptidyl-dipeptidase A) 1	−2.36	−1.44
AK098058	6300	Mitogen-activated protein kinase 12	−2.3	−1.54
N90777	8828	Neuropilin 2	−2.24	−1.23
AF416922	26027	Acyl-CoA thioesterase 11	−2.21	−1.43
AL035661	84532	Acyl-CoA synthetase short-chain family member 1	−2.17	−1.31
AI457620	1002	Cadherin 4, type 1, R-cadherin (retinal)	−2.13	−1.32
NM_153042	221656	Amino oxidase (flavin-containing) domain 1	−2.07	−1.5
U46752	54211	Oxidative stress-induced like	−2.04	−1.37
CA306222	23028	Amine oxidase (flavin-containing) domain 2	−2.03	−1.46
AU148326	54756	Interleukin 17 receptor D	−2.02	−1.47
BC030972	9970	Nuclear receptor subfamily 1, group 1, member 3	−2.02	−1.23
AB045831	63970	p53-regulated apoptosis-inducing protein 1	−1.92	−1.45
AF009664	154754	Protease, serine, 1 (trypsin 1)	−1.92	−1.31
BF196320	84818	Interleukin 17 receptor C	−1.79	−1.27
NM_018725	55540	Interleukin 17 receptor B	−1.75	−1.37
NM_000586	3558	Interleukin 2	−1.73	−1.2
AF497548	131450	CD200 receptor 1	−1.54	−1.26
AI885128	220296	Hepatocyte cell adhesion molecule	−1.51	−1.2
AW296309	926	CD8 antigen, β polypeptide 1 (p37)	−1.5	−1.23
NM_000277	5053	Phenylalanine hydroxylase	−1.48	−1.22

For a complete list, please refer to the Web site referenced in the text.

FC, Fold change; LCB, lower confidence bound; ATPase, adenosine triphosphatase; Acyl-CoA, acyl-coenzyme A.