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Preoperative Gene Expression May be Associated with Neurocognitive Decline after Cardiopulmonary Bypass

Ashraf A. Sabe[1], **Rahul S. Dalal**[1], **Louis M. Chu**[1], **Nassrene Y. Elmadhun**[1], **Basel Ramlawi**[2], **Cesario Bianchi**[1], and **Frank W. Sellke**[1]

[1]Division Of Cardiothoracic Surgery, Cardiovascular Research Center, Warren Alpert Medical School of Brown University, Providence, RI

[2]Methodist DeBakey Heart & Vascular Center, The Methodist Hospital, Houston, Texas, USA

Abstract

OBJECTIVE—Despite advances in surgical techniques, neurocognitive decline (NCD) after cardiopulmonary bypass (CPB) remains a common and serious complication. We have previously demonstrated that patients with NCD have unique genetic responses 6 hours after CPB when compared with normal patients (NORM). We used genomic microarray to objectively investigate whether patients with NCD had associated preoperative gene expression profiles, and how these profiles changed up to four days after surgery.

METHODS—Cardiac surgery patients underwent neurocognitive assessments preoperatively and four days after surgery. Skeletal muscle was collected intra-operatively. Whole blood collected pre-CPB, 6 hours post-CPB, and on post-operative day four was hybridized to Affymetrix Gene Chip U133 Plus 2.0 microarrays. Gene expression in patients with NCD was compared with gene expression in the NORM group using JMP Genomics. Only genes that were commonly expressed in the two groups with a false discovery rate of 0.05 and a fold change of >1.5 were carried forward to pathway analysis using Ingenuity Pathway Analysis. Microarray gene expression was validated by Green real–time polymerase chain reaction and western blotting.

RESULTS—17 out of 42 patients developed NCD. 54,675 common transcripts were identified on microarray in each group across all time points. Preoperatively there were 140 genes that were significantly altered between the NORM and NCD groups ($p < 0.05$). Pathway analysis demonstrated that preoperatively patients with NCD had increased regulation in genes associated with inflammation, cell death, and neurologic dysfunction. Interestingly, the number of significantly regulated genes between the two groups changed over each time point, and decreased from 140 preoperatively, to 64, six hours after CPB, and 25, four days after surgery. There was no correlation in gene expression between the blood and skeletal muscle.

Corresponding Author: Frank W. Sellke, MD, Division of Cardiothoracic Surgery, Cardiovascular Research Center, Warren Alpert Medical School of Brown University, 2 Dudley Street, MOC 360, Providence, RI 02905, fsellke@lifespan.org, Ph: (401) 444-2732, Fax: (401) 444-2380.

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CONCLUSIONS—Patients who developed NCD post-CPB had increased differential gene expression before surgery versus patient who did not develop NCD. While significant differences in gene expression also existed post-operatively, these differences gradually decreased over time. Preoperative gene expression may be associated with neurologic injury after CPB. Further investigation into these genetic pathways may help predict patient outcome and guide patient selection.

Keywords

Neurocognitive Decline; Microarray; Inflammation; Gene Expression; Cardiopulmonary Bypass

Introduction

Neurocognitive dysfunction (NCD) is a common but poorly understood complication of cardiopulmonary bypass (CPB). Depending on the definition, as many as 80% of patients undergoing CPB may manifest neurologic complications postoperatively [1]. Neurologic deficits are commonly divided into two categories: Type 1 deficits include focal neurologic events such as stroke, stupor, and coma, while type 2 deficits are more global cognitive deficits such as memory loss, confusion, and deterioration in intellectual function [2]. While type 1 deficits can usually be attributed to a specific cause, such as cerebral hypoperfusion or thromboembolic events, the etiology of type 2 events is more vague. However, their incidence is similar to that of type 1 events [3], and they can be equally as devastating. A lack of understanding of the precipitating pathophysiology and inability to predict this type of injury only adds to the strain on both patients and their family members.

A variety of pathologic processes including cerebral hypoperfusion, microembolization, inflammation, temperature changes, genetic predisposition, cerebral edema, or dysfunction of the blood-brain barrier have been implicated in NCD [4, 5]. Cardiopulmonary bypass, while an essential component of the cardiac surgeon's armamentarium, has significant deleterious effects on the human body related to the interaction of blood components with the artificial surfaces of the circuit, including activation of leukocytes, cytokine release, and increase in reactive oxygen species. Our group, as well as others, has previously demonstrated the association between systemic inflammation and NCD after CPB [6, 7]. However, a comprehensive understanding of the precipitating and predisposing causes of NCD remains elusive, making accurate diagnosis and treatment difficult.

High-throughput microarray analysis provides insight into the response of nearly the entire human genome to a particular disease, and thus is an intriguing technique for identifying regulatory pathways and genes involved in poorly understood disease processes. Microarray technology has progressed exponentially in the past decade with the completion of the human genome project, development of more comprehensive microchips, and introduction of powerful pathway analysis software. We previously used microarray methods to show that genes associated with inflammation, antigen presentation, and cellular adhesion were differentially regulated in patients exhibiting NCD after CPB. In this prior study same-group comparisons were made both in patients with NCD pre- and postoperatively as well in NORM patients pre- and postoperatively [8]. We now compare NORM patients to those with

NCD pre and postoperatively to assess whether there are inherent differences pre-operatively leading to differential gene regulation 6 hours and 4 days post-CPB. The present study uses up-to-date microarray analytic techniques to identify specific cellular functions that may be involved in the development of NCD immediately and four days post-CPB.

Materials and Methods

Patient Enrollment

We enrolled forty-three patients scheduled electively or urgently for coronary artery bypass grafting, valvular surgery (aortic or mitral), or a combination of the two requiring CPB in this single-institution (Beth Israel Deaconess Medical Center, Boston, MA) prospective cohort study. All forms and procedures were approved by the Beth Israel Deaconess Medical Center Institutional Review Board/Committee on Clinical Investigations. Preoperative informed consent was obtained from all study participants for surgical procedures performed and additional blood and tissue collection for the purpose of this investigation. Exclusion criteria included: patients undergoing aortic arch/root procedures, patients with known calcified aortas or high-grade carotid stenosis, and patients with recent stroke, severe neurologic deficits, hepatic cirrhosis, or chronic renal failure (serum creatinine > 2.0 mg/dL). Patients who were unable to complete baseline psychological testing due to severe cognitive impairment, psychiatric disease, substance abuse, blindness, or poor English were also excluded. One enrolled patient was excluded due to inability to complete the neuropsychological assessment prior to discharge. Ultimately, forty-two patients were included in the analysis.

Surgical Technique

All operations followed the conventional approach at our institution with regards to induction of general anesthesia, invasive monitoring, midline sternotomy, and systemic heparinization. CPB was initiated via right atrial and ascending aorta cannulae with a nonpulsatile system, membrane oxygenator, and a 40-μm arterial filter. Crystalloid pump prime was used. In all cases, mild hypothermic CPB (32–34 °C) with intermittent cold blood hyperkalemic (25 mmol/L) cardioplegia was used. Serum glucose levels were monitored, and intermittent intravenous insulin injection or insulin infusion was used to target a level of less than 130 mg/dL. While on CPB, pump flow was maintained at 2 to 2.4 L/min/m2 body surface area. Arterial partial oxygen pressure was maintained between 150–250 mmHg. Mean blood pressure was maintained between 50 and 90 mmHg by using conventional vasoactive medications.

Neurocognitive Assessment

Patients underwent evaluation with a battery of neurocognitive tests preoperatively (1–10 days before surgical intervention), on postoperative day 4, and at 3 months postoperatively. All patients also underwent depression assessment with the Geriatric Depression Scale. All evaluations were carried out by trained, blinded psychometricians. 8 validated tools were used to assess memory, executive function, attention, language, and global cognition:

The Hopkins Verbal Learning Test 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 to measure verbal learning, retention, and recall. The Boston Naming Test was used to measure confrontational naming[9]. Attention shifting ability was measured by recording time to complete Trailmaking A and B. Digit Span was used to measure working memory and sustained attention span. Fluency was assessed by requiring patients to generate words in a category (semantic fluency) or beginning with a specific letter (phonemic fluency). The Wechsler Test of Adult Reading was used as a test of premorbid intelligence. The Stroop Color-Word Inference Test was used to assess executive function, and the Visual Search and Acuity Test assessed visuospatial abilities and executive function.

Patients with NCD were defined as those who demonstrated a 1-standard deviation decline from baseline on 25% of the tasks (2/8 measures), in accordance with the "Statement of consensus on assessment of neurobehavioral outcomes after cardiac surgery"[10].

Sample Collection and Microarray Processing

For all 42 patients, blood samples were collected from a central venous line preoperatively after induction of anesthesia and before skin incision (Pre-CPB), early or 6 hours (6H) post-CPB in the intensive care unit, and late or 4 days (4D) post-CPB. Blood was drawn directly into PAXgene tubes (QIAGEN Inc, Valencia, CA) for mRNA stabilization and extraction, per the manufacturer's recommendation. Skeletal muscle samples were collected from twenty patients from the left intercostal muscle bed after cannulation but before the initiation of CPB, and again after removal of the aortic cross clamp and weaning from CPB. Skeletal muscle samples were snap frozen in liquid nitrogen immediately after collection and stored at −80 °C.

RNA extraction and purification, cDNA synthesis, and production of biotin-labeled cRNA were completed by the Beth Isreal Deaconess Medical Center Proteomics Core according to previously described protocols[11, 12]. cRNA from all samples were hybridized with Affymetrix GeneChip HG-U133 Plus 2.0 (Affymetrix Inc, Santa Clara, CA), which probes for over 38,500 genes. Chips were scanned with an HP G2500A ChipScanner (Affymetrix), and low-level quality control analysis and signal value measurement was performed using dChip software (Wong et al, Boston, MA) [13]. No outliers were identified by dChip, so all samples were carried on for subsequent analysis.

Gene Expression and Pathway Analysis

Gene expression analysis was performed on raw microchip data using JMP Genomics 4.0 (SAS, Cary, NC) for quality control, normalization, and statistical analysis. Composite chip data were normalized and compared using the Robust Multichip Average method, which revealed one blood and one skeletal muscle sample to be outliers. These were excluded from subsequent analysis. Gene expression in Pre-CPB and Post-CPB skeletal muscle samples and Pre-CPB, 6H Post-CPB, and 4D Post-CPB blood samples in patients with NCD were compared to the corresponding samples in patients without NCD using one-way ANOVA. A post-hoc false detection rate algorithm with alpha of 0.05 was applied to control for false

positives. Genes that were considered significantly regulated met two criteria: 1) mean fold change >1.5 or <−1.5 in NCD patients compared to NORM, and 2) −log (p-value) exceeding threshold calculated by the software for each comparison. All significant genes were uploaded into Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Redwood City, CA) which was used to generate the top canonical pathways involving the differentially regulated genes.

Real-time PCR

Gene expression analysis of whole blood-derived mRNA with HGU 133 Plus 2.0 chips was previously validated by real-time PCR[8]. We used real-time PCR to validate gene expression analysis of skeletal muscle-derived mRNA as well. Total RNA was extracted from frozen sections of skeletal muscle using a Trizol-based method following the manufacturer's recommendations (Gibco BRL, Rockville, MD).

Results

Patient Characteristics

As previously reported, 17 out of the 42 patients included for analysis developed early NCD at post-operative day 4. After three months all but one patient returned to their normal cognitive function[8]. As demonstrated in our prior manuscript, patients had similar baseline preoperative characteristics including age, race, sex and co-morbidities. Similarly, patients intraoperative course was well matched including type of procedure, time on CPB, crossclamp time, use of cell saver and cardiotomy suction. Moreover, there were no differences in observed postoperative complications between the two groups, and there were no documented focal neurologic deficits or cerebrovascular events in any of the enrolled patients during this study period[8].

Gene Expression and Confirmation

We have previously published a comprehensive database of gene expression in patients with and without NCD after CPB, including unsupervised hierarchical clustering of samples, and confirmation of microarray gene-expression with real-time PCR[8]. 54,675 transcripts were identified using our described microarray GeneChip.

Preoperative Gene Expression and Pathway Analysis in Patients with NCD compared with NORM Patients

Preoperatively there were 140 genes that were significantly altered between the NORM and NCD groups, of which 108 were named. Notably all 108 of these genes were upregulated in patients with NCD compared with NORM patients (Figure 1; Table 4). Pathway analysis was used to group genes by potential pathophysiologic function. This analysis demonstrated that preoperatively patients with NCD had a significant increase in several genes involved in inflammation, cell death and neurologic dysfunction. Selected genes have been listed in Table 1. Gene expression in the blood was not correlated with gene expression in the skeletal muscle obtained at the time of surgery.

Postoperative Gene Expression and Pathway Analysis in Patients with NCD compared with NORM Patients

Early postoperatively (6H) the number of significantly regulated genes decreased to 64 compared with preoperative gene regulation, of which 51 were named. 21 of these 51 genes were significantly upregulated, while 30 were downregulated in patients with NCD compared with NORM patients (Figure 1; Table 5). Though the selected genes regulated were different than those regulated preoperatively, pathway analysis demonstrated regulation in several genes associated with inflammation, cell death, and neurologic dysfunction in patients with NCD compared with NORM patients (Table 2). Late postoperatively (4D) the number of significantly regulated genes decreased to 25, of which 19 were named (Figure 1; Table 6). Three of these 19 genes were upregulated and the remaining 16 genes were downregulated in patients with NCD compared with the NORM patients (Table 6). Selected genes involved with inflammation, cell death and neurologic dysfunction are listed in Table 3, of which all were all actually downregulated in patients with NCD compared with NORM patients.

Discussion

The current study demonstrates that patients who developed NCD post-CPB have differential gene expression before surgery versus patients who did not develop NCD. While significant differences in gene expression exist post-CPB they decreased over time. These findings suggest that patients may be inherently predisposed to NCD after CPB independent of surgical or anesthetic technique. This notion is certainly supported by the failure to reduce the incidence of Type 2 NCD, despite improvements in operative techniques [14]. In order to improve these outcomes novel diagnostic and therapeutic techniques will need to be employed with a focus on identifying individual genetic variants associated with disease susceptibility and therapeutic response. The use of up-to-date microarray and bioinformatics analysis is an important step in beginning to address these challenges.

Pre-CPB, 108 named genes were significantly regulated in patients with neurocognitive dysfunction. Several genes involved with inflammation, cell death and neurologic dysfunction were increased in patients who would later develop NCD. Systemic inflammation has been shown to contribute to neurocognitive decline after CPB [7, 15, 16]. In a previous study we demonstrated that while an increase in preoperative inflammatory chemokines did not affect outcome, postoperative elevations in chemokines were associated with the development of delirium after CPB [17]. Chemokines act as potent immune mediators and may attract inflammatory cells, resulting in a disruption of the blood-brain barrier and cognitive dysfunction. In our current study we demonstrate an elevation in several genes associated with T-cell activation and signaling preoperatively in patients that would later develop NCD. For instance, patients who developed NCD postoperatively had significantly elevated regulation in genes implicated in T-cell activation, maturation, and cytokine signaling including ADA, CD3E, CD3G, IL2RG, IL32, NFATC2, and STAT4[18–20]. Perhaps these inherent elevations result in accentuated inflammatory response and resultant increase in chemokine production. These patients also had a significant increase in genes associated with cell death and oxidative stress, like E2F2, EIF2AK1, and FOX03[21–23]. Furthermore,

patients who developed NCD also had an increase in genes more directly associated with neurologic dysfunction like SNCA, FTO, TUBB2A, YY1 and SNAP29[24–28]. Though these genes are not directly related to one-another in a specific pathway, bioinformatics analysis demonstrates that they do share important roles in neurologic function and cognition. SNCA is abundantly expressed in the brain and a major component of amyloid plaques in Alzheimer's disease[24]. FTO, which has been shown to be inversely associated with brain volume, is also associated with Alzheimer's disease as well as reduced verbal fluency in obese patients [25, 26]. While TUBB2A is involved in microtubule and axonal guidance, SNAP29 has been shown to mediate synaptic membrane docking and may slow neurotransmitter release [28]. YY1 has many roles in neuronal development and dysfunction and often plays a larger role in activating or repressing gene expression[27].

Interestingly, there was a relative decrease in the number of genes regulated postoperatively when comparing patients with NCD and those without. Again, these findings suggest that patients may be inherently predisposed to developing NCD after CPB. Further investigations may reveal predictive patterns in gene expression and ultimately result in improved preoperative planning and care of patients undergoing cardiac surgery.

Limitation and Future Directions

There are limitations to this study. Though our baseline patient characteristics and operative techniques were similar in this single institution study, the number of patients in the study was limited. A larger sample of patients would help provide greater insight into the unique gene expression profiles associated with NCD and would allow for a more extensive mapping of gene pathways, as opposed to just placing genes in functional groups, as we have done. Another limitation is that we did not directly sample brain tissue for our mRNA extraction. We could not biopsy brain tissue in patients, and even if this were done it would not be feasible as a regular diagnostic or screening tool in a clinical setting. Of note, many of the regulated genes, which have been discussed in this, are associated with on inflammatory processes in the blood, which could secondarily affect the brain. We did sample skeletal muscle, which like brain tissue would not be exposed to CP, but CPB alone, however there were actually no correlations in gene regulation between the blood and muscle samples.

It is also important to note that this study would need to be repeated before any claim can be made as to whether the aforementioned genes were indeed predictive of NCD in patient populations. Although the results of this current study highly suggest that preoperative gene expression is associated with postoperative NCD, we must also be cautious with the interpretation of microarray. In order to actually demonstrate predictive gene expression patterns another study would need to be designed with a new group of patients, where genes would be checked in a prospective manner preoperatively to determine whether any of the genes identified in the current study were actually a predictor of later NCD in new patient cohorts.

Another common pitfall with the interpretation of microarray is errors with the statistical treatment of the data. Since microarray identifies tens-of-thousands of individual genes, random chance alone can often result in significant p-values when simple statistical analysis is performed. To account for this potential error in false discovery, using specialized

statistical software we performed ANOVA testing with multiple comparison correction and limited our false discovery rate (FDR) to less than 0.05. This is widely accepted as a stringent method to help prevent an error in multiple comparisons, and though it is not a universal application in the interpretation of microarray, it does improve the likely reproducibility of the results.

Conclusions

This work represents a follow-up study of microarray database compiled in 2007. While our prior study identified differences in gene expression after CPB in patients with NCD and in patients without, the current study is the first to directly investigate differences in genetic regulation of patients with NCD compared with NORM pre- and post-CPB. Currently, these studies should serve primarily as a database to guide further genetic studies in different patient cohorts. The overarching goal of this project is to guide novel diagnostic techniques to help identify inherent genetic variations associated with susceptibility of disease, and ultimately to improve preoperative patient selection and individualized therapeutic techniques.

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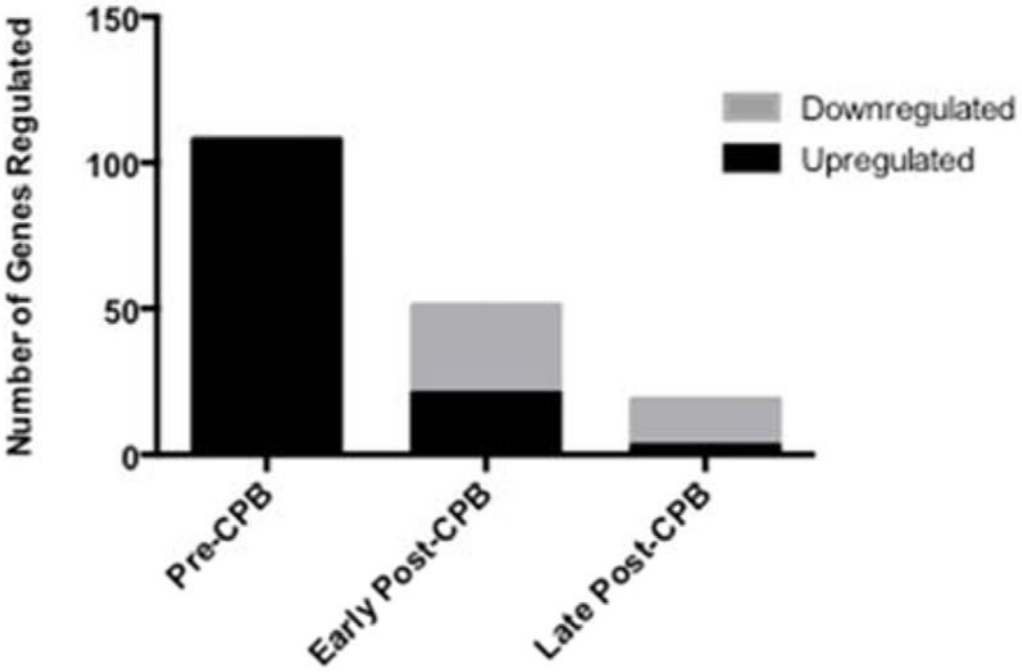

Figure 1. Number of Genes Significantly Regulated in Patients with NCD versus NORM

These represent named genes on pathway analysis. Early Post-CPB: represents gene expression 6 hours after cardiopulmonary bypass (CPB); Late Post CPB: represents gene expression 4 days after CPB. NCD: Neurocognitive Decline Post-CPB; NORM: Patient without Neurocognitive Decline Post-CPB.

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Table 1

Preoperative Gene Expression Exhibiting Significant Regulation in Patients with NCD compared with NORM - Selected Genes Grouped by Potential Pathophysiologic Function

Accession ID	Gene Name	FC	LCI	UCI	p value
Inflammation					
ADA	adenosine deaminase	1.54	1.14	2.08	0.0059
CD3E	CD3e molecule, epsilon (CD3-TCR complex)	1.85	1.30	2.63	0.0009
CD3G	CD3g molecule, gamma (CD3-TCR complex)	1.77	1.28	2.44	0.0008
IL2RG	interleukin 2 receptor, gamma	1.96	1.23	3.14	0.0058
IL32	interleukin 32	2.06	1.29	3.30	0.0032
NFATC2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	1.83	1.20	2.79	0.0058
STAT4	signal transducer and activator of transcription 4	1.71	1.17	2.51	0.0062
Cell death					
CTSB	cathepsin B	2.44	1.43	4.16	0.0015
E2F2	E2F transcription factor 2	1.66	1.23	2.25	0.0014
EIF2AK1	eukaryotic translation initiation factor 2-alpha kinase 1	2.25	1.25	4.03	0.0075
FOXO3	forkhead box O3	1.99	1.26	3.15	0.0038
Neurologic dysfunction					
SNCA	synuclein, alpha (non A4 component of amyloid precursor)	2.01	1.22	3.30	0.0068
FTO	fat mass and obesity associated	1.63	1.15	2.32	0.0069
TUBB2A	tubulin, beta 2A class IIa	4.58	1.64	12.80	0.0041
YY1	YY1 transcription factor	1.97	1.31	2.97	0.0015
SNAP29	synaptosomal-associated protein, 29kDa	1.59	1.22	2.08	0.0009

Gene expression listed as fold change (FC) in neurocognitive decline (NCD) patients compared with normal patients (NORM).

All values represented are significant ($p < 0.05$). LCI: Lower confidence interval; UCI: Upper confidence interval

Table 2

Early Post-CPB (6 hours post-CPB) Gene Expression in Patients with NCD compared with NORM – Selected Genes Grouped by Potential Pathophysiologic Function

Accession ID	Gene Name	FC	LCI	UCI	p value
Inflammation					
CLEC1B	C-type lectin domain family 1, member B	1.56			0.0013
TLR4	toll-like receptor 4	1.78	1.18	2.67	0.0070
TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	0.61	0.43	0.87	0.0075
MALT1	mucosa associated lymphoid tissue lymphoma translocation gene 1	0.63	0.51	0.78	0.0001
Cell death					
EPAS1	endothelial PAS domain protein 1	1.73	1.31	2.27	0.0002
ZBTB16	zinc finger and BTB domain containing 16	1.94	1.32	2.86	0.0010
Neurologic dysfunction					
CDK5RAP2	CDK5 regulatory subunit associated protein 2	1.51	1.12	2.04	0.0074

Gene expression listed as fold change (FC) in neurocognitive decline (NCD) patients compared with normal patients (NORM).

All values represented are significant ($p < 0.05$). LCI: Lower confidence interval; UCI: Upper confidence interval

Table 3

Late Post-CPB (4 days post-CPB) Gene Expression in Patients NCD compared with NORM – Selected Genes Grouped by Potential Pathophysiologic Function

Accession ID	Gene Name	FC	LCI	UCI	p value
Inflammation					
GIMAP4	GTPase, IMAP family member 4	0.45	0.27	0.79	0.0059
PTMA	prothymosin, alpha	0.56	0.38	0.83	0.0045
GPR183	G protein-coupled receptor 183	0.56	0.39	0.80	0.0021
Cell death					
DIABLO	diablo, IAP-binding mitochondrial protein	0.61	0.43	0.87	0.0045
HDAC9	Histone deacetylase 9	0.67			0.0075
Neurologic dysfunction					
MEF2C	myocyte enhancer factor 2C	0.47	0.28	0.77	0.0039

Gene expression listed as fold change (FC) in neurocognitive decline (NCD) patients compared with normal patients (NORM).

All values represented are significant ($p < 0.05$). LCI: Lower confidence interval; UCI: Upper confidence interval

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Table 4

Preoperative Gene Expression in Patients with NCD compared with NORM – Complete List

Accession ID	Gene Name	FC	LCI	UCI	p value
Upregulated					
ADA	adenosine deaminase	1.54	1.14	2.08	0.0059
ANAPC2	anaphase promoting complex subunit 2	1.53	1.12	2.08	0.0081
APOBEC3C	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C	1.73	1.24	2.40	0.0015
ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	1.63	1.19	2.22	0.0027
ARHGEF2	Rho/Rac guanine nucleotide exchange factor (GEF) 2	1.73	1.21	2.46	0.0031
ARID1A	AT rich interactive domain 1A (SWI-like)	1.59	1.24	2.04	0.0004
ARID2	AT rich interactive domain 2 (ARID, RFX-like)	1.55	1.14	2.11	0.0060
ARL4C	ADP-ribosylation factor-like 4C	1.51	1.15	1.99	0.0040
ASB8	ankyrin repeat and SOCS box containing 8	1.73	1.23	2.43	0.0022
ASXL2	additional sex combs like 2 (Drosophila)	1.55	1.15	2.08	0.0049
ATP2B4	ATPase, Ca++ transporting, plasma membrane 4	1.72	1.21	2.44	0.0032
BPTF	bromodomain PHD finger transcription factor	1.68	1.16	2.43	0.0073
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase	1.65	1.19	2.29	0.0034
CD3E	CD3e molecule, epsilon (CD3-TCR complex)	1.85	1.30	2.63	0.0009
CD3G	CD3g molecule, gamma (CD3-TCR complex)	1.77	1.28	2.44	0.0008
CIZ1	CDKN1A interacting zinc finger protein 1	1.57	1.20	2.05	0.0014
CNPPD1	cyclin Pas1/PHO80 domain containing 1	2.02			0.0033
CTSB	cathepsin B	2.44	1.43	4.16	0.0015
DCAF12	DDB1 and CUL4 associated factor 12	2.48	1.46	4.23	0.0011
E2F2	E2F transcription factor 2	1.66	1.23	2.25	0.0014
EIF2AK1	eukaryotic translation initiation factor 2-alpha kinase 1	2.25	1.25	4.03	0.0075
ELOF1	elongation factor 1 homolog (S. cerevisiae)	1.57	1.13	2.18	0.0080
EML3	echinoderm microtubule associated protein like 3	1.51	1.21	1.87	0.0004
EPB41	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	2.07	1.30	3.30	0.0026
FAM104A	family with sequence similarity 104, member A	2.20	1.43	3.38	0.0006
FAM117A	family with sequence similarity 117, member A	1.86	1.20	2.89	0.0068

Accession ID	Gene Name	FC	LCI	UCI	p value
FAM134A	family with sequence similarity 134, member A	1.62	1.14	2.30	0.0083
FAM46C	family with sequence similarity 46, member C	2.62	1.31	5.26	0.0074
FAXDC2	fatty acid hydroxylase domain containing 2	1.67			0.0044
FBXO9	F-box protein 9	3.05	1.77	5.26	0.0001
FECH	ferrochelatase	2.81	1.42	5.56	0.0036
FKBP1B	FK506 binding protein 1B, 12.6 kDa	2.65	1.75	4.03	0.0000
FOXO3	forkhead box O3	1.99	1.26	3.15	0.0038
FTO	fat mass and obesity associated	1.63	1.15	2.32	0.0069
FUNDC2	FUN14 domain containing 2	1.86	1.20	2.87	0.0059
GDE1 (includes EG:393213)	glycerophosphodiester phosphodiesterase 1	2.06	1.29	3.28	0.0030
GSPT1	G1 to S phase transition 1	3.16	1.51	6.61	0.0041
HBZ	hemoglobin, zeta	1.72	1.16	2.56	0.0082
HECA	headcase homolog (Drosophila)	1.50	1.12	2.02	0.0079
HECTD3	HECT domain containing E3 ubiquitin protein ligase 3	1.89	1.46	2.45	0.0000
HEMGN	hemogen	2.04	1.23	3.38	0.0066
IBA57	IBA57, iron-sulfur cluster assembly homolog (S. cerevisiae)	1.69			0.0007
IL2RG	interleukin 2 receptor, gamma	1.96	1.23	3.14	0.0058
IL32	interleukin 32	2.06	1.29	3.30	0.0032
ITM2A	integral membrane protein 2A	2.05	1.30	3.23	0.0027
JHDM1D	jumonji C domain containing histone demethylase 1 homolog D (S. cerevisiae)	1.74	1.17	2.59	0.0067
JUND	jun D proto-oncogene	1.57	1.18	2.10	0.0024
KIAA1143	KIAA1143	1.78	1.22	2.59	0.0032
KIAA1919	KIAA1919	1.90	1.36	2.64	0.0003
KPNA1	karyopherin alpha 1 (importin alpha 5)	1.54	1.16	2.06	0.0037
KPNA6	karyopherin alpha 6 (importin alpha 7)	1.51	1.15	1.99	0.0041
MARCH8	membrane-associated ring finger (C3HC4) 8, E3 ubiquitin protein ligase	2.52	1.43	4.43	0.0030
MINK1	misshapen-like kinase 1	1.56	1.20	2.02	0.0013
MKRN1	makorin ring finger protein 1	2.26	1.30	3.93	0.0046
MPHOSPH9	M-phase phosphoprotein 9	1.51	1.20	1.90	0.0006

Accession ID	Gene Name	FC	LCI	UCI	p value
NDUFV3	NADH dehydrogenase (ubiquinone) flavoprotein 3, 10kDa	1.53	1.12	2.07	0.0076
NFATC2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	1.83	1.20	2.79	0.0058
NTAN1	N-terminal asparagine amidase	1.94	1.27	2.97	0.0029
OLA1	Obg-like ATPase 1	1.83	1.26	2.64	0.0018
PAQR8	progestin and adipoQ receptor family member VIII	1.58	1.20	2.09	0.0015
PCGF5	polycomb group ring finger 5	1.77	1.17	2.67	0.0081
PCSK5	proprotein convertase subtilisin/kexin type 5	1.52	1.13	2.05	0.0066
PIP4K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	2.23	1.28	3.88	0.0055
PITHD1	PITH (C-terminal proteasome-interacting domain of thioredoxin-like) domain containing 1	2.33			0.0011
PNISR	PNN-interacting serine/arginine-rich protein	1.85			0.0030
PRDX2	peroxiredoxin 2	2.41	1.34	4.31	0.0039
PSME4	proteasome (prosome, macropain) activator subunit 4	1.98	1.25	3.13	0.0043
PSMF1	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)	1.82	1.22	2.72	0.0043
PTPN4	protein tyrosine phosphatase, non-receptor type 4 (megakaryocyte)	1.57	1.15	2.14	0.0054
RAB2B	RAB2B, member RAS oncogene family	2.95	1.72	5.08	0.0002
RALGDS	ral guanine nucleotide dissociation stimulator	1.50	1.13	2.00	0.0064
RAPGEF6	Rap guanine nucleotide exchange factor (GEF) 6	1.89	1.30	2.75	0.0011
RGCC	regulator of cell cycle	2.11			0.0021
RNF10	ring finger protein 10	2.41	1.24	3.43	0.0059
RNF123	ring finger protein 123	2.03	1.25	3.31	0.0052
RUNDC3A	RUN domain containing 3A	1.95	1.34	2.82	0.0006
SCML4	sex comb on midleg-like 4 (Drosophila)	1.67	1.15	2.43	0.0082
SEC16A	SEC16 homolog A (S. cerevisiae)	1.86	1.20	2.89	0.0069
SECISBP2	SECIS binding protein 2	1.97	1.21	3.20	0.0070
SEPT6	septin 6	1.50	1.14	1.97	0.0040
SESN3	sestrin 3	2.25	1.32	3.84	0.0035
SF3A2	splicing factor 3a, subunit 2, 66kDa	2.03	1.46	2.84	0.0001
SLC25A37	solute carrier family 25 (mitochondrial iron transporter), member 37	2.50	1.37	4.55	0.0034
SLC38A5	solute carrier family 38, member 5	1.57	1.15	2.14	0.0055

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Accession ID	Gene Name	FC	LCI	UCI	p value
SLC41A1	solute carrier family 41, member 1	1.51	1.32	1.73	0.0000
SLC48A1	solute carrier family 48 (heme transporter), member 1	1.63	1.32	2.03	0.0000
SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	2.08	1.22	3.54	0.0080
SNAP29	synaptosomal-associated protein, 29kDa	1.59	1.22	2.08	0.0009
SNCA	synuclein, alpha (non A4 component of amyloid precursor)	2.01	1.22	3.30	0.0068
SPTAN1	spectrin, alpha, non-erythrocytic 1	2.05	1.30	3.24	0.0026
SSBP3	single stranded DNA binding protein 3	1.62	1.18	2.22	0.0036
ST6GALNAC4	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 4	1.74	1.24	2.45	0.0020
STAT4	signal transducer and activator of transcription 4	1.71	1.17	2.51	0.0062
SUN2	Sad1 and UNC84 domain containing 2	1.63			0.0024
TMEM245	transmembrane protein 245	1.93			0.0031
TMEM86B	transmembrane protein 86B	1.56	1.14	2.14	0.0068
TMOD1	tropomodulin 1	1.75	1.18	2.58	0.0059
TNS1	tensin 1	3.03	1.53	6.03	0.0033
TOLLIP	toll interacting protein	1.74	1.17	2.59	0.0070
TPGS2	tubulin polyglutamylase complex subunit 2	2.02			0.0068
TRIM58	tripartite motif containing 58	2.49	1.35	4.60	0.0041
TSPAN5	tetraspanin 5	2.74	1.57	4.76	0.0006
TUBB2A	tubulin, beta 2A class IIa	4.58	1.64	12.80	0.0041
WDR26	WD repeat domain 26	2.44	1.46	4.10	0.0010
WDR45	WD repeat domain 45	1.89	1.22	2.87	0.0048
WNK1	WNK lysine deficient protein kinase 1	1.92	1.22	3.02	0.0056
YY1	YY1 transcription factor	1.97	1.31	2.97	0.0015
ZMAT2	zinc finger, matrin-type 2	1.82	1.20	2.76	0.0058

Gene expression listed as fold change (FC) in neurocognitive decline (NCD) patients compared with normal patients (NORM).

All values represented are significant ($p < 0.05$). LCI: Lower confidence interval; UCI: Upper confidence interval

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Table 5

Early Post-CPB (6 hours post-CPB) Gene Expression in Patients with NCD compared with NORM - Complete List

Accession ID	Gen Name	FC	LCI	UCI	p value
Upregulated					
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase	1.61	1.17	2.23	0.0046
CCNJL	cyclin J-like	1.70	1.21	2.37	0.0025
CDK5RAP2	CDK5 regulatory subunit associated protein 2	1.51	1.12	2.04	0.0074
CEP19	centrosomal protein 19kDa	1.50			0.0071
CLEC1B	C-type lectin domain family 1, member B	1.56			0.0013
DACH1	dachshund homolog 1 (Drosophila)	1.74	1.33	2.28	0.0008
DHRS12	dehydrogenase/reductase (SDR family) member 12	1.67	1.25	2.23	0.0002
EPAS1	endothelial PAS domain protein 1	1.73	1.31	2.27	0.0039
FKBP1B	FK506 binding protein 1B, 12.6 kDa	2.04	1.35	3.09	0.0010
GPSM3	G-protein signaling modulator 3	2.02	1.37	2.99	0.0006
GRB10	growth factor receptor-bound protein 10	1.54	1.26	1.88	0.0001
KBTBD6	kelch repeat and BTB (POZ) domain containing 6	1.78	1.27	2.48	0.0010
MARCH8	membrane-associated ring finger (C3HC4) 8, E3 ubiquitin protein ligase	1.64	1.16	2.32	0.0058
METTL21D	methltransferase-like protein 21D	1.51			0.0032
OLAH	oleoyl-ACP hydrolase	1.88	1.41	2.50	0.0000
OSBP2	oxysterol binding protein 2	1.85	1.31	2.62	0.0008
PDZK1IP1	PDZK1 interacting protein 1	2.37	1.32	4.24	0.0045
TLR4	toll-like receptor 4	1.78	1.18	2.67	0.0070
TPK1	thiamin pyrophosphokinase 1	1.67	1.24	2.24	0.0011
TSPAN5	tetraspanin 5	2.17	1.25	3.77	0.0065
ZBTB16	zinc finger and BTB domain containing 16	1.94	1.32	2.86	0.0010
Downregulated					
ACADM	acyl-CoA dehydrogenase, C-4 to C-12 straight chain	0.58	0.40	0.85	0.0065
ALG13	ALG13, UDP-N-acetylglucosaminyltransferase subunit	0.51	0.36	0.73	0.0005
ANKRD10	ankyrin repeat domain 10	0.58	0.41	0.81	0.0021
ARGLU1	arginine and glutamate rich 1	0.39	0.20	0.76	0.0067

Accession ID	Gen Name	FC	LCI	UCI	p value
ATF1	activating transcription factor 1	0.63	0.45	0.88	0.0079
C2orf49	chromosome 2 open reading frame 49	0.66	0.51	0.86	0.0025
CLIP4	CAP-GLY domain containing linker protein family, member 4	0.65	0.48	0.88	0.0059
CPEB2	cytoplasmic polyadenylation element binding protein 2	0.54	0.37	0.79	0.0020
CRISP2	cysteine-rich secretory protein 2	0.63	0.50	0.79	0.0001
CSE1L	CSE1 chromosome segregation 1-like (yeast)	0.64	0.48	0.86	0.0035
GPR84	G protein-coupled receptor 84	0.53	0.34	0.83	0.0062
KANSL2	KAT8 regulatory NSL complex subunit 2	0.64			0.0032
MALT1	mucosa associated lymphoid tissue lymphoma translocation gene 1	0.63	0.51	0.78	0.0001
MBNL2	muscleblind-like splicing regulator 2	0.65	0.50	0.85	0.0017
MIR22HG	MIR22 host gene (non-protein coding)	0.62			0.0008
MON2	MON2 homolog (S. cerevisiae)	0.63	0.47	0.84	0.0024
MPHOSPH6	M-phase phosphoprotein 6	0.59	0.46	0.76	0.0001
NADSYN1	NAD synthetase 1	0.60	0.42	0.85	0.0051
PIK3C2A	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha	0.60	0.42	0.86	0.0057
RALGAPA1	Ral GTPase activating protein, alpha subunit 1 (catalytic)	0.65			0.0015
RNF144B	ring finger protein 144B	0.66	0.51	0.84	0.0012
RWDD4	RWD domain containing 4	0.65	1.20	2.65	0.0013
SH3GL3	SH3-domain GRB2-like 3	0.56	0.50	0.84	0.0045
SREK1	splicing regulatory glutamine/lysine-rich protein 1	0.62			0.0011
TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	0.61	0.43	0.87	0.0075
TFEC	transcription factor EC	0.62	0.45	0.84	0.0025
TMEM168	transmembrane protein 168	0.66	0.52	0.84	0.0011
ZCCHC2	zinc finger, CCHC domain containing 2	0.57	0.39	0.82	0.0033
ZCCHC8	zinc finger, CCHC domain containing 8	0.65	0.49	0.86	0.0033
ZMYND11	zinc finger, MYND-type containing 11	0.65	0.49	0.85	0.0027

Gene expression listed as fold change (FC) in neurocognitive decline (NCD) patients compared with normal patients (NORM).

All values represented are significant ($p < 0.05$). LCI: Lower confidence interval; UCI: Upper confidence interval

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Table 6

Late Post-CPB (4 days post-CPB) Gene Expression in Patients NCD compared with NORM – Complete List

Accession ID	Gen Name	FC	LCI	UCI	p value
Upregulated					
DDX17	DEAD (Asp-Glu-Ala-Asp) box helicase 17	1.89	1.34	2.26	0.0001
MLLT10	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	1.57	1.31	1.88	0.0000
PGM2L1	phosphoglucomutase 2-like 1	1.69	1.19	2.41	0.0042
Downregulated					
C11orf31	chromosome 11 open reading frame 31	0.65	0.49	0.88	0.0057
DIABLO	diablo, IAP-binding mitochondrial protein	0.61			0.0013
GIMAP4	GTPase, IMAP family member 4	0.46	0.43	0.87	0.0067
GPR183	G protein-coupled receptor 183	0.56	0.27	0.79	0.0059
HDAC9	histone deacetylase 9	0.67	0.39	0.80	0.0021
MAT2A	methionine adenosyltransferase II, alpha	0.64			0.0075
MEF2C	myocyte enhancer factor 2C	0.47	0.47	0.88	0.0075
PPIA	peptidylprolyl isomerase A (cyclophilin A)	0.66	0.28	0.77	0.0060
PTMA	prothymosin, alpha	0.56	0.38	0.83	0.0045
RPL10	ribosomal protein L10	0.60	0.41	0.87	0.0073
RPL12	ribosomal protein L12	0.60	0.41	0.87	0.0079
RPL15	ribosomal protein L15	0.57	0.38	0.86	0.0078
RPS13	ribosomal protein S13	0.56	0.39	0.81	0.0030
Sept9	septin 9	0.62	0.44	0.87	0.0072
SPCS3	signal peptidase complex subunit 3 homolog (S. cerevisiae)	0.64	0.48	0.85	0.0031
WWP1	WW domain containing E3 ubiquitin protein ligase 1	0.66	0.52	0.83	0.0008

Gene expression listed as fold change (FC) in neurocognitive decline (NCD) patients compared with normal patients (NORM).

All values represented are significant ($p < 0.05$). LCI: Lower confidence interval; UCI: Upper confidence interval