Atrial Fibrillation, Neurocognitive Decline and Gene Expression After Cardiopulmonary Bypass

Rahul S. Dalal1, MD; Ashraf A. Sabe1, MD; Nassrene Y. Elmadhun1, MD; Basel Ramlawi2, MD; Frank W. Sellke1, MD

Abstract

Objective: Atrial fibrillation and neurocognitive decline are common complications after cardiopulmonary bypass. By utilizing genomic microarrays we investigate whether gene expression is associated with postoperative atrial fibrillation and neurocognitive decline.

Methods: Twenty one cardiac surgery patients were prospectively matched and underwent neurocognitive assessments preoperatively and four days postoperatively. The whole blood collected in the pre-cardiopulmonary bypass, 6 hours after cardiopulmonary bypass, and on the 4th postoperative day was hybridized to Affymetrix Gene Chip U133 Plus 2.0 Microarrays. Gene expression in patients who developed postoperative atrial fibrillation and neurocognitive decline (n=6; POAF+NCD) was compared with gene expression in patients with postoperative atrial fibrillation and normal cognitive function (n=5; POAF+NORM) and patients with sinus rhythm and normal cognitive function (n=10; SR+NORM). Regulated genes were identified using JMP Genomics 4.0 with a false discovery rate of 0.05 and fold change of >1.5 or <-1.5.

Results: Eleven patients developed postoperative atrial fibrillation. Six of these also developed neurocognitive decline. Of the 12 patients with sinus rhythm, only 2 developed neurocognitive decline. POAF+NCD patients had unique regulation of 17 named genes preoperatively, 60 named genes six hours after cardiopulmonary bypass, and 34 named genes four days postoperatively (P<0.05) compared with normal patients. Pathway analysis demonstrated that these genes are involved in cell death, inflammation, cardiac remodeling and nervous system function.

Conclusion: Patients who developed postoperative atrial fibrillation and neurocognitive decline after cardiopulmonary bypass may have differential genomic responses compared to normal patients and patients with only postoperative atrial fibrillation, suggesting common pathophysiology for these conditions. Further exploration of these genes may provide insight into the etiology and improvements of these morbid outcomes.

Keywords: Atrial Fibrillation. Cardiopulmonary Bypass. Genes. Microarray Analysis.

INTRODUCTION

Surgical advancements have allowed an increasingly older population to undergo cardiac surgery and cardiopulmonary bypass (CPB) with a low mortality risk. Efforts have therefore focused on reducing postoperative morbidity. Neurocognitive decline (NCD), up to 80% incidence) and atrial fibrillation (AF, 20-45% incidence) remain two of the most common complications after CPB[1,2]. Coronary artery bypass graft (CABG) guidelines by the American College of Cardiology/ American Heart Association describe two types of neurocognitive deficits, with type 2 representing the vast majority[3]. Type 2 deficits are global and may include confusion and intellectual and memory decline without a known focal lesion and may significantly impair patients’ quality of life. The etiology of these deficits is likely related to multiple factors including age, procedure, CPB time, hypoxia, and inflammation[4-6]. Up to 30% of type 2 deficits persist for at least one year and early NCD appears to predict long-term deficits[7].

Like NCD, the high incidence of postoperative AF (POAF) has persisted. POAF generally occurs by postoperative day four and may precipitate heart failure and cerebrovascular emboli[8-10]. Because of increased hospital stay and readmissions, it is estimated that healthcare costs for patients who develop POAF are $10,000 higher than for those who do not[11]. Though several factors have been correlated with POAF after cardiac surgery, our inability to eliminate its incidence may be related to unknown pathophysiologic mechanisms. Studies have proposed that oxidation and inflammation after CPB induce cardiomyocyte damage and predispose to the development of atrial arrhythmias[12]. Experiments in a canine model of rapid atrial pacing demonstrated that statins, which are known for their anti-inflammatory}

Abbreviations, acronyms & symbols

AF = Atrial fibrillation
CABG = Coronary artery bypass graft
CPB = Cardiopulmonary bypass
NCD = Neurocognitive decline
POAF = Postoperative atrial fibrillation
SR = Sinus rhythm

1Division of Cardiothoracic Surgery, Cardiovascular Research Center, Warren Alpert Medical School of Brown University, Providence, RI, USA.
2Methodist DeBakey Heart & Vascular Center, Methodist Hospital, Houston, Texas, USA.

Financial Support: Funding for this research was provided by the National Heart, Lung, and Blood Institute (R01HL46716, Dr. Sellke; T35HL094308, Dr. Dalal) and NIH Training grant ST32-HL094303-03 (Drs. Sabe and Elmadhun).

DOI: 10.5935/1678-9741.20150070

Article received on May 2nd, 2015
Article accepted on September 20th, 2015

Braz J Cardiovasc Surg 2015;30(5):520-32

Article accepted on September 20th, 2015

No conflicts of interest exist for any of the authors.

Correspondence Address:
Frank W. Sellke
2 Dudley Street, MOC 360
Providence, RI, USA 02905
E-mail: fsellke@lifespan.org

Braz J Cardiovasc Surg 2015;30(5):520-32
and anti-oxidant properties, reduced shortening of the atrial effective refractory period and thus POAF susceptibility[10]. In a case-control study, our group previously demonstrated that patients with POAF had elevated serum peroxide levels, excess myocardial oxidation, and an increased oxidative genomic response compared with patients in sinus rhythm (SR)[11].

While these complications have been studied independently, prior research suggests an association between POAF and neurologic abnormalities[12]. In a prospective observational study, Stanley et al.[13] found significantly more cognitive deficits in patients who developed POAF, which was also associated with worse cognitive functioning six weeks after surgery. While it is thought that the paroxysmal nature of POAF, embolization, and decreased cardiac output increase risk for neurologic dysfunction, it remains unknown if there are common pathways by which both NCD and POAF arise.

High-throughput microarray provides a practical approach to investigate genomic changes and disease development. Microarrays can screen the entire human genome for regulated genes and bring light to the underlying pathways that may promote morbidities like NCD and POAF. We previously utilized microarray to demonstrate increased expression of genes involved with inflammation and neurologic dysfunction in patients who developed NCD after CPB compared to patients without NCD (NORM)[14]. We now examine gene expression changes in patients who develop both POAF and NCD (POAF+NCD) compared to patients spared of these complications (SR+NORM) and those who develop POAF alone (POAF+NORM). To further investigate the underlying pathophysiology of these disease processes we utilize modern microarray and bioinformatics techniques to identify genes that may be associated with the combined incidence of these complications.

METHODS
Patient Enrollment and Matching
We performed a single-institution, prospective cohort study approved by the Beth Israel Deaconess Medical Center Institutional Review Board/Committee on Clinical Investigations in Boston, MA. Forty-two consecutive patients were scheduled for urgent or elective primary CABG, valve replacement (mitral or aortic), or a combination of both requiring CPB. All study participants were provided informed written consent for surgical procedures and blood collection for this investigation. Patients with pre-operative documented AF, high-grade carotid stenosis, known calciﬁed aortas, recent cerebrovascular accident, severe neurologic deﬁcits, serum creatinine>2.0 mg/dL, and hepatic cirrhosis were excluded. Subjects undergoing aortic root/arch procedures, on antiarrhythmic medications, or unable to complete neurocognitive assessments, or unable to complete neurocognitive assessments were also excluded.

POAF was deﬁned as sustained AF conﬁrmed by electrocardiogram before postoperative day ﬁve that required anticoagulation or cardioversion. Of the 42 subjects enrolled, only the subset that developed both POAF and NCD was prospectively matched with selected SR+NORM and POAF+NORM patients based on pre-operative baseline characteristics (i.e. sex, age, hypercholesterolemia, hypertension, diabetes mellitus, white blood cell count, β-blocker use), intraoperative characteristics (i.e. CPB and aortic cross-clamp time, cardiomyotomy suction and antiﬁbrinolytic use, procedure type), and postoperative characteristics (i.e. β-blocker use and time to extubation). Subsequent serologic and molecular studies were performed in a blinded fashion.

Surgical Technique
We followed our institution’s conventional operative approach regarding general anesthesia induction, midline sternotomy, systemic heparinization, CPB, and invasive monitoring as previously described[14].

Neurocognitive Assessment
Patients underwent neurocognitive assessments performed by trained, blinded psychometricians between 1 and 10 days pre-operatively, on postoperative day 4, and in the 3rd month of the postoperative period. Patients were also evaluated for depression using the Geriatric Depression Scale. Memory, attention, language, global cognition, and executive functioning were assessed using 8 validated tools:

The Hopkins Verbal Learning Test measured verbal learning, recall, and retention by assessing the maximum number of items learned, the number of items recalled after 20 minutes divided by the maximum number learned, and the number of items correctly named from a list. Working memory and attention span were measured using Digit Span. Attention shifting ability was assessed by recording the time needed to complete Trailmaking A and B. Confrontational naming was measured using the Boston Naming Test. Fluency was evaluated by requiring patients to generate words beginning with a specific letter (phonemic ﬂuency) or in a category (semantic ﬂuency). The Visual Search and Acuity Test and Stroop Color-Word Inference Test measured visuospatial abilities and executive function. Premorbid intelligence was measured using the Wechsler Test of Adult Reading. In accordance with the “Statement of consensus on assessment of neurobehavioral outcomes after cardiac surgery,” NCD was deﬁned as a 1-standard deviation deﬁcit from baseline on 25% of tasks[15].

Blood Collection and Microarray Processing
Blood samples were drawn from patients via central venous catheter pre-operatively immediately after anesthesia induction (pre-CBP), 6 hours postoperatively in the intensive care unit (post-CBP), and on postoperative day four (4D). Whole blood was drawn into PAXgene tubes (Qiagen Inc, Valencia, CA) for extraction and mRNA stabilization per the manufacturer’s instructions.

RNA extraction and puriﬁcation from whole blood, cDNA synthesis, and generation of biotin-labeled cRNA were performed by the Beth Israel Deaconess Medical Center Proteomics Core according to prior protocol[16,17]. All cRNA samples were hybridized to Affymetrix GeneChip HG-U133 Plus 2.0 microarrays (Affymetrix Inc, Santa Clara, CA). Chips were scanned using the HP G2500A ChipScanner (Affymetrix) and dChip software (Wong et al.[18], Boston, MA) was used for quality control analysis and signal measurement. No outliers were identified and all samples underwent subsequent pathway analysis.

Gene Expression and Pathway Analysis
Raw microarray data underwent gene expression analysis using JMP Genomics 4.0 (SAS, Cary, NC) for normalization, quality control, and statistical analysis. The Robust Multichip Average method normalized and compared composite chip data. Gene expression in Pre-CBP, Post-CBP, and 4D blood samples for POAF+NCD patients were compared to corresponding samples from SR+NORM and POAF+NORM using one-way ANOVA. A post-hoc false discovery rate algorithm with alpha of 0.05 minimized false positive results. Signiﬁcantly, regulated genes met two criteria: 1) –log(P-value) exceeding the threshold calculated by JMP Genomics for each comparison and 2) fold change in gene expression >1.5 or <1.5 between groups. A 1.5-fold change cutoff was chosen here and in a prior study of this patient population to reduce background noise while not limiting results to the most labile genes[14,19]. Signiﬁcantly regulated genes were uploaded into Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Redwood City, CA) to generate top canonical pathways regulated by the selected genes.

Real-time PCR
Gene expression analysis of whole blood-derived mRNA with Affymetrix GeneChip HG-U133 Plus 2.0 microarrays was validated previously by real-time PCR[20].
RESULTS

Patient Characteristics

Patients with POAF+NCD (n=6) were prospectively matched with SR+NORM (n=10) and POAF+NORM (n=5). Table 1 lists well-matched baseline characteristics of these subjects and shows no significant differences in race, sex, age, and co-morbidities as calculated by one-way ANOVA. Patients underwent similar intraoperative courses with regard to anesthesia, CPB technique, temperature, and perioperative monitoring. There were no differences in other postoperative complications, such as focal neurologic deficits or cerebrovascular events in patients with POAF compared to SR during the study period. Of 11 total POAF patients, 6 developed NCD (54.5%), and of 12 SR patients, only 2 developed NCD (16.7%). After three months, all but one patient returned regained normal cognitive function[20].

Gene Expression and Confirmation

We previously published comprehensive gene expression databases of patients with POAF or SR before and after CPB as well as patients with and without NCD after CPB, including unsupervised hierarchical sample clustering, and confirmation of microarray gene-expression data with real-time PCR[11,20]. Our described microarray GeneChip identified 54,675 transcripts. Complete lists of genes regulated in the comparisons of POAF+NCD vs. SR+NORM or POAF+NORM are provided in Tables 2 to 7.

Table 1. Characteristics for matching of patients who developed POAF and NCD with controls.

| Characteristic | A | B | C | P-value |
|----------------|---|---|---|---------|
| Pre-operative data | | | | |
| Age (y)a | 66.5±7.4 | 69.2±7.1 | 73.4±5.8 | 0.28 |
| Sex (% male) | 83.3 (5/6) | 100 (10/10) | 80.0 (4/5) | 0.40 |
| Hypertension (% of group) | 83.3 (5/6) | 70.0 (7/10) | 40.0 (2/5) | 0.34 |
| Hypercholesterolemia (% of group) | 50.0 (3/6) | 50.0 (5/10) | 20.0 (1/5) | 0.54 |
| Diabetes mellitus (% of group) | 50.0 (3/6) | 30.0 (3/10) | 40.0 (2/5) | 0.76 |
| Leukocytes (103 cells/μL)a | 7.4±2.1 | 7.2±2.0 | 10.3±2.9 | 0.05 |
| Hematocrit (%) | 35.6±4.3 | 34.5±4.0 | 37.7±7.6 | 0.53 |
| Glucose (mg/dL) | 193±131 | 163±68 | 118±38 | 0.38 |
| Intraoperative data | | | | |
| Procedure (% CABG) | 83.3 (5/6) | 70.0 (7/10) | 80.0 (4/5) | 0.84 |
| CPB time (min)a | 78.3±32.6 | 78.9±26.3 | 70.6±20.1 | 0.84 |
| Cross-clamp time (min)a | 57.7±23.9 | 63.0±21.0 | 46.4±21.3 | 0.40 |

Values are mean ± SD
CABG=coronary artery bypass graft; CPB=cardiopulmonary bypass; POAF=post-operative atrial fibrillation; SR=sinus rhythm

Table 2. Pre-CPB gene expression in patients with POAF+NCD compared with SR+NORM – complete list.

| Accession ID | Gene Name | FC | P-values |
|--------------|-----------|----|----------|
| ADM2 | adrenomedullin 2 | 1.66 | 1.12E-05 |
| CA11 | carbonic anhydrase XI | 1.58 | 4.47E-05 |
| CD101 | CD101 molecule | 2.19 | 1.15E-04 |
| COMTD1 | catechol-O-methyltransferase domain containing 1 | 1.81 | 2.29E-05 |
| GAS6-AS1 | GAS6 antisense RNA 1 | 1.54 | 5.37E-05 |
| KCNKP3 | Kv channel interacting protein 3, calsenilin | 1.56 | 2.51E-22 |
| MCF2L | MCF2 cell line derived transforming sequence-like | 1.52 | 1.00E-04 |
| MECR | mitochondrial trans-2-enoyl-CoA reductase | 1.52 | 2.19E-07 |
| MMP11 | matrix metalloproteinase 11 (stromelysin 3) | 1.71 | 5.01E-15 |
| NUTM2F/NUTM2G | NUT family member 2G | 1.89 | 6.31E-13 |
| PHF20 | PHD finger protein 20 | 0.65 | 1.78E-05 |
| PYCR1 | pyrroline-5-carboxylate reductase 1 | 1.53 | 5.37E-05 |
| RGS12 | regulator of G-protein signaling 12 | 1.52 | 9.77E-06 |
| TOM1L2 | target of mybl-like 2 (chicken) | 1.60 | 1.15E-04 |
| VGLL1 | vestigial like 1 (Drosophila) | 1.87 | 3.02E-07 |
| WIZ | widely interspaced zinc finger motifs | 1.83 | 8.91E-10 |
| ZBED5 | zinc finger, BED-type containing 5 | 1.63 | 1.07E-04 |

FC=fold change
### Table 3. Post-CBP gene expression in patients with POAF+NCD compared with SR+NORM – complete list.

| Accession ID | Gene Name                                                                 | FC   | P-values   |
|--------------|---------------------------------------------------------------------------|------|------------|
| ABHD13       | abhydrolase domain containing 13                                         | 1.92 | 1.00E-05   |
| ACOX1        | acyl-CoA oxidase 1, palmitoyl                                             | 2.75 | 1.23E-04   |
| ARPC1A       | actin related protein 2/3 complex, subunit 1A, 41kDa                     | 2.23 | 7.41E-05   |
| BMX          | BMX non-receptor tyrosine kinase                                         | 7.32 | 1.00E-14   |
| C1GALT1C1    | C1GALT1-specific chaperone 1                                             | 1.63 | 1.82E-08   |
| C2orf76      | chromosome 2 open reading frame 76                                       | 2.38 | 5.25E-08   |
| C5orf30      | chromosome 5 open reading frame 30                                       | 2.56 | 6.17E-06   |
| CDS2         | CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2       | 1.95 | 4.07E-10   |
| CEACAM21     | carcinoembryonic antigen-related cell adhesion molecule 21               | 3.02 | 8.71E-05   |
| CLEC12A      | C-type lectin domain family 12, member A                                 | 1.57 | 2.09E-05   |
| CLEC2B       | C-type lectin domain family 2, member B                                  | 1.62 | 1.95E-05   |
| CREBBP       | CREB binding protein                                                     | 1.83 | 1.29E-06   |
| DAB2         | Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)           | 2.23 | 8.51E-05   |
| DACH1        | dachshund homolog 1 (Drosophila)                                         | 3.27 | 5.01E-11   |
| DNAJC5       | Dnal (Hsp40) homolog, subfamily C, member 5                              | 1.51 | 8.32E-07   |
| EPAS1        | endothelial PAS domain protein 1                                         | 2.43 | 6.17E-05   |
| FAM114A2     | family with sequence similarity 114, member A2                           | 1.64 | 2.04E-06   |
| FAM200B      | family with sequence similarity 200, member B                            | 2.12 | 1.86E-05   |
| FBXO28       | F-box protein 28                                                         | 1.59 | 9.33E-05   |
| FKBP9        | FK506 binding protein 9, 63 kDa                                         | 5.72 | 1.58E-12   |
| GNG2         | guanine nucleotide binding protein (G protein), gamma 2                  | 1.69 | 6.03E-05   |
| GFP2H2       | general transcription factor IIH, polypeptide 2, 44kDa                    | 3.37 | 2.09E-10   |
| HGF          | hepatocyte growth factor (hepapoietin A; scatter factor)                 | 1.79 | 3.47E-05   |
| HIST1H2BE    | histone cluster 2, H2be                                                  | 1.52 | 5.01E-28   |
| HOOK3        | hook homolog 3 (Drosophila)                                              | 2.19 | 1.12E-04   |
| KIDINS220    | kinase D-interacting substrate, 220kDa                                   | 1.54 | 7.08E-06   |
| KLHL7        | kelch-like family member 7                                               | 1.63 | 1.17E-04   |
| KPN1A        | karyopherin alpha 1 (importin alpha 5)                                   | 2.45 | 1.78E-05   |
| LEMD2        | LEM domain containing 2                                                  | 1.59 | 1.66E-05   |
| LOC100506229 | uncharacterized LOC100506229                                            | 2.15 | 3.09E-05   |
| LOC100506328 | uncharacterized LOC100506328                                            | 9.36 | 2.51E-11   |
| LOC285835    | uncharacterized LOC285835                                                | 1.51 | 6.31E-05   |
| MAPK14       | mitogen-activated protein kinase 14                                      | 1.95 | 2.51E-28   |
| MAP35C5      | membrane-associated ring finger (C3HC4) 5                                | 3.07 | 5.50E-06   |
| MLLT4        | myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to 4 | 1.57 | 9.77E-05   |
| MTIF3        | mitochondrial translational initiation factor 3                           | 2.60 | 4.07E-05   |
| NIKAP3       | NFKB activating protein                                                  | 3.08 | 3.63E-05   |
| OXSR1        | oxidative stress responsive 1                                            | 2.84 | 1.62E-08   |
| PDS51        | prenyl (decaprenyl) diphosphate synthase, subunit 1                       | 3.08 | 1.45E-05   |
| PFKFB2       | 6-phosphofructo-2-kinase/Fructose-2,6-biphosphatase 2                    | 3.60 | 2.51E-06   |
| PRKAG1       | protein kinase, AMP-activated, gamma 1 non-catalytic subunit             | 2.73 | 5.13E-05   |
| RASGEF1A     | RasGEF domain family, member 1                                           | 4.12 | 1.78E-05   |
| RILPL1       | Rab interacting lysosomal protein-like 1                                  | 1.89 | 6.03E-06   |
| SFXN1        | sideroflexin 1                                                           | 2.59 | 1.02E-04   |
| SLC39A8      | solute carrier family 39 (zinc transporter), member 8                    | 1.73 | 3.98E-13   |
| ST6GALNAC3   | ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3 | 2.77 | 5.50E-06   |
| STAG1        | stromal antigen 1                                                        | 1.54 | 2.00E-16   |
| SULT1B1      | sulfotransferase family, cytosolic, 1B, member 1                          | 5.57 | 3.47E-10   |

continue
Table 4. 4D gene expression in patients with POAF+NCD compared with SR+NORM – complete list.

| Accession ID | Gene Name                                                                 | FC  | P-values |
|--------------|----------------------------------------------------------------------------|-----|---------|
| AGPAT6       | 1-acylglycerol-3-phosphate O-acyltransferase 6                             | 1.54| 5.61E-04|
| ATP13A4      | ATPase type 13A4                                                           | 1.63| 6.46E-06|
| BCL2L1       | BCL2-like 1                                                               | 3.17| 1.58E-13|
| C20orf203    | chromosome 20 open reading frame 203                                      | 0.45| 4.37E-07|
| CASC7        | cancer susceptibility candidate 7 (non-protein coding)                     | 1.70| 3.98E-11|
| CDC42BPA     | CDC42 binding protein kinase alpha (DMPK-like)                             | 1.94| 1.86E-08|
| CDC2A7       | cell division cycle associated 7                                          | 1.72| 5.75E-05|
| CTSD         | cathepsin O                                                                | 0.54| 8.71E-05|
| DDX17        | DEAD (Asp-Glu-Ala-Asp) box helicase 17                                     | 7.10| 5.01E-27|
| DLD          | dihydrolipoamide dehydrogenase                                             | 2.13| 3.16E-14|
| DOCK1        | dedicator of cytokinesis 1                                                 | 2.09| 6.31E-50|
| DSC2         | desmocollin 2                                                             | 2.01| 4.07E-06|
| FRMD8        | FERM domain containing 8                                                  | 2.42| 2.45E-06|
| GLCCI1       | glucocorticoid induced transcript 1                                       | 2.61| 3.16E-16|
| GRB10        | growth factor receptor-bound protein 10                                    | 1.79| 3.16E-11|
| HNMT         | histamine N-methyltransferase                                              | 1.65| 1.05E-04|
| IDE          | insulin-degrading enzyme                                                   | 1.52| 6.61E-06|
| LOC284080    | uncharacterized LOC284080                                                 | 1.51| 8.13E-05|
| MMD          | monocyte to macrophage differentiation-associated                          | 1.57| 2.00E-15|
| NCR1         | natural cytotoxicity triggering receptor 1                                 | 1.90| 2.57E-05|
| NEDD4L       | neural precursor cell expressed, developmentally down-regulated 4-like, E3| 1.60| 6.31E-11|
| PLXNB1       | plexin B1                                                                 | 1.53| 1.00E-15|
| PRKAA2       | protein kinase, AMP-activated, alpha 2 catalytic subunit                   | 1.54| 5.37E-05|
| PRRT1        | proline-rich transmembrane protein 1                                      | 1.98| 3.98E-05|
| REEP1        | receptor accessory protein 1                                               | 1.52| 2.63E-07|
| RHCE/RHD     | Rh blood group, D antigen                                                  | 1.69| 8.13E-05|
| RIOK3        | RIO kinase 3                                                               | 4.54| 9.55E-05|
| RPL10        | ribosomal protein L10                                                      | 0.40| 8.91E-05|
| RPL18        | ribosomal protein L18                                                      | 0.47| 1.95E-05|
| SMC3         | structural maintenance of chromosomes 3                                   | 1.53| 4.47E-06|
| SRSF1        | serine/arginine-rich splicing factor 1                                     | 2.04| 4.37E-05|
| ST7          | suppression of tumorigenicity 7                                           | 1.58| 6.76E-05|
| TFAP2E       | transcription factor AP-2 epsilon (activating enhancer binding protein 2 epsilon) | 1.93| 5.89E-06|
| UBE2H        | ubiquitin-conjugating enzyme E2H                                           | 2.94| 7.76E-06|

FC=fold change
### Table 5. Pre-CPB gene expression in patients with POAF+NCD compared with POAF+NORM – complete list.

| Accession ID | Gene Name                                                                 | FC  | P-values     |
|--------------|---------------------------------------------------------------------------|-----|--------------|
| ACTR3BP5     | ARP3 actin-related protein homolog B (yeast) pseudogene                    | 0.57| 3.55E-09     |
| A5PS1        | adaptor-related protein complex 5, sigma 1 subunit                        | 1.87| 1.00E-04     |
| C14orf166B   | chromosome 14 open reading frame 166B                                    | 1.54| 1.58E-12     |
| CA11         | carbonic anhydrase XI                                                     | 1.79| 8.91E-06     |
| CCDC36       | coiled-coil domain containing 36                                        | 0.35| 5.01E-21     |
| CIZ1         | CDKN1A interacting zinc finger protein 1                                  | 2.42| 1.10E-04     |
| FHAD1        | forkhead-associated (FHA) phosphopeptide binding domain 1                | 1.51| 6.31E-06     |
| FKRP         | fukutin related protein                                                  | 0.54| 4.17E-05     |
| GTPBP3       | GTP binding protein 3 (mitochondrial)                                   | 0.49| 2.75E-05     |
| KCNIP3       | Kv channel interacting protein 3, calsenil                               | 1.58| 6.31E-19     |
| KHSRP        | KH-type splicing regulatory protein                                       | 1.56| 4.79E-10     |
| LOC100507477 | uncharacterized LOC100507477                                            | 1.99| 3.98E-05     |
| MCF2L        | MCF.2 cell line derived transforming sequence-like                        | 1.66| 4.17E-05     |
| MMP11        | matrix metalloproteinase 11 (stomelysin-3)                              | 1.67| 5.01E-12     |
| NFATC1       | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | 1.61| 6.46E-05     |
| NUTM2F/NUTM2G | NUT family member 2G                                                  | 1.89| 3.16E-11     |
| PHAX         | phosphorylated adaptor for RNA export                                  | 0.59| 2.45E-05     |
| Pycler       | pyrroline-5-carboxylate reductase                                         | 1.75| 5.89E-06     |
| RIN1         | Ras and Rab interactor                                                  | 1.78| 7.94E-05     |
| SLC24A6      | solute carrier family 24 (sodium/lithium/calcium exchanger), member 6    | 0.45| 1.20E-04     |
| SYT17        | synaptotagmin XVII                                                       | 0.57| 3.98E-14     |
| TAC2         | transforming, acidic coiled-coil containing protein 2                    | 0.57| 3.16E-18     |
| TMEM259      | transmembrane protein 259                                                | 1.64| 7.41E-06     |
| TUBG1        | tubulin, gamma 1                                                         | 1.79| 8.71E-05     |
| VGLL1        | vestigial like 1 (Drosophila)                                            | 1.90| 1.74E-06     |
| WZ           | widely interspaced zinc finger motifs                                    | 1.74| 1.48E-07     |
| WNK2         | WNK lysine deficient protein kinase 2                                    | 0.48| 3.98E-31     |
| XYL12        | xylosyltransferase II                                                    | 0.65| 1.05E-04     |
| ZNF528       | zinc finger protein 528                                                  | 0.36| 8.51E-05     |

*FC=fold change*

### Table 6. Post-CPB gene expression in patients with POAF+NCD compared with POAF+NORM – complete list.

| Accession ID | Gene Name                                                                 | FC  | P-values     |
|--------------|---------------------------------------------------------------------------|-----|--------------|
| ANKMY2       | ankyrin repeat and MYND domain containing 2                               | 0.61| 1.58E-12     |
| ANKRD6       | ankyrin repeat domain 6                                                  | 0.54| 1.38E-08     |
| AP4E1        | adaptor-related protein complex 4, epsilon 1 subunit                     | 0.50| 4.68E-05     |
| BCS1L        | BC1 (ubiquinol-cytochrome c reductase) synthesis-like                    | 0.53| 7.76E-05     |
| CD52         | CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2       | 2.03| 3.09E-09     |
| CEBPG        | CCAAT/enhancer binding protein (C/EBP), gamma                            | 0.41| 1.26E-05     |
| CLEC2B       | C-type lectin domain family 2, member B                                  | 1.97| 1.70E-06     |
| DACH1        | dachshund homolog 1 (Drosophila)                                         | 2.47| 8.32E-07     |
| FKBP9        | FK506 binding protein 9, 63 kDa                                         | 2.38| 8.32E-05     |
| GOL1B        | golgi transport 1B                                                       | 0.42| 3.98E-13     |
| GTF2H2       | general transcription factor IIH, polypeptide 2, 44kDa                   | 2.54| 1.35E-06     |
| HIST2H2BE    | (includes others) histone cluster 2, H2be                                | 1.52| 3.98E-26     |
| HIVEP2       | human immunodeficiency virus type I enhancer binding protein 2           | 0.62| 6.03E-05     |
| KMO          | kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)                     | 0.33| 1.00E-31     |
| LOC100506328 | uncharacterized LOC100506328                                             | 5.99| 1.38E-07     |
| LOC728613    | programmed cell death 6 pseudogene                                       | 0.48| 6.92E-05     |
| MAPK14       | mitogen-activated protein kinase 14                                       | 1.98| 6.31E-24     |

*continued...*
**Table 7.** 4D gene expression in patients with POAF+NCD compared with POAF+NORM – complete list.

| Accession ID | Gene Name                                                                 | FC    | P-values       |
|--------------|---------------------------------------------------------------------------|-------|----------------|
| ACSL6        | acyl-CoA synthetase long-chain family member 6                            | 1.62  | 8.13E-06       |
| ADAMTS6      | ADAM metalloprotease with thrombospondin type 1 motif, 6                  | 0.65  | 4.37E-05       |
| ADRBK2       | adrenergic, beta, receptor kinase 2                                        | 0.22  | 1.29E-09       |
| AGPAT6       | 1-acylglycerol-3-phosphate O-acyltransferase 6                            | 1.63  | 1.58E-12       |
| BCL2L1       | BCL2-like 1                                                               | 2.75  | 3.09E-06       |
| C20orf203    | chromosome 20 open reading frame 203                                      | 0.31  | 3.16E-14       |
| CASC7        | cancer susceptibility candidate 7 (non-protein coding)                    | 1.75  | 1.05E-10       |
| CBL          | Cbl proto-oncogene, E3 ubiquitin protein ligase                            | 0.64  | 3.02E-10       |
| CDC42BPA     | CDC42 binding protein kinase alpha (DMPK-like)                            | 2.24  | 2.24E-10       |
| CDC27        | cell division cycle associated 7                                           | 1.88  | 1.70E-09       |
| CHD2         | chromodomain helicase DNA binding protein 2                               | 0.48  | 2.40E-05       |
| CHERP        | calcium homeostasis endoplasmic reticulum protein                         | 0.46  | 8.91E-07       |
| CLIC2        | chloride intracellular channel 2                                           | 2.08  | 5.01E-27       |
| DCAF15       | DDB1 and CUL4 associated factor 15                                         | 0.48  | 5.89E-05       |
| DDX17        | DEAD (Asp-Glu-Ala-Asp) box helicase 17                                     | 6.55  | 2.29E-06       |
| DLD          | dihydrolipoamide dehydrogenase                                             | 1.89  | 2.51E-25       |
| DOCK1        | dedicator of cytokinesis 1                                                | 2.10  | 1.00E-10       |
| EPB41L4B     | erythrocyte membrane protein band 4.1 like 4B                             | 0.65  | 7.94E-49       |
| FRMD8        | FERM domain containing 8                                                  | 3.33  | 2.51E-17       |
| GLCC1        | glucocorticoid induced transcript 1                                       | 2.43  | 1.29E-08       |
| GRB10        | growth factor receptor-bound protein 10                                   | 1.72  | 7.94E-14       |
| HEMGN        | hemogen                                                                   | 2.75  | 1.45E-09       |
| IDE          | insulin-degrading enzyme                                                  | 1.56  | 2.82E-05       |
| L1CAM        | L1 cell adhesion molecule                                                 | 1.60  | 1.05E-05       |

**Continued...**
| Gene Symbol | Description                                                                 | FC | p-value       |
|------------|------------------------------------------------------------------------------|----|--------------|
| LOC100505812 | uncharacterized LOC100505812                                                 | 0.55 | 5.01E-11     |
| MED1       | mediator complex subunit 1                                                    | 0.45 | 6.03E-06     |
| MMD        | monocyte to macrophage differentiation-associated                             | 1.53 | 3.09E-09     |
| MS4A6A     | membrane-spanning 4-domains, subfamily A, member 6A                          | 2.48 | 7.94E-14     |
| NCR1       | natural cytotoxicity triggering receptor 1                                    | 2.45 | 1.07E-04     |
| NEDD4L     | neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase | 1.57 | 2.04E-07     |
| ODF4       | outer dense fiber of sperm tails 4                                           | 0.56 | 1.00E-09     |
| OSBPL11    | oxysterol binding protein-like 11                                            | 0.28 | 2.51E-26     |
| PRDM2      | PR domain containing 2, with ZNF domain                                      | 0.48 | 3.09E-05     |
| PTAR1      | protein prenyltransferase alpha subunit repeat containing 1                  | 0.53 | 8.13E-06     |
| PTPLB      | protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b | 0.46 | 8.71E-05     |
| PTPN9      | protein tyrosine phosphatase, non-receptor type 9                            | 0.61 | 4.68E-05     |
| RAB32      | RAB32, member RAS oncogene family                                           | 0.58 | 1.15E-04     |
| RASSF1     | Ras association (RalGDS/AF-6) domain family member 1                         | 0.46 | 3.24E-06     |
| RBM12B     | RNA binding motif protein 12B                                                | 1.52 | 3.98E-11     |
| REEP1      | receptor accessory protein 1                                                 | 1.72 | 2.45E-07     |
| RPL10      | ribosomal protein L10                                                        | 0.36 | 7.08E-09     |
| SERPINE1   | serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 | 0.59 | 4.79E-05     |
| SGOL1      | shugoshin-like 1 (S. pombe)                                                 | 0.56 | 2.69E-06     |
| SLC22A7    | solute carrier family 22 (organic anion transporter), member 7              | 0.54 | 7.94E-12     |
| SLC5A4     | solute carrier family 5 (low affinity glucose cotransporter), member 4       | 0.50 | 3.02E-05     |
| SMC3       | structural maintenance of chromosomes 3                                      | 1.72 | 3.16E-27     |
| TCF4       | transcription factor 4                                                       | 0.63 | 1.38E-07     |
| UBE2H      | ubiquitin-conjugating enzyme E2H                                              | 2.64 | 1.58E-06     |
| VPS37A     | vacuolar protein sorting 37 homolog A (S. cerevisiae)                        | 1.71 | 1.10E-04     |
| WHAMMP2    | WAS protein homolog associated with actin, golgi membranes and microtubules pseudogene 2 | 0.58 | 5.75E-08     |
| YOD1       | YOD1 deubiquitinase                                                          | 1.53 | 3.47E-05     |
| ZEB1       | zinc finger E-box binding homeobox 1                                         | 0.56 | 1.26E-04     |
| ZNF395     | zinc finger protein 395                                                     | 0.53 | 1.10E-06     |

**Gene Expression and Pathway Analysis in POAF+NCD vs. SR+NORM.**

Figure 1 shows the distribution of regulated genes by fold-change for each time point in this comparison. Pre-CPB, 19 genes were significantly regulated in the POAF+NCD group compared to NORM+SR, of which 17 were named. Notably, 16 of these 17 genes were up-regulated, while 1 was down-regulated. Pathway analysis used to group genes by potential pathophysiologic functions demonstrated that these genes are related to cardiovascular disease, nervous system function, and cell death, as described in Table 8. Post-CPB, the number of genes increased to 65, of which 60 were named. All 60 were up-regulated, and while distinct from those regulated pre-operatively, pathway analysis demonstrated that many of these genes are associated with cardiovascular disease and remodeling, inflammation, and nervous system disorders, as seen in Table 9. At 4D, the number of genes decreased to 41, of which 34 were named. Of these, 30 were up-regulated while 4 were down-regulated. Several genes, as listed in Table 10, are similarly involved with cardiovascular disease, nervous system function, inflammation, and protein degradation.

**Gene Expression and Pathway Analysis in Patients with POAF+NCD vs. POAF+NORM.**

Figure 2 shows the distribution of regulated genes by fold-change for each time point. Pre-CPB 42 genes were significantly regulated in the POAF+NCD group compared to POAF+NORM, of which 29 were named. Of these, 18 were up-regulated, while 11 were down-regulated. These genes were associated with cardiovascular disease, nervous system function, and inflammation. Post-CPB, the number of regulated genes was 39, of which 37 were named. Sixteen of these 37 were up-regulated, while 21 were down-regulated. Pathway analysis demonstrated that these genes serve roles in cardiovascular disease and inflammation. At 4D, the number of regulated genes increased to 72, of which 54 were up-regulated, while 18 were down-regulated.
were named. Twenty-seven of these were up-regulated, while 27 were down-regulated. IPA analysis again revealed that several genes affect cardiovascular disease, inflammation, and cell death. Selected genes grouped by pathophysiologic function for the POAF+NCD vs. POAF+NORM comparisons are found in Tables 11-13. While the majority of the genes identified for these comparisons were distinct from that of POAF+NCD vs. SR+NORM across all time points, multiple genes overlap and are listed in Table 14.

Table 8. Pre-CPB Gene Expression in Patients with POAF and NCD compared with SR and NORM – selected genes grouped by potential pathophysiologic function.

| Accession ID | Gene Name                               | FC  | P-values |
|--------------|-----------------------------------------|-----|----------|
| Cardiovascular disease | ADM2 adrenomedullin-2 | 1.66 | 1.00E-04 |
| Nervous system function | KCNIP3 Kv channel interacting protein 3, calsenilin | 1.56 | 2.51E-22 |
| Cell death and survival | MMP11 matrix metallopeptidase 11 (stromelysin 3) | 1.71 | 5.01E-15 |

FC=fold change

Table 9. Post-CPB gene expression in patients with POAF and NCD compared with SR and NORM – selected genes grouped by potential pathophysiologic function.

| Accession ID | Gene Name                               | FC  | P-values |
|--------------|-----------------------------------------|-----|----------|
| Cardiovascular disease | BMX BMX non-receptor tyrosine kinase | 7.32 | 1.00E-14 |
|                  | EPAS1 endothelial PAS domain protein 1   | 2.43 | 6.17E-05 |
|                  | HGF hepatocyte growth factor (hepapoietin A; scatter factor) | 1.79 | 3.47E-05 |
|                  | MAPK14 mitogen-activated protein kinase 14 | 1.95 | 2.51E-28 |
| Nervous system function | KIDINS220 kinase D-interacting substrate, 220kDa | 1.54 | 7.08E-06 |
|                  | SYNE1 spectrin repeat containing, nuclear envelope 1 | 3.11 | 3.09E-06 |
|                  | YKT6 YKT6 v-SNARE homolog (S. cerevisiae) | 1.72 | 3.39E-05 |
| Inflammation     | CREBBP CREB binding protein              | 1.83 | 1.29E-06 |
| Psychological disorders | TMLHE trimethyllysine hydroxylase, epsilon | 1.95 | 3.47E-05 |

FC=fold change
Table 10. 4D Gene expression in patients with AF and NCD compared with SR and NORM – selected genes grouped by potential pathophysiologic function.

| Accession ID | Gene Name                                      | FC   | P-values |
|--------------|------------------------------------------------|------|----------|
|              | Cardiovascular disease                         |      |          |
| BCL2L1       | BCL2-like 1                                    | 3.17 | 1.58E-13 |
| PRKAA2       | protein kinase, AMP-activated, alpha 2 catalytic subunit | 1.54 | 5.37E-05 |
|              | Nervous system function                        |      |          |
| IDE          | insulin-degrading enzyme                        | 1.52 | 6.61E-06 |
| CDC42BPA     | CDC42 binding protein kinase alpha (DMPK-like)  | 1.94 | 1.86E-08 |
| PLXNB1       | plexin B1                                      | 1.53 | 1.00E-15 |
|              | Inflammation                                   |      |          |
| NCR1         | natural cytotoxicity triggering receptor 1      | 1.90 | 2.57E-05 |
| DOCK1        | dedicator of cytokinesis 1                     | 2.09 | 6.31E-50 |
| SMC3         | structural maintenance of chromosomes 3        | 1.53 | 4.47E-06 |
|              | Protein degradation                             |      |          |
| DLD          | dihydrolipoamide dehydrogenase                 | 2.13 | 3.16E-14 |
| NEDD4L       | neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase | 1.60 | 6.31E-11 |
| UBE2H        | ubiquitin-conjugating enzyme E2H               | 2.94 | 7.76E-06 |

FC=fold change

Table 11. Pre-CPB gene expression in patients with POAF+NCD compared with POAF+NORM – selected genes grouped by potential pathophysiologic function.

| Accession ID | Gene Name                                      | FC   | P-values |
|--------------|------------------------------------------------|------|----------|
|              | Cardiovascular disease/function                 |      |          |
| NFATC1       | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | 1.61 | 6.46E-05 |
| TUBG1        | tubulin, gamma 1                                | 1.79 | 8.71E-05 |
| MCF2L        | MCF.2 cell line derived transforming sequence-like | 1.66 | 4.17E-05 |
|              | Nervous system function                         |      |          |
| FKRIP        | fukutin related protein                         | 0.54 | 4.17E-05 |
| KCNIP3       | Kv channel interacting protein 3, calsenilin    | 1.58 | 6.31E-19 |

FC=fold change
**Table 12.** Post-CPB gene expression in patients with POAF+NCD compared with POAF+NORM – selected genes grouped by potential pathophysiologic function.

| Accession ID | Gene Name                                      | FC  | P-values       |
|--------------|------------------------------------------------|-----|---------------|
|              | Cardiovascular disease/function                |     |               |
|              | MAPK14 mitogen-activated protein kinase 14     | 1.98| 6.31E-24      |
|              | SYNE1 spectrin repeat containing, nuclear envelope 1 | 2.74| 1.26E-04      |
|              | CDS2 CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2 | 2.03| 3.09E-09      |
|              | Inflammation                                  |     |               |
|              | HIVEP2 human immunodeficiency virus type I enhancer binding protein 2 | 0.62| 6.03E-05      |

*FC=fold change*

**Table 13.** 4D Gene expression in patients with POAF+NCD compared with POAF+NORM – selected genes grouped by potential pathophysiologic function.

| Accession ID | Gene Name                                      | FC  | P-values       |
|--------------|------------------------------------------------|-----|---------------|
|              | Cardiovascular disease                         |     |               |
|              | CBL Cbl proto-oncogene, E3 ubiquitin protein ligase | 0.64| 2.24E-10      |
|              | SERPINE1 serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 | 0.59| 2.69E-06      |
|              | BCL2L1 BCL2-like 1                             | 2.75| 3.09E-06      |
|              | MED1 mediator complex subunit 1                | 0.45| 3.09E-09      |
|              | RASSF1 Ras association (RalGDS/AF-6) domain family member 1 | 0.46| 3.98E-11      |
|              | Cell death/survival                            |     |               |
|              | IDE insulin-degrading enzyme                    | 1.56| 1.05E-05      |
|              | RAB32 RAB32, member RAS oncogene family         | 0.58| 3.24E-06      |
|              | CDC42BPA CDC42 binding protein kinase alpha (DMPK-like) | 2.24| 1.70E-09      |
|              | DOCK1 dedicator of cytokinesis 1               | 2.10| 7.94E-49      |
|              | L1CAM L1 cell adhesion molecule                | 1.60| 5.01E-11      |
|              | PTPN9 protein tyrosine phosphatase, non-receptor type 9 | 0.61| 1.15E-04      |
|              | SMC3 structural maintenance of chromosomes 3 | 1.72| 1.38E-07      |
|              | DDX17 DEAD (Asp-Glu-Ala-Asp) box helicase 17   | 6.55| 2.51E-25      |
|              | GRB10 growth factor receptor-bound protein 10  | 1.72| 1.45E-09      |
|              | PRDM2 PR domain containing 2, with ZNF domain | 0.48| 8.13E-06      |
|              | TCF4 transcription factor 4                     | 0.63| 1.58E-06      |
|              | ZEB1 zinc finger E-box binding homeobox 1      | 0.56| 1.10E-06      |
|              | Inflammation                                  |     |               |
|              | ADRB2 adrenergic, beta, receptor kinase 2      | 0.22| 1.29E-09      |
|              | NCR1 natural cytotoxicity triggering receptor 1| 2.45| 2.04E-07      |

*FC=fold change*

**Table 14.** Significantly regulated genes overlapping across multiple comparisons.

| Comparisons                                        | Overlapping Regulated Genes |
|----------------------------------------------------|------------------------------|
| POAF+NCD vs. SR+NORM (Pre-CPB)                     | ca11, kcnip3, mcf2l, mmp11, nutm2f/nutm2g, pycr1, vgl1, wiz |
| POAF+NCD vs. AF+NORM (Pre-CPB)                     | c6ds2, clec2b, dach1, fkbp9, gtf2h2, hist2h2be, mapk14, slc39a8, sult1b1, syne1, timm23, tor1aip2, yplf4, zmf350 |
| POAF+NCD vs. SR+NORM (Post-CPB)                    | apgap6b, bcl2l1, c20orf2f23, casc7, cdc42bpa, cdc4a7, ddx17, dld, dock1, frmd8, glc1c1, grb10, ide, mmd, ncr1, nedd4l, reep1, rpl10, smc3, ube2h |
| POAF+NCD vs. AF+NORM (Post-CPB)                    | UBE2H |
**DISCUSSION**

AF and NCD after cardiac surgery have each been extensively studied. Much of the literature for POAF has pointed to inflammation and oxidative stress as promoting factors. Indeed, prior work from our group demonstrated significantly elevated genomic markers of oxidative stress in the blood of patients who develop POAF after CPB\[^{[11]}\]. We similarly used microarray to study NCD patients and found increased expression of blood inflammatory mediators from those undergoing CPB\[^{[14]}\]. Given that the genomic regulation of systemic cytotoxic insults such as oxidation and inflammation appear to promote POAF and NCD when studied individually, we sought to determine if genomic responses differ in patients who develop both complications.

Our current microarray study shows that the expression profiles of patients who develop both POAF and NCD after CPB differ from those who develop neither complication nor POAF alone. The greatest amount of gene regulation occurred postoperatively, suggesting that CPB may induce a differential genomic response in susceptible patients. Furthermore, POAF+NCD vs. POAF+NORM had the most gene regulation at 4D, while POAF+NCD vs. SR+NORM had the most gene regulation post-CPB with a largely different set of genes identified. This suggests that POAF and NCD after CPB may be linked pathophysiologically through mechanisms distinct from those inducing POAF alone, with more genmic changes occurring at an earlier stage.

Many genes regulated post-CPB in POAF+NCD vs. SR+NORM are associated with pathologic cardiac remodeling. One such gene includes BMX, a non-receptor tyrosine kinase. Mitchell-Jordan et al.\[^{[21]}\] demonstrated that BMX-knockout mice were resistant to massive cardiac hypertrophy following transverse aortic constriction relative to wild type, indicating a significant role for BMX in cardiac remodeling. If the impressive 7.32-fold up-regulation of BMX in the blood of our POAF+NCD patients also reflects their myocardial expression, excess cardiac remodeling after CPB may be a predisposing factor for POAF and NCD. Additional up-regulated genes identified in this group with reported roles in cardiac remodeling include EPAS1, HGF, and MAPK14\[^{[22-24]}\]. While there is much evidence for oxidative stress in cardiac remodeling and AF\[^{[25]}\], our study found genes implicated in remodeling but not oxidative stress, perhaps due to our limited sample size. However, while Ramlawi et al.\[^{[20]}\] demonstrated genomic regulation of oxidative stress in POAF patients, they did not report genes directly related to cardiac remodeling. This difference may lie in the fact that our patients developed NCD in addition to POAF, introducing a potential association of cardiac remodeling with secondary neurologic effects.

Several genes identified in the POAF+NCD vs. SR+NORM comparison are also directly implicated in neurologic dysfunction. KIDINS220 was up-regulated post-CPB and has been shown to accumulate with tau protein in the brains of Alzheimer Disease patients\[^{[26]}\]. At 4D, there was also increased expression of PLXNB1, which controls the behavior of microtubule tips and dendrite morphology\[^{[27]}\]. Given its critical role in regulating the cytoskeleton and dendrite growth, it is postulated to be involved in the pathogenesis of several neurological disorders.

Genes related to inflammation and cell death were also identified in POAF+NCD vs. SR+NORM. KIDINS200, discussed above, has a known role in T-cell receptor-mediated T-cell activation in addition to its neurologic functions\[^{[28]}\]. At 4D, up-regulated pro-inflammatory genes include NCR1 and DOCK. NCR1 encodes a natural killer cell receptor that triggers cytotoxicity, while DOCK1 is involved in cytoskeletal rearrangements required for phagocytosis\[^{[29,30]}\].

**CONCLUSION**

Our findings may expand what is known about the pathophysiology underlying POAF and NCD. While we cannot assert a true genetic association between POAF and NCD given our limited sample size, our results suggest that differential genomic responses existed in our study sample of patients who developed both complications after cardiac surgery. There may have been an influence of pathologic cardiac remodeling and involvement of genes with known roles in inflammation, cell death, and nervous system function that may have promoted POAF and NCD in our patient population. We hope that the database of regulated genes provided by this work sparks further study of differentially expressed pathways that may deepen our understanding of these important and costly complications and potentially offer means of risk stratification and improved patient management.

**Authors' roles & responsibilities**

| Author | Role |
|--------|------|
| RSD    | Analysis or interpretation of data; statistical analysis; final approval of the manuscript; study design |
| AAS    | Study design; final approval of the manuscript |
| Nye    | Study design; final approval of the manuscript |
| Br     | Study design; final approval of the manuscript |
| FWS    | Final approval of the manuscript; study design; implementation of projects/experiments |
REFERENCES

1. Gao L, Taha R, Gauvin D, Othmen LB, Wang Y, Blaise G. Postoperative cognitive dysfunction after cardiac surgery. Chest. 2005;128(5):3664-70.
2. Auer J, Weber T, Berent R, Ng CK, Lamm G, Eber B. Risk factors of postoperative atrial fibrillation after cardiac surgery. J Card Surg. 2005;20(5):425-31.
3. Eagle KA, Guyton RA, Davidoff R, Edwards FH, Evy GA, Gardner TJ, et al.; American College of Cardiology, American Heart Association. ACC/AHA 2004 guideline update for coronary artery bypass graft surgery: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1999 Guidelines for Coronary Artery Bypass Graft Surgery). Circulation. 2004;110(14):e340-437.
4. Murkin JM. Etiology and incidence of brain dysfunction after cardiac surgery. J Cardiothorac Vasc Anesth. 1999;13(suppl 1):12-7; discussion 36-7.
5. Newman MF, Kirchner JL, Phillips-Butte B, Gaver B, Grocott H, Jones RH, et al.; Neurological Outcome Research Group and the Cardiothoracic Anesthesiology Research Endeavors Investigators. Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. N Engl J Med. 2001;344(6):395-402.
6. Creswell LL, Schuessler RB, Rosenblum M, Cox JL. Hazards of postoperative atrial arrhythmias. Ann Thorac Surg. 1993;56(3):539-49.
7. Aranki SF, Shaw DP, Adams DH, Rizzo RJ, Couper GS, VanderVliet M, et al. Predictors of atrial fibrillation after coronary artery surgery. Current trends and impact on hospital resources. Circulation. 1996;94(3):390-7.
8. Ommen SR, Odel JA, Stanton MS. Atrial arrhythmias after cardiothoracic surgery. N Engl J Med. 1997;336(20):1429-34.
9. Fontes ML, Mathew JP, Rinder HM, Zelterman D, Smith BR, Rinder CS; Multicenter Study of Perioperative Ischemia (McSPI) Research Group. Atrial fibrillation after cardiac surgery/cardiopulmonary bypass is associated with monocyte activation. Anesth Analg. 2005;101(1):17-23.
10. Shirosita-Takeshita A, Schram G, Lavoie J, Nattel S. Effect of simvastatin and antioxidant vitamins on atrial fibrillation promotion by atrial-tachycardia remodeling in dogs. Circulation. 2004;110(16):2313-9.
11. Ramlawi B, Otu H, Rudolph JL, Mieno S, Kohane IS, Can H, et al. Genomic expression pathways associated with brain injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg. 2007;134(4):996-1005.
12. Mitchell-Jordan SA, Holopainen T, Ren S, Wang S, Warburton S, Zhang M, et al. Loss of Bmx nonreceptor tyrosine kinase prevents pressure overload-induced cardiac hypertrophy. Circ Res. 2008;103(12):1359-62.
13. Scortegagna M, Ding K, Otay G, Gaur A, Thurmond F, Yan LJ, et al. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Eps1-l/- mice. Nat Genet. 2003;35(4):331-40.
14. Chen AL, Ou CW, He ZC, Liu QC, Dong Q, Chen MS. Effect of hepatocyte growth factor and angiogenisin II on rat cardiomyocyte hypertrophy. Braz J Med Biol Res. 2012;45(12):1150-6.
15. Ren J, Zhang S, Kovacs A, Wang Y, Muslin AJ. Role of p38alpha MAPK in cardiac apoptosis and remodeling after myocardial infarction. J Mol Cell Cardiol. 2005;38(4):617-23.
16. Korantzopoulos P, Kolettis T, Siogas K, Goudevenos J. Atrial fibrillation and electrical remodeling: the potential role of inflammation and oxidative stress. Med Sci Monit. 2003;9(9):RA225-9.
17. López-Menéndez C, Gamar-Moralla A, Jurado-Arjona J, Higuero AM, Campanero MR, Ferrer I, et al. Kidins220 accumulates with tau in human Alzheimer’s disease and related models: modulation of its calpain-processing by GSK3β/RIP1 imbalance. Hum Mol Genet. 2013;22(3):466-82.
18. Laht P, Ottsus M, Remm J, Veske A. B-plexins control microtube dynamics and dendrite morphology of hippocampal neurons. Exp Cell Res. 2014;326(1):174-84.
19. Deswal S, Meyer A, Fiala GJ, Eisenhardt AE, Schmitt LC, Salek M, et al. Kidins220/ARMs associates with B-Raf and the TCR, promoting sustained Erk signaling in T cells. J Immunol. 2013;190(5):1927-35.
20. Pessino A, Sivori S, Bottino C, Malaspina A, Morelli L, Moretta L, et al. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. J Exp Med. 1998;188(5):953-60.
21. Wu YC, Horvitz HR. C. elegans phagocytosis and cell-migration protein CED-5 is similar to human DOK180. Nature. 1998;392(6675):501-4.
22. Zhou R, Snyder PM. Nedd4-2 phosphorylation induces serum and glucocorticoid-regulated kinase (SGK) ubiquitination and degradation. J Biol Chem. 2005;280(6):4518-23.
23. Kaiser P, Seufert W, Höfferer L, Kofler B, Sachsenmaier C, Herzog H, et al. A human ubiquitin-conjugating enzyme homologous to yeast UBC8. J Cell Res. 2014;326(1):174-84.
24. Deswal S, Meyer A, Fiala GJ, Eisenhardt AE, Schmitt LC, Salek M, et al. Kidins220/ARMs associates with B-Raf and the TCR, promoting sustained Erk signaling in T cells. J Immunol. 2013;190(5):1927-35.
25. Pessino A, Sivori S, Bottino C, Malaspina A, Morelli L, Moretta L, et al. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. J Exp Med. 1998;188(5):953-60.
26. Wu YC, Horvitz HR. C. elegans phagocytosis and cell-migration protein CED-5 is similar to human DOK180. Nature. 1998;392(6675):501-4.
27. Zhou R, Snyder PM. Nedd4-2 phosphorylation induces serum and glucocorticoid-regulated kinase (SGK) ubiquitination and degradation. J Biol Chem. 2005;280(6):4518-23.
28. Kaiser P, Seufert W, Höfferer L, Kofler B, Sachsenmaier C, Herzog H, et al. A human ubiquitin-conjugating enzyme homologous to yeast UBC8. J Biol Chem. 1994;269(12):8797-802.
29. Ramlawi B, Rudolph JL, Mieno S, Feng J, Boodhvnani M, Khabbaz K, et al. C-Reactive protein and inflammatory response associated to neurocognitive decline following cardiac surgery. Surgery. 2006;140(2):221-6.
30. Hogan AM, Shipolini A, Brown MM, Hurley R, Cormack F. Fixing hearts and protecting minds: a review of the multiple, interacting factors influencing cognitive function after coronary artery bypass graft surgery. Circulation. 2013;128(2):162-71.
31. Fontes ML, Amar D, Kulak A, Koval K, Zhang H, Shi W, et al. Increased preoperative white blood cell count predicts postoperative atrial fibrillation after coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth. 2009;23(4):4518-23.
32. Canbaz S, Erbas H, Huseyin S, Duran E. The role of inflammation in atrial fibrillation following open heart surgery. J Int Med Res. 2006;34(4):331-40.