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Original Article

Corrective surgery alters plasma protein profiling in congenital heart diseases and clinical perspectives

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Abstract: The final goal for treatment of congenital heart diseases (CHD) is to resume not only the normal heart structure but also physiology. The present study evaluates surgical results at molecular basis on the proteomic pattern in the pre- and post-operative period in tetralogy of Fallot (TOF) and ventricular septal defect (VSD) in order to find whether structure repair is associated with clinically important molecular changes in CHD. Differential protein analysis by using two-dimensional gel electrophoresis and mass spectrometry followed by ELISA validation was performed in the plasma samples of patients with TOF (n=82) or VSD (n=82) preoperatively, 6-month postoperatively, and in normal controls (n=82). A total of 473 protein spots in preoperative patients and 515 in postoperative patients were detected. Significantly (P<0.01) downregulated or upregulated proteins were detected. Validation of proteins in the new cohort of patients demonstrated that in VSD patients, postoperative complement component C3c (P<0.05) was partially and serum amyloid P-component (P<0.05) was completely recovered. In TOF patients, postoperative gelsolin (P<0.05) was partially recovered. In contrast, the elevated fibrinogen gamma chain level (P<0.01) in preoperative patients became normal postoperatively (P=0.1 vs. control). Thus, we have for the first time by using proteomic methods demonstrated that repair surgery for CHD not only corrects the structure malformation but also resumes the normality of certain altered proteins at molecular level. Identification of the recovered or unchanged proteins may facilitate the evaluation of the surgical results and the personalized management in postoperative period and long-term.

Keywords: Congenital heart disease, surgery, repair, tetralogy of fallot, ventricular septal defect

Introduction

The traditional way to evaluate the result of a therapeutic method is mainly from the patient's clinical symptoms and signs. With new technology developed, it may extend to more precise ways by detecting or evaluating the biological specimens (cell populations, proteins, metabolites, RNA, and DNA- including whole-genome sequencing) in the era of precision medicine [1]. Better biomarkers are needed to assist with disease detection and to help guide treatment, particularly for common acquired conditions without a strong genetic predisposition [2]. In view of this, to evaluate repair surgery for congenital heart diseases (CHD), in addition to the traditional method, such as using echocardiography to detect whether the heart structure is normal after heart surgery, new technologies based on multi-omics may play a role.

CHD are the most common birth defects affecting nearly 1% of human live births and are the leading causes of death in infants in the last decades [3]. Ventricular septal defect (VSD) is one of the most prevalent types of CHD, occurring in 30-60% of all neonates with various kinds of CHD [4]. Tetralogy of Fallot (TOF), on the other hand, represents the most common type of cyanotic CHD. Surgical repair remains the major method of treatment although cathe-

ter-based repair is now used in some simple CHD cases.

Genetic causes of CHD have been increasingly reported. Multiple mutations such as in GATA4, TBX5, NKX2-5, Homez, PLAGL1 genes were identified on the VSD patients [5-9]. Similarly, a number of loci of variations have been identified in TOF patients [10]. In addition to the genetic causes, the differentially expressed plasma proteins that may reflect pathogenesis during development of the disease are clinically important. We have recently reported such differential plasma proteins in both VSD and TOF patients in the pre-operative period [11, 12]. These studies revealed plasma protein changes in CHD that may indicate the possible mechanisms for the prolonged bleeding time in TOF patients, the susceptibility to pulmonary infections in patients with CHDs [11], and altered acute phase proteins in VSD patients [12]. Further, we also demonstrated the proteomics changes in CHD with complications such as pulmonary arterial hypertension [13] and other heart diseases [14-16].

The final goal for treatment of CHD is to resume not only the normal heart structure but also normal life. Corrective surgery may turn the structure malformation of the heart to normal but it is unknown whether such repair is associated with molecular changes towards normal. In order to explore the surgical results at molecular basis in individuals towards "Precision Medicine" in the postoperative rehabilitation and the long term outcome, the knowledge regarding basic molecular changes after surgery is of importance. We therefore designed the present study to compare the proteomic profiling in the pre- and post-operative period in patients with TOF and VSD in order to find whether structure repair is associated with clinically important molecular changes in CHD.

Materials and methods

From Aug 2011 to 2015, blood samples were taken from patients with TOF (n=82) and VSD (n=82) at TEDA International Cardiovascular Hospital, Tianjin, China. The blood samples from 82 children with same ethnic, gender, and age for body-checking or in the CHD-screening program that demonstrated normal heart and no other diseases were used as control. All control subjects were confirmed by clinical screening plus echocardiography that confirmed no cardiac diseases. The protocol was approved by the Ethics Committee of TEDA-International Cardiovascular Hospital, Tianjin, China and informed consent was obtained from their parents or guardians. The demographics of the CHD patients and controls are shown in **Table 1**. Blood were taken before operation (n=41) in TOF and VSD (n=41) patients. During postoperative follow-up at the outpatient clinic, usually 6 months or longer after operation, blood was also taken in patients with TOF (n=41) or VSD (n=41). Routine clinical assessment of the patients was performed. All patients underwent corrective surgery and the diagnosis was verified by preoperative echocardiography and corrective surgery. Other diagnostic procedures such as MRI or CT scan were also used to help the definitive diagnosis when appropriate. Other complex CHDs were excluded. The enrolled VSD phenotypes included perimembranous (68), subarterial infundibular (7), and infracristal or intracristal type (7).

Sample preparation

Blood samples were taken from patients before surgery and 6 months after surgery as described above. From each sample, 2 ml blood was allowed to clot at 4°C for at least 2 h and then centrifuged at 1500 g for 10 min to reach sediment of the clotted cells. Plasma was then collected, divided into aliquots, and stored frozen at -80°C until the analysis was carried out.

Grouping of the samples

There were totally two groups and 6 subgroups: Preoperative group: I: VSD (N=41). II: TOF (N=41). III: the control (N=56). Postoperative group: Ia: VSD (N=41). IIa: TOF (N=41). IIIa: the control (N=41).

These subgroups of samples were studied in either proteomics or ELISA validations.

Proteomic study

Plasma high-abundance protein depletion: For the proteomic study, the plasma samples from TOF patients (n=15), VSD patients (n=15), and healthy controls (n=30 in pre-operative and n=15 in post-operative, respectively) were collected in these periods. These samples were separately pooled in each group and processed

Table 1. Demographics of study population

Stage	Groups	Number	Sex (M/F)	Age (years)
2-DE	VSD (preop)	15	8/7	3.4 ± 4.6
	TOF (preop)	15	8/7	2.2 ± 9.9
	Controls	30	16/14	4.5 ± 11.5
	VSD (postop)	15	8/7	1.3 ± 1.1
	TOF (postop)	15	8/7	2.2 ± 2.0
	Controls	15	8/7	2.6 ± 1.0
ELISA (VSD)	VSD (preop)	26	13/13	2.8 ± 1.6
	VSD (postop)	26	13/13	1.0 ± 0.9
	Controls	26	13/13	2.8 ± 1.5
ELISA (TOF)	TOF (preop)	26	18/8	2.6 ± 2.6
	TOF (postop)	26	18/8	1.6 ± 1.7
	Controls	26	18/8	2.3 ± 0.8

2-DE, two-dimensional electrophoresis; ELISA, enzyme linked immunosorbent assay; TOF, tetralogy of Fallot; VSD, ventricular septal defect; M, male; F, female.

to deplete high abundance proteins (albumin, immunoglobulin [Ig] G) using the ProteoExtract™ Albumin/IgG Removal Kit (Calbiochem, La Jolla, CA, USA). Samples were processed according to the manufacturer's instructions. Use of pooled serum samples from multiple patients is a well-accepted approach to better detect the differential proteins between the patients and controls [17]. The pre-operative and post-operative samples had their own healthy controls.

Two-dimensional gel electrophoresis (2-DE): The first-dimensional gel separation was carried out with Ready Strip immobilized pH gradient (IPG) strips (Bio-Rad, CA, USA), following the manufacturers protocol. About 500 μg protein of plasma for gel was diluted to 170 μL with rehydration solution (5 M urea, 2 M thiourea, 2% for 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate, 100 mM dithiothreitol, 0.5% v/v pH 3-10 IPG buffer, 40 mM Tris base, 2% sulfobetaine 3-10, and trace bromophenol blue), and applied to immobilized 17 cm pH 3-10 nonlinear gradient strips by overnight rehydration at 50 V.

Isoelectric focusing (IEF) was performed with an Ettan IPGphor II apparatus (GE Healthcare, Waukesha, WI) as followed steps: 0-500 V (500 Vh), 500 V (2,500 Vh), 500-3,500 V (10,000 Vh), 3,500 V (50,000 Vh), and 3,500-500 V (8,000 Vh) for a total of 7.1 kVh. All IEF steps were done at 20°C. After the first-dimensional IEF, IPG gel strips were placed in an equilibration solution (6 M urea, 2% sodium dodecyl sulfate, 30% glycerol, 50 mM Tris-HCl, pH 8.8) containing 1% dithiothreitol for 10 minutes with shaking at 50 rpm on an orbital shaker. The gels were then transferred to the equilibration solution containing 2.5% iodoacetamide and shaken for another 10 minutes before placing on 12% polyacrylamide gel slab.

The 2-DE separation was performed by using Protean II electrophoresis equipment and Tris-glycine buffer (25 mM Tris, 192 mM glycine) containing 0.1% sodium dodecyl sulfate at a current setting of 5 mA/gel for the initial first hour, and 10 mA/gel thereafter. The second-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis was developed until the bromophenol blue dye marker had reached the bottom of the gel. This process was repeated 3 times in each group as previously reported [11, 12, 15].

Image analysis

After 2-DE separation, there were totally 18 gels to be stained including the 3 technical repeats for each subgroup of the samples (there were 6 subgroups as mentioned above). The gels were stained with Coomassie blue R-250 (Sigma, St Louis, MO, USA). Spot detection and quantification were done using PD Quest 2-DE analysis software (Bio-Rad, Hercules, CA). Spot intensity was quantified automatically by calculation of spot volume after normalization of the image by taking the ratio of intensity of one spot to the total spots, and expressed as a fractional intensity. Only those spots with significant change between the disease group (TOF or VSD) and control ($P<0.01$ by unpaired t test) in expression intensity were selected for MS analysis.

Tryptic digestion

Protein spots were excised from the gel with the Ettan Spot Picker and destained with 25 mM ammonium bicarbonate, 50% acetonitrile. Gels were then dried completely by centrifugally ophilization. Each spot was digested overnight in trypsin for 16 hours at 37°C. The pep-

tides were extracted 3 times with 50% acetonitrile, 0.1% trifluoroacetic acid, and dried completely by centrifugal lyophilization.

MALDI/TOF MS

Before the analysis, external calibration with angiotensin II and ACTH 18-39 was used. This procedure typically results in mass accuracies of 100 ppm or better. The peptide mixture (0.3 μl) was then mixed with a-cyano-4-hydroxycinnamic acid matrix (1:1, v/v) and analyzed on an ABI 4700 MALDI-TOF/TOF Plus mass spectrometer (Applied Biosystems, USA), operated in the delayed extraction and reflector mode. The laser wavelength was 337 nm, and the laser repetition rate was 3 Hz. The MALDI spectra were averaged over 200-400 laser shots.

MALDI/TOF MS data analysis and bioinformatics analysis

Data were integrated and processed by using GPS Explorer software (Version 2.4.1, Applied Biosystems, USA). The peak list generated by 4000 Series Explorer software (Version 2.0, USA) was analyzed using the MASCOT (Version 2.4.1, Matrix Science, London, UK) search engine, which searched the 202,522 entries in the protein databases of the National Center for Biotechnology Information (NCBI) non-redundant human database (downloaded on 11/05/2014) Protein identification was carried out by peptide mass finger-printing (PMF). Search parameters were set as follows: precursor tolerance: ± 0.1 Da; missed cleavages: 1; fixed modifications: carbamidomethyl; variable modifications: oxidation. Threshold score/expectation value for accepting individual spectra was set to ion score 10. The MASCOT score from the sample with ammonium phosphate was higher than that from the sample without ammonium phosphate, thus increasing the confidence in the correct identification of the proteins.

Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) [18], and STRING were performed for downstream analysis. GO contains biological process, cellular component, and molecular function categories. Enrichment analyses of KEGG pathways were performed with same hypergeometric algorithm used in gene ontology enrichment analysis. The pathways were clustered into the following sub-categories: metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases. In GO analysis, some previously reported preoperative proteomic study [11, 12] was incorporated for a complete view.

Enzyme-linked immunosorbent assay

Further validation of candidate proteins was performed by using human ELISA kits (CUSABIO BIOTECH, Life Sciences Advanced Technologies Inc., USA) to detect plasma levels in a new cohort of pre- and post-operative patients (VSD or TOF) and their own control (N=26 for each group). The methods followed the manufacturer's instructions. The identification of candidate proteins for further validation was based on 1) expressed differently between VSD or TOF patients and the control; 2) potential functional or pathological significance in CHD; and 3) more than 1 peptide was identified by LC-MS/MS.

Statistical analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL) and GraphPad Prism 5 Demo software (GraphPad Software, San Diego, CA) were used for statistical analysis. GO, KEGG, and STRING analyses were performed by using multi-omics data analysis tool (omicsbean, http://www.omicsbean.cn). The spot intensity was compared by unpaired t test. One-way ANOVA was used for comparing the ELISA verification among three groups. Data were expressed as mean ± SD. $P<0.05$ was considered as significant.

Results

2-DE analysis, MALDI/TOF MS identification

In proteomic studies, a total of 473 protein spots in preoperative patients (Table S1) and 515 in postoperative patients (Table S2) were detected. Significantly ($P<0.01$ vs. control) downregulated (11 in preoperative and 15 in postoperative group) and upregulated (14 in preoperative and 7 in postoperative group) protein spots were detected.

All 25 protein spots of interest in preoperative patients and 22 in postoperative patients were trypsin-digested and MALDI-TOF MS was used for the identification of the protein spots. In pre-

operative study, 20 protein spots and 10 corresponding proteins or polypeptides could be identified whereas 5 protein spots did not have PMF or did not have corresponding proteins in the MASCOT database. In postoperative study, 15 protein spots and 11 corresponding proteins or polypeptides could be identified (**Table 2**) whereas 7 protein spots did not have PMF or did not have corresponding proteins in the MASCOT database.

GO, KEGG, and STRING analysis of the differentially expressed proteins (DEPs)

GO enrichment and KEGG pathway analysis were performed for an overview of the function of all of the DEPs and the potential linkage among them. **Figure 1A** and **1B** display the amount of GO enrichment analyses (biological process, cellular component, and molecular function) and KEGG pathways based on the DEPs (**Figure 1C**, **1D**). *P* value <0.05 represents the significant GO terms and pathways for the DEPs between the disease group compared to the control in homo sapiens from GO and KEGG database. The DEPs were most significantly involved in regulation of heterotypic cell-cell adhesion in biological process, blood microparticle in cellular component, and protein binding-bridging in molecular function in preoperative proteomics results (**Figure 1E**). However, the DEPs were most significantly involved in apoptotic cell clearance in biological process, blood microparticle in cellular component, and C5a anaphylatoxin chemotactic receptor binding in molecular function in postoperative results (**Figure 1F**). Further KEGG pathway enrichment and STRING analysis (**Figure 2**) revealed that these proteins were mainly involved in complement and coagulation cascades and staphylococcus aureus infection in pre- (**Figure 2A**, **2C**) and post- (**Figure 2B**, **2D**) operative patients respectively. In the analysis, the common pathway was complement and coagulation cascade that is closely related to the pathology of CHD.

Significantly altered protein expression in patients with TOF and VSD

The present study emphasizes the proteomic profile after surgery. **Table 2** shows the altered proteins in the postoperative period that were most significant and confirmed with mass spectrometry. In the post-operative proteomic study in TOF group, three up-regulated proteins or polypeptides (Alpha-1-antichymotrypsin, Alpha-1-antitrypsin, Fibrinogen gamma chain) and five down-regulated proteins or polypeptides (ceruloplasmin, C4B, serotransferrin, inter-alpha-trypsin inhibitor, H-ficolin) were identified (**Table 2**). In the postoperative VSD group, three down-regulated proteins or polypeptides (macroglobulin alpha2, complement component C3c, fibrinogen beta chain) were identified (**Table 2**).

Figures 3 and **4** demonstrate the differentially expressed proteins that were most significant in the proteomic study in preoperative patients with VSD (**Figure 3A**) or TOF (**Figure 4A**) and in postoperative patients with repaired VSD (**Figure 3B**) or TOF (**Figure 4B**). In details, **Figure 3** shows the 5 protein spots that were identified in either pre- or post-operative patients with VSD in the proteomic analysis. These are ficolin-3, alpha-1-glycoprotein 2, haptoglobin, serum amyloid P-component, and complement component C3c. Similarly, **Figure 4** shows the 3 protein spots that were identified in either pre- or post-operative patients with TOF in the proteomic analysis. Those are ficolin-3, gelsolin, and fibrinogen gamma chain. All the proteins were identified by mass spectrometry and the maps are shown in the lower middle panel in **Figures 3** and **4**.

Validation of the altered plasma proteins by ELISA

Further validation of the chosen proteins (ficolin-3, gelsolin, alpha-1-acid glycoprotein 2, haptoglobin, serum amyloid P-component, fibrinogen gamma chain, and complement component C3c) was performed by ELISA in 78 new patients (**Table 3**). **Figure 5** shows the results of ELISA validation of the differential proteins in pre-operative and post-operative patients with VSD (A-E, n=26), TOF (F-H, n=26), and in normal controls (n=26).

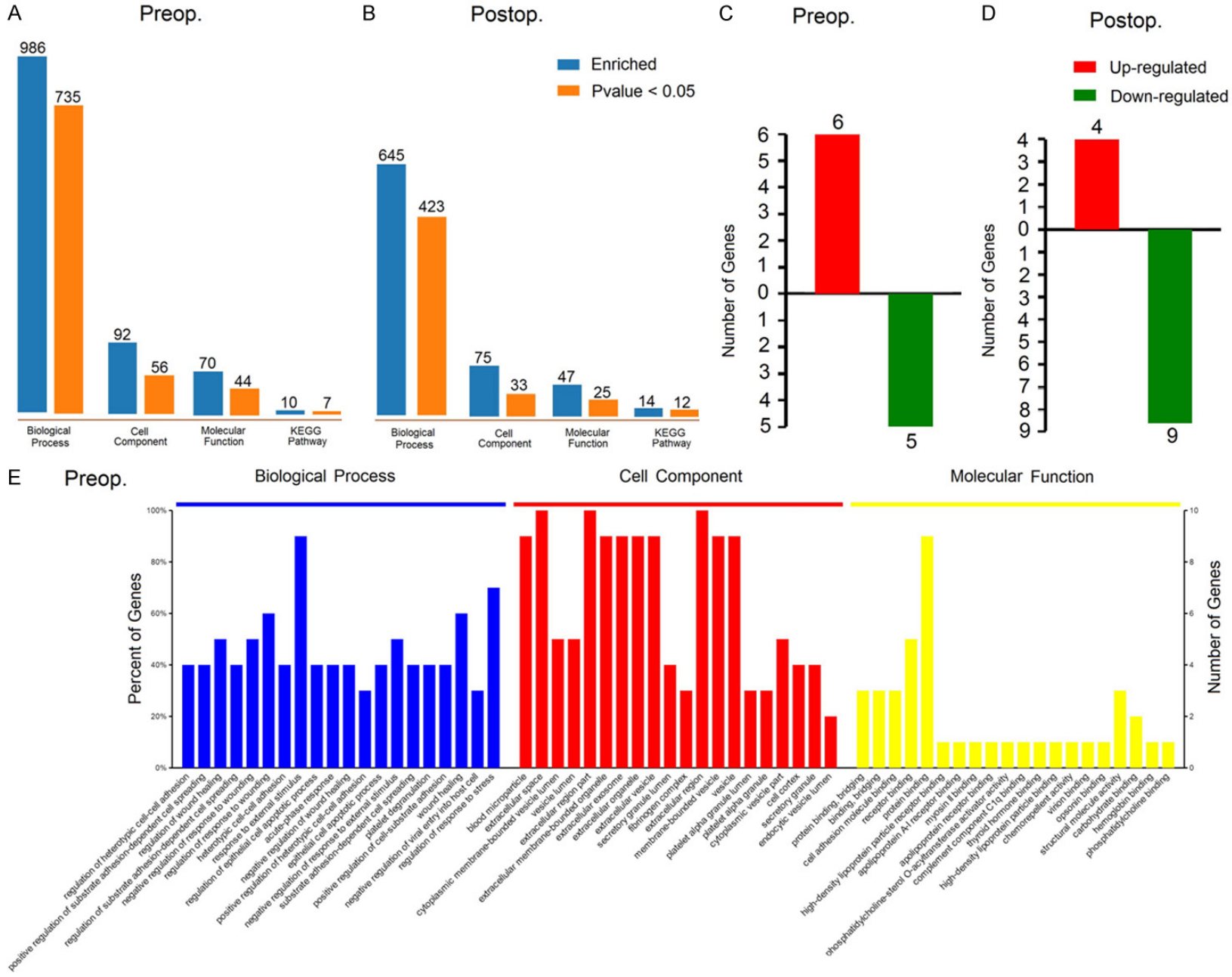
Discussion

The present study for the first time demonstrates that repair surgery in CHD changes the protein profile and that some of the preoperatively-changed proteins may be altered towards normal level after surgery. This study shows that repair surgery for either complex cyanotic CHD such as TOF or simple CHD such as VSD may change the pathological process of the disease to resume normal physiology.

Table 2. Differentially expressed proteins identified by the matrix-assisted laser desorption-ionization time-of-flight mass spectrometry in postoperative patients

Protein Identification in the Plasma of Patients	Groups	MW (Da)[a]	pI[b]	Protein Score CI (%)	Representative Amino Acid Position	Representative Sequence	Numbers of mass values matched	Sequence coverage (%)
Down-regulated								
Ceruloplasmin	TOF	116196.7	5.43	99.088	178-187	IYHSHIDAPK	9	8
C4B	TOF	48064.5	5.78	99.276	148-153	LPMSVR	10	22
Macroglobulin alpha2	VSD	162072.5	5.95	99.998	331-336	VDSHFR	20	15
Serotransferrin	TOF	79280.5	6.81	100.000	565-571	NPDPWAK	31	40
Complement component C3c	VSD	40204.4	4.79	100.000	56-61	RPQDAK	17	52
Complement component C3c	VSD	21596.3	5.84	100.000	87-93	LPYSVVR	11	56
Inter-alpha-trypsin inhibitor	TOF	103549.0	6.51	100.000	289-294	KIQQTR	17	16
H-Ficolin	TOF	25410.1	6.14	99.963	208-214	GVGHPYR	8	29
Fibrinogen beta chain	VSD	40167.1	6.95	100.000	229-234	ISQLTR	12	26
Up-regulated								
Alpha-1-antichymotrypsin	TOF	49985.7	5.5	99.970	172-178	FTEDAKR	13	27
Alpha-1-antitrypsin	TOF	44839.0	5.31	100.000	388-394	VVNPTQK	21	60
Alpha-1-antitrypsin	TOF	44839.0	5.31	100.000	388-394	VVNPTQK	24	60
Fibrinogen gamma chain	TOF	47971.1	5.54	100.000	385-389	WYSMK	20	46
Fibrinogen gamma chain	TOF	47971.1	5.54	100.000	385-389	WYSMK	20	51
Fibrinogen gamma chain	TOF	50077.2	5.61	100.000	402-406	WYSMK	17	41

MW (Da)[a] molecular weight; pI[b], isoelectric point.


A
Preop.
986
735
92
56
70
44
10
7
Biological Process
Cell Component
Molecular Function
KEGG Pathway
B
Postop.
Enriched
Pvalue < 0.05
645
423
75
33
47
25
14
12
Biological Process
Cell Component
Molecular Function
KEGG Pathway
C
Preop.
Number of Genes
6
5
D
Postop.
Up-regulated
Down-regulated
Number of Genes
4
9
E
Preop.
Biological Process
Cell Component
Molecular Function
Percent of Genes
Number of Genes
regulation of heterotypic cell-cell adhesion
positive regulation of substrate adhesion-dependent cell spreading
regulation of substrate adhesion-dependent cell spreading
regulation of wound healing
negative regulation of response to wounding
regulation of response to wounding
heterotypic cell-cell adhesion
response to external stimulus
regulation of epithelial cell apoptotic process
acute-phase response
negative regulation of wound healing
positive regulation of heterotypic cell-cell adhesion
epithelial cell apoptotic process
negative regulation of response to external stimulus
epithelial cell proliferation
substrate adhesion-dependent cell spreading
positive regulation of cell-substrate adhesion
platelet degranulation
negative regulation of viral entry into host cell
wound healing
regulation of response to stress
blood microparticle
extracellular space
extracellular vesicle lumen
vesicle lumen
extracellular region part
extracellular organelle
extracellular exosome
extracellular vesicle
secretory granule lumen
fibrinogen complex
extracellular region
membrane-bounded vesicle
vesicle
platelet alpha granule lumen
platelet alpha granule
cytoplasmic vesicle part
cell cortex
secretory granule
endocytic vesicle lumen
protein binding, bridging
binding, bridging
cell adhesion molecule binding
receptor binding
protein binding
high-density lipoprotein particle receptor binding
apolipoprotein A-I receptor binding
myosin II binding
apolipoprotein receptor binding
phosphatidylcholine-sterol O-acyltransferase activator activity
complement component C1q binding
thyroid hormone binding
high-density lipoprotein particle binding
chemorepellent activity
virion binding
opsonin binding
structural molecule activity
carbohydrate binding
hemoglobin binding
phosphatidylcholine binding

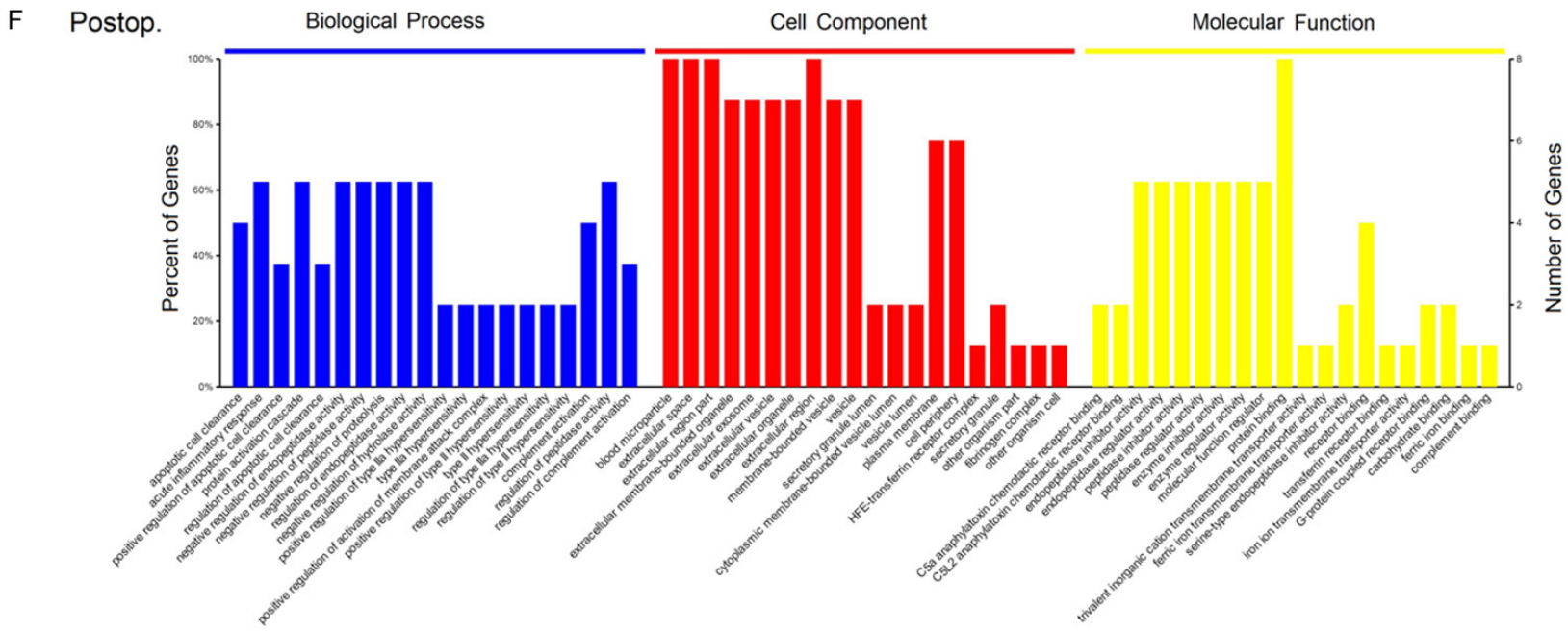
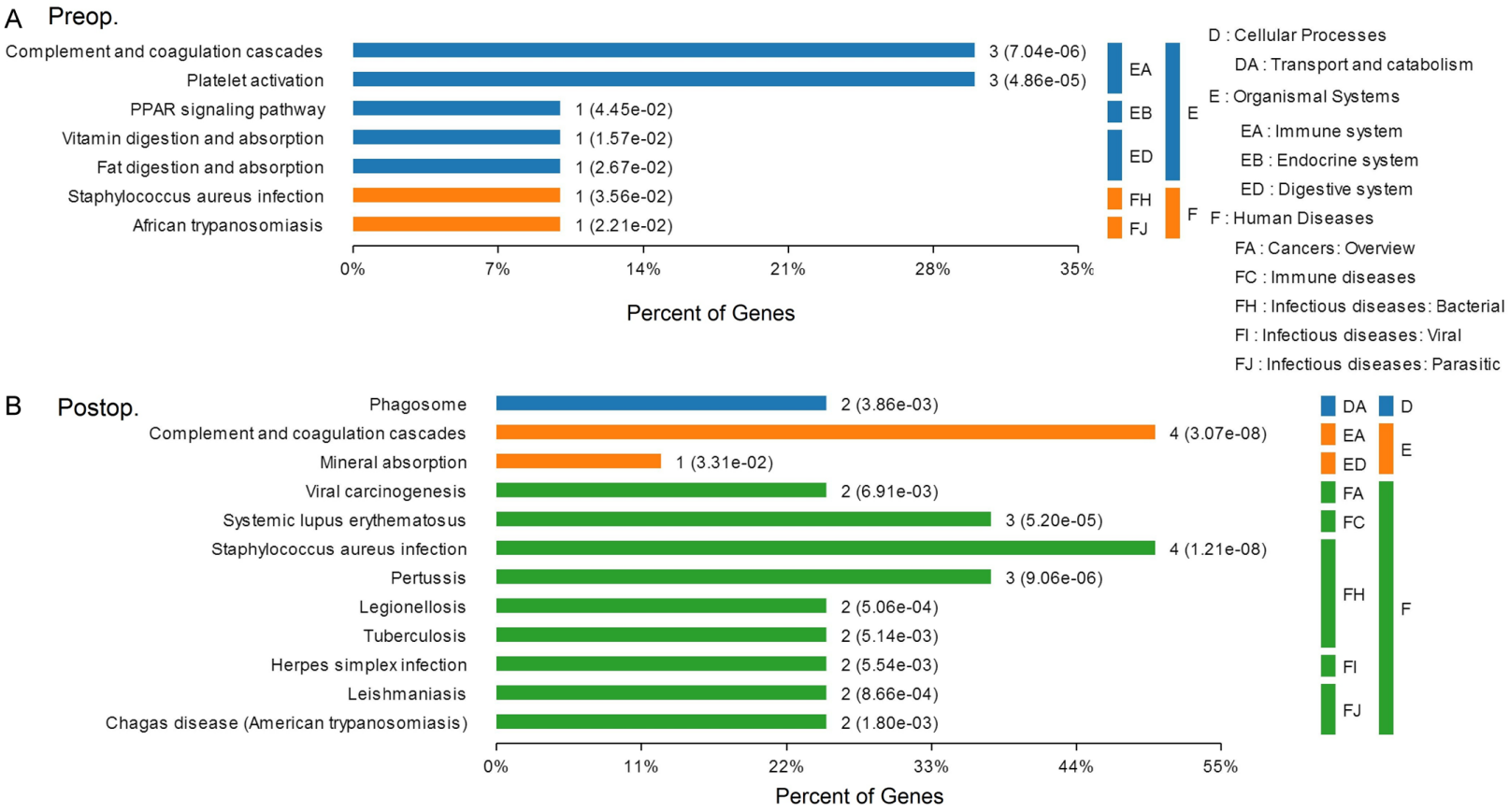


Figure 1. Summary of the protein profile in preoperative (Preop.), postoperative (Postop.) patients, and controls. (A and B) show the enriched number of Gene Ontology terms (biological process, cellular component, and molecular function) and Kyoto Encyclopedia of Genes and Genomes pathways associated with the query proteins. Each category with $P<0.05$ is statistically significant. There were 11 and 13 differentially expressed proteins identified in preop and postop patients, respectively (C, D). (E and F) show the top twenty significant Gene Ontology terms. Y-axis represents the percent and number of genes.


A Preop.
Complement and coagulation cascades
Platelet activation
PPAR signaling pathway
Vitamin digestion and absorption
Fat digestion and absorption
Staphylococcus aureus infection
African trypanosomiasis
3 (7.04e-06)
3 (4.86e-05)
1 (4.45e-02)
1 (1.57e-02)
1 (2.67e-02)
1 (3.56e-02)
1 (2.21e-02)
0%
7%
14%
21%
28%
35%
Percent of Genes
EA
EB
ED
FH
FJ
E
F
D : Cellular Processes
DA : Transport and catabolism
E : Organismal Systems
EA : Immune system
EB : Endocrine system
ED : Digestive system
F : Human Diseases
FA : Cancers: Overview
FC : Immune diseases
FH : Infectious diseases: Bacterial
FI : Infectious diseases: Viral
FJ : Infectious diseases: Parasitic
B Postop.
Phagosome
Complement and coagulation cascades
Mineral absorption
Viral carcinogenesis
Systemic lupus erythematosus
Staphylococcus aureus infection
Pertussis
Legionellosis
Tuberculosis
Herpes simplex infection
Leishmaniasis
Chagas disease (American trypanosomiasis)
2 (3.86e-03)
4 (3.07e-08)
1 (3.31e-02)
2 (6.91e-03)
3 (5.20e-05)
4 (1.21e-08)
3 (9.06e-06)
2 (5.06e-04)
2 (5.14e-03)
2 (5.54e-03)
2 (8.66e-04)
2 (1.80e-03)
0%
11%
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33%
44%
55%
Percent of Genes
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EA
ED
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FC
FH
FI
FJ
D
E
F

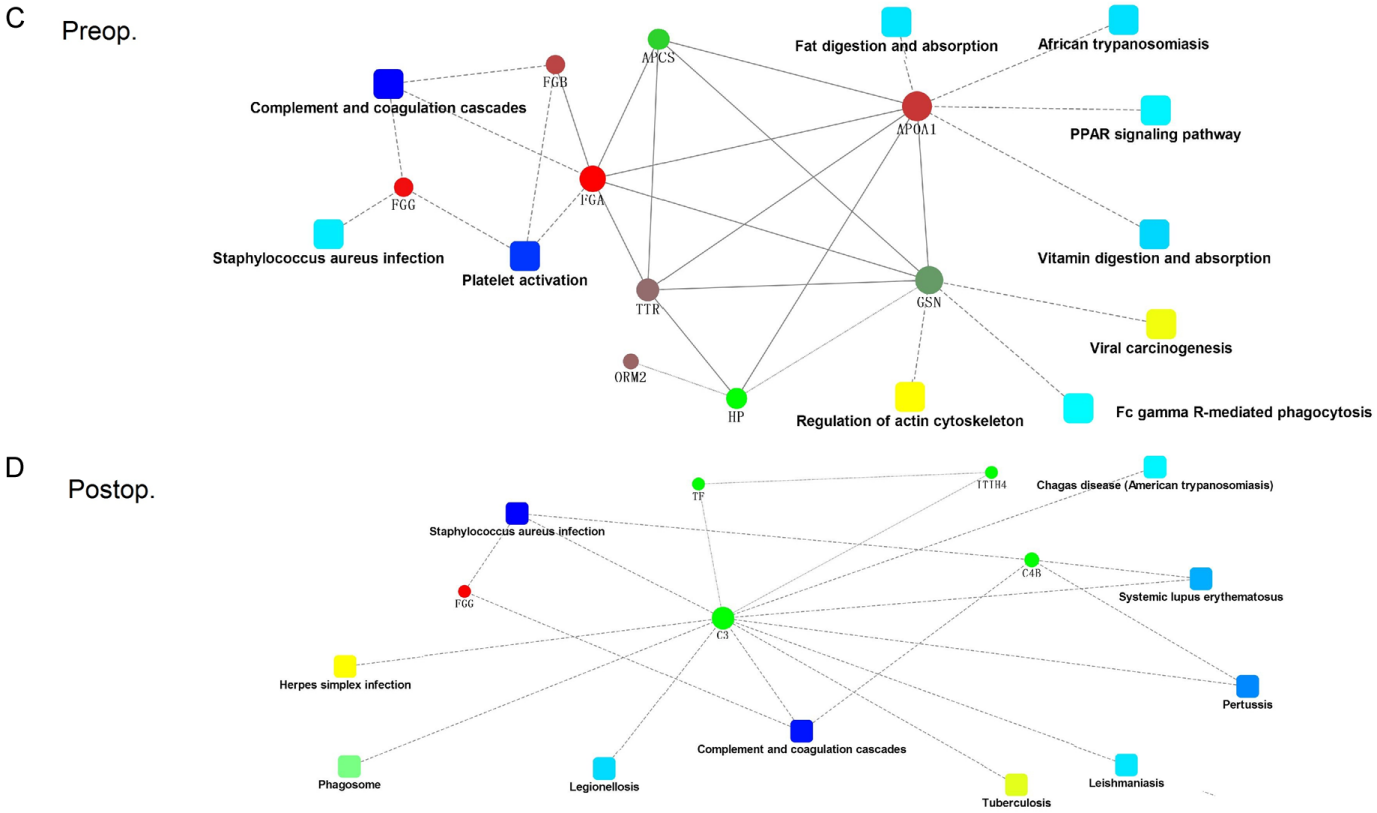
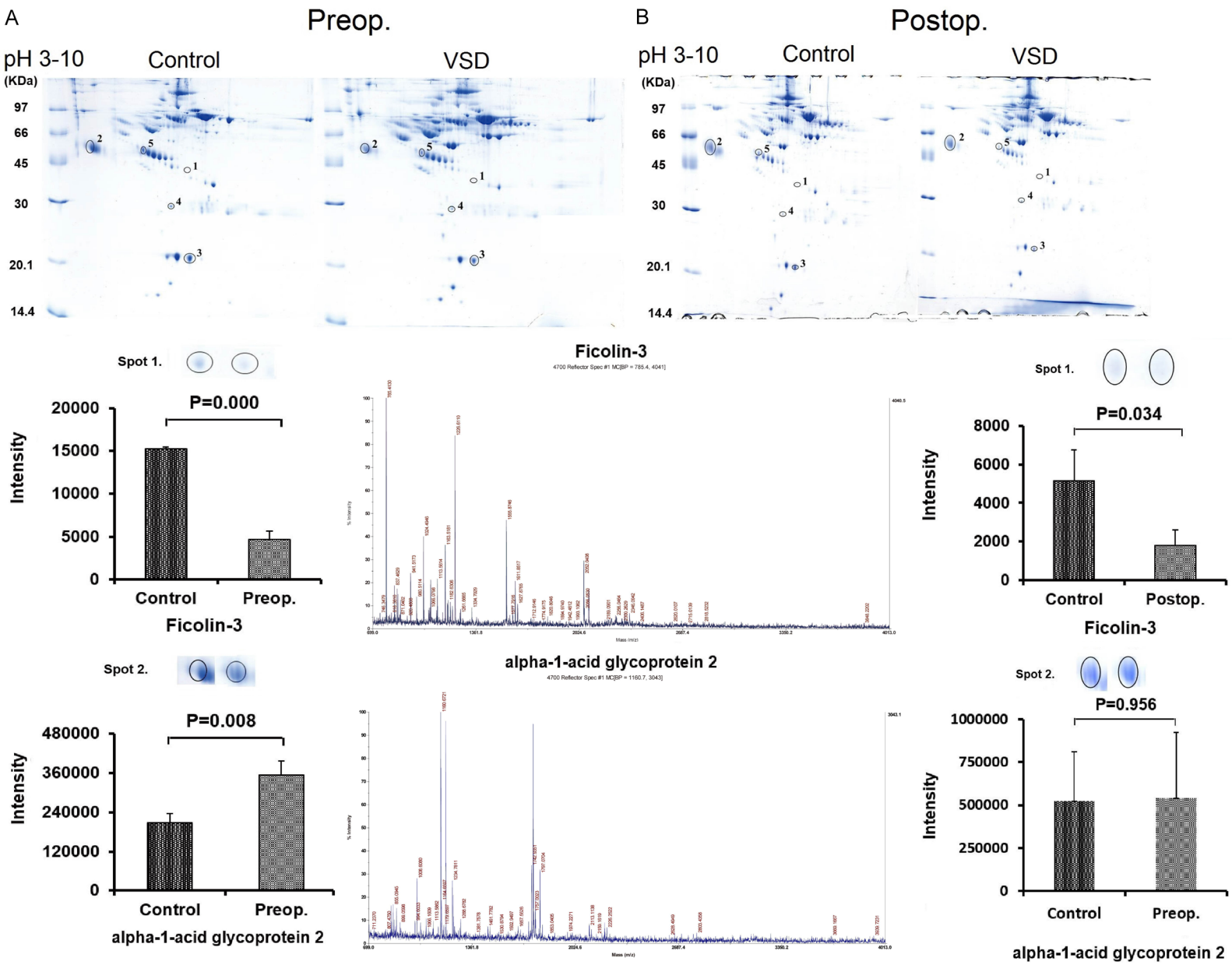


Figure 2. Most significantly associated Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of differentially expressed proteins and key regulation network model of protein-protein interaction in preoperative (A, C. Preop.), postoperative (B, D. Postop.) patients, and controls. In the model, circle nodes indicate proteins; filled color represents expression fold change using gradient color from blue to red; rectangles indicate KEGG pathways; filled color represents *P*-value using gradient color from yellow to blue. A solid line between two proteins indicates a known interaction annotated in the STRING database; a dashed line between proteins indicates indirect interaction.


A
Preop.
pH 3-10
Control
VSD
(KDa)
97
66
45
30
20.1
14.4
B
Postop.
pH 3-10
Control
VSD
(KDa)
97
66
45
30
20.1
14.4
Spot 1.
P=0.000
Intensity
20000
15000
10000
5000
0
Control
Preop.
Ficolin-3
Ficolin-3
4700 Reflector Spec #1 MC[BP = 785.4, 4041]
alpha-1-acid glycoprotein 2
Spot 1.
P=0.034
Intensity
8000
6000
4000
2000
0
Control
Postop.
Ficolin-3
Spot 2.
P=0.008
Intensity
480000
360000
240000
120000
0
Control
Preop.
alpha-1-acid glycoprotein 2
4700 Reflector Spec #1 MC[BP = 1160.7, 3043]
Spot 2.
P=0.956
Intensity
1000000
750000
500000
250000
0
Control
Preop.
alpha-1-acid glycoprotein 2

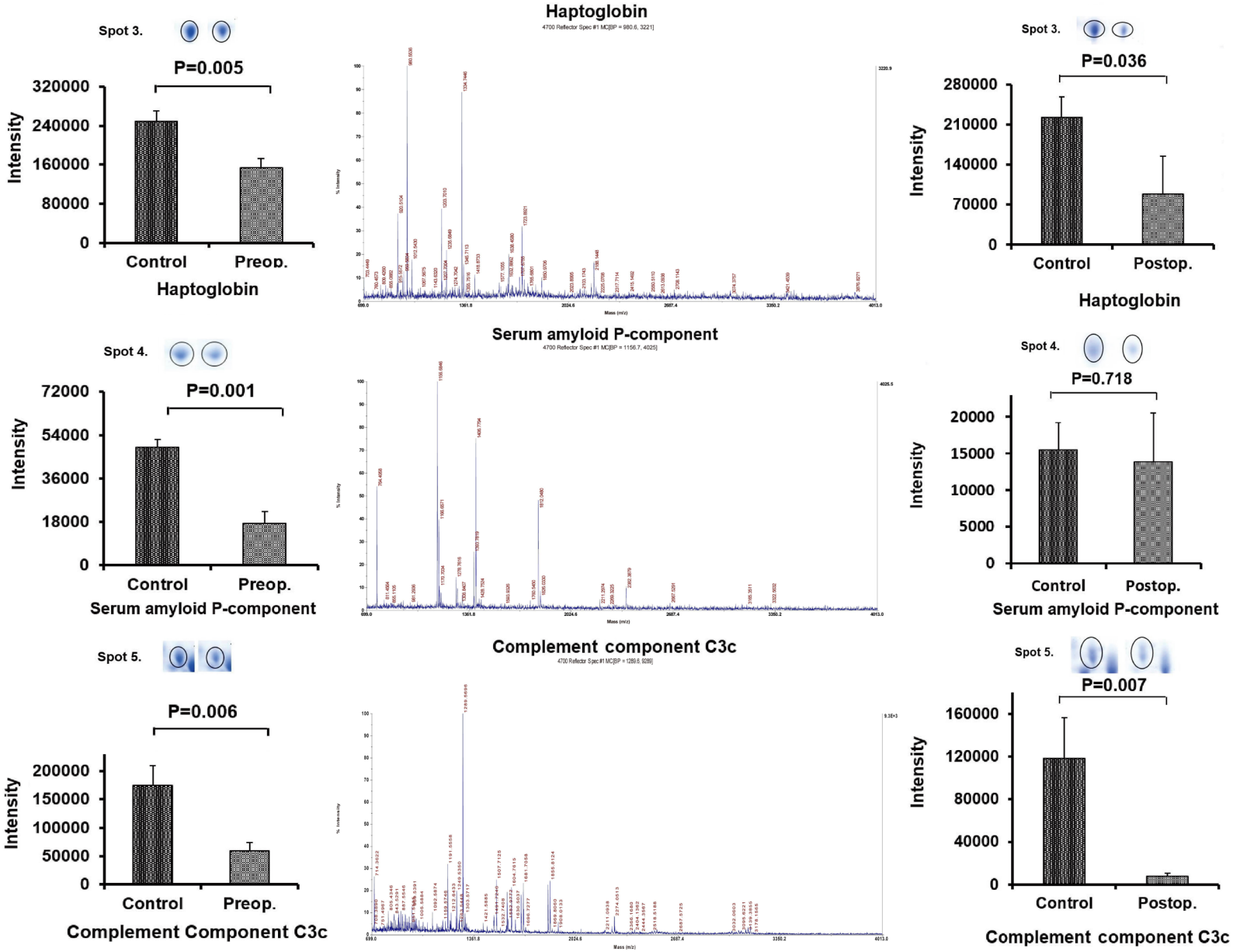
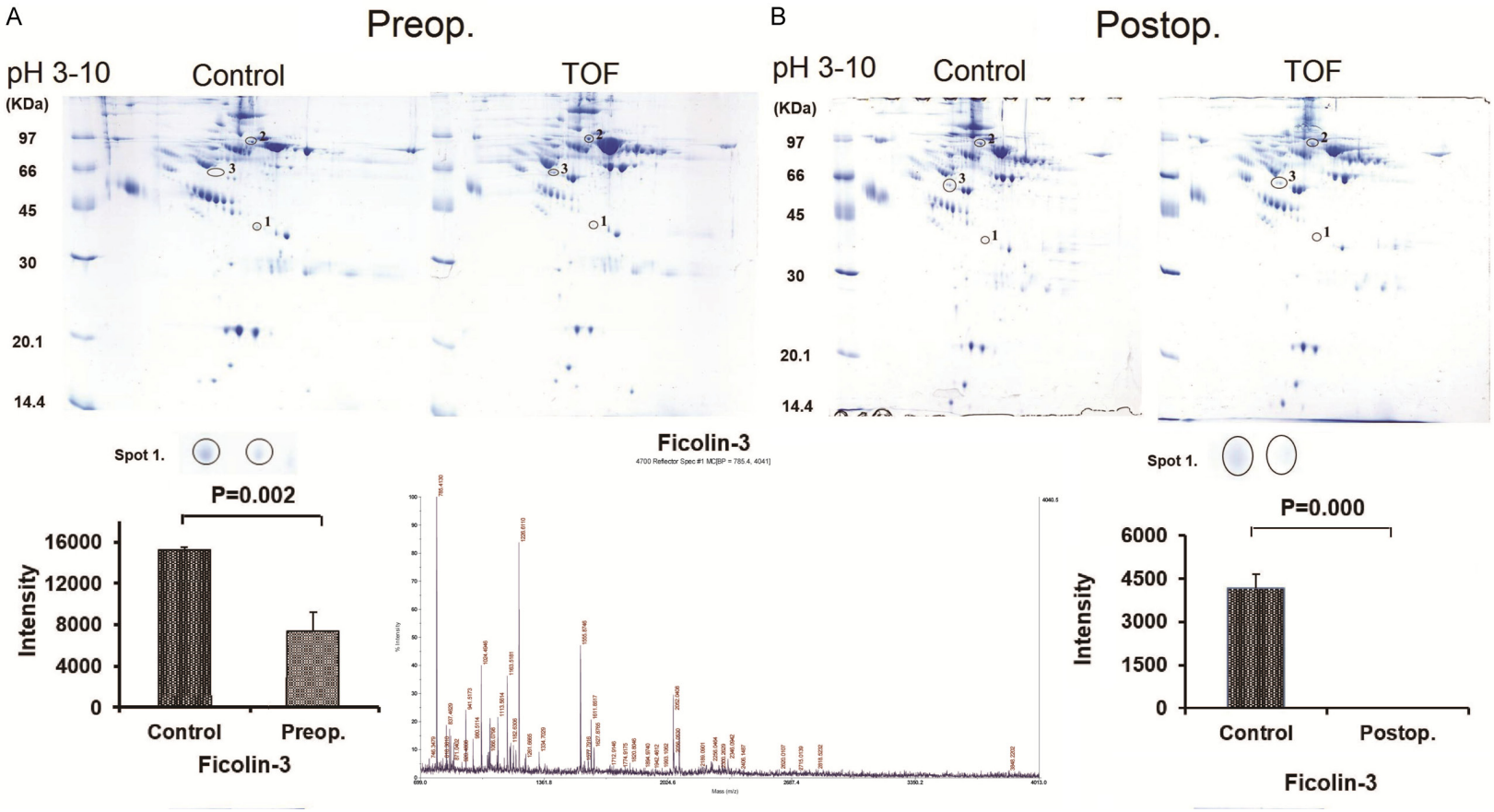

Spot 3.
P=0.005
Intensity
320000
240000
160000
80000
0
Control
Preop.
Haptoglobin
Haptoglobin
4700 Reflector Spec #1 MC[BP = 980.6, 3221]
Spot 3.
P=0.036
Intensity
280000
210000
140000
70000
0
Control
Postop.
Haptoglobin
Spot 4.
P=0.001
Intensity
72000
54000
36000
18000
0
Control
Preop.
Serum amyloid P-component
Serum amyloid P-component
4700 Reflector Spec #1 MC[BP = 1156.7, 4025]
Spot 4.
P=0.718
Intensity
20000
15000
10000
5000
0
Control
Postop.
Serum amyloid P-component
Spot 5.
P=0.006
Intensity
200000
150000
100000
50000
0
Control
Preop.
Complement Component C3c
Complement component C3c
4700 Reflector Spec #1 MC[BP = 1289.6, 9286]
Spot 5.
P=0.007
Intensity
160000
120000
80000
40000
0
Control
Postop.
Complement component C3c

Figure 3. Most significant differential proteins identified in proteomic study in preoperative (Preop.) patients with VSD (A) and in postoperative (Postop.) patients with VSD (B). Five spots with significant change (P<0.01) in expression intensity were selected for mass spectrometry analysis. The upper panel shows two-dimensional gel electrophoresis. The lower left and right panels show the spot intensity analyzed by unpaired t test. The lower middle panel shows the identified mass spectrum maps of each of these 5 proteins.



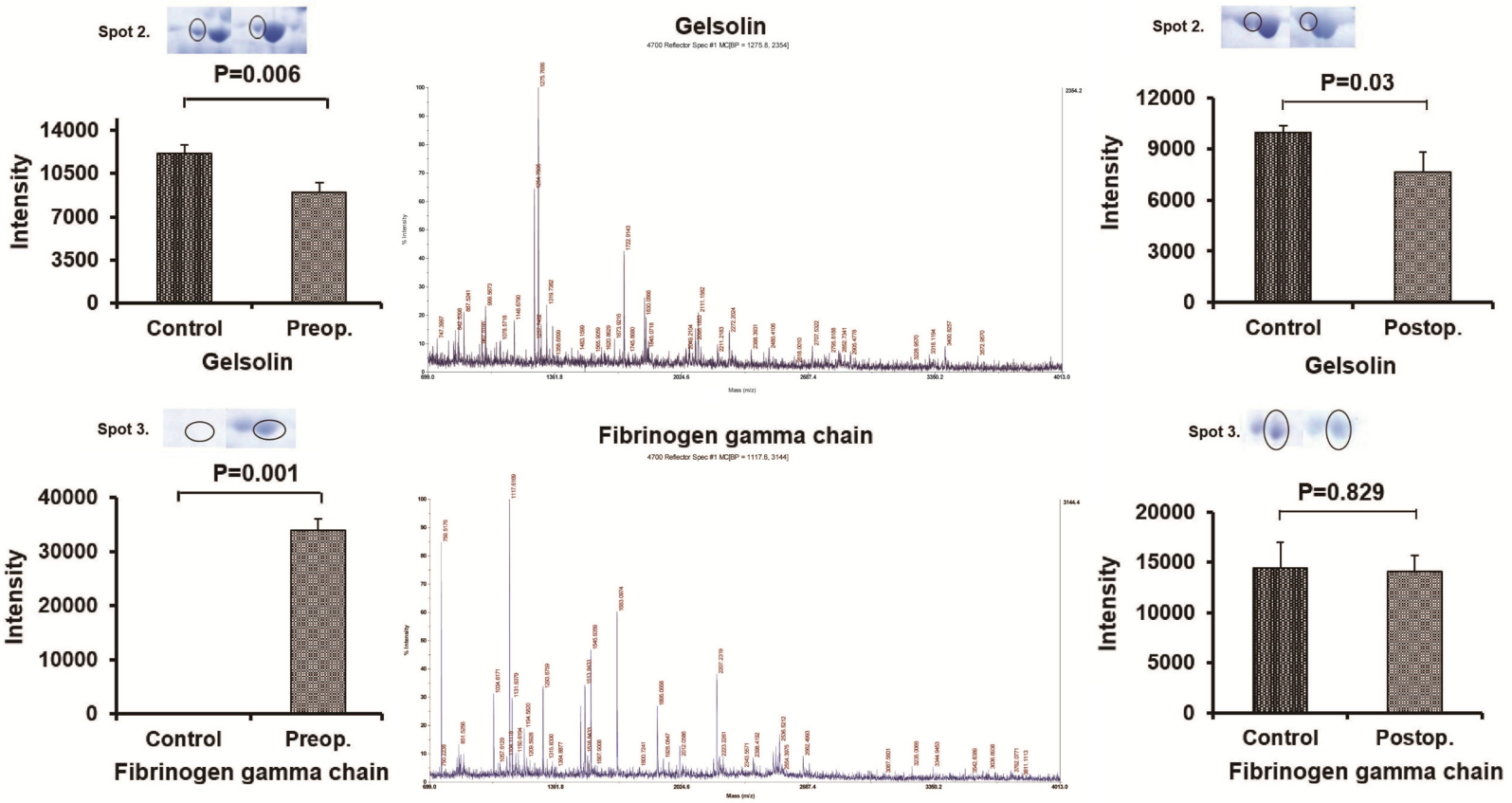


Figure 4. Most significant differential proteins identified in proteomic study in preoperative (Preop.) patients with TOF (A) and in postoperative (Postop.) patients with TOF (B). Three spots with significant change (P<0.01) in expression intensity were selected for mass spectrometry analysis. The upper panel shows two-dimensional gel electrophoresis. The lower left and right panels show the spot intensity analyzed by unpaired t test. The lower middle panel shows the identified mass spectrum maps of each of the three proteins. Note: The zero value of ficolin-3 and fibrinogen gamma chain is due to too low levels in the proteomic study.

Table 3. Validation of the altered Proteins by ELISA

Protein	Concentration			P		
	Control	Preop	Postop	Preop vs Control	Preop vs. Postop	Postop vs. Control
VSD						
ficolin-3 (ng/ml)	33692.9 ± 7493.9	11023.8 ± 2299.4	14986.7 ± 4784.8	0.006	0.459	0.040
alpha-1-acid glycoprotein 2 (ng/ml)	186115.3 ± 12565.1	244516.2 ± 20248.0	211709.7 ± 8263.5	0.020	0.148	0.102
Haptoglobin (ng/ml)	415544.2 ± 71821.6	175498.0 ± 39685.7	202442.7 ± 41816.3	0.005	0.542	0.013
serum amyloid P-component (ng/ml)	33.7 ± 1.8	29.0 ± 0.3	30.6 ± 0.7	0.011	0.043	0.105
complement component C3c (ng/ml)	70644.3 ± 5572.8	39591.3 ± 1411.9	45945.6 ± 1553.1	0.000	0.04	0.000
TOF						
ficolin-3 (ng/ml)	27092.2 ± 5226.9	14375.4 ± 2397.1	14852.5 ± 2251.4	0.032	0.885	0.037
gelsolin (ng/ml)	135133.7 ± 23422.9	41897.4 ± 2885.2	74115.3 ± 13110.2	0.000	0.027	0.02
fibrinogen gamma chain (ng/ml)	476079.1 ± 21659.5	575046.0 ± 27460.6	523043.1 ± 18162.5	0.007	0.121	0.103

Our study therefore provides important information regarding postoperative management after repair surgery in CHD.

We have previously demonstrated that in patients with CHD the protein profile is different from the normal subjects and this is true not only in complex CHD but also in simple CHD such as VSD [11, 12]. Moreover, as mentioned previously, the proteomic changes are also seen in CHD associated with complications such as pulmonary arterial hypertension [13] and other heart diseases [14, 15].

A clinically important question towards precision medicine is whether repair or corrective surgery may resume the physiological status towards normal in CHD, particularly in complex cyanotic CHD. This is an important issue in postoperative management of the patient as well as in evaluation of the patient to be a "normal" person for social activities in order to facilitate late education and personal life. The present study gives strong evidence that there are significant changes in the plasma protein profile after the repair surgery even as fast as 6 months postoperatively. The GO and KEGG analyses convincingly show that the protein profile and the top pathways have significant changes after surgery (**Figures 1**, **2**). The validation of the proteins demonstrates that the proteins altered preoperatively, either up-regulated or down-regulated, changed towards normal after surgery. These results clearly show that surgery has changed the pathology of the disease and at least partially resumes the normal physiological level of these proteins. In VSD patients, these proteins include alpha-1-acid glycoprotein 2, serum amyloid P-component, and complement component C3c whereas in TOF patients, these proteins include fibrinogen gamma chain and gelsolin. It is obvious that the changed or "recovered" proteins are not limited to those validated proteins in the present study. Considering the function of these proteins, the clinical implications of these findings are significant.

Alpha-1-acid glycoprotein 2 (**Figure 5B**), also named orosomucoid 2 and serum amyloid P-component (**Figure 5D**) in VSD patients were down-regulated before operation and completely recovered after the repair surgery. Orosomucoid 2 is one of the major acute phase proteins in humans [12, 19] whereas haptoglobin functions to bind free plasma hemoglobin [20] and plays a role in modulating many aspects of the acute phase response of the innate immune system [21]. Serum amyloid P-component is a member of the short pentraxin family. It was found that human amyloid P component may play a key role in the negative regulation of the innate immune response to DNA vaccines under physiological conditions [22]. The decrease of amyloid P component level in the plasma of VSD patients may facilitate innate immune response activation for stress response of organism against infection. Interestingly, the decrease of this protein in VSD patients resumed normal level after repair surgery for VSD.

We have previously shown that gelsolin was down-regulated in the plasma of patients with TOF (a typical cyanotic CHD) but was normal in VSD patients [11]. The finding in the present study that the content of gelsolin in plasma is partially recovered after surgery supports the notion that corrective surgery makes the coagulation system towards normal in TOF patients. As we previously discussed [11], gelsolin is required for rapid platelet activation [23, 24].

A similar result was found for complement component C3c in VSD patients. It was down-regulated before operation and partially recovered after the repair surgery. The complement system is an important mediator of natural and acquired immunity. It consists of approximately 30 proteins that can exhibit catalytic activity, function as regulators, or act as cellular surface receptors. These components normally circulate in inactive forms and are activated by the classical, alternative, or lectin pathways. Complement component C3c plays a central role in all three activation pathways [25].

We previously also found that ficolin-3 was down-regulated in the plasma of children with TOF and VSD [11]. Studies have demonstrated that children with CHD are more susceptible to infections [26] especially of the respiratory tract [22]. The finding of immuno-deficiency in patients with CHD in the present study may have important clinical implication in the management of infections and may be developed as a biomarker of susceptibility to infections in CHD patients. In the present study, in both diseases ficolin-3 was down-regulated and not recovered after surgery. There are two possibili-

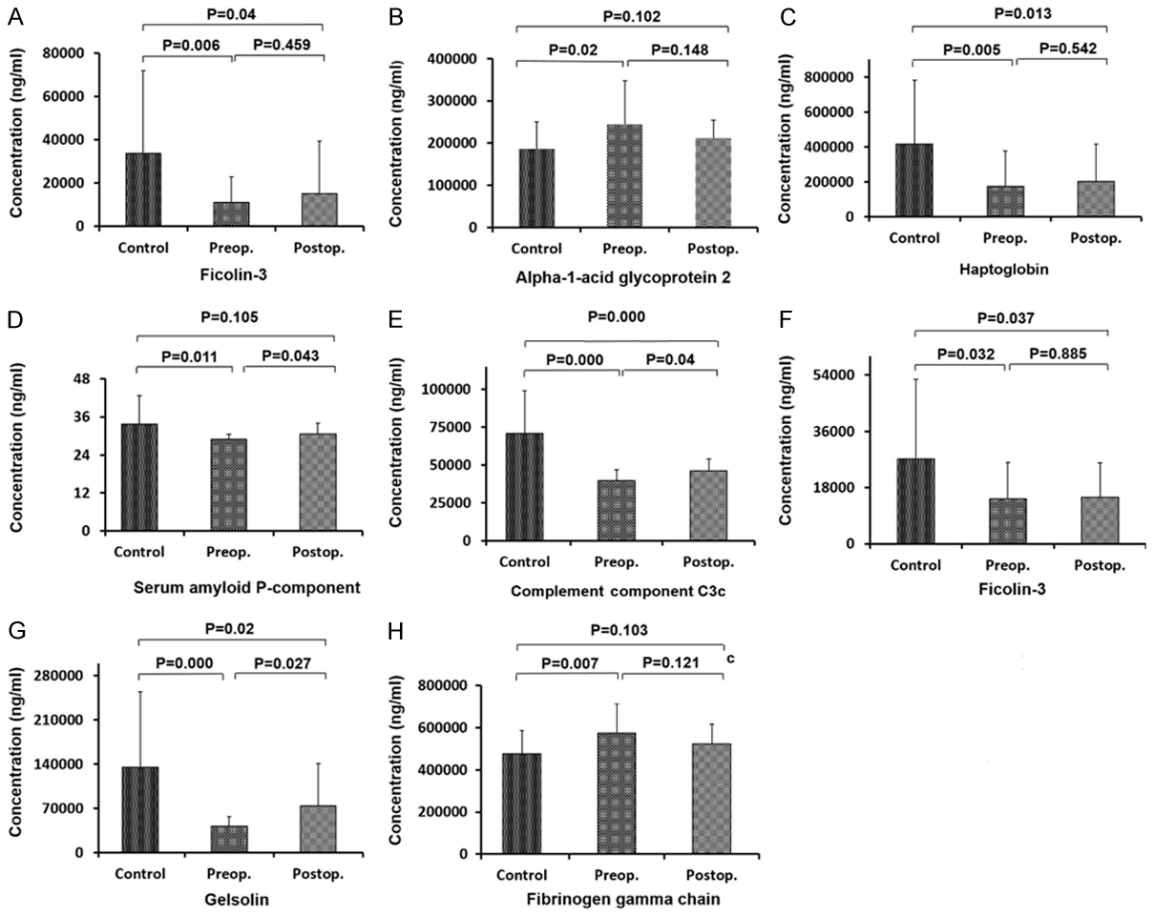


Figure 5. Validation of differentially expressed proteins in preoperative (Preop.) and postoperative (Postop.) patients with VSD (A-E, n=26), TOF (F-H, n=26), and normal controls (n=26). In VSD patients, postoperative complement component C3c ($P<0.05$) was partially and serum amyloid P-component ($P<0.05$) was completely recovered. In TOF patients, postoperative gelsolin ($P<0.05$) was partially recovered and the elevated fibrinogen gamma chain level ($P<0.01$) in preoperative patients became normal postoperatively ($P=0.1$).

ties to explain this result. Firstly, it suggests that the deficiency of ficolin-3 in CHD may be related to ficolin-3 gene deficiency and secondly, the deficiency of ficolin-3 in CHD may take longer than 6 months to recover. This result may suggest that careful management of the lung after repair surgery in CHD is necessary, at least in the early postoperative period.

Similarly, haptoglobin in VSD patients had no difference before and after surgery (**Figure 5C**). These results provide evidence that at least in the first 6 months after surgery, there are proteins that remain at abnormal levels.

It is interesting whether the findings from the present study are applicable in other CHDs. CHDs are complex diseases that have many different phenotypes. For example, some of the left-to-right-shunt related CHDs are associated with pulmonary arterial hypertension that also has protein changes as we already demonstrated [13]. As to the mechanism of how each identified protein is affected by surgical repair is a more complicated issue and needs further studies. Nevertheless, the present study provides evidence for changes of the protein profiling after repair surgery in CHD. This translational significance needs all clinicians to be aware of.

In summary, CHD is associated with significant plasma protein changes that may well reflect the pathological nature of the disease. Repair surgery in CHD significantly changes the protein profiling. In particular, corrective surgery for TOF and VSD may change the abnormal pro-

tein profile towards normal. However, some of these altered proteins such as ficolin-3, haptoglobin, complement component C3c, and gelsolin may take longer time to reach the normal level after the repair surgery, or the deficiency may be related to the genetic basis in these patients.

Clinical perspectives

CHD are the most common birth defects affecting nearly 1% of human live births and are the leading causes of death in infants in the last decades. VSD is one of the most prevalent types of CHD, occurring in 30-60% of all neonates with various kinds of CHD, and TOF represents the most common type of cyanotic CHD. Surgical repair remains the major method of treatment.

This study reports that CHD is associated with significant plasma protein changes that may well reflect the pathological nature of the disease. Repair surgery in CHD significantly changes the protein profiling. In particular, corrective surgery for TOF and VSD may change the abnormal protein profile towards normal. However, some of these altered proteins such as ficolin-3, haptoglobin, complement component C3c, and gelsolin may take longer time to reach the normal level after the repair surgery, or the deficiency may be related to the genetic basis in these patients.

Our findings suggest that identification of the recovered or unchanged proteins postoperatively may facilitate the evaluation of the surgical results and the personalized management in the postoperative period and in the long-term in CHD patients.

In conclusion, by using proteomic methods we have demonstrated that repair surgery for CHD not only corrects the structure malformation but also resumes normal level of certain altered proteins. To identify the recovered or unchanged proteins postoperatively may facilitate the evaluation of the surgical results and the personalized management in the postoperative period and in the long-term in CHD patients.

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Disclosure of conflict of interest

None.

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Table S1. Total protein spots in preoperative patients vs. control

Number	SSP	MR	PI	Control 1	Control 2	Control 3	TOF 1	TOF 2	TOF 3	VSD 1	VSD 2	VSD 3	P<0.01
				Intensity	Intensity	Intensity	Intensity	Intensity	Intensity	Intensity	Intensity	Intensity	★ represent 18 differentially expressed protein spots iddentified by MALDI-TOF MS
1	201	55.23497	3.14681	0	0	165848	0	189932	0	0	0	0	
2	304	69.448494	3.35783	0	0	0	0	0	0	0	0	3324.4	
3	501	85.258507	3.13142	0	6612.38	9102.79	0	0	0	0	0	15566	
4	502	83.113731	3.13363	0	0	0	0	0	0	0	0	9468.5	
5	503	85.052841	3.33662	0	3741.41	18382.4	0	0	0	0	0	9781.7	
6	504	88.09774	3.34113	0	0	4810.96	0	0	0	0	32638	2915.7	
7	603	109.27892	3.05196	0	0	0	0	0	0	22316	0	36373	
8	605	94.3526	3.06595	0	20756.07	4617.85	0	0	0	0	0	15792	
9	606	89.163689	3.12103	0	21133.84	11366.3	0	0	0	0	57500	18371	
10	608	92.952637	3.32186	0	242292.5	231155	70425	77611	84285	82384	0	201207	
11	609	89.31749	3.4321	0	3993.36	0	67512	0	0	0	0	6104	
12	610	89.526688	3.62888	0	0	2808.49	0	0	0	0	0	0	
13	707	134.5	3	0	36353.5	5317.56	0	0	0	0	8246	90693	
14	708	121.17358	3.03531	0	0	0	0	0	0	0	6639.9	17581	
15	801	134.95479	3.26817	0	32212.54	0	0	0	0	0	69803	12683	
16	802	149.7639	3.41624	0	24879.09	0	0	0	0	0	0	0	
17	803	150.66039	3.29503	0	37215.81	3911.4	0	0	0	0	0	0	
18	804	133.70949	3.35719	0	12306.02	0	0	0	0	0	0	0	
19	805	133.51012	3.41935	0	7925.84	0	4555.5	0	0	0	0	0	
20	907	160.18858	3.26379	0	0	7239.52	0	0	0	0	0	0	
21	1201	54.745365	3.53148	193020	187824.3	238951	229088	0	208321	317374	401248	342041	
22	1202	59.210426	4.44875	4916.33	13182.24	4414.23	0	0	0	1963	3085.9	5967.4	
23	1203	62.934322	4.50118	76909.9	185270.2	67008.4	20578	97040	113078	231514	152869	195292	
24	1204	57.938438	4.56418	5671.77	18809.15	3895.11	0	0	0	4951	0	3713.5	
25	1205	62.5723	4.60255	7288.82	107113.3	39307.8	12583	50466	62901	144363	90345	143076	
26	1206	61.713844	4.6968	0	0	0	0	10675	15458	9714.3	0	4401.3	
27	1207	60.540882	4.34412	4690.27	0	2476.73	0	0	7918.5	0	0	5391.6	
28	1208	53.57444	3.77449	0	292558.8	321299	79655	0	0	0	0	66402	
29	1209	56.359238	4.69198	0	24091.71	10193.7	0	0	0	4514.2	0	3914.2	
30	1210	44.15736	4.45345	4296.84	0	0	0	0	0	0	0	0	
31	1211	45.82346	4.85699	0	0	0	0	0	6619	0	0	0	
32	1301	63.080383	4.42101	33400.6	177003.1	71875.4	120642	51360	84135	15257	49583	25723	
33	1302	73.937164	4.71227	0	36252.8	43322	0	0	0	0	0	16637	
34	1303	70.267822	4.73607	0	0	0	0	41640	0	0	357.85	8782.4	

35	1304	68.753548	4.74718	0	0	10075.3	0	8284.6	0	0	0	5954.5
36	1305	65.428535	4.35301	47555.3	0	38954.4	25602	0	0	149022	37026	100486
37	1306	63.609825	4.69024	0	0	0	0	0	0	15027	0	0
38	1307	61.388165	4.60986	0	0	0	0	0	21687	0	0	366.15
39	1308	68.539658	4.61016	0	0	0	0	0	11394	0	0	0
40	1309	70.915794	3.82925	0	0	0	0	0	15568	0	0	0
41	1401	80.755569	4.28188	75982.1	0	0	0	0	0	0	0	31202
42	1402	80.020943	4.34978	0	147768.3	0	0	0	93334	15202	59365	27260
43	1403	78.463928	4.43323	83366.3	69919.69	101120	79460	19092	79913	60954	36906	118039
44	1405	76.291878	4.52267	31503.7	69819.36	0	41329	42910	42862	43826	33787	27484
45	1406	76.84687	4.58009	0	0	62574.3	0	32241	0	0	0	33582
46	1407	75.257507	4.63817	0	82811.52	0	0	31881	0	29027	32384	27749
47	1501	81.883217	4.14676	0	0	0	0	0	0	61028	29789	15781
48	1502	88.20063	4.18531	0	0	0	0	0	2969.6	0	0	8864.3
49	1503	88.405365	4.32311	0	0	0	0	0	7436.5	0	0	7866.6
50	1504	88.37674	4.43496	0	0	0	0	0	0	0	0	9194.3
51	1505	84.121506	4.63155	0	11467.68	0	0	0	57813	0	0	7654.8
52	1506	88.134056	4.65115	0	0	0	0	0	0	0	0	4946.8
53	1507	88.054832	4.74666	0	0	0	44776	6195.5	35569	32978	0	4874.2
54	1508	83.286133	4.05493	0	0	0	0	0	0	28153	0	0
55	1509	84.222282	3.96196	0	0	0	0	0	0	19012	0	0
56	1510	84.494469	4.81026	0	52888.71	29914.2	0	0	41469	0	0	45742
57	1605	88.548752	4.56648	0	5494.53	0	0	0	41050	3226.7	0	10157
58	1606	111.50487	4.59288	0	0	0	0	0	0	7607.8	0	9023.3
59	1607	93.201012	3.58498	0	56346.17	0	0	0	0	11155	0	9027.5
60	1608	89.256561	3.75948	0	0	0	0	0	0	0	0	3596.3
61	1609	95.213943	4.17115	0	0	0	0	0	0	2793.9	0	0
62	1610	93.46785	3.78301	0	3953.45	0	0	0	0	0	0	0
63	1611	92.338158	4.76137	0	7213.67	0	6902.8	0	10639	0	0	0
64	1612	88.701042	4.89633	0	10837.92	0	0	0	0	0	0	0
65	1613	89.363617	4.83738	0	7133.09	0	0	0	0	0	0	0
66	1704	133.46918	4.3108	0	0	0	0	0	0	0	0	7595.2
67	1705	113.39864	4.34934	0	0	0	0	0	0	6371.6	0	0
68	1706	107.24583	4.70574	0	0	0	0	0	0	14214	0	0
69	1707	117.97164	4.68844	0	0	0	4768.7	0	0	0	0	0
70	1708	117.12176	4.21649	0	0	0	5065.3	0	0	0	0	0
71	1801	133.51012	3.60895	0	8047.62	0	0	0	0	0	0	0
72	1909	148.5121	4.21394	0	24181.04	0	0	0	0	0	0	3849.4
73	1910	148.25104	3.57435	0	90730.97	73467.6	50222	64158	88483	5193.1	0	60434
74	1911	150.36317	3.77142	0	0	3004.2	0	0	0	0	0	0

75	2101	43.320789	4.96314	15398.7	50406.28	65010	27516	48755	33978	28726	36876	64260	
76	2103	42.175709	5.06452	17417.8	20370.61	33555.1	18701	36631	27735	5376.8	13951	33723	
77	2105	41.050941	5.18149	11988.9	19511.55	10724.7	11596	16370	13215	1208.8	5803.1	21667	
78	2106	16.211849	5.1472	11141.7	29068.4	18325.5	0	0	0	0	0	611.31	
79	2107	44.714851	4.83553	0	11567.11	0	0	0	0	7988.5	0	15700	
80	2201	50.147118	4.9566	57354.9	127867.3	88662.2	21519	41837	18262	15571	1290.1	29405	
81	2202	52.53857	5.00204	134414	193366.5	196483	51218	81827	54910	43952	57804	74285	★
82	2203	49.022678	5.05397	15584.4	0	0	4951.6	29636	17834	7125.9	14683	20519	
83	2204	50.346386	5.15842	217502	306175.8	236867	134387	174778	126765	54253	95086	234193	
84	2205	58.861317	5.21067	0	0	0	11858	0	12156	0	0	21709	
85	2206	61.192863	5.21827	0	0	0	7029.6	6238.9	0	0	0	8536.1	
86	2207	54.896164	4.83605	33946	68186.26	118957	0	0	0	17146	12954	19257	
87	2208	53.733646	5.07676	0	0	0	11635	23711	0	1325.3	0	10627	
88	2301	68.524361	4.82294	0	0	943.87	13016	0	21882	0	0	1815.5	
89	2302	70.225845	4.83348	0	0	48828.8	0	44286	0	0	11429	15766	
90	2303	74.981445	4.88236	0	45979.62	58462.4	45048	29708	10789	15988	11385	33774	
91	2304	68.706345	4.92569	0	17950.68	14586.6	0	0	0	0	0	16562	
92	2305	74.20472	4.94696	30397.7	63989.15	43634.7	0	62451	17572	18567	54773	33700	
93	2306	71.873047	5.0149	48686	53342.35	66028.3	0	10143	49711	13605	17615	80398	
94	2307	67.22065	5.16976	148403	185617.5	136845	44747	38936	299638	22016	121509	57504	
95	2308	73.740646	5.16441	16164.5	27580.51	0	0	16957	0	0	0	28219	
96	2309	69.756332	5.08357	127190	111801.4	0	31107	261625	95435	28012	0	61129	
97	2401	79.425735	5.00187	0	0	0	0	0	38382	12456	0	38195	
98	2402	78.394844	5.08679	0	0	0	0	57494	12261	0	0	43881	
99	2403	77.45295	5.17974	0	0	0	0	26086	0	0	0	47127	
100	2501	83.686378	4.92585	24103.7	38518.58	87993.7	26257	90976	55827	39575	86265	89616	
101	2502	88.173912	4.95541	9130.67	4273.21	29185.3	0	0	0	0	0	5549.1	
102	2503	82.941254	5.09807	0	24603.11	0	0	0	0	2226.9	40252	1334.1	
103	2504	87.823227	5.13837	0	11676.65	0	69592	9386.1	119861	16425	0	53029	
104	2505	83.057411	5.2789	0	60742.89	0	0	69908	0	24693	8192.3	20275	
105	2506	87.829231	5.25434	16928.5	25290.98	12573.2	0	58243	0	29111	67329	58306	
106	2507	83.133629	5.1923	0	11499.34	0	19303	18990	0	9405.6	0	21474	
107	2601	91.61718	4.82557	3824.8	5494.27	16142	0	16658	11894	15327	29293	32144	
108	2602	93.460953	5.27786	0	0	0	0	0	0	6096.6	0	0	
109	2603	87.781708	5.00337	0	3530.69	0	0	0	0	0	0	0	
110	2604	90.152367	5.36594	0	0	2722.59	0	0	0	0	0	0	
111	2605	92.237045	5.1945	0	0	0	0	5315.6	0	0	0	0	
112	2701	120.11557	5.18311	0	0	0	17528	7277.9	0	0	0	13220	
113	2703	107.21475	5.3489	0	28160.91	0	0	11997	0	11855	0	8650.8	
114	2704	107.90418	5.25477	0	19202.35	0	0	9783.5	0	12023	0	23030	

115	2705	111.59942	5.32648	0	3277.58	0	0	0	0	0	0	0	
116	2706	115.58678	5.24123	0	8956.28	0	0	0	0	0	0	0	
117	2707	124.81827	5.34364	0	4789.32	0	0	0	0	0	0	0	
118	2708	119.76539	5.09231	0	0	0	0	6502.8	0	0	0	5381.5	
119	2907	151.65312	5.34624	0	0	11535.8	0	0	0	0	0	0	
120	2908	152.16222	5.20895	0	0	11764	0	0	0	0	0	0	
121	3102	27.955202	5.46226	0	15626.59	0	5694.7	18557	0	0	0	14795	
122	3103	44.974888	5.48343	9263.62	0	9029.87	1386.8	1524.5	1619.4	7162.1	0	9095.7	
123	3105	16.281061	5.51223	73836.4	197717.2	107278	44197	72015	44843	74626	38894	87770	
124	3106	29.853062	5.53104	0	7408.82	0	0	4068.2	0	2031.7	0	4346.8	
125	3107	43.394863	5.30412	0	16498.23	0	0	0	0	1749.6	0	4862.3	
126	3108	27.149191	5.44977	3090.65	5354.94	0	0	0	0	0	0	0	
127	3201	60.937336	5.30878	0	0	0	16467	28605	16837	8971.5	10034	17764	
128	3202	49.103771	5.31545	185138	360083.1	125406	154619	234819	150201	147342	136258	264018	
129	3203	52.117218	5.37305	0	0	0	8546.1	10144	7865.7	0	2548	24873	★
130	3205	60.55217	5.38638	0	0	0	31794	35246	35146	11425	20461	38138	★
131	3207	47.862659	5.48051	107487	240049.3	93613.1	130354	228607	171091	96535	124196	230675	
132	3208	58.642719	5.39337	0	0	0	0	0	0	0	0	11538	
133	3209	60.631966	5.51104	0	0	0	0	0	4530.4	4308.4	0	4931.8	
134	3210	50.474804	5.54982	0	0	0	0	0	21640	0	2078.3	5347.7	
135	3301	64.167099	5.29764	13136.7	39094.1	142510	22814	68074	148541	6201.7	12237	37556	
136	3302	72.391884	5.31075	10718.9	53917.14	18587.3	13178	15628	15604	16535	7732.8	47447	
137	3303	66.287376	5.359	13026.3	66687.11	12087.3	12681	77873	110783	24202	66636	78062	
138	3304	65.633667	5.4366	21194.8	72484.55	20886.3	43359	46743	27167	14946	16840	55398	
139	3305	68.298843	5.47557	12558.6	85664.02	5196.32	9711.5	6685.4	0	7143.9	3705.3	30334	
140	3306	70.893845	5.47927	26382.6	51254.25	36767.4	74533	101319	131463	115104	72826	14602	
141	3307	72.669518	5.36592	77009.8	0	0	0	0	52613	44197	0	4873	
142	3308	69.04335	5.42975	0	0	5934.59	0	0	0	0	0	0	
143	3309	63.646061	5.48162	0	0	0	5485.5	0	8608.5	0	0	0	
144	3401	81.244301	5.3666	119018	226426.9	68741.1	110375	76225	124137	70628	83511	197052	
145	3402	79.016495	5.54175	4403.21	4150.39	0	0	3616.1	2604.3	0	0	6384.5	
146	3403	81.013794	5.4673	0	41484.91	44244	25915	58457	65656	88660	54967	49613	
147	3501	88.088326	5.41318	0	11150.74	0	0	0	4632.9	10924	0	10605	
148	3502	87.482292	5.50241	6347.93	13511.08	0	0	7767.4	12213	10885	5260.4	14844	
149	3503	81.948349	5.52745	0	0	12803.1	0	7554.5	0	0	7929.6	23678	
150	3504	82.121109	5.62618	0	19385.35	0	0	5777.1	0	0	0	0	
151	3601	106.82647	5.30402	13745.9	37910.38	0	28797	10220	54403	9914.4	34950	28240	
152	3602	93.102226	5.32341	0	39231.17	8933.79	17150	7871.3	6737.9	6041.7	8490.6	10456	
153	3604	92.587364	5.38151	35383.9	43026.22	6310.38	0	13519	11238	9744	13709	14215	
154	3605	107.91123	5.40004	0	47849.39	5883.41	17780	11321	0	49471	19956	46382	

155	3607	92.600937	5.47603	5817.42	0	9600.41	13793	8474.3	6080.5	30717	6956.4	7652.8	
156	3608	92.255478	5.53289	12987.4	18123.72	0	5485.5	8824.4	10564	0	2238.6	10096	
157	3609	89.212006	5.69703	0	5086.11	0	0	0	0	0	0	0	
158	3702	118.97967	5.45186	0	70841.73	15245.8	46890	10642	73017	0	34099	43250	
159	3704	118.20879	5.53224	78543.3	67428.64	10246.1	43251	48675	53218	244366	42282	89926	
160	3705	108.47164	5.4572	0	5472.8	0	11611	8202.2	0	0	14250	15250	
161	3801	150.61723	5.61747	65937.7	0	2247.93	0	11389	0	0	0	0	
162	3802	124.77396	5.50091	0	0	4606.81	0	0	0	0	0	0	
163	3803	122.83788	5.5777	0	0	5156.24	0	0	0	0	0	0	
164	3804	139.80858	5.48839	0	0	0	0	0	11521	0	0	0	
165	3805	143.2728	5.45099	0	0	0	0	0	5068.2	0	0	0	
166	3806	137.20958	5.54448	0	0	0	0	0	5440.5	0	0	0	
167	3807	135.31966	5.60736	0	0	0	0	0	26377	0	0	0	
168	3907	151.48386	5.43234	0	0	8021.51	0	0	0	0	0	0	
169	4101	27.768263	5.55348	7573.63	14571.44	6929.32	33116	39785	54855	8166.5	14431	26125	
170	4102	24.267517	5.58328	6517.13	14062.99	8276.52	11241	32037	13449	12584	5494.2	33192	
171	4104	43.749615	5.65034	28828.1	19913.7	62994.2	5515.2	3653.2	8922.6	12823	6066.7	14773	
172	4107	28.935293	5.82631	46530.7	52539.29	47932.8	17934	22043	16336	12933	16285	22937	★
173	4108	43.055237	5.83446	66561.8	113040.3	96607.8	16904	25480	20274	29320	12882	35251	★
174	4109	17.459076	5.85514	71016.8	79826.47	90026.8	117100	125586	109150	113758	100966	129186	
175	4111	38.54731	5.85037	0	1699.84	0	0	0	0	0	0	1783.8	
176	4112	25.977785	5.89782	0	2778.29	0	0	0	0	0	0	3145.4	
177	4113	28.259743	5.79635	0	0	0	0	5656.7	0	0	0	1419.2	
178	4114	20.753252	5.75576	128960	109851.3	145377	9152.9	64142	18526	40392	26103	30875	
179	4115	32.04356	5.69065	0	0	0	0	9799.2	7275.8	2572.6	2045.3	6894.1	
180	4116	30.816439	5.69528	0	0	0	0	0	0	5764.6	0	0	
181	4117	37.844578	5.73834	0	0	0	0	2536.2	0	0	0	0	
182	4202	58.369308	5.62301	0	0	0	22843	19485	49736	51163	21528	56202	
183	4203	47.06358	5.64571	136096	116187.4	158710	41892	41434	54884	39757	42282	132135	★
184	4204	57.520432	5.73741	17390.6	28164.9	17477.9	114960	398972	171956	270057	217820	234528	★
185	4205	53.084576	5.7425	0	0	0	0	4853.1	2889.5	1480	1260.1	2570.9	
186	4206	46.806248	5.82897	74844.3	101294.9	79891.2	22688	41801	32024	29378	27069	47931	★
187	4207	57.193134	5.88795	22931.2	25872.58	25132.8	222910	368633	235349	372150	327965	157380	
188	4208	60.694786	5.72084	0	0	0	0	0	0	17616	0	0	
189	4209	60.577026	5.87671	0	0	0	0	0	0	28075	0	0	
190	4301	71.717003	5.56842	39343.5	45702.39	59953.9	26569	48161	59930	39549	31711	70093	
191	4302	67.860207	5.5685	0	56334.76	0	0	2410.8	0	2724.2	0	2402.9	
192	4304	72.426277	5.71671	86675.6	90053.73	114815	43764	102589	59601	164588	59174	67236	
193	4305	72.649788	5.80771	81038.7	0	0	51099	44202	0	0	69656	46230	
194	4306	73.374245	5.91413	68070.5	3153.46	43611.7	31072	39010	0	0	19759	10596	

195	4307	67.890594	6.0031	0	0	0	0	0	8677.5	0	0	0
196	4401	78.512329	5.60204	3755.79	5113.39	7667.15	4340.4	9200.1	14243	30090	1929.1	11880
197	4402	70.291283	5.66433	0	0	0	3938.9	0	2190	0	1802.5	1372.1
198	4403	76.094681	5.81837	8166.52	1994.06	0	0	0	0	0	0	0
199	4404	76.24321	5.86807	0	3892.44	0	0	0	0	0	0	0
200	4501	85.458862	5.59043	18959.7	21054.02	58895.9	15709	24349	46636	66220	27572	45083
201	4502	84.362114	5.67516	82085.8	55296.41	0	29811	107305	73357	137738	70276	61476
202	4503	82.900757	5.7698	107782	190515.5	146101	139100	138641	120657	185448	131407	213842
203	4504	82.172295	5.8797	184486	107166.6	117508	167325	247440	173865	305183	268742	121534
204	4601	92.049484	5.5887	9179.87	0	44573.5	5829	8547.1	8036.9	3252.7	17710	10543
205	4602	116.70799	5.59281	0	49756.85	0	7685.1	25597	0	0	15629	5293.5
206	4604	93.76474	5.6752	7161.1	14583.48	17841.5	3475	8772.8	8287.9	0	3959.6	7508
207	4605	93.736099	5.73449	6581.92	0	15665.6	5487.3	13111	7345.7	0	8834.5	11312
208	4606	93.440651	5.79965	5859	9451.34	0	10373	6004.6	0	31242	6036.2	6440.8
209	4607	109.02878	5.88427	0	0	0	0	0	0	0	0	15680
210	4608	107.03375	5.93656	0	0	0	0	0	0	0	0	8150.4
211	4609	91.447594	5.66696	0	0	0	0	6243.7	0	0	3493.4	0
212	4610	93.460052	5.90837	8721.36	0	0	0	18494	0	0	15224	0
213	4611	103.75181	5.76449	7946.66	0	0	12516	0	0	0	0	0
214	4612	89.011322	5.96368	0	26119.67	0	0	0	0	0	0	0
215	4613	89.251427	5.84013	0	7025.63	0	0	0	0	0	0	0
216	4707	131.87347	5.79286	12146.5	16221.37	0	34771	10953	95595	0	219887	38505
217	4710	131.94904	5.7064	58983.7	33796.63	5948.04	0	8321.7	0	0	0	34771
218	4711	130.69487	5.85965	40080	0	156695	76066	0	0	0	0	0
219	4712	98.896133	5.99111	0	0	83887.9	0	0	0	0	0	0
220	4713	114.85765	5.9325	0	0	0	19333	0	111155	0	0	0
221	4801	132.00205	5.75353	0	39997.43	0	0	0	0	0	0	0
222	4802	148.40054	6.03738	0	0	0	61042	22647	0	0	0	18178
223	4904	148.51059	5.71216	0	29471.6	64713.2	14169	0	0	0	0	0
224	5102	42.318436	6.00596	18323.1	40418.96	34045.4	4017.4	8572.1	4425.1	3840.7	4577.1	8854.9
225	5103	20.630774	6.0451	315178	609190.6	341211	207678	224234	187422	184749	226521	310666
226	5104	16.027409	6.07654	0	0	0	15641	8642.3	10189	6677.3	0	15563
227	5201	58.229675	5.98804	0	0	0	54528	87388	35869	0	48208	50064
228	5202	49.583363	6.1113	3217.52	2406.24	1744.58	2453.3	2863.7	2131.6	997.51	0	1749.6
229	5203	59.258335	6.11581	0	0	0	0	3586.5	9793.7	0	0	12309
230	5204	46.493561	5.99457	4575.09	6329.01	1925.19	4807.8	8224.6	2824.2	5551.5	835.67	4030.3
231	5205	38.747658	6.21276	2651.5	1816.55	964.61	0	0	0	0	0	0
232	5206	41.767563	6.20147	3869.91	0	2200.28	0	0	0	0	0	0
233	5207	56.5355	6.41887	0	0	0	2862.3	0	3798.4	0	0	0
234	5301	73.276207	5.99705	1570.32	35103.28	0	65910	91930	59741	17472	54040	44707

235	5302	72.49559	6.19717	0	0	0	0	0	42253	29696	21316	36548	
236	5303	70.586487	6.46758	0	0	0	7540.7	0	0	0	0	0	
237	5401	79.982742	5.98035	188338	191366.7	261199	239888	254886	201443	336018	254199	165211	
238	5402	81.009583	6.07535	80147.6	66389.43	0	116054	60726	165390	141498	104871	85388	
239	5403	77.354813	6.09202	51313.8	31878.58	35246.7	54096	44167	0	3032.3	14562	25607	
240	5404	78.306305	6.1873	118236	94192.77	93418.4	147404	221835	192463	113739	229516	146321	
241	5405	72.126205	6.32321	0	0	0	28451	78354	62138	0	0	0	
242	5406	72.948723	6.45317	0	0	0	7329.5	0	0	0	0	0	
243	5502	83.737045	6.13606	77172.3	35919.04	89539.3	0	0	0	0	0	60627	
244	5503	88.280106	6.15481	18961.1	28269.74	14599.7	15096	0	4766.6	36136	28994	4957.3	
245	5504	83.486893	6.21923	39483.1	39476.88	50455.8	17771	21347	29751	0	21155	42744	
246	5601	106.76424	5.98875	87616.8	112003	77121	70583	71159	59429	263882	244091	102062	
247	5603	92.610703	6.08666	5561.14	3751.32	6846	3884.8	7463.8	0	5258.5	4263.4	3679.8	
248	5604	91.769356	6.16393	11487.5	12003.66	12865.7	8317.6	8752.4	9818.1	9772	11172	9944.2	★
249	5606	92.789558	6.02815	7284.12	0	0	0	9317.4	0	56971	1947.1	0	
250	5607	85.897369	6.26207	0	0	0	4450.8	0	0	0	5236.5	3200.2	
251	5608	93.421623	5.98786	11006	0	0	0	0	0	0	0	0	
252	5609	95.434395	6.27371	10970.7	6476.38	17665.5	0	0	0	0	0	0	
253	5610	96.045876	6.2102	6637.36	0	5188	0	0	0	0	0	0	
254	5611	88.422951	6.05054	0	17739.36	0	0	0	19324	0	0	0	
255	5612	86.856155	6.41591	0	0	17968	0	0	0	0	0	0	
256	5613	99.210762	6.31333	0	0	0	0	15798	0	0	0	0	
257	5702	121.37341	6.00347	52760.9	0	16120.4	0	0	0	0	121192	32460	
258	5703	131.38329	6.01125	324043	211449.8	195833	287972	643574	520384	304468	432437	170633	
259	5801	146.32088	6.27077	0	0	0	0	0	0	9668.3	0	0	
260	6104	20.488344	6.41748	227171	270390	249779	148723	142818	231663	143757	141362	176085	★
261	6107	38.014965	6.40518	14969.1	15274.06	15476.3	5450.1	7961.4	9020	5113.1	3458.2	5315.8	★
262	6108	28.627853	6.7066	55066.2	22852.68	77267.8	25565	25317	52467	8991.6	16354	11069	
263	6109	27.916252	6.7116	51045.6	26881.66	46581.5	18021	18428	2260	8433.4	0	13344	
264	6110	29.618685	6.53549	0	0	0	0	0	0	7508.2	0	0	
265	6111	28.388916	6.45302	0	15620.89	24819.4	6659.8	10673	19476	9059.3	0	0	
266	6112	14.420136	6.55363	4610.31	0	6375.18	0	4499.4	0	0	0	0	
267	6113	20.362097	6.71233	12446.1	0	22711.5	0	4315.5	0	0	0	0	
268	6114	30.199705	6.87771	11813.8	6400.38	20759.1	0	0	0	0	0	0	
269	6115	28.102427	6.88244	0	0	29480.8	20087	0	0	0	0	0	
270	6201	59.232845	6.35888	0	0	0	0	0	0	0	0	6627.7	
271	6202	60.164967	6.54107	11825.6	15162.14	17816.7	6295.7	6294.9	9422.5	11512	5728.2	8361.5	
272	6203	61.398743	6.62044	0	0	0	9683.4	7980.7	16797	10932	4606.2	8799.9	
273	6204	61.31818	6.34392	0	0	0	7329.6	7452.9	9983.4	3207.2	0	4202.6	
274	6205	52.889694	6.67993	4344.74	3317.03	0	3248.1	2483.6	4758.3	0	1990	2793.9	
275	6206	54.369751	6.26998	0	0	2198.8	2466.6	2604	4414.7	1317	0	2187.7	

276	6207	51.212521	6.27945	0	0	0	2431.6	2225	3899	604.05	0	1705	
277	6208	49.872551	6.6909	0	0	2559.84	3449.3	3191.3	6163.1	0	0	3702.6	
278	6209	53.646042	6.44447	6053.94	4210.97	6078.08	4535.5	4187.1	7107.9	6452.1	0	0	
279	6210	31.255579	6.91521	26901.8	12785.27	43330.2	0	0	0	0	0	0	
280	6211	37.329033	6.56388	5915.09	4337.07	5624.74	0	0	5001.4	0	0	1952.1	
281	6212	40.607544	6.69232	0	0	1244.33	0	0	0	0	0	1522.4	
282	6213	41.111897	7.12902	0	0	4172.68	0	0	0	0	0	0	
283	6214	52.062283	6.56049	0	0	0	0	0	3958.3	0	0	0	
284	6215	59.777271	6.72486	0	0	0	0	6655.6	0	0	0	0	
285	6216	59.842468	6.81601	0	0	0	11786	6454.5	0	0	0	0	
286	6301	67.750359	6.34419	0	0	0	7483.5	4316.7	6874.1	0	35731	12405	
287	6302	66.727028	6.49129	18271	0	49289.9	13202	6715.8	5882.5	0	24343	15323	
288	6305	65.552338	6.67807	24693.8	32876.11	29598.3	92527	86013	108119	189015	97000	117824	★
289	6306	73.487991	6.80042	23175.9	65814.44	16031.1	10494	24382	37009	0	37967	55698	
290	6307	57.872063	6.73908	6024.01	5264.31	6365.94	0	6234.3	10739	0	5938.7	0	
291	6308	62.562634	6.69739	0	0	0	0	0	0	12046	0	0	
292	6309	62.189663	6.43418	6710.51	0	10067.3	0	0	0	0	0	0	
293	6310	63.026733	6.49293	0	0	0	4791.7	0	6962.5	0	0	0	
294	6311	69.186996	6.52293	0	0	0	32938	30311	65804	0	0	29499	
295	6312	66.17704	6.97941	0	0	0	41348	36467	28069	0	0	0	★
296	6313	61.76437	6.81048	0	0	0	19489	11953	0	0	0	0	
297	6401	69.791321	6.61121	0	0	0	0	0	0	90350	0	0	
298	6402	73.646149	6.61157	15150.4	0	20773.3	35414	0	0	0	0	0	
299	6403	73.219101	6.70829	0	0	0	0	52671	0	0	0	0	
300	6404	74.099609	6.86849	0	0	0	0	14339	0	0	0	0	
301	6501	87.256477	6.33638	52737.4	0	82916.2	40227	45776	56987	0	0	26219	
302	6502	83.940872	6.66043	15892.6	19120.27	344701	417244	80380	708538	1E+06	776080	1E+06	
303	6503	85.475563	6.53838	105457	57939.08	70938.9	0	0	68050	94880	33253	22291	
304	6504	85.857559	6.48964	0	0	122152	0	0	0	0	55038	21778	
305	6505	82.146378	6.28286	8580.06	13859.82	22057.1	22463	4445	48344	29262	12304	20611	
306	6601	90.02182	6.24267	36885.4	62597.1	50757.2	67397	75159	83140	72265	51202	72034	
307	6602	91.957901	6.33999	2170.12	3383.21	7924.06	6940.4	7318.4	7184.9	19671	7992	6791.7	
308	6603	107.9437	6.34008	0	14696.22	0	12815	0	0	0	0	24609	
309	6604	99.32901	6.38663	109713	121816.3	0	46438	26594	10600	0	48984	27448	
310	6605	109.04787	6.41438	0	15223.14	0	9370.1	0	0	0	0	12408	
311	6606	97.375175	6.4749	65543.1	0	0	53966	21034	17501	0	46030	41243	
312	6607	108.1569	6.50741	0	6547.52	0	0	0	0	0	0	19779	
313	6608	96.228287	6.55079	132248	110647.6	0	89489	95439	64283	150839	63950	79863	
314	6609	97.833344	6.65405	17727.5	24583.83	163264	22843	35550	19430	37680	34351	18274	
315	6610	98.286949	6.77722	0	0	0	9556.1	9417.6	0	0	7567.7	0	

316	6611	89.32206	6.5589	0	0	33449.5	19227	0	36488	0	0	20264	
317	6707	113.63145	6.76538	0	0	3209.89	0	0	0	7362.8	0	0	
318	6708	111.93856	6.59247	0	26693.35	0	0	0	0	0	0	0	
319	6709	121.53126	6.6711	0	23338.36	0	0	0	0	0	0	0	
320	6710	120.71494	6.72445	0	34984.47	0	10297	0	0	0	0	0	
321	6711	126.71697	6.497	0	24130.97	49936.2	0	0	0	0	0	0	
322	6712	107.77052	6.91983	0	0	2348.61	0	0	0	0	0	0	
323	6713	99.853485	6.94102	0	0	2951.77	4888	0	0	0	0	0	
324	6801	137.14648	6.51165	0	0	0	7601.4	0	0	7663.5	0	0	
325	6802	143.64465	6.80164	0	0	2615.88	0	0	0	0	0	0	
326	6909	148.81781	6.77145	0	49591.4	22008.9	12511	57356	0	0	0	3408.3	
327	6910	145.83086	6.21955	0	0	40790	50958	69393	0	20870	67196	19883	
328	6911	145.93138	6.63425	0	55442.06	0	0	0	0	0	0	0	
329	6912	149.4063	6.83001	0	0	19350.9	0	0	0	0	0	7847.1	
330	7105	35.400002	7.1	120051	91645.76	168223	133357	140112	159816	79701	125294	98858	
331	7106	27.773653	7.38232	90330.9	21930.4	88902	44263	42797	52566	15967	74097	20309	
332	7107	36.263718	6.82376	49235.2	17177.41	70088.3	45865	19547	26876	19710	35673	26057	
333	7108	29.563637	6.85726	33498.7	0	53031	25477	22101	0	12871	21643	9104.2	
334	7109	30.158134	7.23032	6905.79	3049.39	7201.14	8027.6	8654	8710.2	1800.3	3204.9	1546.4	
335	7110	27.880016	7.51956	0	0	56905.6	49161	25582	36767	31657	0	0	
336	7111	15.390293	7.59341	0	0	0	64122	0	61952	2637.2	7014.6	0	
337	7112	29.328537	7.04596	23255.6	0	41224.6	17512	27972	0	0	19765	0	
338	7113	15.645049	7.14662	13003.6	0	23412.3	0	61492	0	0	0	0	
339	7114	31.923283	7.48609	0	0	4535.37	0	0	0	0	0	0	
340	7115	29.275604	7.80974	0	0	52971.7	12045	16134	18779	0	0	0	
341	7201	57.641407	7.59082	4617.38	2930.3	5040.38	8125.6	6503.8	8713.3	5864.9	5526.5	2186.5	
342	7202	51.399597	7.15234	0	0	0	0	0	0	518.33	0	0	
343	7203	50.881023	7.34038	0	0	0	0	0	0	2759.6	0	0	
344	7204	46.600269	7.22499	0	0	0	0	0	0	723.95	0	0	
345	7301	64.881561	6.82508	76722.3	48235.34	79404.5	491670	49685	270813	561775	154234	455916	
346	7302	74.442703	7.05169	13561.3	7632.28	12244.1	12586	17243	7675.7	14398	23127	8074.4	
347	7303	64.10672	7.10549	32088.2	16878.64	33246	76056	103467	238665	68764	58134	282197	
348	7306	74.477814	7.24411	0	0	0	5267.7	9240.9	5275.7	6273.9	10896	7186.4	
349	7307	65.81881	7.35373	0	0	3674.48	72818	75112	69108	38699	55722	57938	★
350	7308	66.928642	7.51287	0	0	0	0	0	10324	0	0	23106	
351	7309	67.102097	7.58749	0	0	0	16382	0	0	9512	15954	5031.9	
352	7310	74.430084	7.59735	0	0	0	1071.1	0	0	0	334.47	492.13	
353	7311	73.625084	7.41588	0	0	0	7097	6064.7	0	0	2080.1	14.03	
354	7312	61.749012	7.03247	0	0	0	4581.6	3570.3	3997.5	0	2593.9	0	★
355	7313	60.896942	7.05888	0	0	0	0	3808.4	0	0	2054.8	0	
356	7314	62.613003	7.3664	0	0	0	0	0	0	4193.8	2255.8	0	

357	7315	67.958275	7.26181	0	0	0	0	0	23167	0	0	0	
358	7316	70.220108	7.59057	0	0	0	0	0	0	0	0	0	
359	7401	79.467033	7.02092	6860.67	0	0	34901	0	0	89839	93336	92914	
360	7402	76.774689	7.05603	0	0	0	19162	0	6414.8	13973	7594.7	4704.8	
361	7403	79.297272	7.14918	4437.23	0	0	63522	47231	64419	80844	33954	39772	★
362	7404	81.375748	7.20384	74905.4	40474.91	55858.6	83191	45136	96830	107540	161684	68622	
363	7405	78.453018	7.28302	0	0	0	128374	121431	172087	136664	42601	58463	
364	7406	80.863373	7.41321	217431	158713.2	11643.7	149573	245593	322303	246155	265096	313706	
365	7407	78.705093	7.5316	0	7420.57	8232.86	169639	174981	117473	66835	144039	71897	
366	7409	77.09536	7.60398	0	0	0	102150	64125	102679	102190	105684	44237	
367	7410	81.040489	7.26149	0	0	36027.4	0	35883	0	0	0	20361	
368	7411	76.011215	7.41588	0	0	0	0	34247	8186.8	13354	5558.6	31.2	
369	7412	76.848068	7.22975	0	0	0	0	0	0	0	0	8125.3	
370	7413	66.459229	7.20589	0	0	0	0	12595	57340	0	14633	0	
371	7414	70.651077	7.03616	0	0	0	0	0	0	6870.6	0	0	
372	7415	70.329323	7.10563	0	0	3646.34	0	0	0	0	0	0	
373	7501	84.039116	7.0475	2884.66	36524.24	48685.2	76135	8161.9	49743	33550	38172	9888.1	
374	7502	86.829384	7.03627	0	0	5843.01	0	0	41616	0	36900	164184	
375	7503	87.168144	7.1627	5559.44	0	8956.23	15933	40264	21314	0	0	29402	
376	7504	87.445419	7.32676	10528.4	7424.47	11835	12219	14904	25342	0	10394	14399	
377	7505	87.677559	7.49799	0	0	0	0	0	3075.9	60497	0	36640	
378	7507	83.991867	6.95881	154875	0	0	0	0	0	59245	176576	64855	
379	7508	83.242577	6.83628	437563	262496.2	324600	618332	2E+06	1E+06	345471	21086	18136	
380	7509	84.544434	7.33712	0	0	0	0	0	0	28481	15552	0	
381	7510	79.086769	7.35572	0	0	0	108259	113382	91199	0	131828	10305	★
382	7511	83.191833	7.54055	0	0	20741.1	0	0	0	0	0	0	
383	7512	79.90506	7.62872	0	0	4352.88	0	0	0	0	0	0	
384	7601	109.83738	7.55845	0	5694.15	0	2229	0	0	0	0	3077.1	
385	7602	100.18715	7.33388	0	1909.45	0	0	0	0	0	0	9219.3	
386	7603	110.66248	7.45171	0	0	0	0	0	0	0	0	1300.2	
387	7604	97.68116	7.43118	3881.09	0	0	0	0	0	66021	0	0	
388	7605	101.21985	7.21917	6597.12	0	0	0	0	0	0	0	9582.7	
389	7606	100.62815	7.13712	7738.81	5179.22	0	4854.4	0	0	5739.6	0	6930.9	
390	7607	102.68776	7.56776	0	0	0	0	0	0	1521.2	0	0	
391	7608	101.74812	7.07434	0	0	0	8339.4	7355.4	0	6177.5	0	0	
392	7609	95.209686	7.31674	4966.33	3294.9	2979.68	4116.4	0	0	1902	0	0	
393	7610	95.807861	7.19687	3022.26	3646.04	2858.85	4112	0	0	4992.5	0	0	
394	7702	144.2924	7.30049	0	0	0	0	0	0	0	0	2927.1	
395	7801	123.63935	7.45161	0	0	1723.22	0	0	3307.9	0	0	0	
396	7903	149.57552	6.89292	0	0	7343.21	23171	0	0	5816.2	0	4223.1	

397	8108	35.352913	8.78374	0	0	0	0	0	0	0	0	9518.7	
398	8109	36.151978	8.78629	0	0	0	0	0	0	4525.3	8772.7	28680	
399	8110	27.602818	8.07616	0	96003.91	97497.5	37174	7321.8	0	112933	0	42942	
400	8111	27.66987	8.77844	2975.61	3558.61	20398.2	0	0	0	0	64839	21294	
401	8112	36.275364	7.98569	0	0	0	0	0	0	3756.1	0	746.72	
402	8113	29.043589	7.67508	17332.5	0	0	0	0	0	26254	0	0	
403	8114	29.636883	7.70665	0	0	0	0	0	0	5369.5	0	0	
404	8115	28.309048	8.19591	90281.4	0	0	0	0	69689	15154	0	0	
405	8116	27.668713	8.60201	0	0	0	0	0	0	29765	0	0	
406	8117	36.100384	8.05711	0	0	0	0	0	0	5439	0	0	
407	8118	37.548512	8.00177	0	0	0	0	0	0	2392.1	0	0	
408	8119	35.906036	8.46627	0	0	0	0	0	0	25346	0	0	
409	8120	35.821426	8.39061	0	0	0	0	0	0	3887.9	0	0	
410	8121	37.566177	8.47913	0	0	0	0	0	0	3173.1	0	0	
411	8122	37.652294	8.79946	0	0	0	0	0	0	4808.9	0	0	
412	8123	15.005152	8.48422	0	0	0	0	4549.5	0	98162	0	0	
413	8201	61.530449	8.76964	0	0	0	0	0	0	2936.1	0	4438.7	
414	8202	36.24567	9.26424	0	0	0	0	0	0	0	15494	0	
415	8203	51.92347	7.71218	0	0	0	0	0	0	830.01	0	0	
416	8301	72.080086	8.73049	0	20160.59	34781.4	37045	30961	44790	14088	0	2117.9	
417	8302	68.098633	7.76318	0	0	0	14489	24611	22313	3949.2	12552	6632.9	
418	8303	64.900818	7.68227	0	0	0	0	0	0	1504.5	0	0	
419	8304	62.15699	8.48111	0	0	0	0	0	0	13837	0	0	
420	8305	62.6838	8.22014	0	0	0	0	0	0	12344	0	0	
421	8306	67.398453	8.03155	0	0	0	0	0	0	0	0	1883.8	
422	8307	61.57914	7.94693	0	0	5242.36	0	0	0	0	0	0	
423	8308	64.871216	7.84116	0	0	3828.8	0	0	0	0	0	0	
424	8401	78.884964	7.69055	11898.1	13638.25	8901.71	120628	179397	151924	183792	102479	73202	★
425	8403	77.022858	7.8228	9667.26	0	0	36558	29627	64377	82139	47130	36456	
426	8405	78.529625	7.89888	7745.41	4355.16	19906.3	117552	177361	148859	131650	95952	59003	
427	8406	77.016197	8.07716	9764.85	0	4093.95	0	0	30602	27718	0	11293	
428	8407	76.708733	7.77559	0	0	0	5828.3	0	0	0	14956	0	
429	8408	77.235703	8.19044	0	0	0	0	38271	0	10998	22301	0	
430	8409	69.583427	7.81237	0	0	0	0	0	0	6735.9	0	0	
431	8410	74.174919	7.94994	0	0	0	0	0	0	8687.7	0	0	
432	8411	73.885124	8.05559	0	0	0	0	0	0	6287.5	0	0	
433	8412	73.894485	8.17442	0	0	0	0	0	0	22303	0	0	
434	8413	72.96981	8.26828	0	0	0	0	0	0	17732	0	0	
435	8414	73.130936	8.42034	2648.54	0	0	0	0	0	45611	0	0	
436	8415	77.435127	8.3415	0	0	0	0	0	0	6718.6	0	0	

437	8416	77.740738	7.99695	6452.58	0	0	0	0	0	0	0	0
438	8417	72.016388	7.97386	0	4033.47	0	0	0	0	0	0	0
439	8418	74.902061	9.5111	0	0	3090.81	0	0	0	0	0	0
440	8419	77.766998	8.51358	0	0	3702.13	22054	0	46440	0	0	0
441	8420	72.237465	8.83919	0	0	20501	0	0	0	0	0	0
442	8501	84.178719	7.65381	12048.5	0	7813.02	0	21253	21284	0	12525	6308.7
443	8502	83.957863	7.74439	0	10652.89	15667	16234	0	0	13161	0	8155.5
444	8503	84.086372	7.93349	0	0	0	5233.7	0	0	0	4188.3	2875.7
445	8504	83.683189	8.17378	0	1773.93	1623.59	0	6341.5	0	4689.4	0	1893
446	8505	83.475159	8.7213	0	0	881.71	0	0	3536.6	1974.4	0	3016.9
447	8507	85.112068	9.23679	4815.21	0	1285.96	0	0	1249.7	1938	1298	11853
448	8508	84.606216	9.53479	91213.4	173742.1	18030.8	41953	41472	32801	40517	50682	25124
449	8509	82.710632	9.62492	76177.9	71794.23	5553.41	87421	75397	77286	56282	0	18620
450	8511	80.356674	7.75823	0	0	20714.6	93359	70739	0	0	43094	0
451	8512	81.138268	8.04837	0	0	0	13978	0	7547	9211.4	6111.1	0
452	8513	79.38398	8.07512	0	0	17751.4	0	0	0	0	0	0
453	8514	79.602104	8.14346	0	0	13860.4	0	0	0	0	0	0
454	8515	83.527306	7.84293	0	0	0	18157	0	0	0	0	0
455	8601	91.094879	7.7819	0	4575.1	0	0	0	0	7036.3	745.04	2776.3
456	8602	90.787415	7.91218	2513.23	3441.84	2623.84	0	3455.4	0	16614	1119.1	4001.2
457	8603	90.553169	8.03577	0	0	0	0	0	3072.1	0	0	2120.4
458	8604	91.554161	7.6675	0	0	0	0	0	0	4146.1	0	1602
459	8605	109.21822	7.68245	6830.62	2483.34	0	8132	0	0	0	0	1568.2
460	8606	89.927406	8.17193	0	0	2191.82	0	0	0	0	0	0
461	8607	90.421051	8.30289	0	0	3492.92	0	0	0	0	0	0
462	8608	90.793037	8.65878	0	0	5988.25	0	0	0	0	0	0
463	8704	138.86711	7.9088	0	0	0	0	0	0	0	0	4157.2
464	8705	115.94124	7.93889	0	0	0	0	0	0	1082.6	0	0
465	8801	147.03111	7.68395	0	0	0	3877.3	0	0	0	0	1776.4
466	9105	36.975006	9.8576	0	0	0	0	0	10654	0	28243	4994.5
467	9201	35.53199	9.85111	0	0	0	0	0	0	0	3310.3	0
468	9502	85.281441	9.71756	0	72392.99	70738.9	49751	0	0	27513	86697	13134
469	9503	85.398842	9.82491	8153.33	12092.1	40052.5	25538	45086	49904	5828.9	29868	20499
470	9601	85.213326	9.92274	0	0	0	0	0	12872	2558.3	0	0
471	9602	84.900002	10	0	0	0	0	0	0	4956.5	0	0
472	9603	90.5448	8.80689	0	0	0	0	0	6233.2	0	0	0
473	9604	106.3949	9.0691	0	0	0	0	0	800.48	0	0	0

Table S2. Total protein spots in postoperative patients vs. control

Number	SSP	MR	PI	VSD 1	VSD 2	VSD 3	Contro 1	Control 2	Control 3	P<0.01
				quantity	quantity	quantity	quantity	quantity	quantity	★ represent 4 differentially expressed protein spots identified by MALDI-TOF MS
1	308	53.75098	3.228633	708616.25	192921.48	669706.13	976877.69	268786	373769.94	
2	603	95.291534	3.223195	82978.82	91520.79	22900.32	66250.66	76157.71	55417.69	
3	701	103.4	3	6773.31	2412.72	3305.11	23962.65	713.85	6696.96	
4	702	124.42976	3.5174	1388.5	5167.91	2262.2	4950.92	2120.28	509.16	
5	703	101.45232	3.29454	0	0	0	0	2265.71	0	
6	1304	63.827499	4.489492	103665.41	125635.85	34913.58	49166.27	88090.99	38242.37	
7	1305	63.136322	4.580797	2571.12	2781.05	1196.38	3309.42	4668.3	2472.04	
8	1405	74.610786	4.43752	30653.27	29305.18	11193.64	13875.03	42033.7	7385.87	
9	1408	65.928154	4.31077	34521.29	30636.95	32491.15	11823.77	38850.49	106838.7	
10	1409	64.197731	4.387456	77271.51	15670.78	114574.89	24110.41	54222.5	17757.24	
11	1410	67.768356	4.187554	6235.18	2146.89	18402.75	4203.5	34996.07	15272.53	
12	1501	77.764374	4.232663	14186.39	6159.11	48677.22	7073.57	64941.59	17831.42	
13	1504	76.312645	4.340547	49117.51	25720.56	33612.76	18826.43	41477.64	46493.32	
14	1704	101.12689	4.592475	2600.32	3763.93	3894.51	4619.62	17852.5	5953.14	
15	2310	41.73428	5.032025	3319.89	8102.46	13338.18	9628.6	22772.56	10199.09	
16	2312	52.423134	4.936818	14047.62	19380.92	71177.84	67212.19	87980.61	55169.3	
17	2313	48.752831	4.861201	8093.68	4187.36	10583.2	78943.53	154949.55	119927.85	★
18	2314	53.747078	4.756154	3045.66	56.18	8209.59	22357.47	37867.63	9940.86	
19	2315	47.805485	4.995495	5739.89	9570.23	21337.04	7466.77	8992.99	23974.86	
20	2316	43.929615	4.737859	0	0	1886.55	8360.44	9745.14	5584	
21	2317	42.803608	4.900807	24564.76	12318.65	32387.37	37599.66	16511.11	15659.63	
22	2405	74.966393	4.796458	3162.7	6801.62	5988.09	6160.81	22095.66	1690.17	
23	2410	73.566956	4.954459	27757.49	11514.27	43281.57	14154.42	17585.93	32929.41	
24	2411	68.513908	5.038164	25297.8	42084.48	42139.41	84146.12	190750.25	134387.98	
25	2412	73.311852	4.532381	14712.86	34938.01	4974.98	15469.49	48082.9	25912.6	
26	2414	74.162514	5.14015	6866.13	11955.41	9610.86	7365.25	10039.17	16801.49	
27	2504	80.801262	4.849208	11840.4	9916.5	11267.67	13532.88	35602.68	6923.21	
28	2505	79.120155	4.951534	2931.82	8249.09	14183.32	11038.36	16423.33	30653.27	
29	2509	85.965919	4.682377	34633.72	14174.8	15897.18	19642.45	35186.86	34347.83	
30	2510	83.447426	5.095891	6370.75	8285.88	3185.08	6565.56	14130.31	9226.64	
31	2512	84.093758	4.990587	4635.35	6092.41	18780.42	6555.01	15831.74	9407.02	
32	2513	74.532898	4.876679	20901	12224.96	22513.97	10772.65	11840.56	15420.93	
33	2514	78.317039	5.031161	7157.26	11808.69	9077.67	7734.14	18428.46	15088.22	
34	2601	94.251289	4.71489	12150.99	12820.78	9842.63	11456.07	34981.12	13464.14	
35	2711	100.47666	5.239432	15164.5	41430.67	15960.97	10903.97	18147.42	9033.03	

36	3108	15.084836	5.416075	35013.73	24549.45	99078.09	113158.09	79577.13	49626.89
37	3202	29.960453	5.478655	0	0	0	3934.09	0	0
38	3301	49.349258	5.104475	58959.57	93807.95	54482.71	48263.86	224719.97	683182.13
39	3302	40.221207	5.125803	5118.62	7813.78	5976.07	5923.27	8728.73	4303.61
40	3303	60.49823	5.188143	0	0	0	0	9600	0
41	3305	60.122536	5.292578	31790.08	26751.32	13456.56	15662.5	13412.54	22602.75
42	3306	47.967907	5.295503	259975.41	201713.48	176209.83	102829.09	183869.3	233873.61
43	3307	59.828339	5.388268	23879.16	20290.23	20256.44	21472.59	17335.08	16444.14
44	3403	65.858833	5.237226	121708.08	33096.66	8316.9	45007.97	131845.77	32778.63
45	3404	72.293297	5.266505	21382.97	31653.77	15884.14	8852.52	8601.73	26714.53
46	3407	71.09877	5.49134	46761.07	15568.07	64828.36	83510.85	49941.27	55601.45
47	3409	67.865181	5.137219	19817.44	13039.65	15608.75	109529.15	24857.65	40972.03
48	3410	67.290207	5.373112	41191.28	13513.45	23867.6	50886.43	13692.71	18927.59
49	3411	66.346008	5.438021	13251	13419.71	13592.32	30600.1	24256.9	10677.87
50	3412	73.051529	5.398818	10184.69	17646.09	19150.79	28045.71	18508.82	24183.75
51	3413	70.371063	5.375284	37328.93	11046.63	1450.53	4046.57	5588.78	19814.55
52	3502	77.470528	5.144532	10911.48	12814.62	5800.66	5325.13	7381.92	6687.95
53	3513	88.434464	5.220301	47396.81	60523.42	15985.66	27668.58	42952.45	44135.68
54	3515	83.032303	5.298994	51975.2	81716.83	20885.74	22744.61	14989.4	41168.62
55	3517	79.58551	5.356828	23375.13	16342.81	13767.94	6893.44	13640.69	16464.48
56	3518	81.964844	5.390437	15335.76	95759.1	46968.3	75975.38	60800.54	59623.24
57	3519	79.688545	5.547524	2140.08	8068.38	4543.05	3485.62	5018.74	4652.4
58	3520	82.411636	5.537518	5006.42	11611.94	9179.52	8807.22	15098.64	21642.01
59	3521	77.137138	5.394059	0	0	0	4520.9	4082.69	2655.9
60	3522	77.044891	5.470041	0	0	0	9914.69	6417.91	5220.79
61	3523	76.43856	5.54646	0	0	0	3791.55	4262.69	4596.02
62	3524	82.431396	5.214635	23229.04	30368.95	18438.48	2873.31	13471.94	26118.29
63	3525	79.080887	5.429504	35055.13	5618.57	4035.68	1738.97	3694.1	4716.77
64	3608	93.6642	5.57152	4369.83	12264.08	2696.84	9040.83	19655.83	7914.33
65	3609	93.768669	5.349641	22673.18	13263.6	8088.66	9240.15	4840.39	7006.05
66	3610	93.680283	5.413783	12267.17	23732.53	6826.65	8883.8	8512.37	8187.65
67	3611	89.627892	5.422659	3782.8	11060.83	0	0	3209.34	8847.13
68	3612	81.622826	5.469908	52087.57	12715.42	46140.11	70367.78	42308.96	36278.37
69	3613	94.063164	5.467207	7590.86	11243.98	21913.3	4479.99	5195.6	4914.78
70	3614	87.333076	5.517429	3163.9	8456.54	8842.23	18808.54	10582.36	10780.46
71	3709	99.981239	5.315647	41769.03	37754.21	30310.21	44418.91	14266.04	28189.32
72	3710	99.520332	5.4133	18985.79	16626.39	21548.38	20766.64	24226.98	18748.51
73	4108	14.395176	5.950723	26354.48	6698.71	17124.89	14048.58	17295.86	23376.42
74	4202	27.429754	5.519047	99.87	3769.72	1800.74	9501.65	5556.44	8340.19
75	4203	20.6	5.7	42725.18	53180.66	48790.32	32031.24	54915.34	59332.3

76	4205	16.594303	5.78572	78970.14	169842.58	128802.36	146146.63	95093.3	119110.95
77	4206	28.520304	5.836925	7885.17	21016.67	12674.63	19626.19	14542.33	12506.83
78	4208	25.052305	5.568287	7680.18	7043.63	11101.72	36636.36	4059.18	7944.39
79	4209	30.264193	5.700826	0	0	0	5104.82	8317.32	8162.14
80	4210	29.699821	5.931857	0	0	0	0	1205.07	1243.79
81	4301	44.188007	5.48644	196.49	5750.35	784.96	11751.16	8242.14	119.59
82	4302	46.048759	5.488532	113249.28	191746.16	173718.72	65366.58	55001.5	190708.77
83	4303	59.884758	5.515368	1074.87	3445.05	0	3960.91	2955.8	0
84	4307	43.198597	5.662016	1244.28	10931.87	4148.61	10803.59	8632.56	1625.83
85	4308	46.06057	5.657016	17263.61	36776.54	42878.42	26523.18	38983.59	23157.11
86	4310	45.563591	5.85038	10133.4	26733.41	20376.85	32142.31	25323.54	8501.39
87	4311	42.252262	5.851346	8103.53	12053.9	8616.71	40341.46	34182.55	19276.66
88	4315	56.333012	5.756387	191246.28	296620.47	52916.3	64924.12	277657.91	214323.86
89	4316	56.258102	5.889024	185749.98	69485.48	288805.19	133709.53	66365.05	393698.41
90	4317	57.513206	5.624236	5502.79	15559.83	32346.39	25356.77	26243.4	26073.91
91	4318	53.24268	5.759084	1608.79	5208.5	2607.94	2940.66	3445.62	1771.79
92	4402	67.141808	5.516595	8599.66	7797.64	2404.29	9823.15	10248.95	14493.96
93	4407	72.265656	5.941123	13601.65	18457.21	18794.17	33201.32	31108.83	9522.1
94	4408	72.69458	5.702438	15527.77	31195.04	35398.12	14007.35	3459.33	6722.61
95	4409	72.220673	5.594825	12739.79	20829.29	21312.65	17728.29	18123.22	16514.6
96	4503	85.493698	5.597703	12626.07	23080.7	24706.21	29993.08	32668.68	33851.59
97	4506	83.318222	5.69781	84573.33	96700.64	50655.61	92752.13	67095.74	90669.73
98	4508	81.916275	5.80138	40257.92	238050.25	55827.7	232943.78	121014.55	133318.08
99	4509	75.728333	5.814042	0	44437.66	0	12334.43	4893.32	7576.12
100	4510	80.426552	5.926008	178916.23	253883.98	234658.78	155497.55	418631.03	291455.47
101	4511	79.129402	5.616607	2691.56	5908	2709.13	4854.25	4416.79	1902.77
102	4512	79.750313	6.087329	68610.56	97305.62	96430.77	113094.24	129656.81	168281.88
103	4608	97.392281	5.772105	2977.54	9258.71	4804.41	5564.69	6528.23	5867
104	4610	94.334229	5.858774	7930.54	15103.46	6637.94	6665.14	13367.29	9564.93
105	4613	94.61956	5.779109	6966.51	15889.89	11618.97	8320.37	14691.66	10531.33
106	4614	93.184265	5.660622	9549.2	12133.9	10080.44	7646.42	5121.48	8239.99
107	4615	93.043427	6.107409	48149.89	37701.55	13061.4	2686.18	3256.61	14123.76
108	4616	94.074272	6.047936	3280.92	16163.61	15101.69	10199.65	48832.91	12528.46
109	4702	101.40389	5.525258	19592.63	177646	190520.91	85441.17	134407.7	62666.92
110	4710	126.38646	5.609995	22408.36	25334.26	27433.4	35324.34	5203.4	28680.47
111	4711	126.21771	5.738339	7445.25	12442.91	9995.67	6596.9	10292.34	11116.15
112	4712	96.917664	5.839162	9040.81	15304.19	4025.87	4872.33	5555.53	4995.6
113	4713	96.715744	5.899492	54814.14	8005.6	3548.25	213.03	10098.64	7219.58
114	5201	20.435503	5.983393	153540.36	104797.8	151940.88	143334.88	325534.19	169811.02
115	5202	20.608208	6.333179	159402.55	77426.51	30096.99	204042.47	200777.41	263946.38

116	5203	37	6.4	2115.46	2430.44	921.28	5906.04	6245.54	3264.22	
117	5302	58.73111	6.642779	4146.43	6922.51	2051.38	6181.8	4562.08	4333.92	
118	5304	58.01445	6.058127	20497.29	17329.52	5537	6613.92	16412.57	16301.03	
119	5305	41.986263	6.023441	1781.73	3865.17	3225.49	13924.64	2817.17	2194.3	
120	5306	46.155128	6.023441	914.48	3132.72	2514.81	5603.08	931.93	1741.4	
121	5307	60.133869	6.377947	1784.07	3554.82	887.12	4107.03	2073.27	1564.16	
122	5308	43.394848	6.097419	0	0	0	2568.14	3273	3360.48	
123	5309	51.989727	6.288337	781.74	2156.59	864.99	237.98	1347.69	1381.56	
124	5310	48.829674	6.295348	436.04	2082.24	834.93	812.17	1980.96	585.5	
125	5401	73.219345	6.040141	26330.93	70232.04	73012.45	68299.82	91336.77	4456.72	
126	5403	70.599678	6.292275	26531.46	18569.37	19079.47	18859.3	16789.88	24510.12	
127	5405	67.619011	6.63299	39568.13	33603.54	18402.03	40183.16	26264.39	23358.82	
128	5407	62.337059	6.592499	2613.29	3540.18	0	7193.1	1838.96	6248.02	
129	5511	79.51622	6.21361	154386.64	89009.4	142907.86	91390.7	28910.26	51608.03	
130	5512	84.027908	6.14075	171831.73	26908.12	56557.75	31058.66	15650.58	37287.3	
131	5513	83.246933	6.225085	45856.51	41613.77	51489.51	27281.81	6554.43	60906.7	
132	5514	82.479393	6.327631	21953.48	3336.15	14252.4	13148.51	7019.06	37314.62	
133	5515	79.326431	6.02411	153703.38	182926.78	213196.8	203663.47	50525.46	215096.83	
134	5516	76.745178	6.090461	29479.42	7729.99	26757.31	30812.02	48469.41	19158.41	
135	5609	96.193886	6.667656	72862.29	23437.32	59867.04	117159.6	57078.44	52927.82	
136	5611	92.391083	6.156599	41765.02	15344.9	19032.3	14123.72	2930.35	14413.11	
137	5612	98.419449	6.024427	15536.48	60531.42	150808.48	7875.63	16126.55	165738.3	
138	5614	92.205299	6.381066	5899.17	7696.04	2760.81	2932.27	4612.73	1725.76	
139	5615	85.920647	6.561874	35074.04	17055.8	16958.52	22725.43	19095.58	74691.85	
140	5616	87.21991	6.336704	61616.65	13877.75	17834.52	19863.54	15151.2	16839.89	
141	5617	86.201828	6.447507	34179.35	16785.16	19577.01	18094.01	39232.89	27817.28	
142	5618	87.546425	6.259118	12546.95	17871.91	23988.45	10333.61	1593.56	33262.4	
143	5619	90.868614	6.265385	76476.2	43578	41731.22	35048.5	34233.21	70868.61	
144	5703	109.3625	5.975655	25065.26	219211.11	580647.25	280814.69	501305.34	1730781.13	
145	5705	100.88771	6.004876	82800.11	20938.96	144824.17	615739.38	402617.13	169625.78	
146	5713	100.33884	6.390278	22784.49	25918.45	28897.65	64901.38	101877.34	66396.52	★
147	5721	96.47451	6.409903	82848.35	36376.05	21093.91	99415.67	68612.37	151508.52	
148	5722	96.073822	6.567084	92798.35	31495.64	34514.46	76019.97	95069.59	78045.49	
149	5723	96.873901	6.767138	37863.03	12934.1	26497.9	15793.66	19071.88	30289.99	
150	5724	103.55337	6.397872	0	0	0	0	6762.15	42337.04	
151	6201	28.277126	6.807684	41690.37	18379.94	13303.68	18411.06	12855.48	11456.88	
152	6204	34.641285	7.21489	87186.16	78492.27	157186.89	114674.54	112080.62	101273.87	
153	6205	35.722145	6.922492	1483.81	3266.24	1375.56	3591.03	3763	11616.47	
154	6206	39.580093	6.814374	2240.03	838.59	379.05	2041.73	2042.29	2950.68	
155	6207	36.366077	6.622732	2594.22	4050.27	0	3968.43	877.78	1141.34	
156	6208	29.502281	6.972403	4013.82	13641.15	18015.44	10609.37	8468.47	5524.74	

157	6209	23.800369	6.935859	0	0	0	10415.95	7226.46	6746.81	
158	6210	29.618456	7.337079	2924.17	3967.89	2029.22	2664.06	1030.59	1935.14	
159	6211	27.593985	6.817189	34799.98	16627.5	18094.35	9428.65	8950.11	5295.26	
160	6212	28.236675	7.008859	958.02	8717.54	13750.74	9527.15	4474.59	1521.93	
161	6213	20.448137	6.778597	7640.2	6289.83	0	12395.75	8722.26	9087.19	
162	6301	59.511997	6.729281	13235.78	11547.21	6766.87	16486.76	12732.14	10815.21	
163	6303	55.933678	6.884992	4060.43	6776.48	3638.47	5843.93	6811.4	6199.54	
164	6304	60.334377	6.92458	589.16	5031.47	3112.3	19507.51	24109.88	12388.42	
165	6306	63.079105	7.186164	0	0	0	3133.06	2340.97	2115.52	
166	6307	59.448261	7.209593	1108.77	2824.39	1835.16	13073.79	13382.65	15257.51	★
167	6309	60.933651	7.176187	3530.12	4439.02	4685.38	4566.28	3851.9	4378.3	
168	6310	51.099998	6.8	1628.91	3080.33	1185.12	2269.72	1516.88	2221.02	
169	6311	57.178787	6.762667	2168.03	2505.9	0	5116.65	5630.78	2631.63	
170	6402	64.887802	6.818441	109136.51	84145.63	145554.64	142403.72	99833.91	104790.77	
171	6404	64.616508	6.969593	101624.56	139742.23	24636.14	66242.02	202150.59	295353.94	
172	6406	65.380852	7.1813	0	0	0	0	11414.62	0	
173	6410	64.119705	7.304842	315382.06	228174.81	51850.52	68436.87	42929.54	367170.53	
174	6411	73.357475	7.205573	5708.18	6688.74	6749.74	7175.45	7012.22	10194.88	
175	6412	65.928764	6.573778	5387.76	6469.79	2880.94	10091.52	15482.91	16604.43	
176	6413	62.156204	7.035269	0	0	0	0	7149.54	0	
177	6414	67.885803	7.023471	0	22342.58	6051.02	0	7723.66	14590.77	
178	6508	76.962341	7.453789	62335.51	33435.9	362793.75	16045.93	239452.59	45696.31	
179	6510	78.336288	7.286368	50549.98	26041.66	66535.33	12036.11	37101.44	43904.36	
180	6511	82.943192	7.207672	30861.71	15673.66	25116.72	6874.84	41036.57	36577.05	
181	6512	75.947685	7.21355	2738.14	14188.58	32620.04	43656.33	31704.82	16498.32	
182	6513	88.877571	6.698854	46617.14	11046.63	75796.16	26344.88	19697.87	91430.5	
183	6514	85.160515	6.638875	74240.42	81777.06	43748.41	64350.46	43922.96	47129.59	
184	6515	83.653275	6.715314	110569.84	14302.04	164319.09	173812.06	2915.04	68956.44	
185	6516	78.975075	7.229045	63395.12	12831.86	27143.71	6127.2	28362.13	57598.17	
186	6518	83.141479	6.83231	52700.6	1387919.3	92069.61	45149.46	177963.3	336845.88	
187	6519	84.600113	7.121251	116654.83	15313.12	33091.3	163914.19	97101.2	32130.87	
188	6520	82.673576	6.992909	612344.5	22549.17	872035.75	893061.69	408464.28	524969.38	
189	6613	89.593246	6.621112	20837.48	15237.97	14121.08	44373.44	25682.42	29144.1	
190	6614	85.589996	7.458669	9146.84	9599.55	4886.47	11236.55	14001.76	8464.85	
191	6713	98.63195	7.218313	3280.42	5733.22	4841.23	8853.85	10670.64	3985.99	
192	6714	98.13829	7.292304	7172.56	6718.92	9946.33	4592.6	11937.13	6922.25	
193	6715	98.543167	7.372056	5673.17	5407.9	4665.74	3663.83	8021.13	6216.91	
194	6716	98.130402	7.47302	2438.92	3391.85	2430.04	2408.65	3917.44	2510.23	
195	6717	97.340378	7.417755	841.04	4612.72	5945.27	9487.32	7150.29	4717.58	
196	6718	96.966408	7.512439	3301.1	4626.61	3404.98	4395.11	5145.31	4398.5	

197	7201	27.182077	7.639426	64359.52	38171.36	83295.54	34256.94	22576.61	20842.04	
198	7202	30.6	7.7	0	0	0	43473.04	28006.02	28636.16	★
199	7204	27.325821	8.364484	89982.05	69327.85	88624.46	41448.29	37057.39	15485.19	
200	7205	32.296295	8.457034	0	0	0	13075.78	5784.8	0	
201	7206	35.372967	8.253143	2546.34	9095.82	12114.84	6520.05	7670.33	0	
202	7207	29.03377	7.932686	19752.45	13336.62	15911.53	21100.32	10857.45	2942.06	
203	7208	24.762571	7.78394	0	0	0	4955.74	3598.17	4568.77	
204	7209	28.006899	8.443879	24701.36	11265.17	18543.93	10389.33	8265.2	4508.57	
205	7301	63.118958	7.511454	0	0	0	4560.56	5680.65	3147.42	
206	7302	56.416065	7.832244	3368.85	5984.63	5150.55	6574.57	6211.45	5477.81	
207	7304	63.753345	7.951801	2417.72	2853.26	3861.05	4417.24	10723.26	3717.17	
208	7305	63.453228	8.333614	0	0	0	5500.15	4228.67	3893.26	
209	7401	65.053551	7.56965	0	0	0	0	7709.64	0	
210	7402	64.307251	7.599546	33932.26	24611.79	55517.36	46544.65	35704.58	28180.15	
211	7404	65.402756	7.788447	8147.32	6157.01	3515.33	6885.71	6770.11	4689.4	
212	7409	74.232178	8.222845	48141.87	30512.06	16407.14	41400.29	118012.89	93326.27	
213	7414	74.189011	7.879743	35351.96	27346.56	72318.67	112162.09	98485.64	69182.84	
214	7415	66.625259	8.0939	4938.17	5960.69	7446.3	5865.56	5822.23	3436.09	
215	7416	73.044197	7.421786	5048.91	5856.03	6105.89	12377.6	6801.19	7836.03	
216	7417	72.433388	7.645866	42.62	3847.06	2617.67	4477.6	2821.19	4330.31	
217	7418	74.775635	7.63139	522.16	17394.25	54753.76	41409.63	40434.22	20085.96	
218	7419	75.074867	7.419796	3310.03	21330.46	18538.33	21186.61	13787.65	24538.95	
219	7420	71.785446	8.304024	21045.97	9986.74	13377.2	12880.82	8757.35	4435.8	
220	7421	64.884621	8.099698	7965.69	4779.05	2510.78	2258.19	4957.73	3113.23	
221	7422	67.593979	7.771114	2214.89	5127.29	3857.75	7501.25	6398.95	3913.65	
222	7502	77.61232	7.703588	396398.75	135664.25	38242.83	191157.84	295202.44	159278.95	
223	7504	76.192513	8.001943	403625.88	26332.29	151647.17	31128.71	172301.3	21328.5	
224	7506	79.273132	8.110284	4134.81	9449.8	6798.67	20989.27	11968.91	7922.28	
225	7510	77.0504	7.813771	47097.68	107475.87	20639.3	154585.02	26290.38	17984.47	
226	7511	76.769501	8.245502	44526.77	15078.12	126719.14	24060.82	8247.64	12868.82	
227	7512	77.51606	7.563348	78756.73	24486.71	32457.7	96380.19	30098.16	104238.48	
228	7514	78.88224	7.400971	75216.04	28041.26	48679.54	226849.61	83817.85	64294	
229	7608	90.198784	8.377399	1591.45	3918.22	2752.9	4165.35	13097.72	2085.4	
230	7609	90.489876	8.115012	2930.78	4201.25	3078.99	6219.86	3641.48	254.3	
231	7610	90.545395	8.271983	12563.04	6025.95	5996.59	5322.01	5360.67	5167.21	
232	7611	90.737373	7.950207	2428.11	2546.11	1486.05	3013.12	2204.22	923.13	
233	7711	97.9086	7.722767	13008.05	3501.04	2736.09	8779.58	3967.13	5391.13	
234	7712	97.277237	7.60442	5715.06	6454.45	3454.05	2734.56	3916.84	2928.36	
235	8201	34.029339	8.767346	7823.37	8179.81	9864.45	7997.55	7935.16	0	
236	8202	34.733913	8.769247	3343.68	22347.74	28758.28	31082.6	17681.28	0	
237	8204	34.766781	9.150815	8078.19	6522.93	9036.12	11688.69	8039.4	8226.29	

238	8209	31.468376	9.238902	0	0	0	17250.53	14269.82	0
239	8210	28.545645	8.789179	0	0	0	4328.4	5221.6	0
240	8211	28.370546	9.184835	0	0	0	5793.02	5007.61	0
241	8212	27.420809	8.810859	9727.58	11832.33	24351	0	0	0
242	8401	69.939674	8.752387	50403.34	9595.43	37872.09	33140.05	19743.88	4406.29
243	8402	73.994881	8.446354	21531.04	225688.08	286858.44	35010.73	11538.07	40979.11
244	8403	69.813484	8.953663	1633.35	11139.1	25003.62	2601.21	2572.26	3851.56
245	8506	81.699997	10	33982.08	22244.75	146739.48	63396.97	100834.17	20221.15
246	8507	79.202614	8.550418	2216.57	2541	1840.2	5310.78	3258.24	4093.73