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A New Trauma Frontier: Exploratory Pilot Study of Platelet Transcriptomics in Trauma Patients

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Abstract

Background: The earliest measurable changes to post-injury platelet biology may be in the platelet transcriptome, as platelets are known to carry *messenger* ribonucleic acids (RNAs), and there is evidence in other inflammatory and infectious disease states of differential and alternative platelet RNA splicing in response to changing physiology. Thus, the aim of this exploratory pilot study was to examine the platelet transcriptome and platelet RNA splicing signatures in trauma patients compared to healthy donors.

Methods: Pre-resuscitation platelets purified from trauma patients (n=9) and healthy donors (n=5) were assayed using deep RNA sequencing. Differential gene expression analysis, weighted gene co-expression network analysis, and differential alternative splicing analyses were

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performed. In parallel samples, platelet function was measured with platelet aggregometry, and clot formation was measured with thromboelastography.

Results: Differential gene expression analysis identified 49 platelet RNAs to have differing abundance between trauma patients and healthy donors. Weighted gene co-expression network analysis identified co-expressed platelet RNAs that correlated with platelet aggregation. Differential alternative splicing analyses revealed 1188 splicing events across 462 platelet RNAs that were highly statistically significant (false discovery rate <0.001) in trauma patients compared to healthy donors. Unsupervised principal component analysis of these platelet RNA splicing signatures segregated trauma patients in two main clusters separate from healthy controls.

Conclusions: Our findings provide evidence of finetuning of the platelet transcriptome through differential alternative splicing of platelet RNA in trauma patients, and that this finetuning may have relevance to downstream platelet signaling. Additional investigations of the trauma platelet transcriptome should be pursued to improve our understanding of the platelet functional responses to trauma on a molecular level.

Study type: Basic Science

Level of evidence: N/A

Keywords

Blood Platelets; Wounds and Injuries; Sequence Analysis; RNA

Background

Trauma induced coagulopathy (TIC) is a potentially devastating acquired multi-phenotypic failure of vascular homeostasis caused by severe injury and hemorrhagic shock, and is associated with bleeding, thrombosis, inflammatory complications, and death (1, 2). Platelet dysfunction is often reported as a critical component of TIC and its complications. However, despite a large body of literature identifying platelet dysfunction in up to half of injured patients, the *ex vivo* evidence in support of this is discordant, with increased levels of activated platelets that contribute to hemostasis, yet paradoxically impaired platelet aggregation(1, 3–5). Further, impaired platelet aggregation is commonly identified outside of TIC and in patients with minor injury, is not accounted for by thrombocytopenia, and cannot be rescued with platelet transfusion (5–8). Numerous factors have been implicated in this biology (9–14), however given the contradictory findings and complexities in the *ex vivo* study of platelet function (related to the small size and reactive nature of platelets, as well as limitations of the available assays), it remains unknown whether the described platelet dysfunction in trauma is pathologic or not, and whether it requires treatment (5). As such, there is a clear need for improved methods of studying post-injury platelet biology.

Despite being small and anucleate, platelets participate in an array of physiological functions including clot formation and breakdown, endothelial maintenance, and immune response (15). Platelets do not transcribe genes *de novo*, but they carry megakaryocyte derived *messenger* ribonucleic acids (RNAs) to support these various functions. There is evidence that this platelet transcriptome is stable in health (16). However, it has been identified that

in some infectious and inflammatory disease states, platelet RNA can undergo differential alternative splicing in response to physiological signals as part of the tailored platelet response to that disease state (17–26). Therefore, transcriptomics is emerging as an attractive means of studying the platelet response to disease (25, 27–30). Recently, we identified numerous RNAs potentially involved in platelet function in the plasma of traumatic brain injury patients (31), and hypothesized that platelets themselves were the most likely source of this. However, it remains unknown if the physiology of trauma alters the platelet transcriptome, or if differential alternative splicing of platelet RNA could be responsible for the described changes in platelet function after injury. Thus, the aim of this exploratory pilot study was to examine the platelet transcriptome and platelet RNA splicing signatures in trauma patients compared to healthy donors.

Methods

Participant Enrollment and Sample Collection

Whole blood was collected in standard laboratory vacuum-sealed tubes containing 3.2% (0.109mol/L) sodium citrate (Becton Dickinson, Franklin Lakes, NJ) from a convenience sample of trauma patients who met trauma activation criteria on arrival to the emergency department prior to resuscitation, and from healthy donors over a two year period. From the citrated whole blood, platelets were immediately isolated for RNA sequencing, and functional assays of clot formation and platelet aggregation were performed by the below described techniques. Clinical characteristics of the trauma patients were collected in parallel, however given the exploratory pilot nature of the study, no power calculations were performed, and no *a priori* clinical hypotheses were made. Healthy donors were recruited during the same two year time period, underwent the same sampling, and limited clinical characteristics were collected in parallel (age, sex, and use of anti-coagulants and anti-platelets). Samples were not analyzed if participants were determined to be under age 18, pregnant, in custody, or receiving anti-coagulant or anti-platelet medications. This study was approved by the Institutional Review Board of the University of California, San Francisco (IRB#19–28933) and informed consent was obtained from all participants.

Platelet Isolation

Platelets were immediately isolated for RNA sequencing, based on previously described methods (18, 22, 24). Briefly, fresh whole blood was centrifuged at 200Xg for 20 minutes. The resultant platelet rich plasma supernatant was treated with Prostaglandin-E1 (PG-E1, 600nM, Cayman Chemical, Ann Arbor, MI), and centrifuged at 800xg for 20 minutes to pellet platelets. Platelets were resuspended in PIPES Saline Glucose buffer (PSG, 5mM PIPES, 145mM NaCl, 4mM KCl, 50μM Na_2HPO_4, 1mM $MgCl_2{\cdot}6H_2O$, 5.5mM glucose, pH 6.8) with PG-E1 and incubated with magnetic bead-conjugated anti-CD45 (Cat. No. 130-045-801, Miltenyi Biotec, Bergisch Gladbach, Germany). Leukocytes were negatively selected by passing the platelet anti-CD45 bead suspension through a magnetic column and platelets were pelleted by centrifugation and resuspended in M199 medium (Lonza, Basel, Switzerland). Platelets were again pelleted by centrifugation, lysed in Trizol (Life Technologies, Carlsbad, CA) and stored at −80°C. All platelet isolates were stored for <2 years to limit storage or RNA degradation effects.

Molecular Biology and RNA Sequencing

From platelet lysates stored in Trizol, RNA was isolated using the Qiamp RNA Blood Mini Kit (Qiagen, Hilden, Germany). Deoxyribonucleic acid (DNA) contamination was removed with DNAse I and sample quality was assessed by Bioanalyzer (Agilent, Santa Clara, CA). Complementary DNA (cDNA) libraries were generated with 1–2ng of RNA using the Ovation random primed isothermal amplification system (NuGen, Redwood City, CA). Sequencing was performed at a depth of 100–400 million reads using a Novaseq 6000 (Illumina, San Diego, CA).

Transcriptomic Analyses

Differential Gene Expression Analysis: Platelet RNA Abundance in Trauma Patients vs. Healthy Donors—First, we performed differential gene expression analysis to compare platelet RNA abundance in trauma patients compared to healthy donors. RNA sequencing data were aligned to the GRCh38 assembly of the human genome and mapped with release 100 of the Ensembl gene annotations of this genome using STAR Aligner (version 2.6.0c) (32). Quality was checked using fastQC (version 0.11.5)(33). To compare overall platelet RNA abundance, gene level expression values were then quantitated from mapped reads using featureCounts within the Subread package (version 2.0.0) (34). Differential gene expression analysis was performed by a generalized linear model within EdgeR (35). We assessed for significance using a false discovery rate (FDR) of 0.05 under the Benjamini-Hochberg (BH) procedure (36, 37).

Weighted Gene Co-Expression Network Analysis: Platelet RNA Module-Trait Relationships in Trauma Patients and Healthy Donors—Next, weighted gene co-expression network analysis (WGCNA)(38, 39) was utilized to further characterize platelet RNA abundance associated with trauma. Briefly, WGCNA constructs a network of genes from patterns of co-expression in an agnostic manner with no *a priori* patient characteristics. Sets of genes sharing co-expression patterns (i.e., modules) are identified in the resultant network. This approach allows for an independent unsupervised interpretation of the gene expression data (38). In doing so, a summary score of the expression of genes in these modules were evaluated for an association with platelet aggregation responses. Following identification of modules in our data we performed a further analysis examining group membership using higher orders of biological organization including gene ontology and protein-protein interaction analyses.

Following alignment, the log counts per million gene lists were filtered with a threshold of 1 count per million in at least five samples in order to remove the lowest expressed reads. From this the resultant genes were used for WGCNA analysis. We selected an empirical soft power threshold of 17, which represented a strong model fit to a scale free topology (signed $R^2 = 0.80$) to generate the signed adjacency matrix. A clustered gene tree was generated using the “average” method. Genes with highly correlated expression were grouped into modules based on topological overlap. Each module was assigned a color, and this color label was used for identification in all subsequent analyses. The module eigengenes (i.e., the first principal component of variation among the co-expressed genes in that module) of each of the co-expression modules was tested for an association with platelet aggregation

responses. Pearson's correlation coefficients and *p*-values were calculated for each pair of eigengene and platelet aggregation response. A significant correlation was assessed using a p-value of <0.05. To evaluate for higher orders of biological organization of genes from modules where traits correlated with module expression levels, we evaluated for functional enrichment using pathway analysis (GO, KEGG and Reactome) using Metascape (40) and the Search Tool for the Retrieval of Interacting Genes (STRING) (41) for protein-protein interaction elucidation. We assessed for significance of the functional enrichment pathway analysis using a p-value <0.01.

Differential Alternative Splicing Analyses: Platelet RNA Splicing in Trauma Patients vs. Healthy Donors—Analysis of differential alternative platelet RNA splicing was performed using alternative splicing mapping tool (rMATS) (42). We assessed significance of the differential alternative splicing with a FDR cutoff of <0.001. For each splicing event, a ratio was calculated for each sample between transcripts found to include that splicing event and transcripts found specifically without that splicing event. Unsupervised principal component analysis was performed on the samples' splice junction inclusion ratios within R (version 4.1.1). K-means clustering was performed to group samples into clusters with maximum similarity, using the 'kmeans' method in R. A pathway overrepresentation test was performed to identify pathways that are enriched for genes showing differential alternative splicing events using Gene Ontology (GO) Enrichment Analysis (43–45).

Functional Assessment of Clot Formation and Platelet Aggregation

Rotational Thromboelastometry: Clot Formation—Rotational Thromboelastometry (ROTEM, Werfen, Barcelona, Spain) was performed to assess global clot formation immediately on whole blood according to manufacturer's directions. Extrinsic clotting pathway function was tested using Extem reagents. The Clot Formation Time in seconds (CFT), α angle in degrees, Maximum Clot Formation in mm (MCF), and percent Maximum Lysis (ML) were quantified.

Multiple Impedance Platelet Aggregometry: Platelet Aggregation—Platelet aggregometry using a Multiplate multiple impedance aggregometer (Roche, Basel, Switzerland) was performed to assess platelet aggregation in response to stimulation immediately on whole blood according to manufacturer's directions. The following platelet surface stimulants were used (all from Hart Biologicals, Hartlepool, UK): ADP (final concentration 6.45μM), thrombin analog (SFLLRN, final concentration 32.3μM), or collagen (final concentration 3.23μg/mL). An extensive panel of platelet aggregation responses to each surface stimulant were included: areas under the aggregation curve (AUC), maximal aggregation value (aggregation units), maximal aggregation velocity (velocity units), baseline (pre-stimulation) aggregation value (impedance units), and endpoint (post-stimulation) aggregation value (impedance units). Deviation from the mean and correlation coefficient served as quality control values.

For all functional assessments of clot formation and platelet aggregation, Kruskal-Wallis tests were used to assess overall statistical significance and Wilcoxon signed rank tests were used to make pairwise comparisons.

Results

Trauma Patients and Healthy Donors

We isolated platelets from a total of nine trauma patients and five healthy donors. The trauma patients suffered from a range of severity of injuries, but all had normal platelet counts (median and interquartile range of 267 and 224–309 $\times 10^9$ platelets/L; additional clinical characteristic details in Supplementary Table 1).

Transcriptomic Analyses

Differential Gene Expression Analysis: Platelet RNA Abundance in Trauma Patients vs. Healthy Donors—Forty-nine platelet RNAs were found with differing abundance in trauma patients compared to healthy donors (Table 1), primarily mitochondrial or annotated as such. All but two platelet RNAs were found to be present in lower abundance in trauma patients than in healthy donors.

Weighted Gene Co-Expression Network Analysis: Platelet RNA Module-Trait Relationships in Trauma Patients and Healthy Donors—Correlative module-trait relationships were identified for sets of co-expressed platelet RNAs and platelet aggregation responses. Multiple sets of co-expressed platelet RNAs correlated with measures of platelet aggregation responses (cyan, brown, light cyan; Figure 1 and Supplementary Figures 1–12).

Differential Alternative Splicing Analyses: Platelet RNA Splicing in Trauma Patients vs. Healthy Donors—We found 1188 splicing events across 462 platelet RNAs (FDR <0.001; Figure 2A) in trauma patients compared to healthy donors. There was relatively uniform platelet RNA splicing across the healthy donors, but unsurprisingly a higher degree of heterogeneity across the trauma patients. Using unsupervised principal components analysis, three main clusters formed: healthy donors (Group 1), and two separate trauma clusters (Group 2 and Group 3; Figure 2B). There was one outlying trauma patient (#7) that did not cluster with others. These clusters were consistent with those identified by K-means clustering when applied to all 1188 significant splicing events (Supplementary Figure 13). We performed a Gene Ontology enrichment analysis across the platelet RNAs that contained significant splicing events to identify associated biological processes (Figure 2C). We found these platelet RNAs were involved in coagulation, platelet activation, and wound healing, as well as a myriad of other processes including immune response, post-transcriptional gene regulation, and other signaling and cell physiologic processes.

Finally, given the identification of splicing of platelet RNAs involved in coagulation and platelet activation in trauma patients compared to healthy donors, we next examined if Group 1, 2, and 3 differed in clot formation and platelet aggregation responses (Figure 3). Relative to Group 1 (healthy donors), Group 2 and Group 3 (trauma) had trends toward more

disordered clot formation (Figure 3A). Specifically, clot formation time was prolonged, while α angle and maximum clot formation were decreased. Further, Group 2 had trends toward the lowest platelet aggregation responses to ADP, thrombin analog, and collagen stimulation (Figure 3B).

Discussion

The results of this exploratory pilot study show promise for applying a transcriptomic approach to the study of post-injury platelet biology. Specifically, we have shown evidence of finetuning of the platelet transcriptome through differential alternative splicing of platelet RNA in trauma. Further, our results preliminarily support that both the static and dynamic platelet transcriptome after trauma has relevance to downstream platelet signaling, as has been identified in other disease states (25, 26, 28–30). In the case of the WGCNA analysis, our findings suggest that co-expressed platelet RNAs correlate with platelet aggregation responses that are specifically perturbed in the context of trauma physiology. In the case of the differential alternative splicing analyses, using unsupervised techniques two main clusters of trauma patients formed based on their platelet RNA splicing signatures, and these clusters appeared to differ in clot formation and platelet aggregation responses. Overall, we believe these findings suggest there may be biologic and physiologic relevance to finetuning of the platelet transcriptome in trauma patients, and substantiate that platelet transcriptomics may be valuable for improving our molecular understanding of the often-described but poorly understood post-injury platelet dysfunction.

The spliced platelet RNAs we identified in trauma patients are known to be involved with cytoskeletal function, vesicle fusion, cell signaling, and intriguingly, *regulation* of splicing. As such, we hypothesize that platelet RNA could be used to further activate or repress additional splicing and downstream functions. In fact, while the number of platelet RNA splicing events that passed very high statistical significance was large (FDR <0.001, 1188 splicing events) the overall percent change of the splicing ratios was relatively small (data not shown), suggesting a significant proportion of platelet RNA is not spliced in circulating platelets early following injury. This could mean that following any degree of injury, there are transcriptionally prepared circulating platelets, and a major signaling event (i.e. shock, intracranial hypertension, etc) may be required to complete maturation of platelet RNA for translation into necessary gene products specific to the signal received.

Several limitations warrant consideration. *First*, while the sample size is small, this was an exploratory pilot transcriptomics study, and our results passed very stringent statistical cutoffs. Further, our sample size is compatible with other exploratory platelet transcriptomic studies (22, 46, 47). *Second*, although unsupervised analyses can be subject to unaccounted for confounders, the unbiased nature of our cDNA preps, mapping efficiency of using paired end Novaseq runs, and incorporation of high read depth of the samples maximized our opportunity to find the differential alternative spliced platelet RNAs in trauma patients. *Third*, we did not power the study to be able to examine differences in clinical characteristics between trauma patients and cannot make any conclusions on the clinical data of the patients. We recognize that the trauma patients studied were heterogeneous, including patients suffering from a range of severity of injury (from minor to severe), and

patients with and without shock. Given platelet dysfunction is identified throughout the trauma literature in up to half of trauma patients regardless of injury severity or shock state, this representative cohort is relevant to an exploratory analysis of the platelet transcriptome in trauma patients compared to healthy donors. Our cohort included patients with low injury severity scores, suggesting that severe injury is not necessary to trigger alterations in the platelet transcriptome. This is relevant given the previous trauma literature has identified platelet dysfunction in patients with minor injury, and may support our hypothesis that any amount of injury could create transcriptionally prepared circulating platelets. In addition, although the healthy donors and trauma patients were relatively well matched in age, there was sex variation in the healthy donors but not the trauma patients. It should be noted however that despite sex variation in the healthy donors, they clustered tightly together, an initial suggestion that the platelet transcriptome may be conserved between men and women. Hypotheses related to the effects of clinical differences of the trauma platelet transcriptome will be testable with large, diverse, and carefully phenotyped sample sets, now that our pilot data supports this line of investigation. *Finally*, there are known other RNA regulatory mechanisms that we did not examine that may also contribute to the trauma platelet transcriptome (48, 49). Furthermore, while we did not perform proteomics, it can be reasonably assumed that a subset of the platelet RNAs we examined are for the purpose of production of protein products (50).

In summary, we have shown evidence of finetuning of the platelet transcriptome through differential alternative splicing of platelet RNA in trauma patients, and that this finetuning may have relevance to downstream platelet signaling. Additional investigations of the trauma platelet transcriptome should be pursued to improve our understanding of the platelet functional responses to trauma on a molecular level and to uncover potential unique therapeutic targets for platelet-based contributions to hemorrhage, thrombosis, development of organ failure, and death after injury. Future studies with larger patient populations are necessary to allow for incorporation of more extensive clinical data and additional measures of post-injury platelet function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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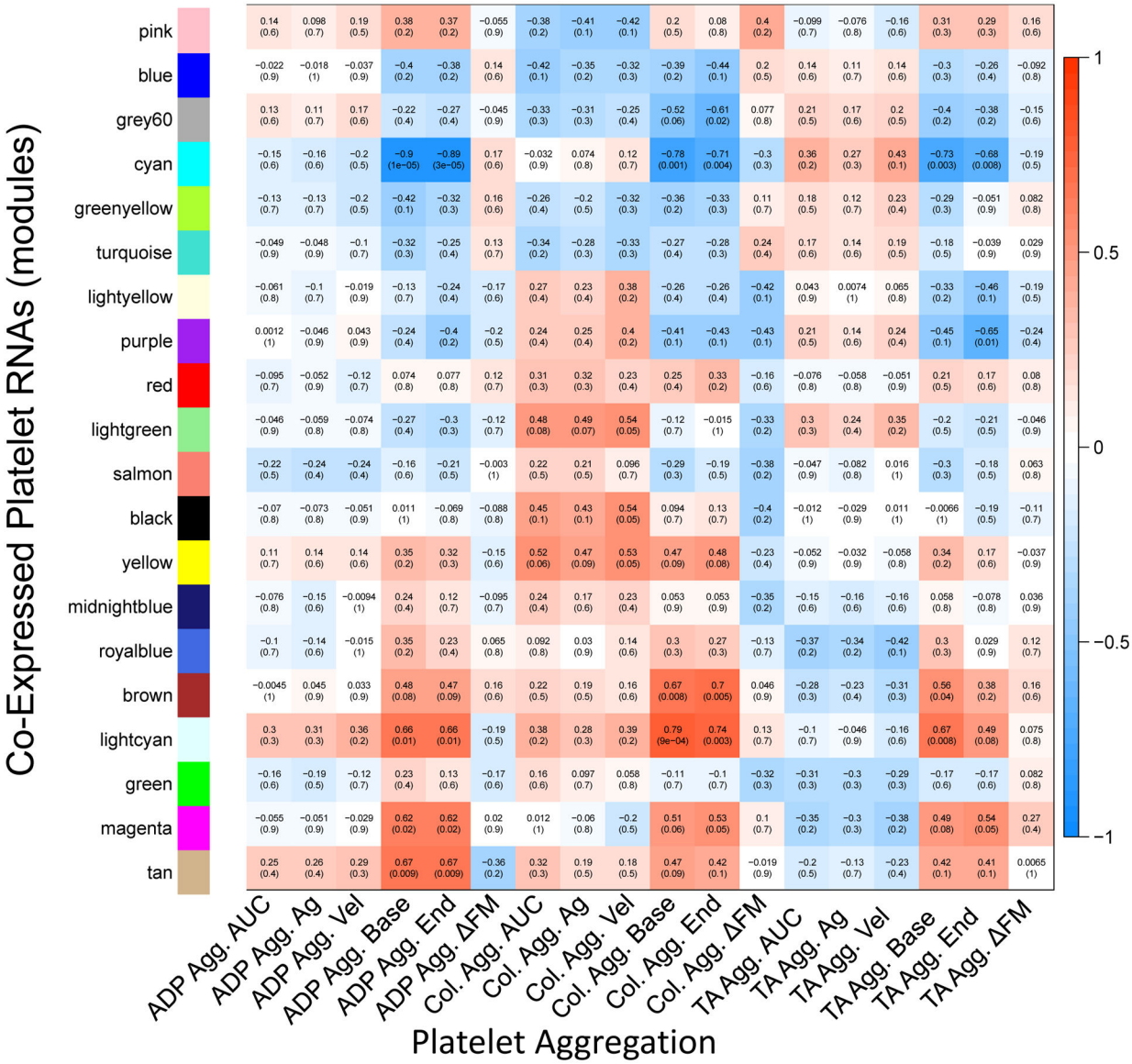

Figure 1. Heatmap of correlations of co-expressed platelet RNAs and platelet aggregation.

Within each box the top number indicates the calculated Pearson's correlation coefficient, and the bottom shows the p-value. Positive correlations are depicted in red and negative correlations are depicted in blue. For example, the cyan set of co-expressed platelet RNAs correlated negatively with baseline aggregation for all three stimulants, suggesting its involvement in platelet aggregation. Agg., aggregometry; Ag, maximal aggregation value in Multiplate aggregometry; Vel, maximal aggregation velocity in Multiplate aggregometry; base, baseline (pre-stimulation) aggregation value; end, endpoint (post-stimulation) aggregation value; ΔFM, deviation from mean in aggregometry (quality control value describing deviation of duplicate aggregometry electrode pairs, included here as a control that appropriately should not correlate with gene expression); TA, thrombin analog.

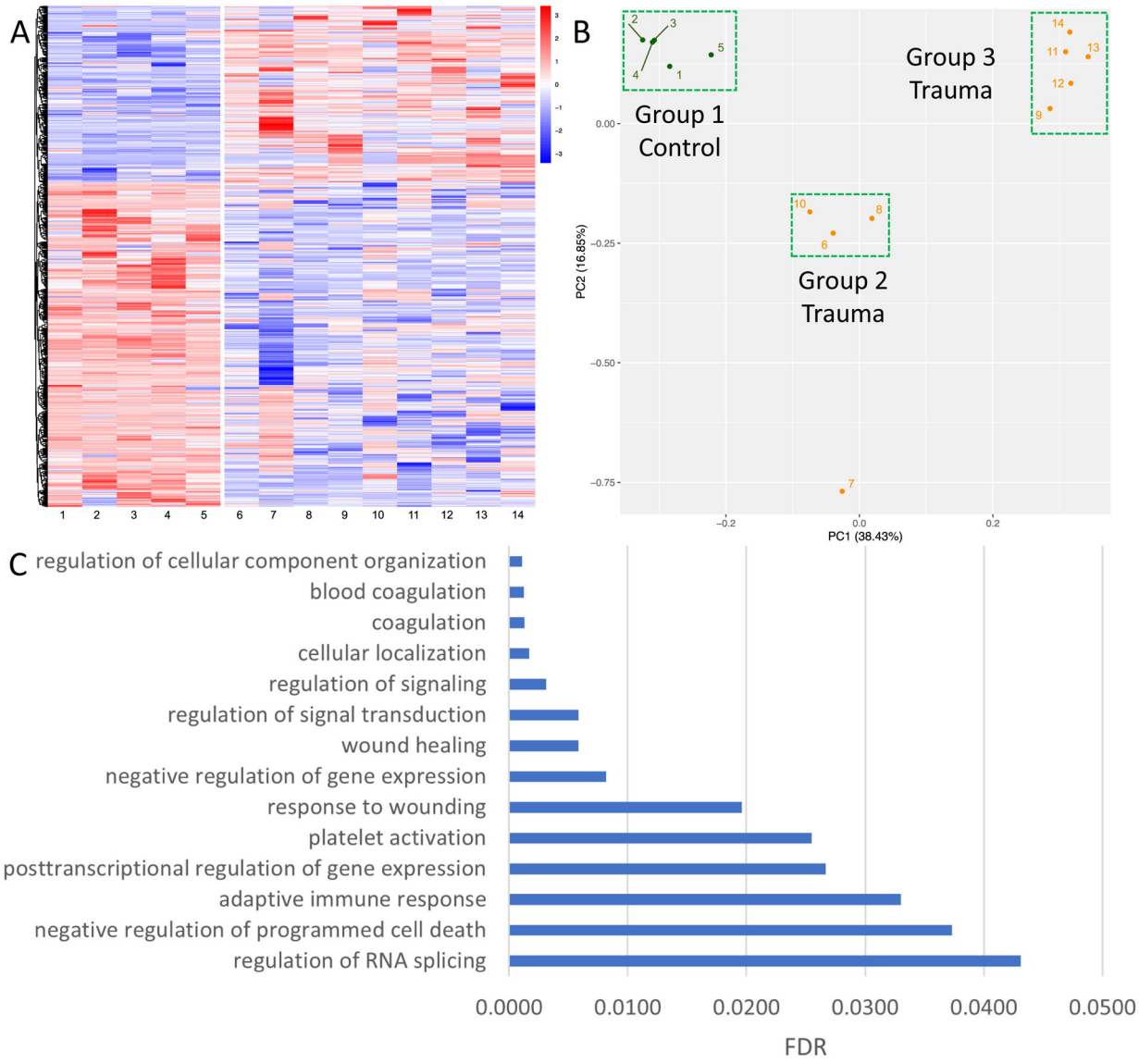

Figure 2. Differential alternative splicing of platelet RNA in trauma patients.

A) Heat map showing differential alternative splicing of platelet RNA in healthy donors (left) versus trauma patients (right). Blue corresponds to decreased inclusion ratio and red corresponds to increased inclusion ratio for each splicing event. In total, 1188 splicing events across 462 platelet RNAs passed a FDR cutoff of <0.001. B) Unsupervised principal component analysis reduced the data into two components explaining 16.85% and 38.43% of the data, respectively. Three main clusters were formed with unsupervised principal component analysis: healthy donors (Group 1), and two separate trauma clusters (Group 2 and Group 3). PC, principal component. C) Selected pathways from Gene Ontology enrichment analysis which are overrepresented among the platelet RNAs found to have differential alternative splicing between healthy donors and trauma patients, along with their FDR. Discovered pathways involve many common functions of platelets, including both coagulation and immune responses.

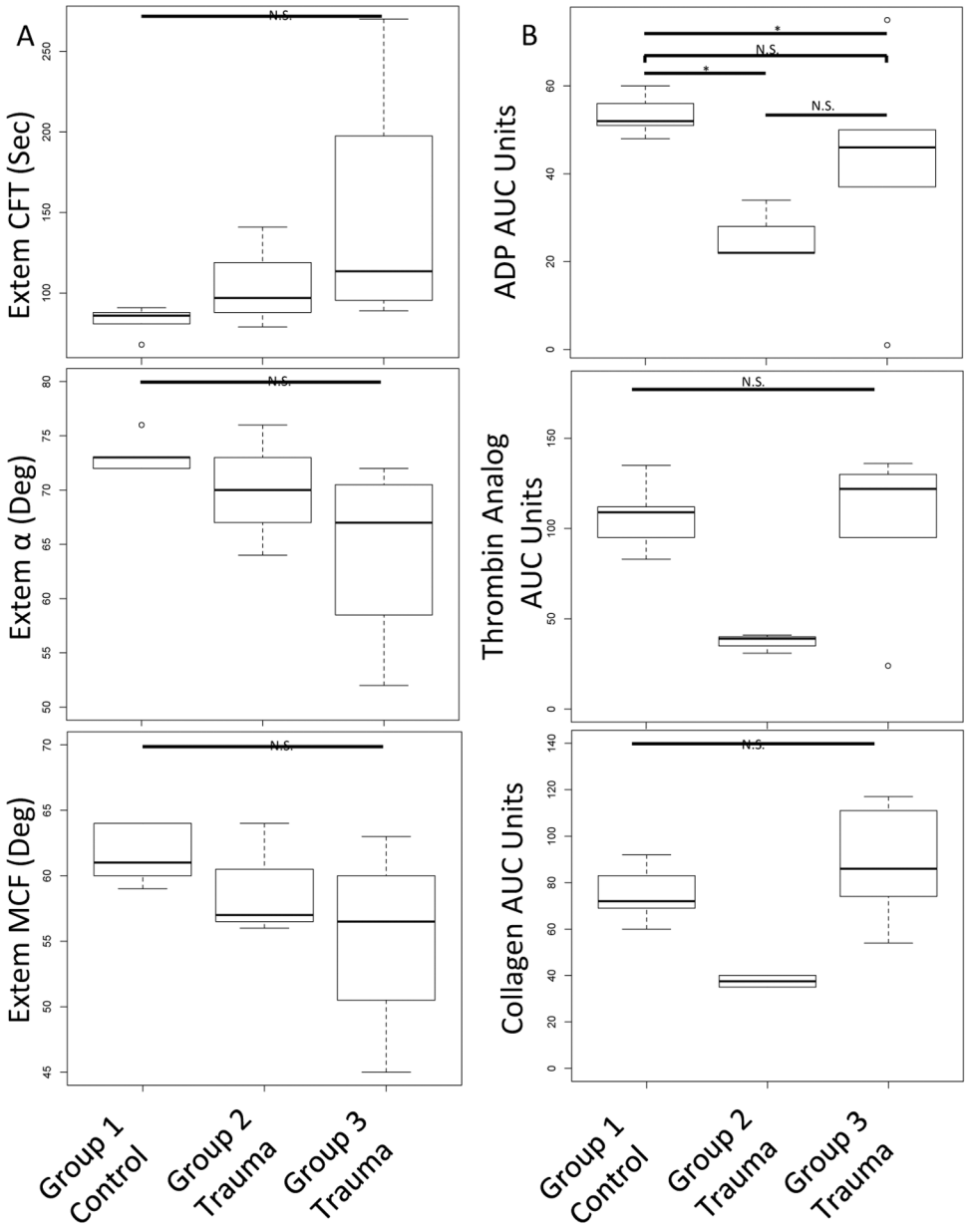

Figure 3. Platelet RNA splicing principal component groups have distinct clot formation and platelet aggregation responses.

A) Compared to Group 1 (healthy donors), Group 3, and to a lesser extent Group 2, had prolonged clot formation time (CFT), decreased α angle and decreased maximum clot formation (MCF) by ROTEM. B) Compared to Group 1 (healthy donors), Group 2 had decreased platelet aggregation responses to ADP, thrombin analog, and collagen stimulation by platelet aggregometry. Kruskal-Wallis tests were used to assess overall statistical significance and Wilcoxon signed rank tests were used to make pairwise comparisons; N.S., not statistically significant; *, $p \leq 0.05$

Table 1.

Platelet RNA Abundance in Trauma Patients Compared to Healthy Donors

Gene	Gene Name	Fold Change	*p*-value	FDR
MYL4	Myosin Light Chain 4	5.82	3.53×10^{-5}	0.01
AC093809.1	Mitochondrially Encoded Cytochrome C Oxidase III Pseudogene	5.74	9.64×10^{-5}	0.03
AGGF1P10	Angiogenic Factor With G-Patch And FHA Domains 1 Pseudogene 10	5.22	9.78×10^{-5}	0.03
REEP1	Receptor Accessory Protein 1	−3.65	2.12×10^{-4}	0.04
AC109635.3	Pantothenate kinase 3 pseudogene	3.32	1.17×10^{-4}	0.03
FBXO48	F-Box Protein 48	3.22	1.96×10^{-4}	0.04
IL6STP1	interleukin 6 signal transducer pseudogene 1	2.99	2.31×10^{-5}	0.01
MTATP8P1	Mitochondrially Encoded ATP Synthase 8 Pseudogene 1	2.86	1.37×10^{-5}	0.01
MTND5P11	Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 5 Pseudogene 11	2.84	2.53×10^{-8}	0.00
MTND6P4	Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 6 Pseudogene 4	2.67	1.01×10^{-6}	0.00
AC107954.1	Capping protein (actin filament) muscle Z-line, alpha 2	2.61	7.45×10^{-5}	0.02
SEPTIN7P3	Septin 7 Pseudogene 3	2.53	1.12×10^{-4}	0.03
H3P1	H3 Histone Pseudogene 1	2.44	2.53×10^{-5}	0.01
MTND4LP30	Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 4L Pseudogene 30	2.41	2.44×10^{-5}	0.01
MTATP8P2	Mitochondrially Encoded ATP Synthase 8 Pseudogene 2	2.39	6.96×10^{-6}	0.01
FTH1P3	Ferritin, Heavy Polypeptide 1 Pseudogene 3	2.32	3.55×10^{-5}	0.01
RN7SL3	RNA Component Of Signal Recognition Particle 7SL3	2.32	2.48×10^{-5}	0.01
MTND4P12	Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 4 Pseudogene 12	2.25	2.56×10^{-7}	0.00
NAP1L1P1	Nucleosome Assembly Protein 1-Like 1 Pseudogene 1	2.23	2.00×10^{-6}	0.00
MTRNR2L8	Humanin-Like 8	2.13	8.27×10^{-5}	0.02
PCNPP5	PEST Containing Nuclear Protein Pseudogene 5	2.12	1.40×10^{-5}	0.01
MTCO3P12	Mitochondrially Encoded Cytochrome C Oxidase III Pseudogene 12	2.11	1.84×10^{-6}	0.00
NOMO2	NODAL Modulator 2	2.07	1.26×10^{-4}	0.03
AP000763.2	Mitochondrially Encoded Cytochrome C Oxidase I Pseudogene	2.07	9.22×10^{-6}	0.01
MTCO1P40	Mitochondrially Encoded Cytochrome C Oxidase I Pseudogene 40	2.03	8.90×10^{-6}	0.01
MTCYBP18	Mitochondrially Encoded Cytochrome B Pseudogene 18	1.98	9.12×10^{-5}	0.03
OVCH1-AS1	OVCH1 antisense RNA 1	1.92	2.64×10^{-6}	0.00
FTH1P5	Ferritin, Heavy Polypeptide 1 Pseudogene 5	1.91	5.61×10^{-5}	0.02
YWHAZP5	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein, Zeta Polypeptide Pseudogene	1.88	2.02×10^{-5}	0.01
ATF4P3	Activating Transcription Factor 4C	1.87	2.73×10^{-5}	0.01
MTCO2P12	Mitochondrially Encoded Cytochrome C Oxidase II Pseudogene 12	1.82	2.62×10^{-5}	0.01
NDUFA4	NADH-Ubiquinone Oxidoreductase MLRQ Subunit	1.81	5.39×10^{-6}	0.01
FUNDC1	FUN14 Domain-Containing Protein 1	1.76	1.04×10^{-4}	0.03
H3P47	H3 Histone Pseudogene 47	1.72	2.20×10^{-4}	0.04
TLK1P1	Tousled Like Kinase 1 Pseudogene 1	1.72	4.31×10^{-5}	0.02

Gene	Gene Name	Fold Change	*p*-value	FDR
FTH1P8	Ferritin, Heavy Polypeptide 1 Pseudogene 8	1.67	1.36×10^{-5}	0.01
H3P16	H3 Histone Pseudogene 16	1.66	6.08×10^{-5}	0.02
TTC1	Tetratricopeptide Repeat Domain 1	1.63	9.52×10^{-6}	0.01
FTH1P23	Ferritin, Heavy Polypeptide 1 Pseudogene 23	1.63	3.83×10^{-5}	0.01
FTH1P16	Ferritin Heavy Chain 1 Pseudogene 16	1.57	1.55×10^{-4}	0.04
CCNYL1	Cyclin Y Like 1	1.55	1.72×10^{-4}	0.04
ITGB3BP	Integrin Subunit Beta 3 Binding Protein	1.54	1.68×10^{-4}	0.04
DNAJC3	DnaJ Heat Shock Protein Family (Hsp40) Member C3	1.54	2.76×10^{-5}	0.01
SNN	Stannin	−1.48	2.30×10^{-4}	0.04
AC113404.3	RAP1B Like (Pseudogene)	1.48	1.21×10^{-5}	0.01
AC084824.2	Nucleosome Assembly Protein 1-Like 1 (NAP1L1) Pseudogene	1.48	2.08×10^{-4}	0.04
FTH1P11	Ferritin Heavy Chain 1 Pseudogene 11	1.47	9.91×10^{-5}	0.03
ROCK2	Rho Associated Coiled-Coil Containing Protein Kinase 2	0.89	5.74×10^{-5}	0.02
RIT1	Ras Like Without CAAX 1	0.89	1.46×10^{-4}	0.03

*Fold change ($\log_2$); FDR, false discovery rate. Gene names for protein-coding RNA transcripts are those maintained by the HUGO Gene Nomenclature Committee. Non-coding RNA transcripts are given names from miRBase and Rfam (an EMBLservice).