

Aspirin Pharmacogenomics

Genomic Medicine Forum

April 21, 2011

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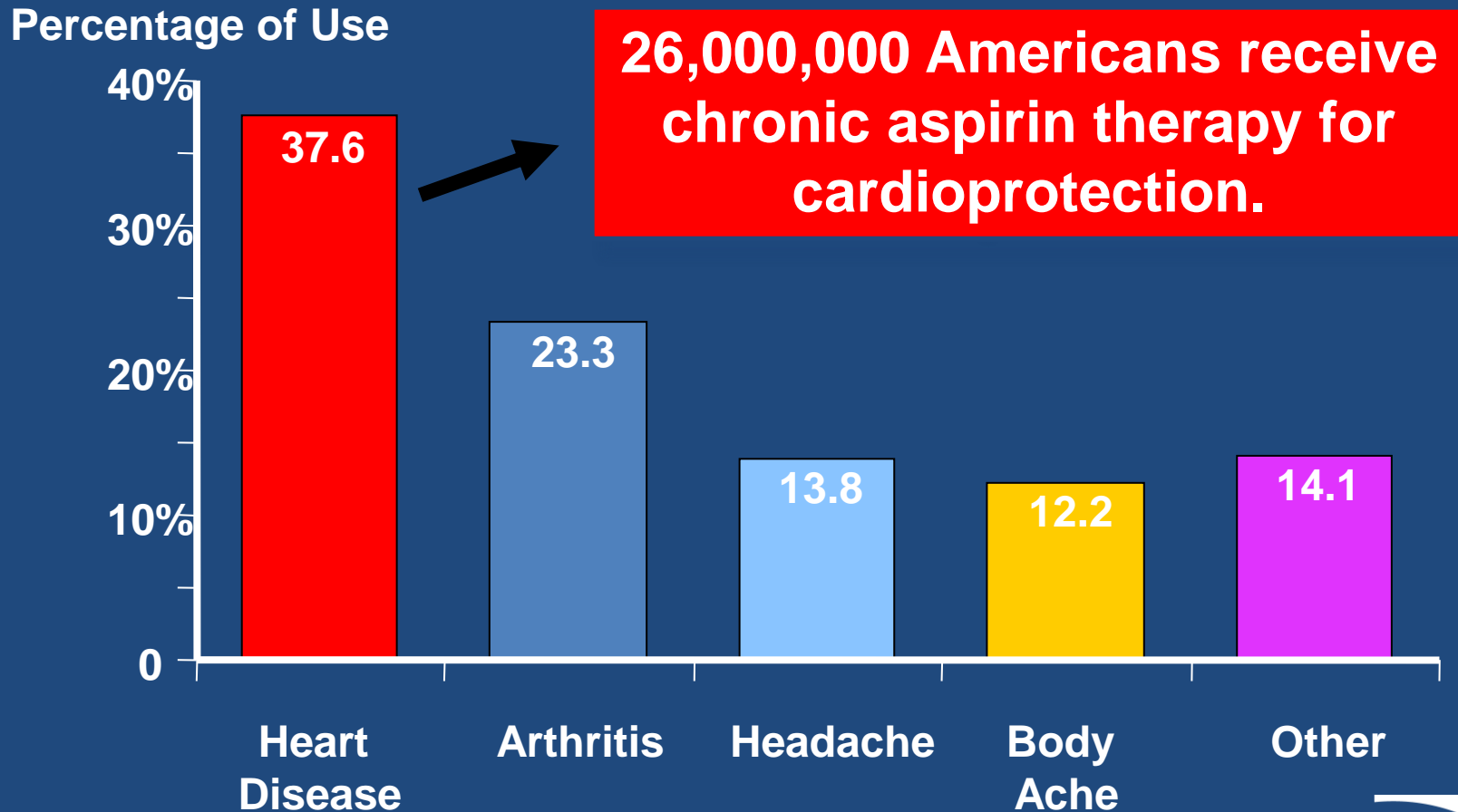
Aspirin Pharmacogenomics

(A Progress Report)

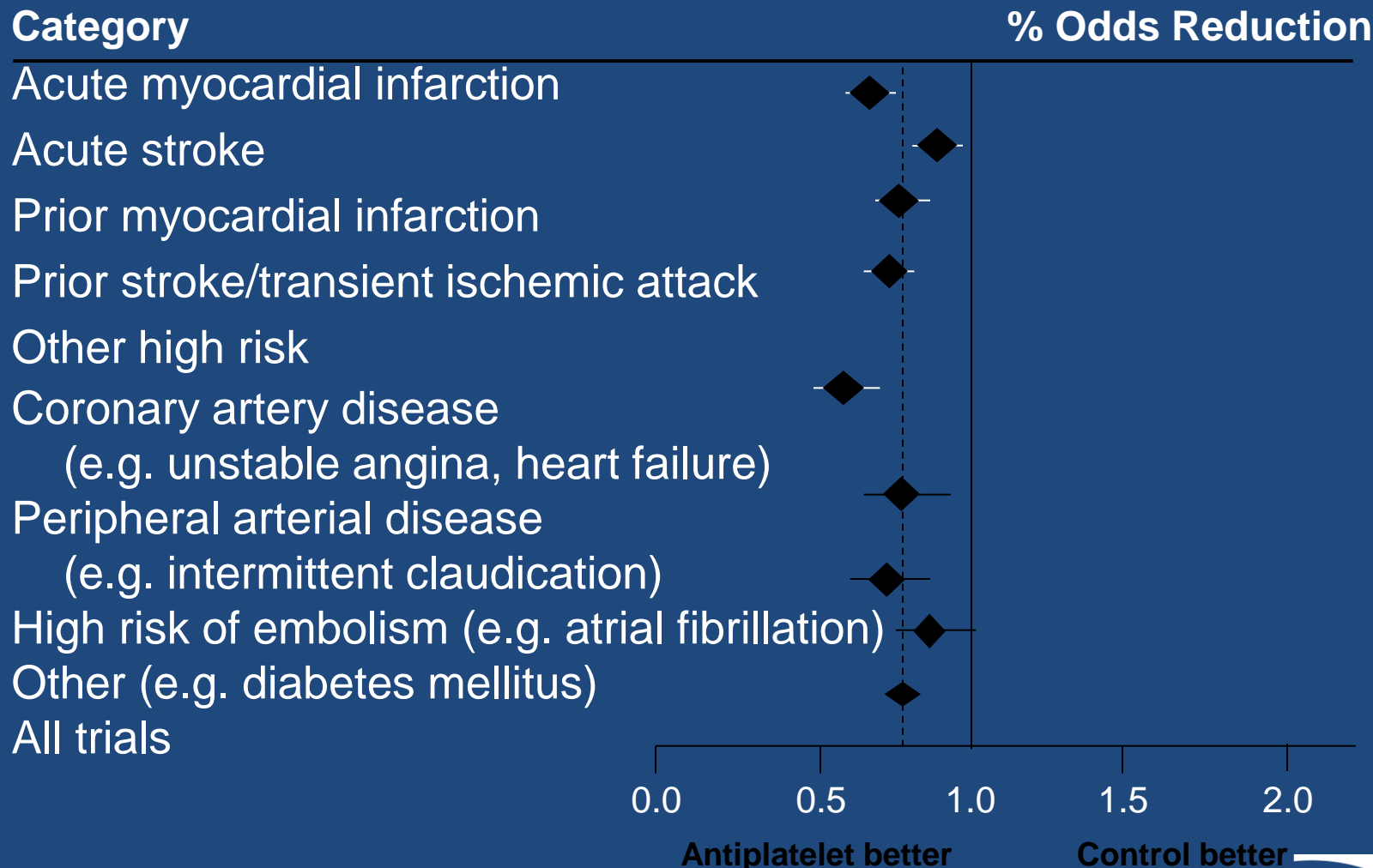
Overview

- Background
- Development and validation of platelet function score (PFS) for non COX-1 dependent platelet function (NCDPF)
- NCDPF on aspirin is not due to uninhibited COX-1
- Whole blood gene-expression analysis of PFS and validation
- Future directions

Aspirin Usage In the US



Efficacy of Antiplatelet Therapy on Vascular Events



*Vascular events = myocardial infarction, stroke or vascular death

Variability in Response to Aspirin

- Variability in the clinical response to aspirin
 - 20-30% will experience an event on aspirin

ATC, Lancet 2002

- Variability in the laboratory response to aspirin

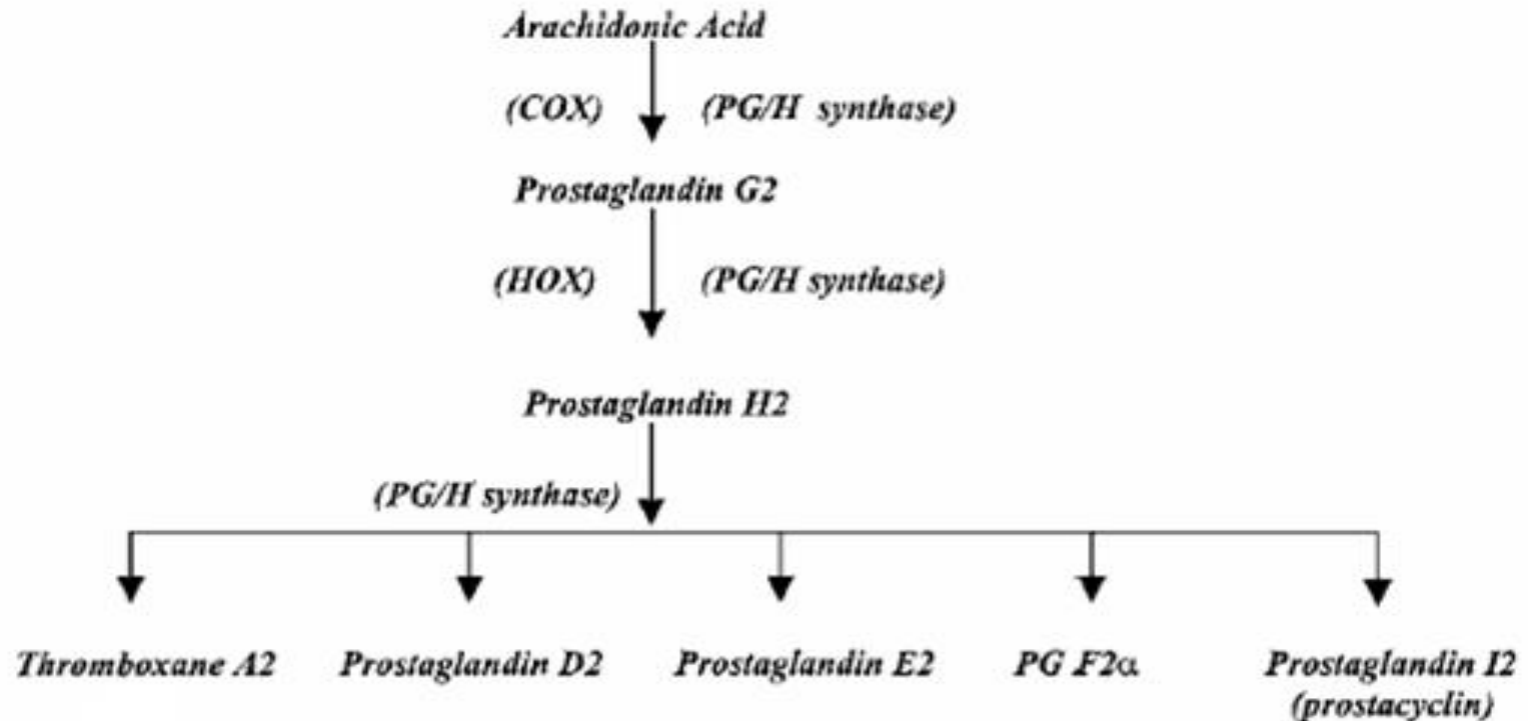
Becker et al, JAMA 2006

- Variability in platelet function assays associated with increased risk of events on aspirin

Frelinger et al, Circulation 2009

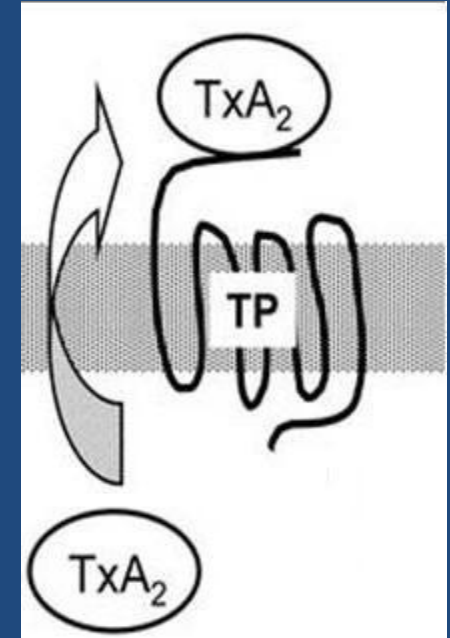
- Limited knowledge of underlying biology

Thromboxane synthesis



Platelet aggregation

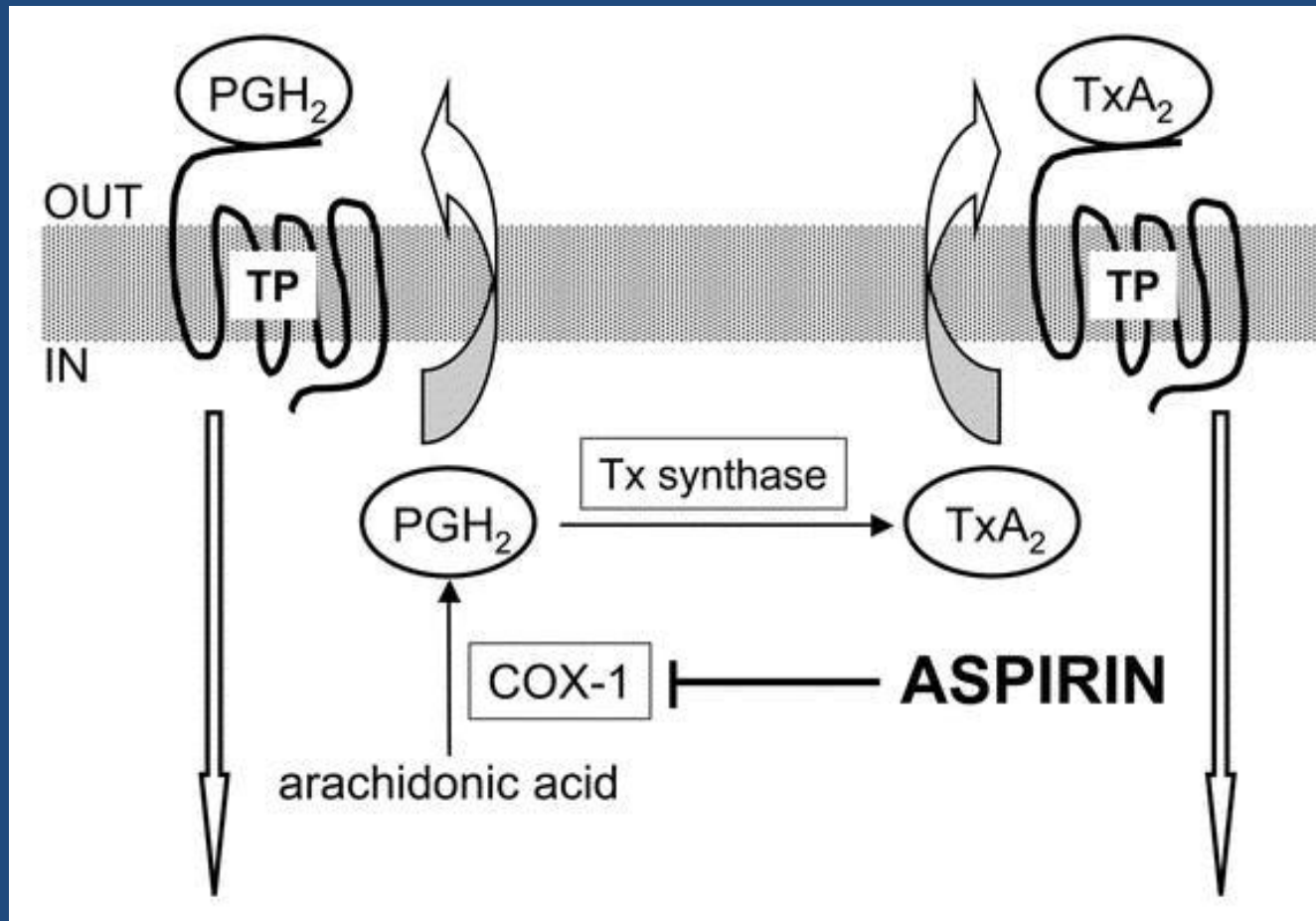
Thromboxane A₂



Thromboxane B₂ metabolites
(serum and urine)

- Platelet aggregation
- Vasoconstriction

Aspirin mechanism



COX-1 dependent platelet function

vs Non COX-1 dependent platelet function (NCDPF)

AA → Thromboxane

- Sensitive to COX-1 inhibition by ASA
- Agonist: Arachidonic acid
- Minimal variability on ASA
- Not heritable
 - no known genetic variants associated with function

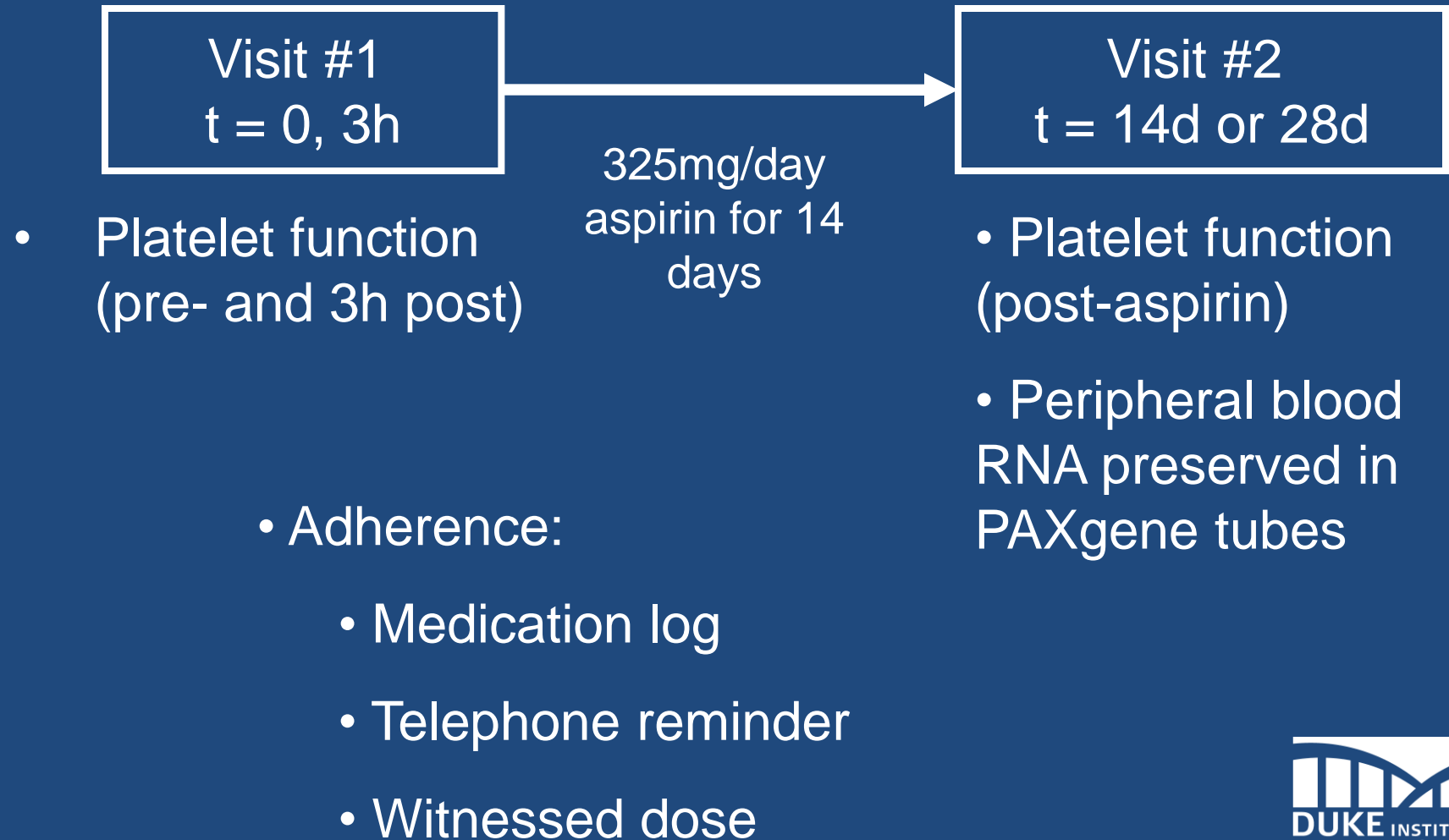
AA → Thromboxane

- Can be robust despite inhibition of COX-1 with ASA
- Agonists: ADP, Collagen, Epi
- Highly variable on ASA
- Highly heritable
 - GWAS identified genomic regions associated with function

Goals

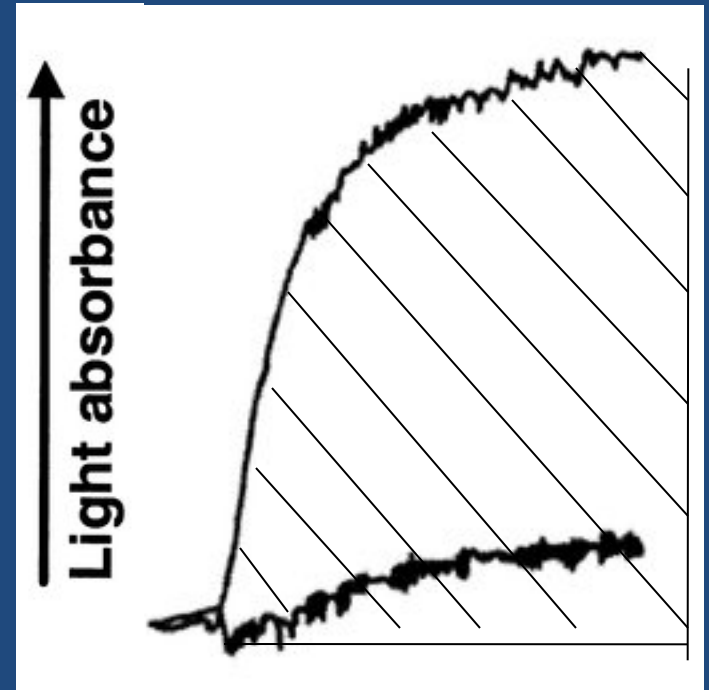
- 1) To study platelet function responses in healthy volunteers under controlled environmental conditions → accurate phenotype
- 2) To identify biological pathways associated with the response to aspirin

Methods – Aspirin challenge study in healthy volunteers (Discovery)



Methods – Measuring NCDPF

- Light transmittance aggregometry
 - Agonists
 - ADP 10, 5, and 1 μM
 - Epinephrine 10, 1, 0.5 μM
 - Collagen 5 and 2 $\mu\text{g}/\text{ul}$
 - Area under the aggregometry curve (AUC)
 - Measured in: % min
- PFA100 closure time (collagen/epi)



High correlation between assays

	PFA	ADP 10 μ M	ADP 5 μ M	ADP 1 μ M	Epi 10 μ M	Epi 1 μ M	Epi .5 μ M	Col 5 μ g	Col 2 μ g
PFA	-	-0.25	-0.29	-0.27	-0.50	-0.50	-0.51	-0.23	-0.29
ADP 10 μ M	-0.25	0.40	0.87	0.61	0.65	0.58	0.58	0.79	0.74
ADP 5 μ M	-	0.83	0.45	0.65	0.65	0.58	0.62	0.77	0.67
ADP 1 μ M	-	0.42	0.54	0.42	0.66	0.66	0.70	0.53	0.58
Epi 10 μ M	-0.28	0.66	0.66	0.49	0.42	0.91	0.92	0.60	0.66
Epi 1 μ M	-0.26	0.47	0.52	0.61	0.79	0.48	0.94	0.53	0.65
Epi 0.5 μ M	-0.22	0.41	0.42	0.58	0.62	0.83	0.38	0.57	0.68
Col 5 μ g	-0.44	0.56	0.41	-	0.45	0.33	0.26	0.25	0.87
Col 2 μ g	-0.55	0.55	0.49	-	0.44	0.32	-	0.75	-

Platelet Function Score (PFS)

- Strong linear correlations → principal components analysis
 - 1st principal component → PFS
- Aspirin significantly reduced PFS
 - 5.5 ± 2.1 vs. 3.4 ± 1.5 , $p < 0.0001$

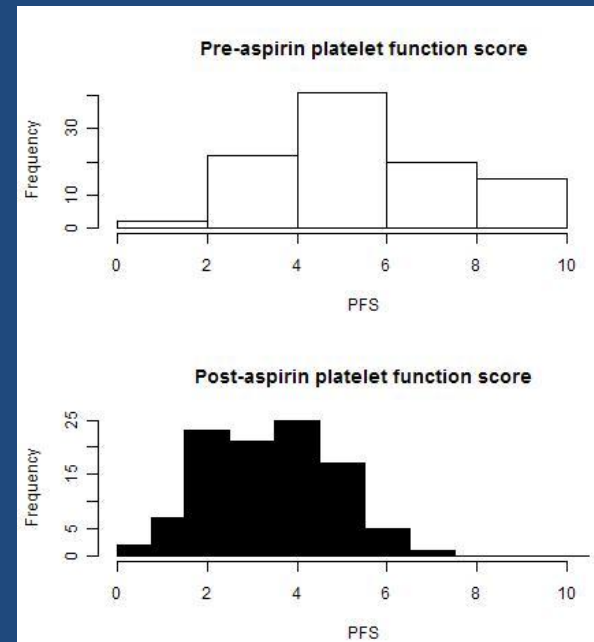


Table 3. Correlations between platelet function score (PFS) and non COX1 dependent measures of platelet function before and after aspirin**

	PFA100	ADP 10 μ M	ADP 5 μ M	ADP 1 μ M	Epi 10 μ M	Epi 1 μ M	Epi 0.5 μ M	Col* 5 μ g	Col* 2 μ g
Pre-Aspirin	-0.33	0.76	0.79	0.72	0.87	0.89	0.81	0.46	0.45
Post-Aspirin	-0.51	0.86	0.88	0.81	0.88	0.85	0.87	0.76	0.79

**p < 0.0001 for all pairs of correlations.

PFS Validation

Correlation between individual measures of non-COX1 dependent platelet function and post-aspirin PFS in discovery and validation groups.

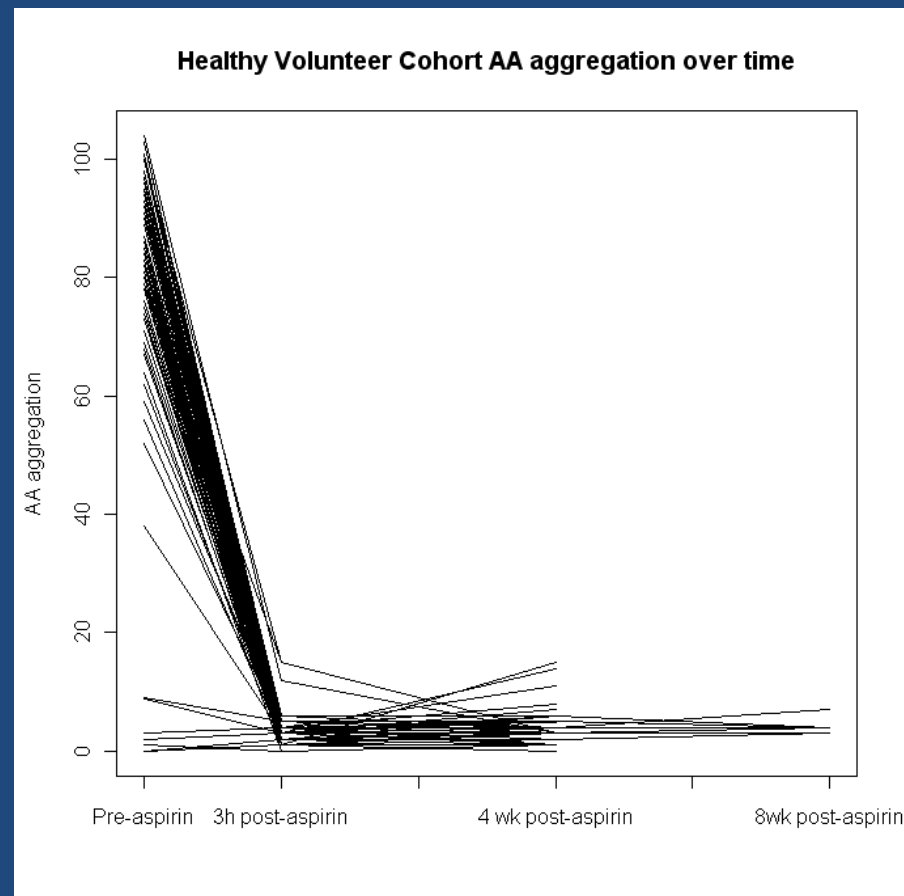
	Epinephrine 10 μ M	ADP 10 μ M	Collagen 5mg/ml	PFA100 closure time
Discovery group (n = 40)	0.87*	0.90*	0.87*	-0.34**
Validation group (n = 98)	0.76*	0.85*	0.80*	-0.34**

* $p < 0.0001$; ** $p < 0.05$

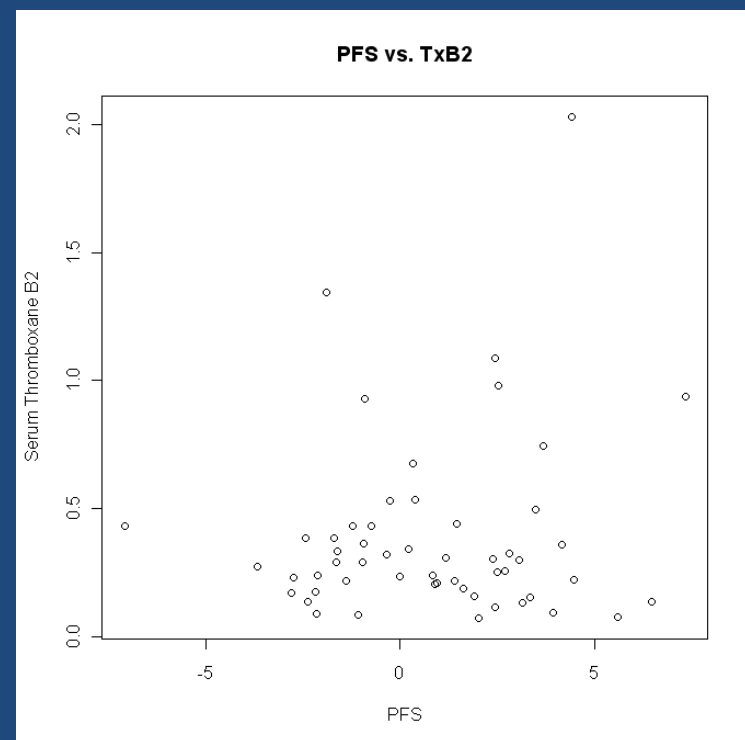
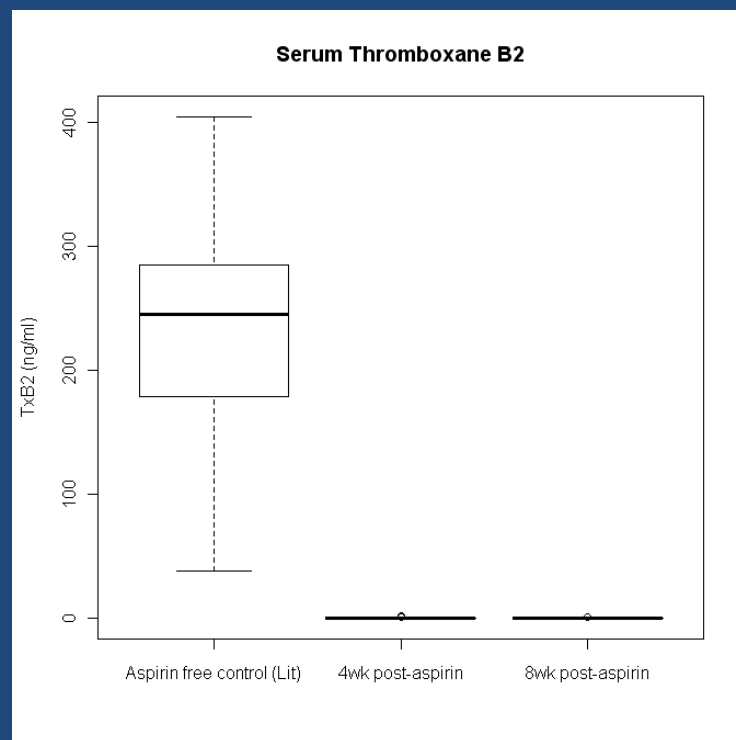
Conclusions - 1

- Non COX-1 dependent platelet function on aspirin can be efficiently summarized via PFS
- Higher PFS relates to higher platelet function
- Aspirin lowers PFS, though variability remains

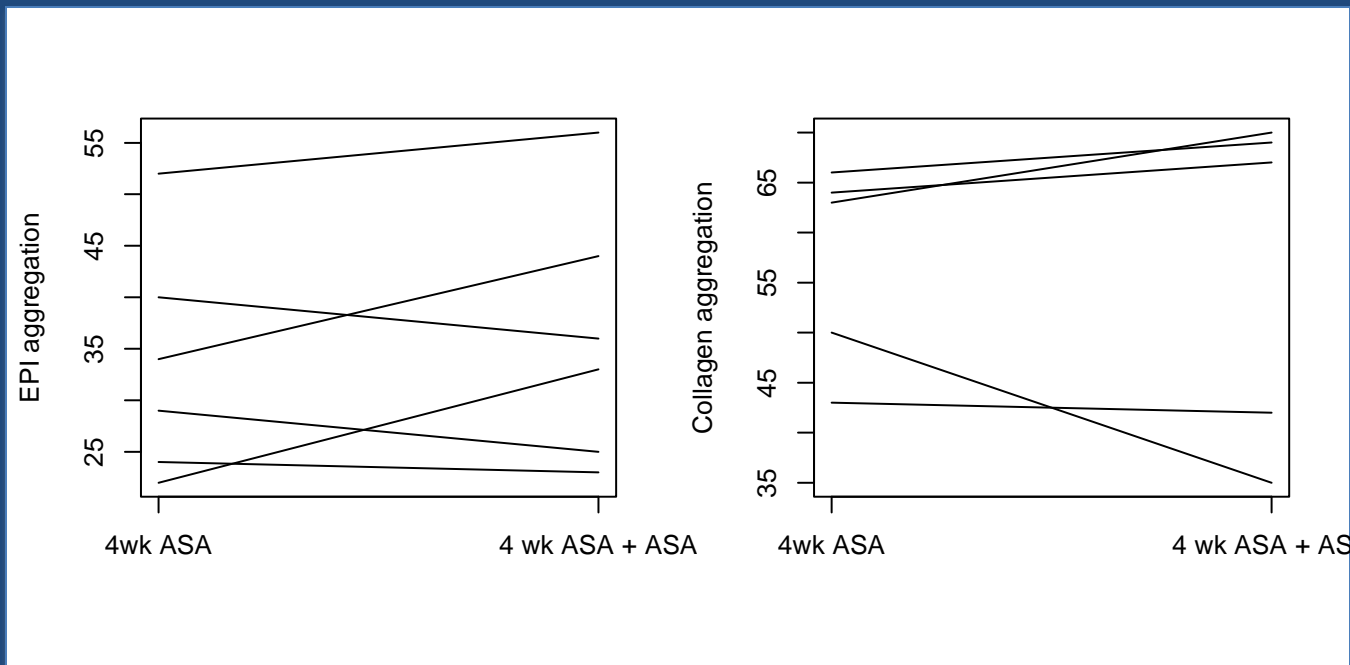
PFS on aspirin is not due to inadequate COX-1 inhibition: Arachdonic Acid



PFS on aspirin is not due to inadequate COX-1 inhibition: serum TxB2



NCDPF not due to residual COX-1 : *in vitro* ASA



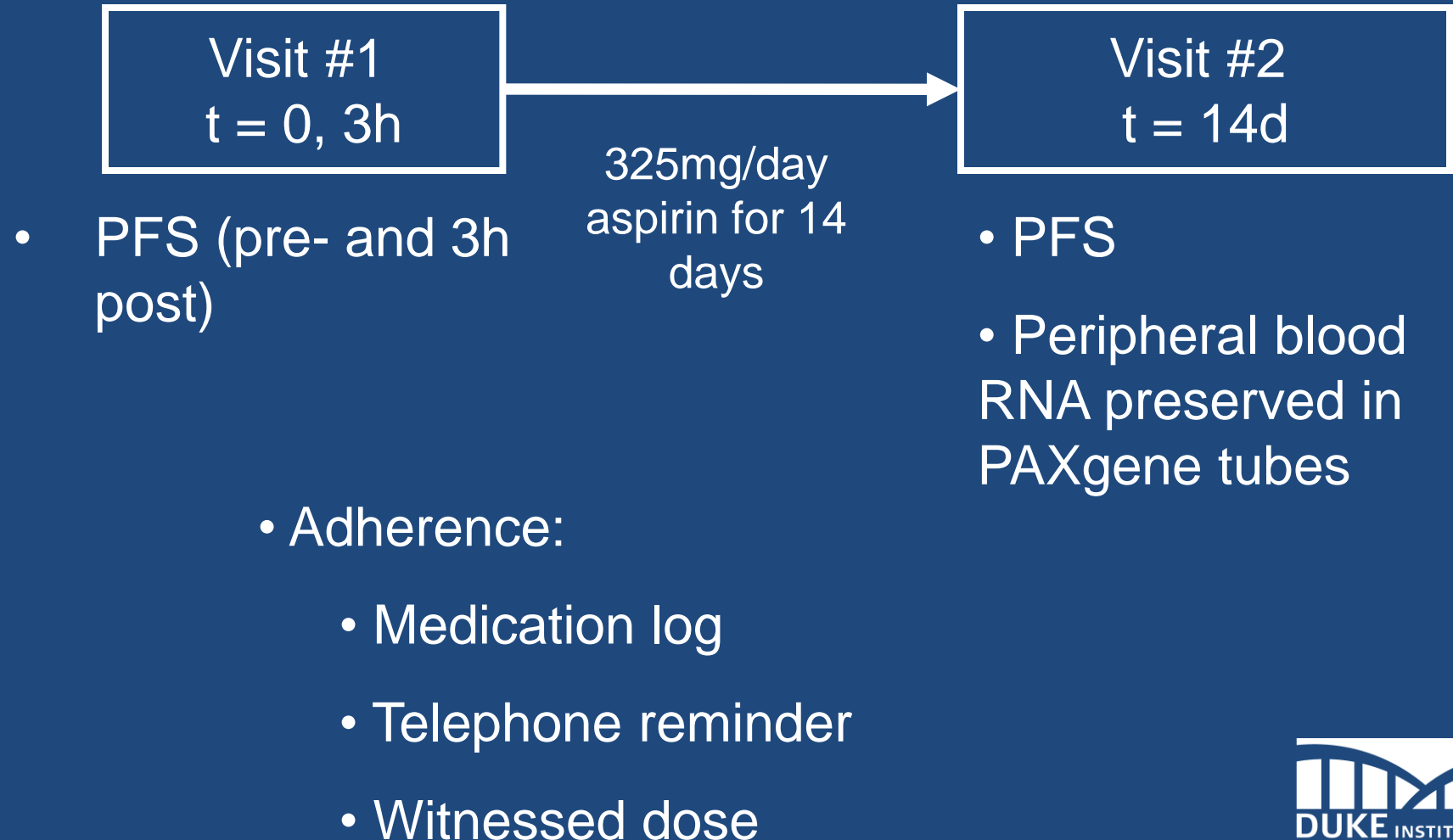
Conclusions - 2

- NCDPF on aspirin, as measured by PFS, is not due to residual platelet COX-1 activity

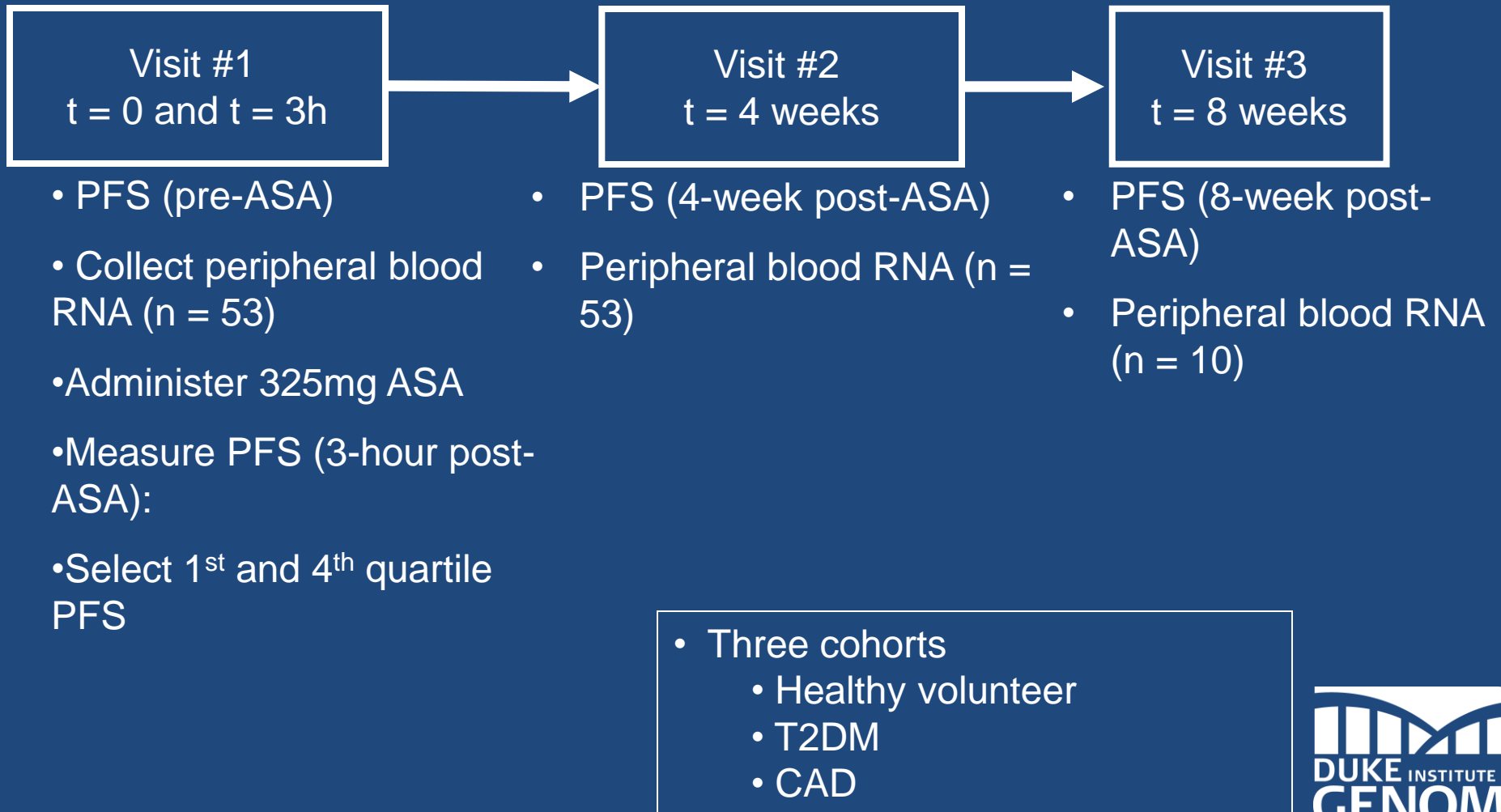
Rationale

- To use peripheral blood gene expression as a tool to identify novel pathways that underlie NCDPF on aspirin.

Methods – Aspirin challenge study in healthy volunteers (Discovery)



Methods – Aspirin challenge study in healthy volunteers (Validation)



Methods – RNA analysis overview

Hypothesis: Peripheral blood RNA analysis can identify pathways associated with platelet function on aspirin

- Affymetrix U133 Plus 2.0 microarray
- Nonspecific filtering (mean, variance)
- Bayesian factor analysis
- Correlation between factors and PFS in Discovery cohort
 - N = 50, $p \leq 0.05$
- Correlation between factors from Discovery cohort in Validation cohort
 - N = 53, $p < 0.05$
- Pathway analysis of genes represented by selected factors using GATHER

54,000 probes

~3500 probes

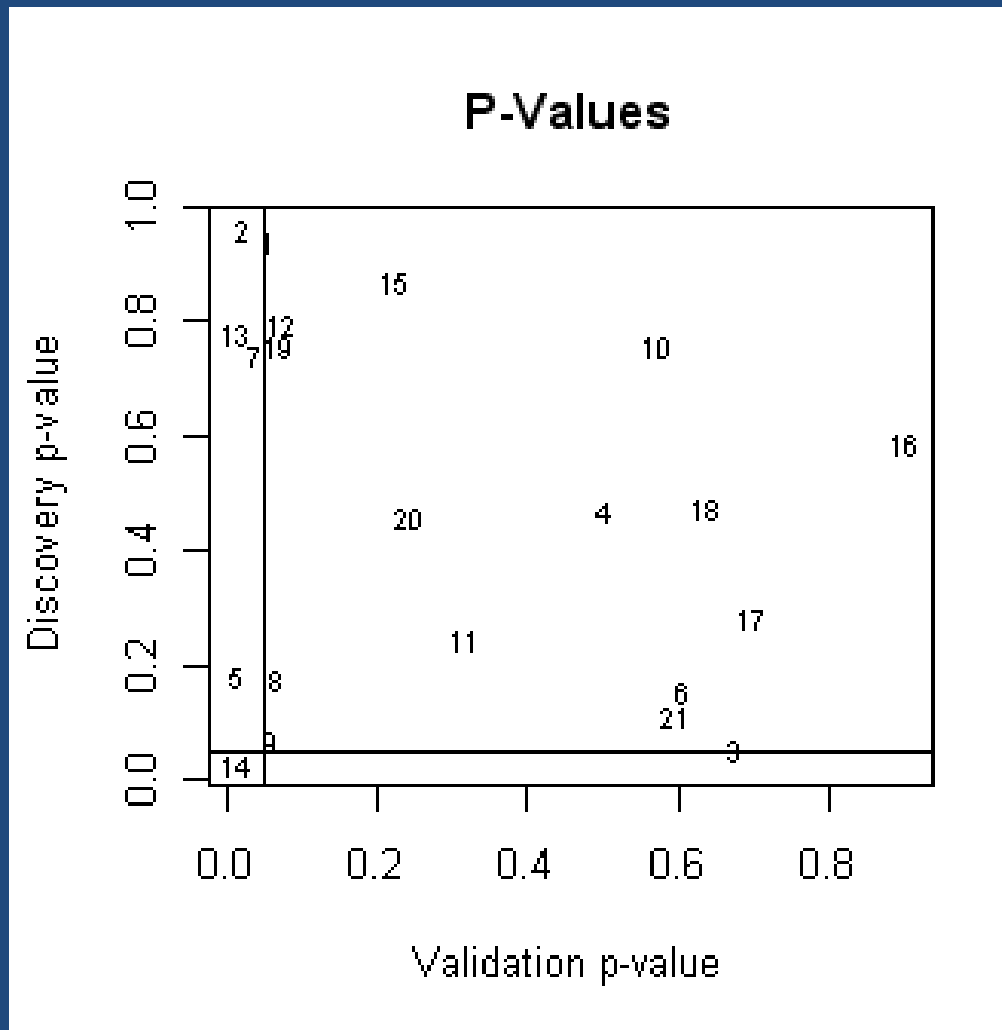
20 factors

2 factors

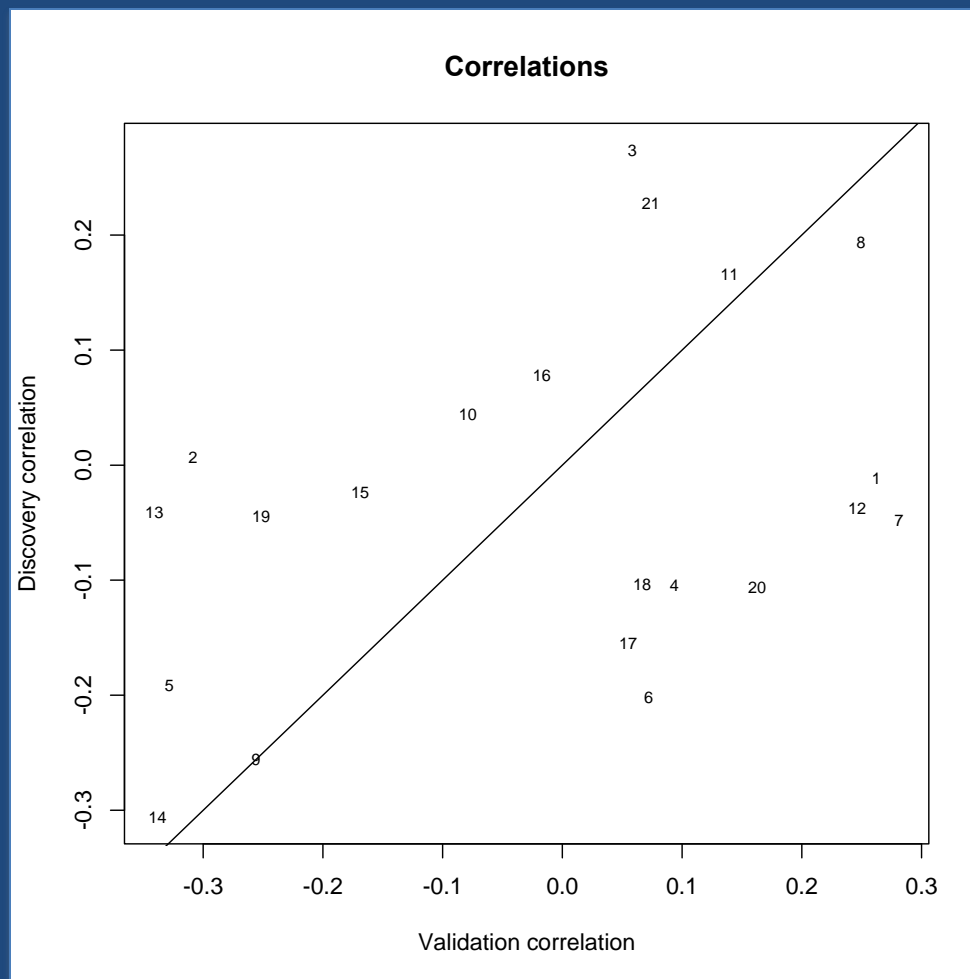
1 factor

1 pathway

Factors that correlate with PFS in discovery and validation cohorts

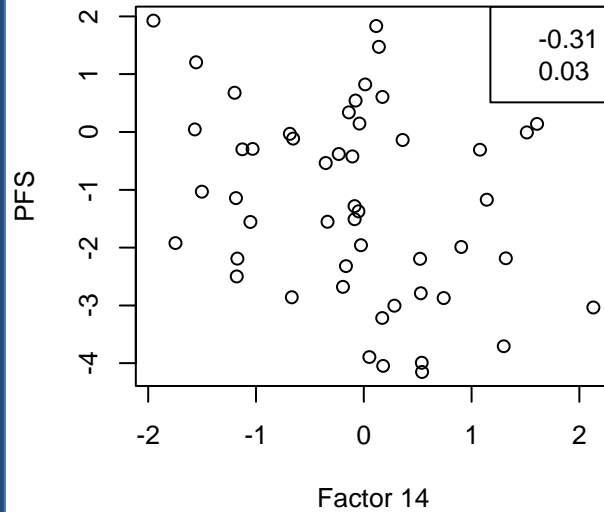


Correlation of factors with PFS

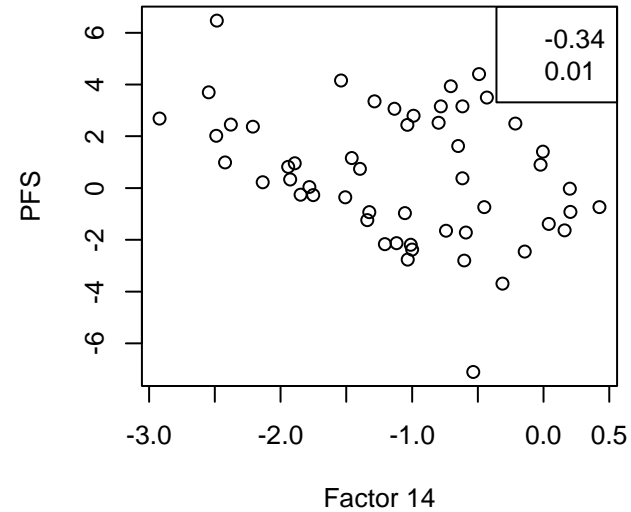


Factor 14 and PFS

Discovery, Factor 14, 2wk PFS



Validation, Factor 14, 4wk PFS

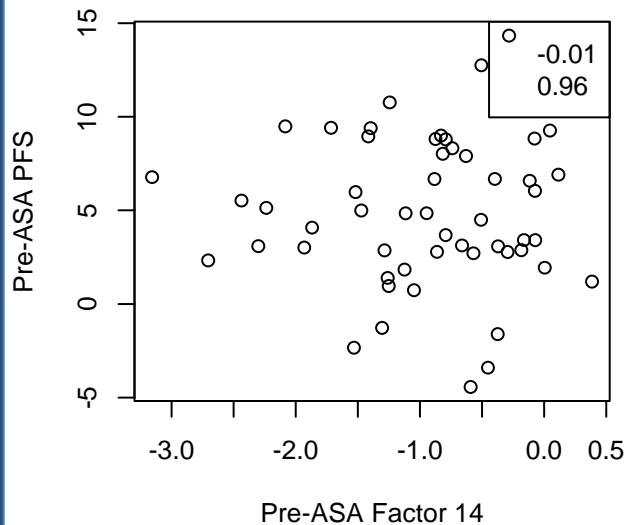


Conclusion -3

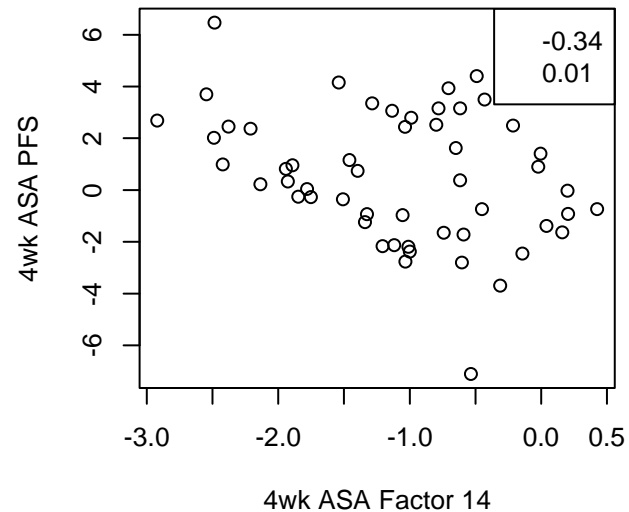
- Factor 14 is inversely and consistently associated with NCDPF as measured by PFS.

Factor 14 pre- vs. post-ASA PFS

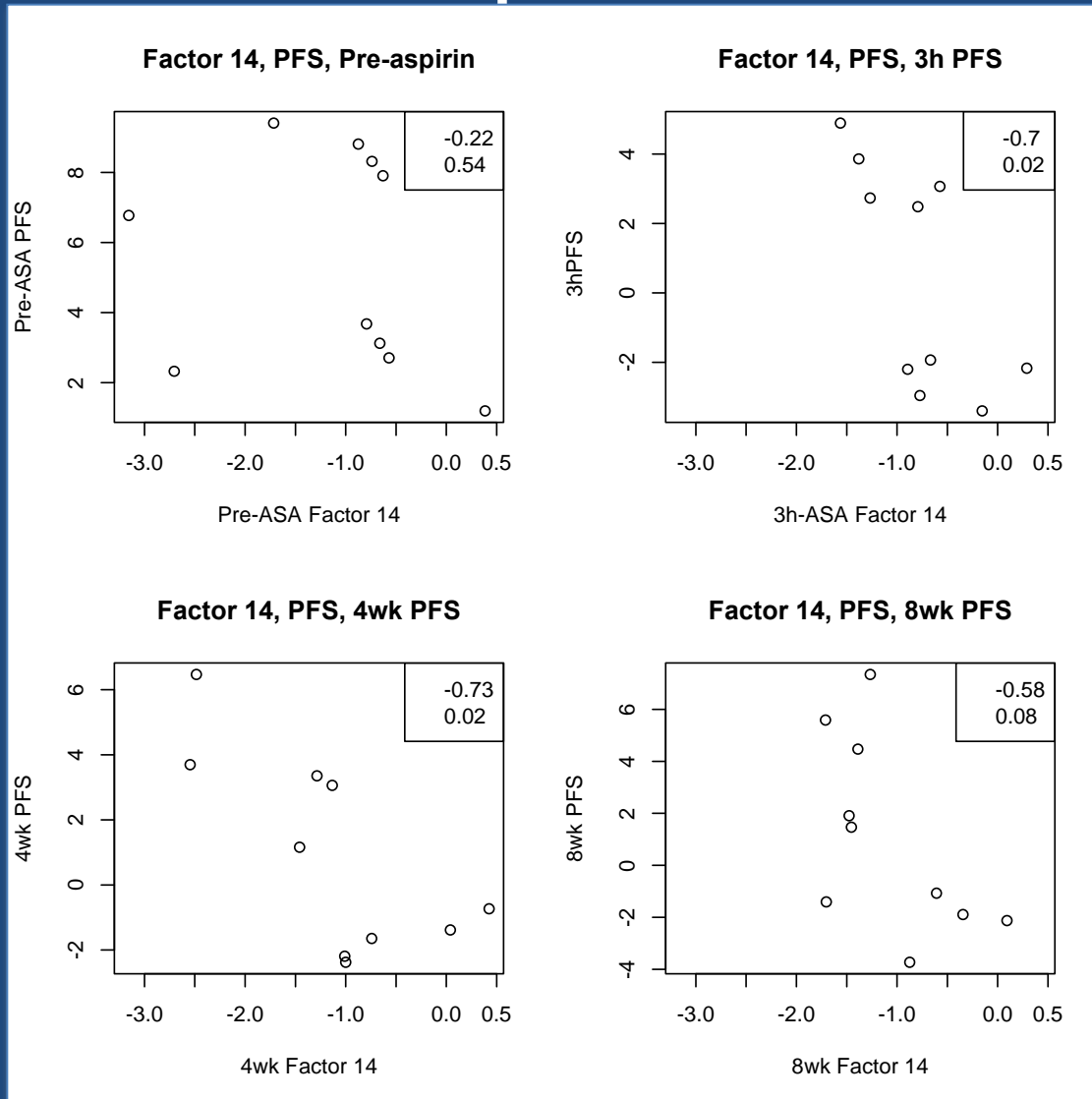
Validation cohort, Factor 14 v PFS Pre-AS.



Validation cohort, Factor 14, 4wk PFS



Factor 14 vs. PFS at multiple time points



Conclusion - 4

- Aspirin exposure appears to alter the relationship between Factor 14 and PFS
 - May be as early as 3h after 1st aspirin dose

Pathway analysis

- GATHER analysis of Factor 14

- 6 genes emerge

- GP1BB: platelet glycoprotein 1B

GPIb-V-IX complex
inhibited by
ARC1779



- ITGA2B: platelet glycoprotein IIb

- ITGB3: platelet glycoprotein IIIa



IIB/IIIa complex
inhibited by
eptifibatide,
tirofiban, abciximab

- PF4

Platelet factor-4 found in platelet
alpha-granules



- PROS1

Vitamin K dependent anticoagulant



- THBS1

Platelet aggregation



Conclusions - 5

- Factor 14 is comprised of several platelet specific genes
 - Drug targets for antiplatelet therapy (GPIBB, ITGA2B, ITGB3)
 - Known role in platelet function (all)
 - When mutated lead to thrombophilia or thrombasthenia (GPIBB, ITGA2B, ITGB3, PROS1)

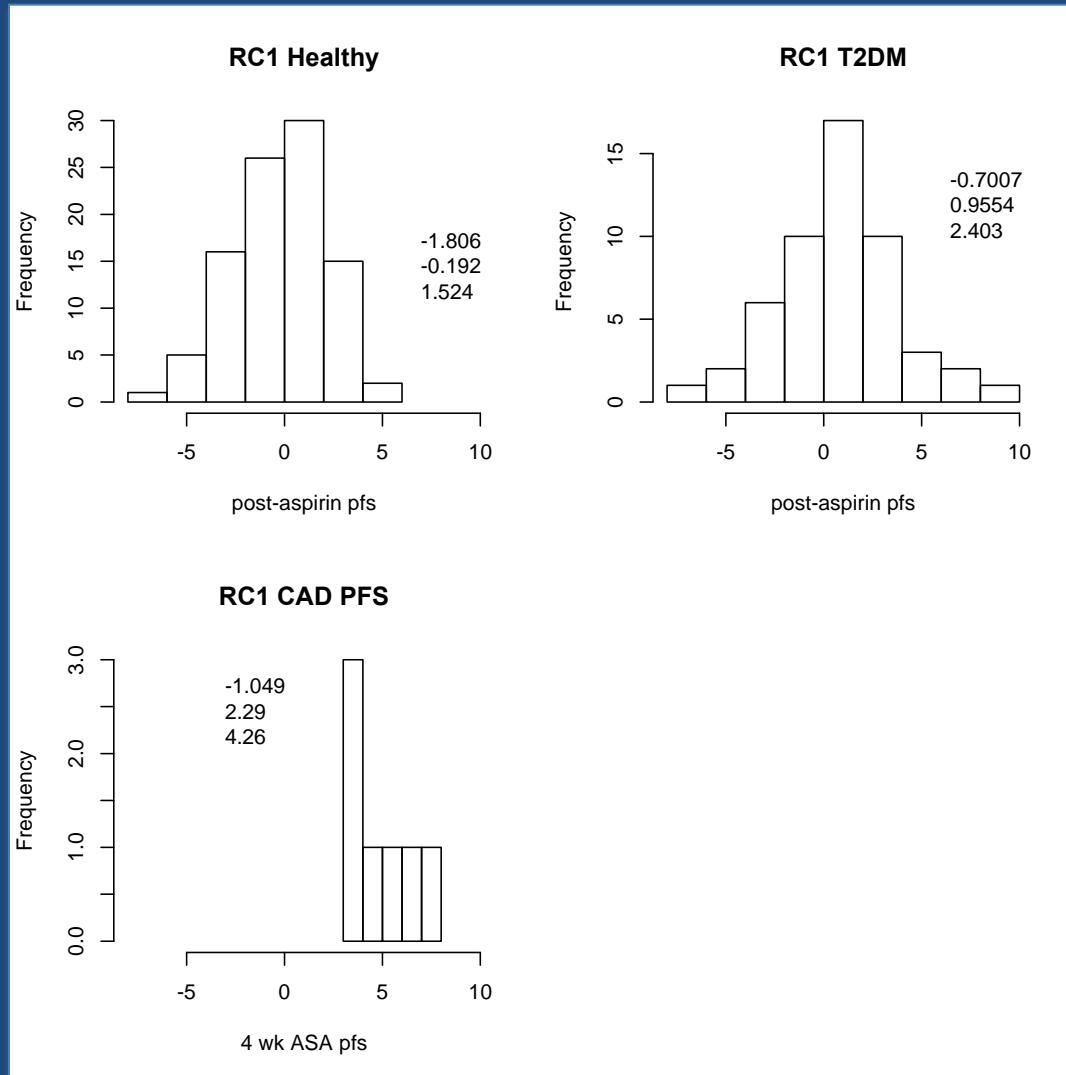
Summary

- Non COX1 dependent platelet function (i.e. ADP, collagen, Epinephrine agonists) can be summarized by a single metric, the PFS
 - A “global” platelet function phenotype.
- NCDPF is not due to inadequate COX-1 inhibition
 - Additional agents needed to reduce platelet function
- Peripheral blood gene expression can probe the underlying biology of the response to aspirin
 - Platelet factors involved in aspirin response

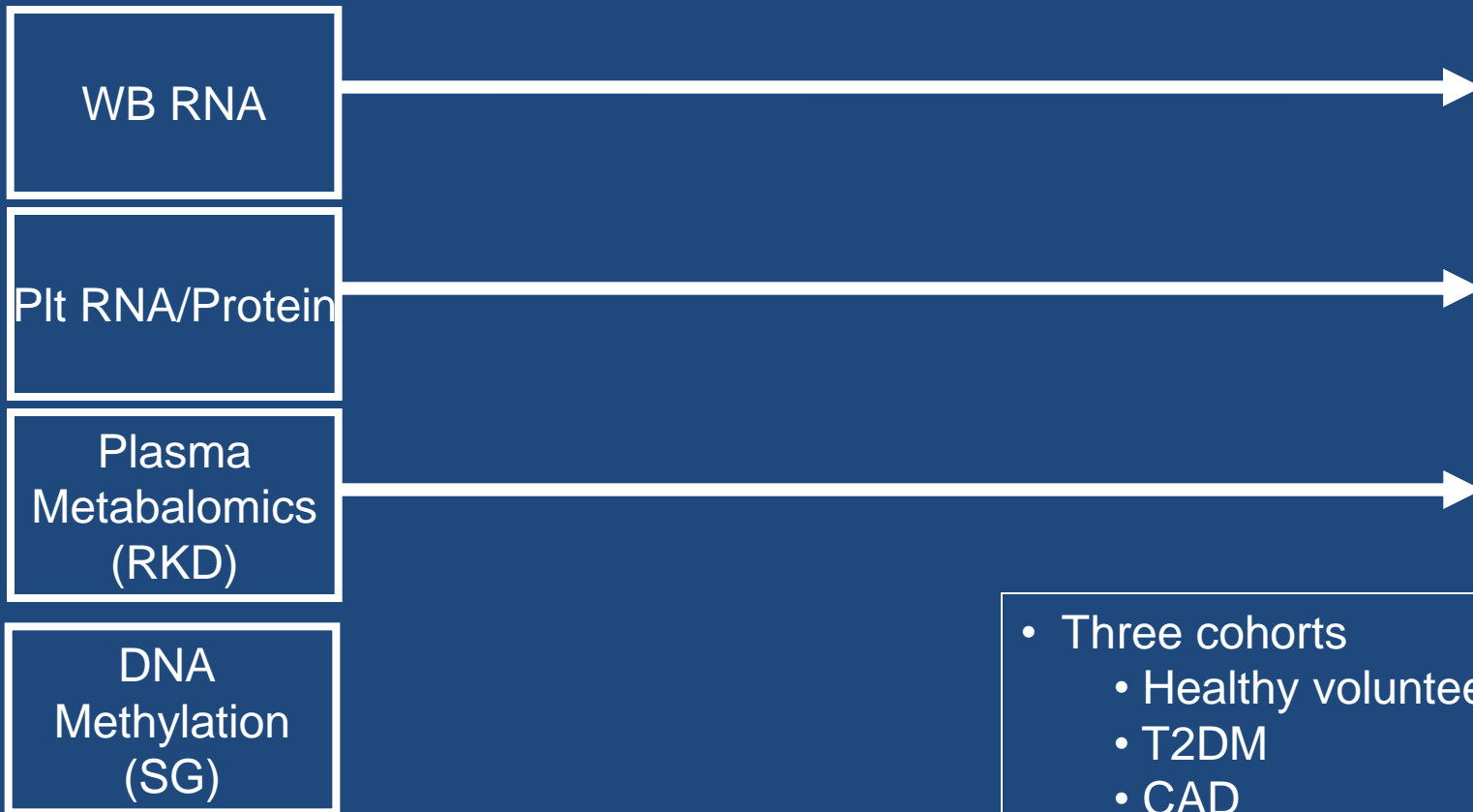
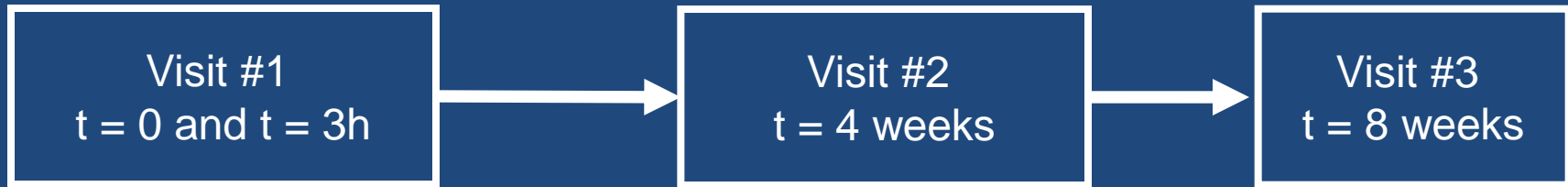
Ongoing studies

- PFS components
 - Epinephrine, ADP, Collagen etc.
- Factor model in pre-aspirin data
 - Prediction of PFS on aspirin
- Correlation between Factor 14 → clinical outcomes in patients with CAD treated with aspirin
 - Duke CATHGEN biorepository

Ongoing studies: From health to disease



Ongoing studies: Parallel platforms



- Three cohorts
 - Healthy volunteer
 - T2DM
 - CAD

Acknowledgments

- Collaborators
 - Geoffrey Ginsburg, MD, PhD (Cardiology, IGSP)
 - Thomas Ortel, MD, PhD and Platelet Function Laboratory staff (Hematology)
 - Richard Becker, MD (Cardiology, Hematology)
 - Jen-Tsan Chi, MD, PhD (IGSP)
 - Joseph Lucas PhD (IGSP)
 - DCRU Staff
- Funding:
 - IGSP, T32HL007101, UL1RR024128, RC1GM091083

Questions/Comments?