INSTITUTE FOR SOCIAL RESEARCH
UNIVERSITY OF MICHIGAN

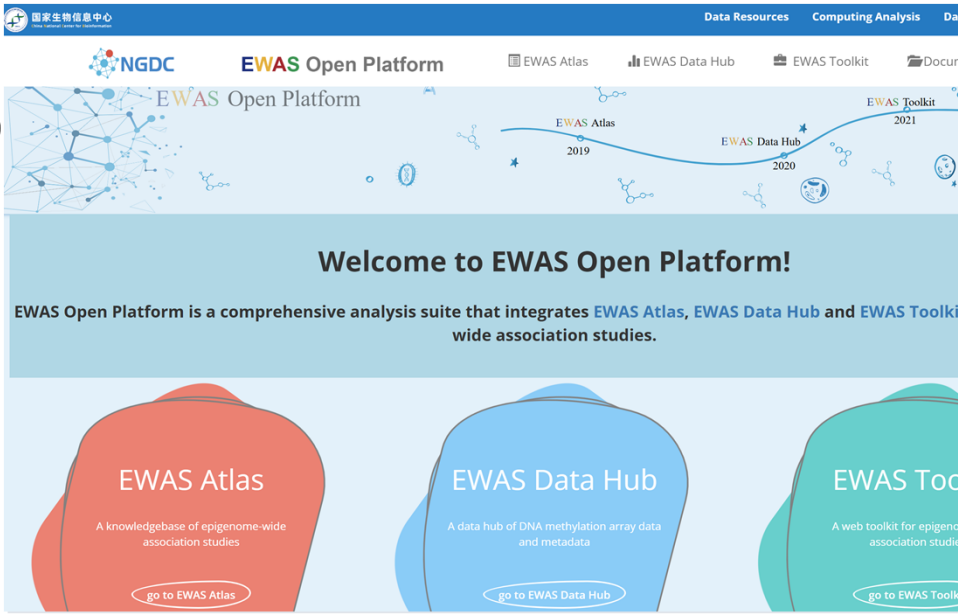
DNA Methylation Lab Experiment

NIA BIOMARKER NETWORK INTERNATIONAL MEETING

April 9, 2025

DNA Methylation and Harmonization

- DNA Methylation is being used in a wide variety of applications
- Most use the Illumina Arrays (450, EPIC 1&2)
- Shared QC and Algorithms
- Does this mean we are harmonized?



Batch Effects

Clin Epigenetics. 2022; 14: 58.
Published online 2022 Apr 29. doi: 10.1186/s13148-022-01277-9
PMCID: PMC9055778
PMID: 35488315

Batch-effect detection, correction and characterisation in Illumina HumanMethylation450 and MethylationEPIC BeadChip array data

Jason P. Ross,[✉1] Susan van Dijk,[1] Melinda Phang,[2] Michael R. Skilton,[2,3,4] Peter L. Molloy,[1] and Yalchin Oytam[5]

BMC Bioinformatics. 2020; 21: 271.
Published online 2020 Jun 30. doi: 10.1186/s12859-020-03559-6
PMCID: PMC7328269
PMID: 32605541

Simulating ComBat: how batch correction can lead to the systematic introduction of false positive results in DNA methylation microarray studies

Tristan Zindler,[✉1] Helge Frieling,[1] Alexandra Neyazi,[1] Stefan Bleich,[1] and Eva Friedel[2,3]

Front. Genet., 16 March 2018
Sec. Epigenomics and Epigenetics
Volume 9 - 2018 | https://doi.org/10.3389/fgene.2018.00083

Adjusting for Batch Effects in DNA Methylation Microarray Data, a Lesson Learned

E. M. Price[1,2,3*] Wendy P. Robinson[1,2]

ORIGINAL RESEARCH article
Front. Genet., 13 October 2014
Sec. Epigenomics and Epigenetics
Volume 5 - 2014 | https://doi.org/10.3389/fgene.2014.00354

Stratified randomization controls better for batch effects in 450K methylation analysis: a cautionary tale

Olive D. Buhule[1] Ryan L. Minster[2] Nicola L. Hawley[3] Mario Medvedovic[4] Guangyun Sun[4] Satupaitea Viali[5] Ranjan Deka[4] Stephen T. McGarvey[6] Daniel E. Weeks[1,2*]

- Significant Attention, Methods of Adjustment, etc
- Typically explains 10-15% of variance even in very good studies
- Randomization helps
- Most longitudinal studies are on different batches
- A different lab is at least a different batch

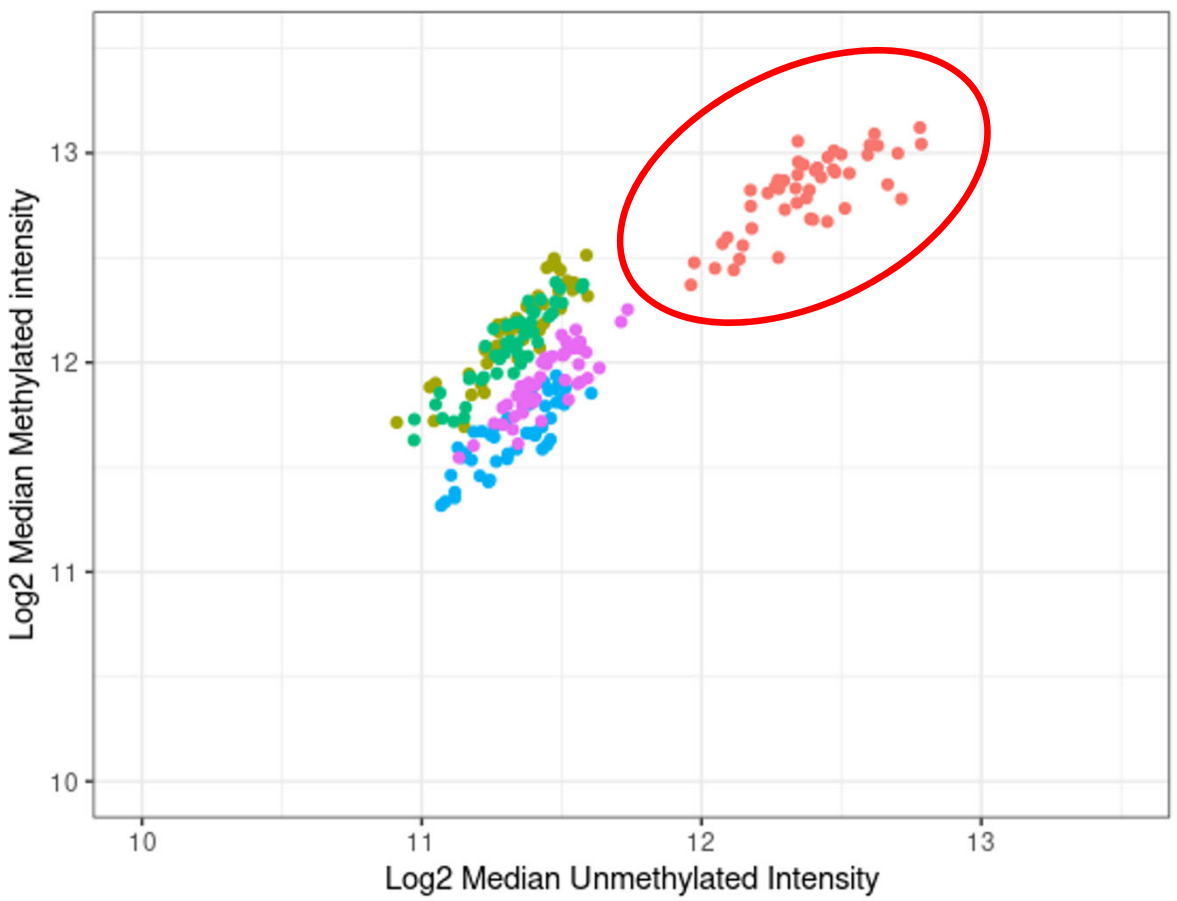
Overview

- 5 sets of 48 samples sent to 4 labs (LASI, HRS, NICOLA, TILDA)
- 2 sets from Lab 1 (1 was shipped and the other stayed)
- Lab 2 and Lab 4 used EPICv2 Beadchip
- Following HRS DNAm QC. All samples were noob normalized, a dye bias background correction method
- Technical replicates included on most samples
- Crimmins and Faul R01 AG068937-Social Circumstances and Epigenomics Promoting Health in Three Countries

Overview of Comparisons

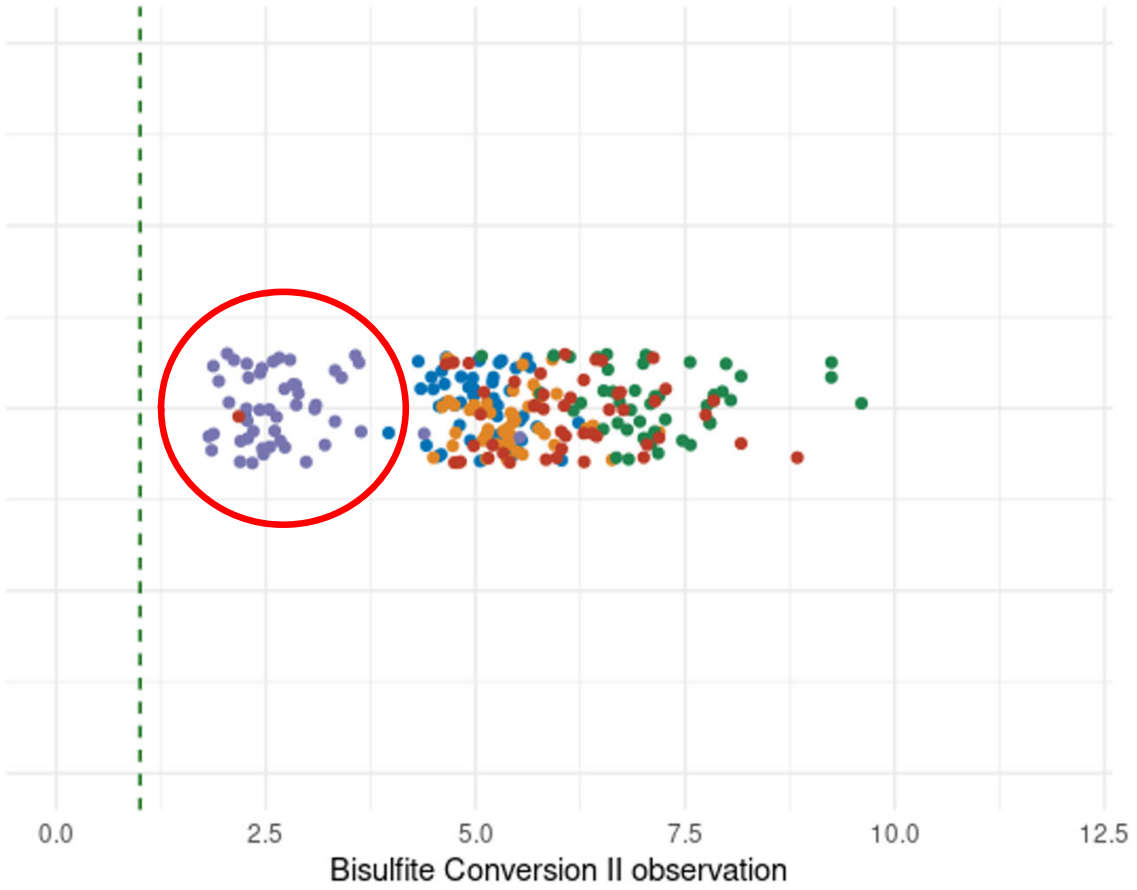
- Quality Control metric comparisons
- Cell Distribution
- Smoking
- Clocks
- Summary and Future (EWAS, Normalization,etc)

Comparing Raw Intensities



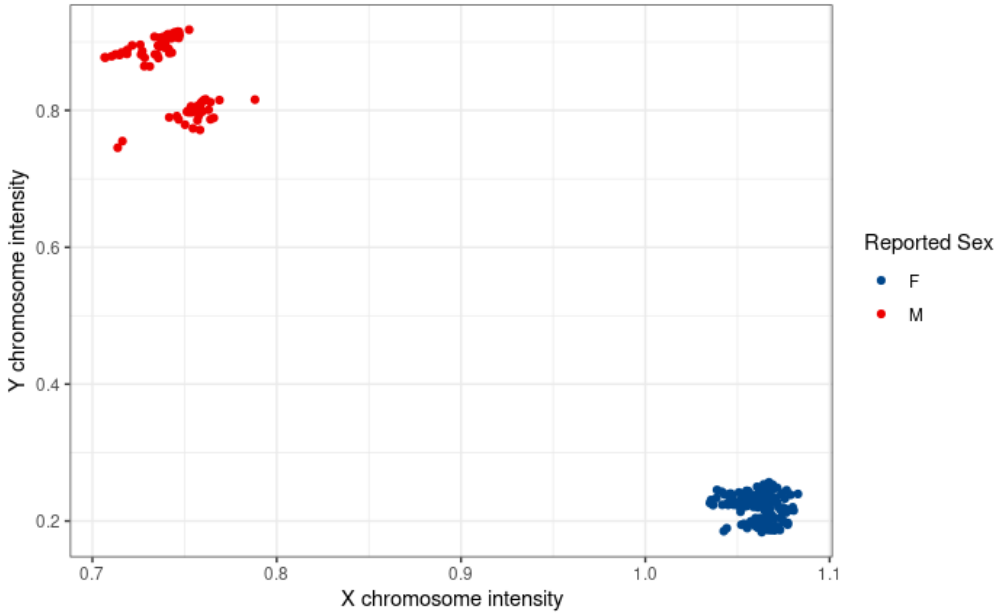
- All 240 samples pass QC
- No samples are low intensity (< 9)

Illumina Control Metrics



- 17 Illumina control metrics, measured using control probes placed on the assay
- All samples passed the 17 metrics
- This metric checks for successful bisulfite conversion

Check Sex



- All samples match reported sex at birth
- Clear difference between Y-Chromosome probes for EPICv1 and v2

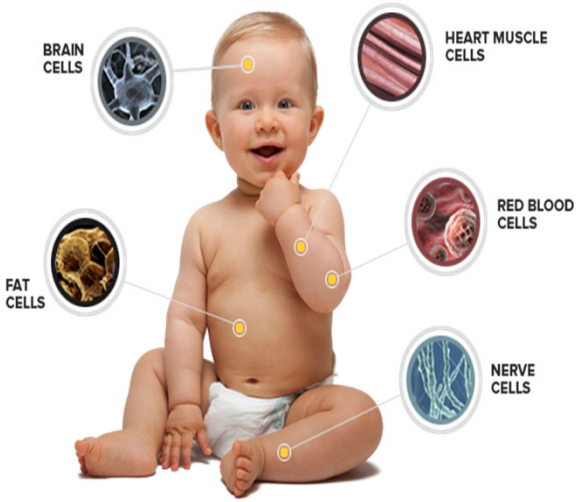
Comparison of cut probes

All values with a detectionP value of > 0.01 and/or a beadcount of under 4 are labeled as unreliable. All probes with over 5% unreliable values are cut.

Lab 1 (Shipped)	Lab 1	Lab 2 (v2)	Lab 3	Lab 4 (v2)
33,478 (3.9%)	33,117 (3.8%)	27,339 (2.9%)	18,904 (2.2%)	18,904 (2.0%)

- 5829 cut probes shared between EPIC 1
- 17835 cut probes shared between EPIC 2

Estimated Cell Distribution



- A primary function of Methylation is cell differentiation
- Often the first PCs of methylation data are related to cell distribution
- Cell type estimates are regularly used as confounders

Estimated Cell Distribution

- We used EpiDish to estimate Cell distributions:

  Neutrophils, Eosinophils, Basophils, Monocytes, and

  Lymphocytes: T cells (cd4 and cd8), B cells, and Natural Killer

  Memory and Naïve subtypes
- All cell type estimates were within 10-15% of each other (i.e. if the estimate was .01 the range was (.009-.011)
- One exception- Monocytes—closer to 22%
- Correlated at 0.92-0.99, except monocytes (0.78)
- Overall very strong concordance

Smoking

- The next largest association in DNAm after cell distribution and sex is typically smoking
- Smoking (including maternal smoking during pregnancy) leaves a strong signature on the methylome
- Within our sample we had 17 current smokers, 14 former smokers, and 17 never-smokers

› Epigenomics. 2019 Oct;11(13):1469-1486. doi: 10.2217/epi-2019-0206. Epub 2019 Aug 30.

EpiSmokEr: a robust classifier to determine smoking status from DNA methylation data

Sailalitha Bollepalli [1 2], Tellervo Korhonen [1 3], Jaakko Kaprio [1 2], Simon Anders [1 4], Miina Ollikainen [1 2]

Smoking

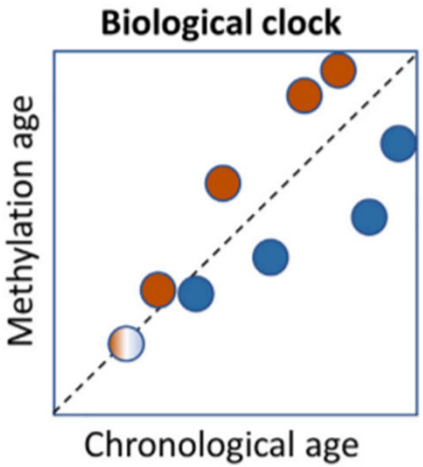
- Of the 17 **Current Smokers** all labs, batches, and technical replicates found 14 of them and most found 16.

- Of the 17 **Never Smokers** all labs, batches, and technical replicates found 15 of them and most found all 17.

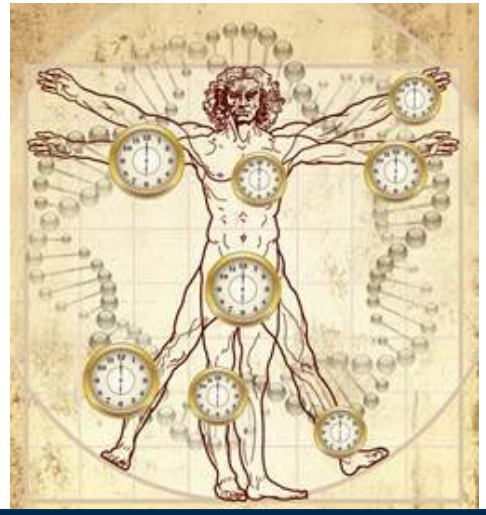
- Of the 14 **Former Smokers** labs and batches matched only 50% of the time. And who matched was not the same study to study.
- Technical replicates matched for Former smokers 93% of the time

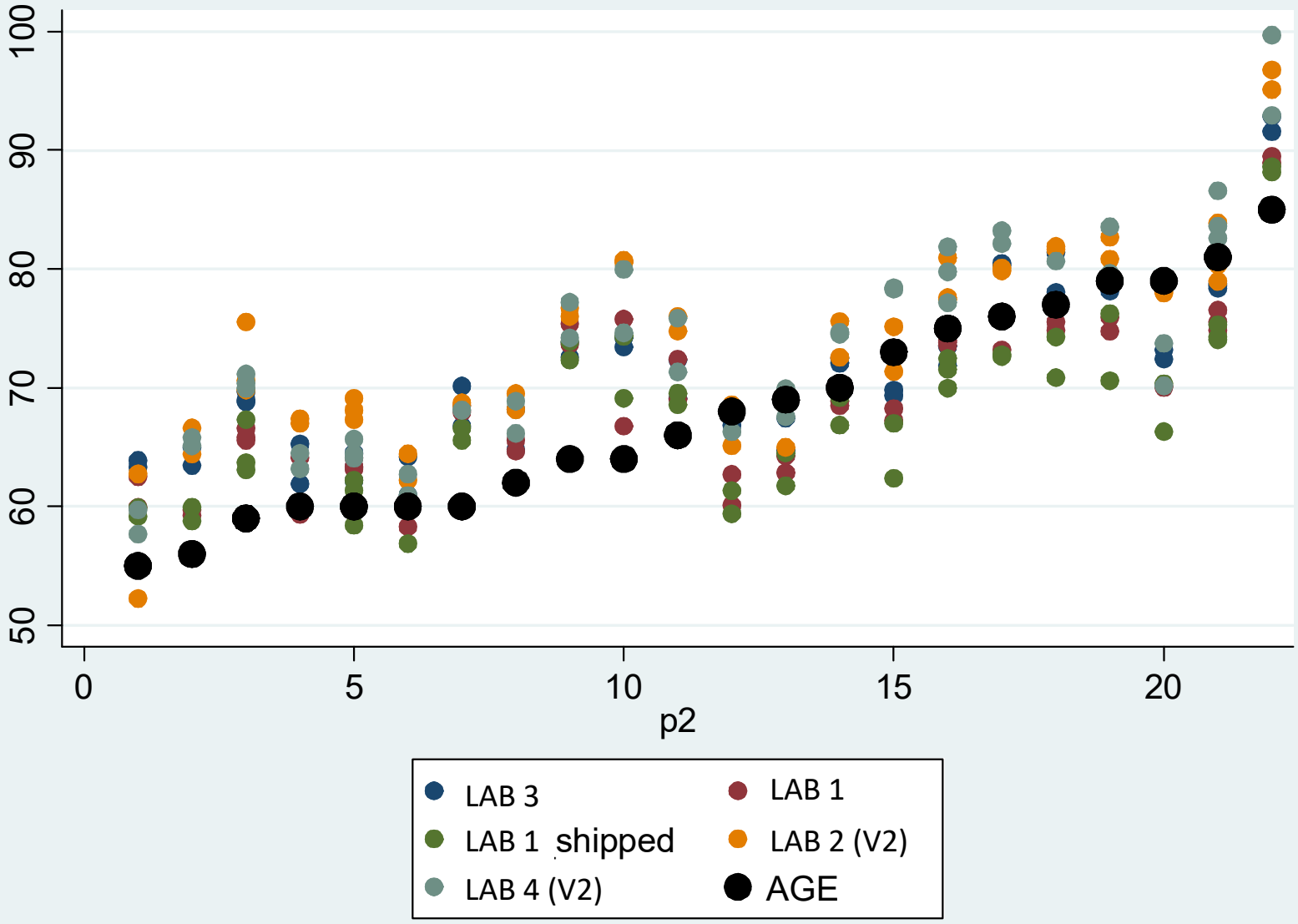
- General smoking probabilities correlated 0.88-0.94

Comparing Epigenetic Clocks



- Most widely used epigenetic measure in social and population health research
- Methylation changes with time and exposures
- 3 generations of clocks





Horvath

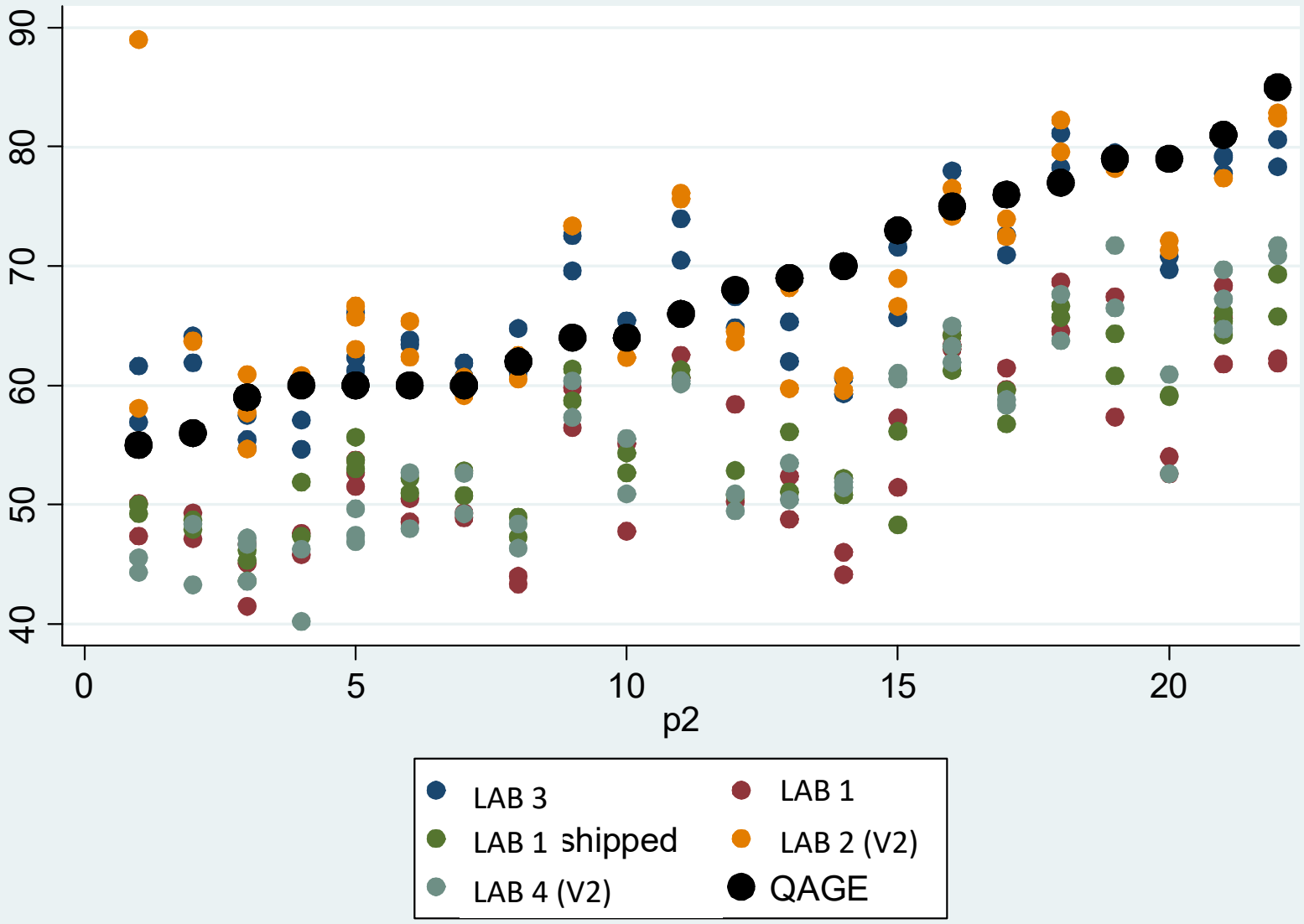
Avg range- 11.3 y

Tech Replicates- p=0.87

Batch-p=0.01
Lab-p=0.001

Corr-0.81

Within-person Variance- 24%



Pheno

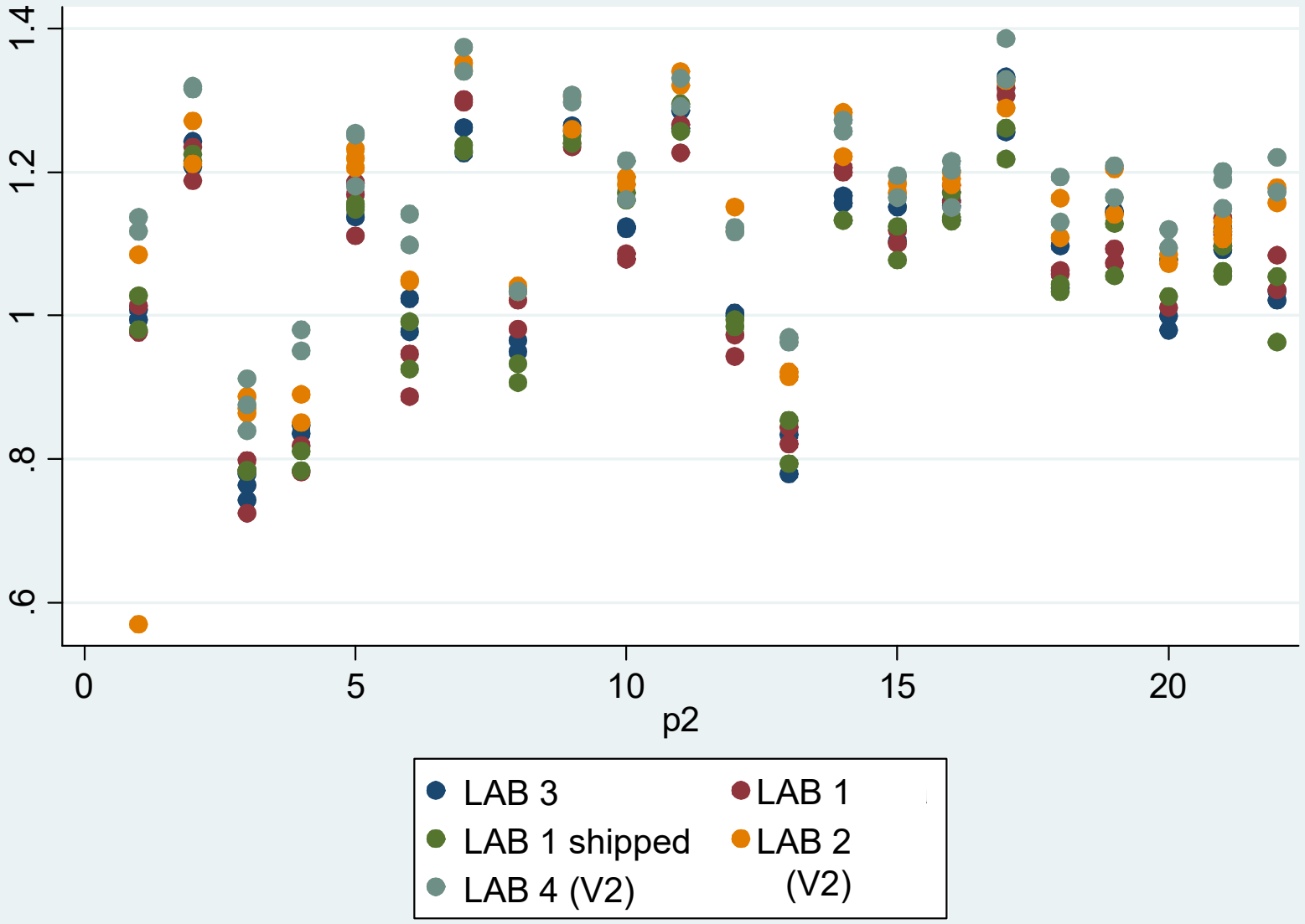
Avg range- 21.8 y

Tech Replicates- p=0.45

Batch-p=0.005
Lab-p<0.001

Corr-0.63

Within-person Variance- 41%



DunedinPACE

Avg range- 0.18

Tech Replicates- p=0.87

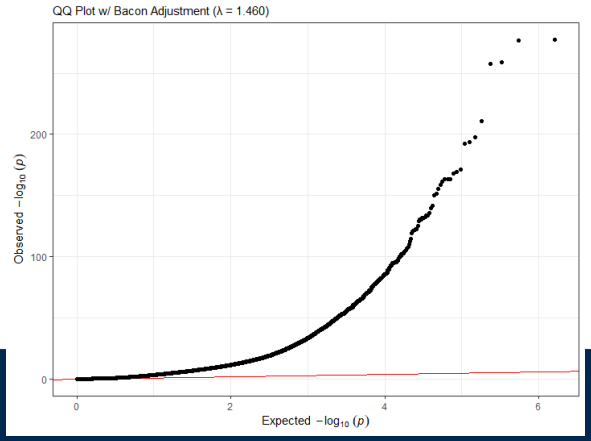
Batch-p=0.009
Lab-p=0.002

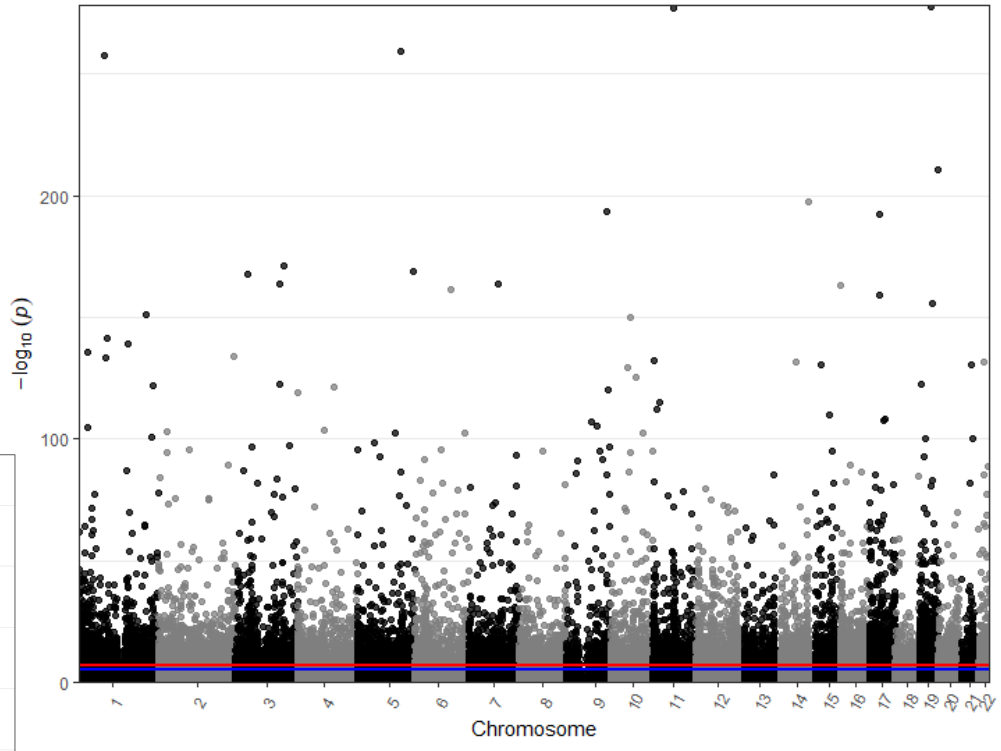
Corr-0.92

Within-person Variance- 18%

EWAS of Within Person Variance

- 24,710 probes with a bacon adjusted p-value < 10^(-7)
- 8,937 probes with an average coefficient of variation greater than 0.250
- 30 probes CV> 1





Summary

- Lab effects are slightly larger than batch effects which are larger than chip differences
- QC differences may seem large but don't seem to have a major effects on many outcomes
- Cell distribution estimates are consistent in both absolute and relative terms
- Smoking—Current and Never very similarly determined, former smoking…is a mess
- Clocks: high relative concordance but absolute differences are large
- Exploring potential lab/batch affected probes
- Need to try other QC pipelines—especially different normalizations

Biomarker Hype Curve