# Comprehensive analysis of transcriptional response upon multiple drug treatments

Yizhen Zhong, BS[1]; Minoli Perera, PhD, PharmD[1]
[1]Department of Pharmacology, Northwestern University Feinberg School of Medicine

## Introduction

- Investigating the drug response at the transcriptional level is powerful for understanding the mechanism of drug adverse effects, elucidating inter-individual drug response variability and developing personalized therapies.
- However, drug perturbation experiments are limited to immortal cell lines which do not reflect the true physiology and gene expression of the liver, a key organ for drug metabolism.
- Moreover, available resources are mostly based on microarray which lacks the broad dynamic range on RNA-sequencing data in capturing transcript abundance.
- Here, we aim to characterize the transcriptional response upon drug treatments in liver powering by the RNA-seq technology.

## Methods and Materials

- We treated 68 primary human hepatocytes derived from donors of African American ancestry with 6 known inducers of drug metabolism, Omeprazole, Phenobarbital, Dexamethasone, Carbamazepine, Phenytoin and Rifampicin and measured the transcriptome via RNA-seq technology before and after treatment.
- We performed pair-wise differential expression (DE) analysis and identified genes that were up- or down-regulated upon drug treatment.
- We utilized Grade of membership (GoM) clustering to capture the heterogeneity among drug response profiles.
- We used weighted co-expression network to compare the expression regulatory architecture among drug treatments.

## Drugs overview

| | Omeprazole | Phenobarbital | Dexamethasone | Carbamazepine | Phenytoin | Rifampicin |
|---|---|---|---|---|---|---|
| **Application** | Stomach ulcers | Seizures | Inflammatory and autoimmune conditions | Seizures | Seizures | Antibiotic |
| **Mechanism of action** | Proton pump inhibitor | Acts on GABA receptor | Glucocorticoid receptor agonist, Immunosuppressant | Potassium channel modulator, Sodium channel blocker | Sodium channel blocker | RNA polymerase inhibitor |
| **Targets** | ABCB1 ABCC3, ATP4A, AHR, CYP2C19, CYP3A4 | ABCB1, GABRA1, CBRNA4, CHRNA7, GRIA2, GRIK2, CYP2C19, CYP2C9, CYP2B6 | ANXA1, CYP3A4, CYP3A5, NOS2, NROB1, NR3C1, NR3C2 | ABCB1, CYP1A2, CYP3A4, SCN1A, SCN3A, SCN5A | ABCB1, CYP2B6, CYP2C19, CYP3A4, SCN1A, SCN2A, SCN5A | ABCB1, CYP2B6, CYP2C19, CYP2C8, CYP3A4, CYP3A5, NR112, SLCO1A2, SLCO1B1, SLCO1B3 |

## Pair-wise differential expression analysis revealed common and specific gene signatures upon drug treatments

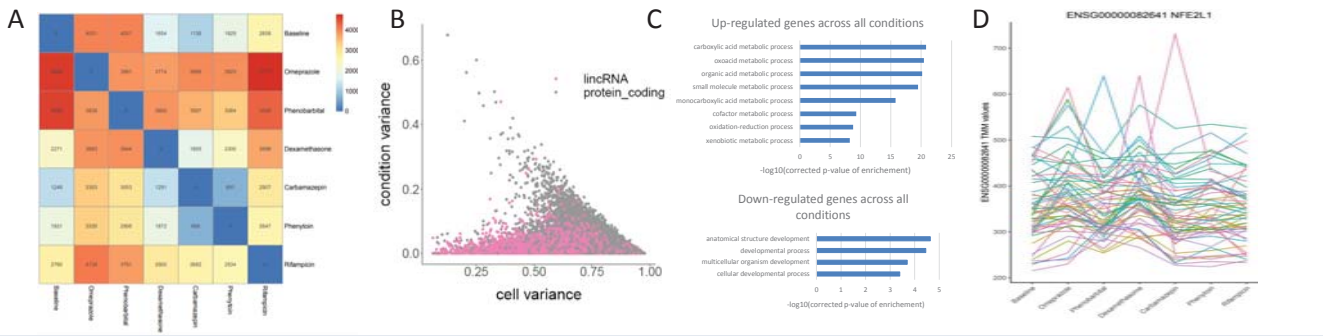


**Figure 1:** Expression signatures upon multiple drug treatments. **A.** Pair-wise differential expression analysis demonstrated treatment with Phenobarbital and Omeprazole induced the strongest response and Carbamazepine induced the lowest response. **B.** More variance in gene expression was attributed to inter-individual variance than inter-condition variance. **C.** Top Gene Ontology terms enriched in genes that were up- or down-regulated across all conditions compared with baseline. **D.** Common up-regulated genes are enriched for a transcription factor, NEF2L1, targets.

## Phenytoin and Carbamazepine have similar differential expression response but different co-expression structure

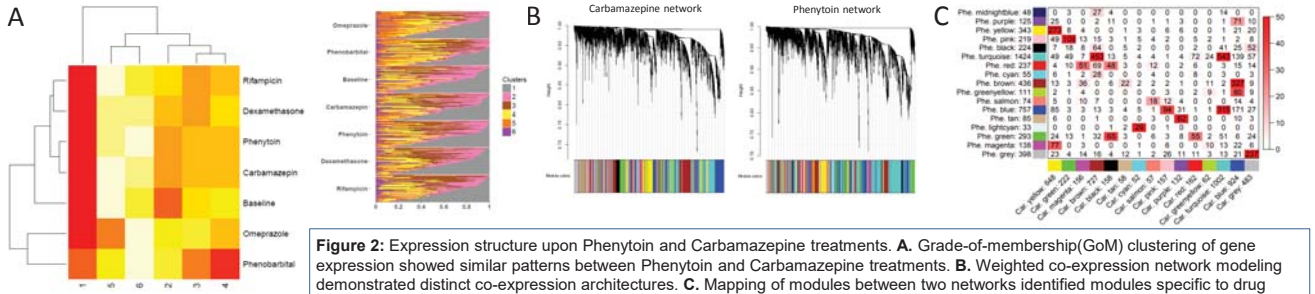


**Figure 2:** Expression structure upon Phenytoin and Carbamazepine treatments. **A.** Grade-of-membership(GoM) clustering of gene expression showed similar patterns between Phenytoin and Carbamazepine treatments. **B.** Weighted co-expression network modeling demonstrated distinct co-expression architectures. **C.** Mapping of modules between two networks identified modules specific to drug treatment (e.g. greenyellow for Carbamazepine and salmon for Phenytoin).

## Future work

- Investigate the role of NFE2L1 in modulating drug response with ChIP-seq and functional analysis.
- Identify pharmacologically relevant genes whose expression or fold-change upon drug treatment are associated with African ancestry.
- Explore the different roles of protein-coding RNA and long non-coding RNA in modulating drug response.
- Integrate the expression with genotype to identify common and drug-specific genetic regulators.

## Conclusions

- Drug perturbation on primary hepatocytes allows us to characterize the drug-specific mechanism of action and identify generic signatures of drug response.
- Different drugs exhibited broad-spectrum transcriptomic responses.
- *NFE2L1* may serve as the general up-stream regulator of drug treatments.
- Both differential expression analysis and GoM clustering demonstrate the similarity between Phenytoin and Carbamazepine, which may result from their common therapeutic application on epilepsy. Co-expression network analysis highlights drug-specific regulatory architecture.

National Institute on Minority Health and Health Disparities
7R01MD009217-04

Reference: Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet J-P, Subramanian A, Ross KN: **The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease.** *science* 2006, **313:**1929-1935.
Chhibber A, French CE, Yee SW, Gamazon ER, Theusch E, Qin X, Webb A, Papp AC, Wang A, Simmons CQ: **Transcriptomic variation of pharmacogenes in multiple human tissues and lymphoblastoid cell lines.** *The pharmacogenomics journal* 2017, **17:**137.
Mangravite LM, Engelhardt BE, Medina MW, Smith JD, Brown CD, Chasman DI, Mecham BH, Howie B, Shim H, Naidoo D: **A statin-dependent QTL for GATM expression is associated with statin-induced myopathy.** *Nature* 2013, **502:**377.