Antihypertensive Response and Precision Medicine: Novel Insights from Genomics and Metabolomics Integration

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Outline

I. Background & Significance
   - Hypothesis
   - Study aims

II. Materials & Methods
   - Study participants
   - Metabolomics & Genomics

III. Approach & Results
   - Analyses framework
   - Study results
   - Pilot study

IV. Conclusion
I. Background & Significance
Hypertension and Cardiovascular Risk

Hypertension Globally

1.1 BILLION PEOPLE worldwide have high blood pressure

Hypertension is a leading cause of cardiovascular disease and mortality worldwide

1/3 OF ADULTS (78 MILLION)
Prevalence of hypertension in the United States

When your blood pressure is high:

You are 4x more likely to die from a stroke

You are 3x more likely to die from heart disease

69% of people who have a first heart attack...

77% of people who have a first stroke...

74% of people with chronic heart failure...

Hypertension Control and Hydrochlorothiazide (HCTZ)

1st Line Treatment

Uncomplicated Essential Hypertension

Hydrochlorothiazide Response

ONLY ABOUT HALF

Of hypertensive patients treated with HCTZ achieve blood pressure (BP) control

Controlled BP Uncontrolled BP

46%

Current Approach for therapy selection and BP control is Suboptimal

Genome Wide Association of European American HCTZ Treated Patients

Many other genetic variants with sub-genome wide p-values (p <5x10^-8) - might be true positive

But

Difficult to ascertain statistically with genomics data alone

PRKCA; Protein Kinase C Alpha, GNAS; G-protein alpha subunit
Pharmacometabolomics

The Pharmacogenomics of Anti-Platelet Intervention (PAPI) Study: Variation in Platelet Response to Clopidogrel and Aspirin

Laura M. Bozzi¹, Braxton D. Mitchell¹,², Joshua P. Lewis¹, Kathy A. Ryan¹, William R. Herzog³, Jeffrey R. O’Connell¹, Richard B. Horenstein¹, Alan R. Shuldiner¹,², and Laura M. Yerges-Armstrong¹,*

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³Geriatric Center, Baystate Medical Center, Springfield, Massachusetts 01199, USA

Pretreatment metabotype as a predictor of response to sertraline or placebo in depressed outpatients: a proof of concept

R Kaddurah-Daouk¹, SH Boyle¹, W Matson², S Sharma², S Matson³, H Zhu⁴, MB Bogdanov⁵, E Churchill¹, RR Krishnan¹,⁶, AJ Rush⁶, E Pickering⁷ and M Delnomdedieu⁸

Metabolomics Reveals Amino Acids Contribute to Variation in Response to Simvastatin Treatment

Miles True⁹, Peter D. Krzeminski⁹

Pharmacometabolomics Reveals Racial Differences in Response to Atenolol Treatment

William R. Wikoff¹*, Reginald F. Frye², Hongjie Zhu³, Yan Gong³, Stephen Boyle³, Erik Churchill³, Rhonda M. Cooper-Dehoff², Amber L. Beitelshes⁴, Arlene B. Chapman⁵, Oliver Fiehn¹, Julie A. Johnson², Rima Kaddurah-Daouk³,⁶, Pharmacometabolomics Research Network

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Pharmacometabolomics-Pharmacogenomics Integration

Glycine and a Glycine Dehydrogenase (GLDC) SNP as Citalopram/Escitalopram Response Biomarkers in Depression: Pharmacometabolomics-informed Pharmacogenomics

Yuan Ji¹,⁎, Scott Hebrring¹,⁎, Hongjie Zhu²,⁎, Gregory D Jenkins³, Joanna Biernacka³, Karen Snyder⁴, Maureen Drews⁴, Oliver Fiehn⁵, Zhaobang Zeng², Daniel Schaid³, David A. Mrazek⁴, Rima Kaddurah-Daouk⁵,⁎⁎, and Richard M. Weinshilboum¹,⁎

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⁵ Metabolomic Center, Mayo Clinic, Rochester, Minnesota, USA

TSPAN5, ERICH3 and Selective Serotonin Reuptake Inhibitors in Major Depressive Disorder: Pharmacometabolomics-informed Pharmacogenomics

Meenal Gupta, PhD¹, Drew Neavin, BSc#¹, Duan Liu, PhD#¹, Joanna Biernacka, PhD², Daniel Hall-Flavin, MD³, William V. Bobo, MD³, Mark A. Frye, MD³, Michelle Skime, MSc, CCRP³, Gregory D. Jenkins, MSc², Anthony Batzler, BSc², Krishna Kalari, PhD², Wayne Matson, PhD⁴, Swati S. Bhasin, BSc⁴, Hongjie Zhu, PhD⁵, Taisei Mushiroda, PhD⁶, Yusuke Nakamura, MD, PhD⁷, Michael H. Connolly, MD, PhD⁸, and Richard M. Weinshilboum¹

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⁷Department of Biostatistics, Mayo Clinic, Rochester, Minnesota, USA
⁸Department of Psychiatry and Behavioral Sciences, Mayo Clinic, Rochester, Minnesota, USA

Purine Pathway Implicated in Mechanism of Resistance to Aspirin Therapy: Pharmacometabolomics-Informed-Pharmacogenomics

Laura M. Yerges-Armstrong¹,⁎, Sandrine Ellero-Simatos²,⁎⁎, Anastasia Georgiades⁴,⁎, Hongjie Zhu⁵, Joshua Lewis¹, Richard B. Horenstein¹, Amber L. Beitzelshes¹, Adrie Dane²,⁎, Theo Reijmers²,⁎, Thomas Hankemeier²,⁎, Oliver Fiehn⁵, Alan R. Shuldiner¹,⁎, Rima Kaddurah-Daouk⁶,⁷,⁎⁎, and Pharmacometabolomics Research Network

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Hypothesis

Integrating metabolomics with genomics would help identifying novel biomarkers and pathways associated with the inter-individual variability in response to thiazide diuretics
Identify metabolites significantly associated with the BP response to HCTZ

Integrate metabolomics with genomics to identify novel pathways and biomarkers associated with HCTZ BP response
II. Materials & Methods
Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR)

Clinical trials.gov # NCT00246519

• A prospective, multi-center, randomized clinical trial that recruited mild to moderate hypertensive participants

• Aimed to investigate the role of genetics on the blood pressure response and adverse metabolic events of HCTZ and/or atenolol

Johnson JA, et al.. Am Heart J 2009, 157:442-449. HCTZ; hydrochlorothiazide
Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR)

Eligibility
- Assess BP, Biological Samples + Labs

Washout Period 4-6 weeks
- Assess BP

Randomization
- HCTZ monotherapy
  - Assess BP, add Atenolol
    - 6-9 weeks (Monotherapy)
    - Assess BP
  - Replication (n=214) (PEAR HCTZ add-on)

- Atenolol monotherapy
  - Assess BP, add HCTZ
    - 6-9 weeks (Combination therapy)
    - Assess BP

Discovery (n=228) (PEAR HCTZ monotherapy)

Collected baseline fasting plasma samples from 123 European Americans (Whites) treated with HCTZ in PEAR

Untargeted metabolomics profiling was conducted on those samples using GC-TOF MS platform

We identified 212 structurally known and 272 unknown metabolites

Metabolomics analysis was performed through Pharmacometabolomics Research Network

HCTZ; hydrochlorothiazide. GC-TOF MS; Gas Chromatography-Time of Flight Mass Spectroscopy.
PEAR Genomics
Experimental Setup

• Genotyping was done for PEAR participants included in this study using Illumina Human Omni1-Quad Bead Chip

• Genotype call rates >95% and SNP call rates >95%

• MaCH software was used to impute SNPs based on HapMap III haplotypes

• SNPs were excluded-MAF < 3%/imputation r²<0.3
III. Approach & Results
Analyses Framework

**STEP1**

**Metabolomics Analysis**
Identify baseline metabolites significantly associated with **HCTZ monotherapy** BP response (FDR<0.05)

**STEP2**

**Genomic Analysis**
Select SNPs with P-value<5x10^{-5} from PEAR **HCTZ monotherapy** BP GWAS Analysis

**STEP3**

Genomics-Metabolomics Integration

**STEP4**

**Replication**
Replicate SNPs in PEAR **HCTZ add-on** therapy

**STEP5**

**Create a Response Score**
Create a HCTZ response score using replicated SNPs

**STEP6**

**Response Score Replication**
Replicate the response score in **GERA** participants treated with HCTZ
Step 1: Metabolomics Analysis

A linear regression analysis was conducted to test the association between each metabolite and HCTZ BP response.

- 212 known metabolites
- Age, gender, and baseline BP
- HCTZ BP response

13 Significant Metabolites (FDR<0.05)

HCTZ; hydrochlorothiazide, FDR; false discovery rate, BP; blood pressure
Analyses Framework

**STEP 1**
Metabolomics Analysis
Identify baseline metabolites significantly associated with HCTZ monotherapy BP response (FDR<0.05)

**STEP 2**
Genomic Analysis
Select polymorphisms with P-value<5x10^{-5} from PEAR HCTZ monotherapy BP GWAS Analysis

**STEP 3**
Genomics-Metabolomics Integration

**STEP 4**
Replication
Replicate SNPs in PEAR HCTZ add-on therapy

**STEP 5**
Create a Response Score
Create a HCTZ response score using replicated genetic SNPs

**STEP 6**
Response Score Replication
Replicate the response score in GERA participants treated with HCTZ

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Step 2: Genomics Analysis

GWAS SNPs (Illumina Omni1-Quad Bead Chip – imputed Hap Map III)

Age, Gender, Baseline BP and principal component 1,2

103 SNPs (p-values <5x10^-5)

HCTZ BP Response
Analyses Framework

**STEP 1**
Metabolomics Analysis
Identify baseline metabolites significantly associated with HCTZ monotherapy BP response (FDR<0.05)

**STEP 2**
Genomic Analysis
Conduct GWAS and select polymorphisms with P-value<5x10^-5 from PEAR HCTZ monotherapy BP GWAS Analysis

**STEP 3**
Genomics-Metabolomics Integration

**STEP 4**
Replication
Replicate SNPs in PEAR HCTZ add-on therapy

**STEP 5**
Create a Response Score
Create a HCTZ response score using replicated genetic SNPs

**STEP 6**
Response Score Replication
Replicate the response score in GERA participants treated with HCTZ
## Step 3: Metabolomics-Genomics Pathway Integration

### Pathway Analysis

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**SNPs at p-values <5x10^{-5} (n=103 SNPs)**

**13 significant metabolites (FDR<0.05)**

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PRKAG2: Protein kinase, AMP-activated, gamma 2 non-catalytic subunit. DCC: Deleted in Colorectal Cancer. EPHX2: Epoxide hydrolase 2
Replication of the 3 identified SNPs with HCTZ BP response

**PEAR HCTZ monotherapy (n=228)**

- **PRKAG2 rs2727563 Genotypes**
  - C/C (n=62)
  - T/C (n=110)
  - T/T (n=42)
  - HCTZ SBP response (mmHg): P=2E-05

- **DCC rs12604940 Genotypes**
  - A/A (n=194)
  - G/A (n=31)
  - G/G (n=3)
  - HCTZ SBP response (mmHg): P=2E-05

- **EPHX2 rs13262930 Genotypes**
  - C/C (n=18)
  - C/G (n=79)
  - G/G (n=131)
  - HCTZ SBP response (mmHg): P=3E-05

**PEAR HCTZ add-on (n=214)**

- **PRKAG2 rs2727563 Genotypes**
  - C/C (n=62)
  - T/C (n=110)
  - T/T (n=42)
  - HCTZ SBP response (mmHg): P=0.01

- **DCC rs12604940 Genotypes**
  - A/A (n=164)
  - G/A (n=50)
  - HCTZ DBP response (mmHg): P=0.01

- **EPHX2 rs13262930 Genotypes**
  - C/C (n=12)
  - C/G (n=63)
  - G/G (n=139)
  - HCTZ SBP response (mmHg): P=0.039

**PRKAG2**: Protein kinase, AMP-activated, gamma 2 non-catalytic subunit. **BP**: blood pressure.
### Analyses Framework

**STEP 1**
**Metabolomics Analysis**
Identify baseline metabolites significantly associated with HCTZ monotherapy BP response (FDR < 0.05)

**STEP 2**
**Genomic Analysis**
Select polymorphisms with P-value < $5 \times 10^{-5}$ from PEAR HCTZ monotherapy BP GWAS Analysis

**STEP 3**
**Genomics-Metabolomics Integration**

**STEP 4**
**Replication**
Replicate polymorphisms in PEAR HCTZ add-on therapy

**STEP 5**
**Create a Response Score**
Create a HCTZ response score using replicated genetic signals

**STEP 6**
**Response Score Replication**
Replicate the response score in GERA participants treated with HCTZ
Thiazide Diuretics Genetics Response Score

• Created based on 3 replicated SNPs: PRKAG2 rs2727563, DCC rs12604940, and EPHX2 rs13262930

• Points were given as follows:
  - Homozygous genotype with the greatest BP lowering effect = 2 points
  - Heterozygous genotype = 1 point
  - Homozygous genotype with the worst BP lowering effect = zero

• Alleles with BP lowering effect were then summed up for inclusion in a regression model

• Adjusted for age, gender, baseline BP, and PC1,2
Thiazide Diuretics Genetics Response Score

**PEAR**

- HCTZ DBP response (mmHg)
  - P = 3 x 10^-9
  - r^2 = 11.9%

**GERA**

- HCTZ DBP response (mmHg)
  - P = 0.03

- HCTZ SBP response (mmHg)
  - P = 1 x 10^-8
  - r^2 = 11.3%

**PEAR**: Pharmacogenomic Evaluation of Antihypertensive Responses.

**GERA**: Genetic Epidemiology of Responses to Antihypertensives
**In Summary**

- Identified **13 metabolites** significantly associated with HCTZ BP response

- Identified novel signals *PRKAG2* rs2727563, *DCC* rs12604940 and *EPHX2* rs13262930 associated with HCTZ BP response

- Replicated in another independent group of patients treated with HCTZ therapy

- Using the **3 replicated SNPs**, we created a response score that explained 11.3%-11.9% of the variability in HCTZ BP response

- Replicated this response score in another independent group of participants treated with HCTZ monotherapy

**PRKAG2**: protein kinase, AMP-activated, gamma 2 non-catalytic subunit. **DCC**: Deleted in Colorectal Cancer.
Follow-up to the genomics-metabolomics findings

Pilot Metabolomics Study (Research in Progress)
Genomics-Metabolomics Integration Findings

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**Genomics:**
- PRKAG2: Protein kinase, AMP-activated, gamma 2 non-catalytic subunit
- DCC: Deleted in Colorectal Cancer
- EPHX2: Epoxide hydrolase 2

**Genomics SNPs:**
- PRKAG2 rs2727563
- DCC rs12604940
- EPHX2 rs13262930

**Correlations:**
- r=0.30, P=9x10^-4
- r=0.26, P=3x10^-3
Arachidonic acid

- Arachidonic acid is a polyunsaturated omega-6 fatty acid

- Arachidonic acid metabolites (eicosanoids) have been involved in diverse signaling cascades associated with:
  - BP regulation
  - Inflammation
  - Renal vascular tone
  - Sodium transport

- Less is known about the association between arachidonic acid metabolites and BP response to HCTZ

# Genomics-Metabolomics Integration Findings

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PRKAG2: Protein kinase, AMP-activated, gamma 2 non-catalogic subunit. DCC: Deleted in Colorectal Cancer. EPHX2: Epoxide hydrolase 2.
EPHX2 (Epoxide Hydrolase 2)

- HCTZ decreases the protein expression of sEH in spontaneously hypertensive rats

- HCTZ might be mediating its antihypertensive BP response via the inhibition of the sEH

Roman RJ. Physiol Rev..2002 Jan;82(1):131-85.

EET: Epoxyeicosatrienoic acid
DHET: Dihydroxyeicosatrienoic acid
sEH: Soluble Epoxide Hydrolase 2
Research in Progress

Pilot Study

“Investigating the Implications of Eicosanoids on the Blood Pressure Response to Thiazide Diuretics”
In Conclusion

• Emphasized the strength of using multiple “omics” for identifying novel pathways and biomarkers associated with drug response.

• Provided multiple levels of replication – which further substantiates the importance of *PRKAG2*, *DCC* and *EPHX2* as potential determinants of the BP response to HCTZ.

• Replication in other studies could provide more evidence for using those signals in guiding the selection of HCTZ therapy.

• Results from the pilot metabolomics project may help confirm the importance of these signals.
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