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

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Outline

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- ❑ Study aims

II. Materials & Methods

- ❑ Study participants
- ❑ Metabolomics & Genomics

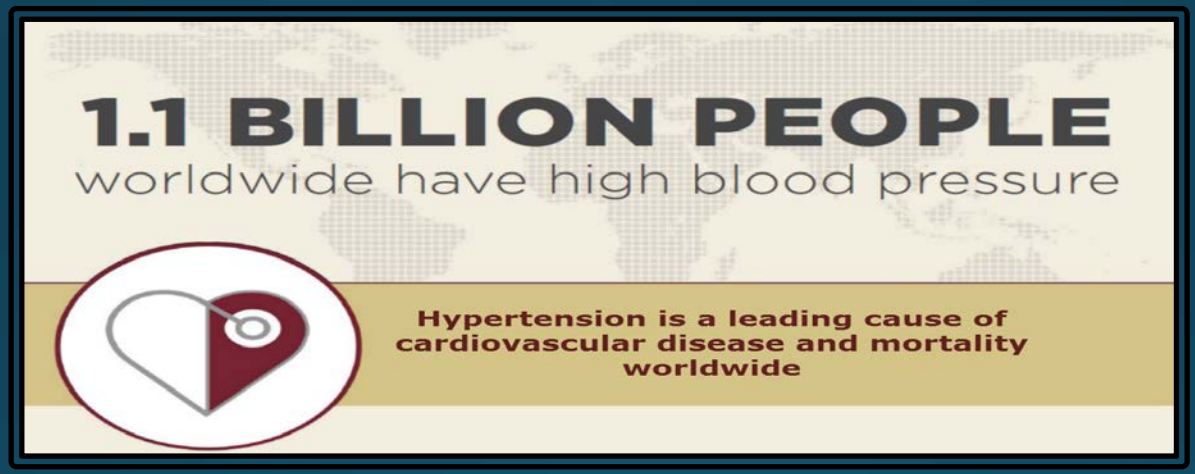
III. Approach & Results

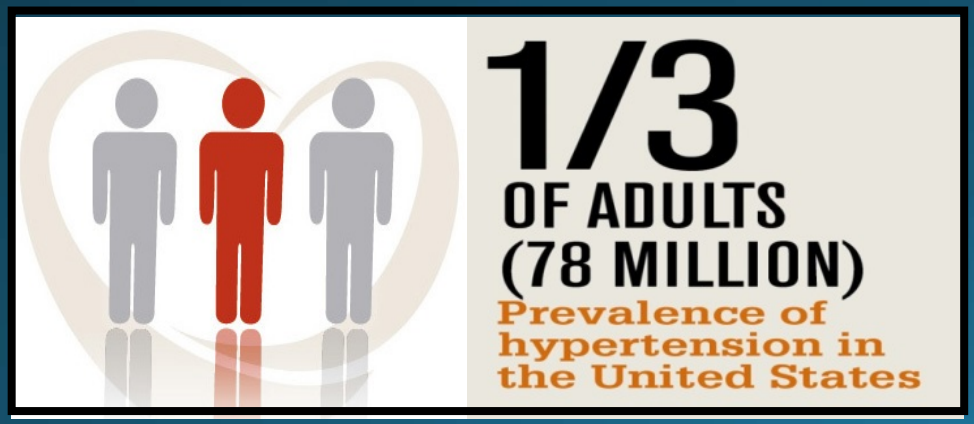
- ❑ Analyses framework
- ❑ Study results
- ❑ Pilot study

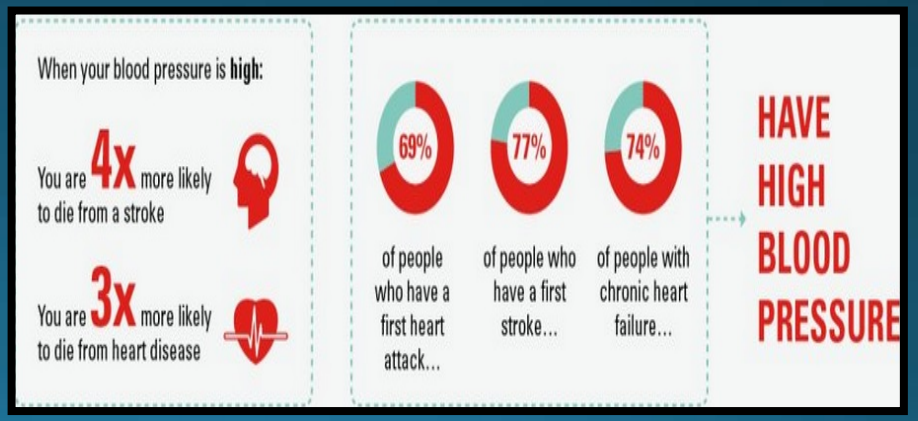
IV. Conclusion

I. Background & Significance

Hypertension and Cardiovascular Risk





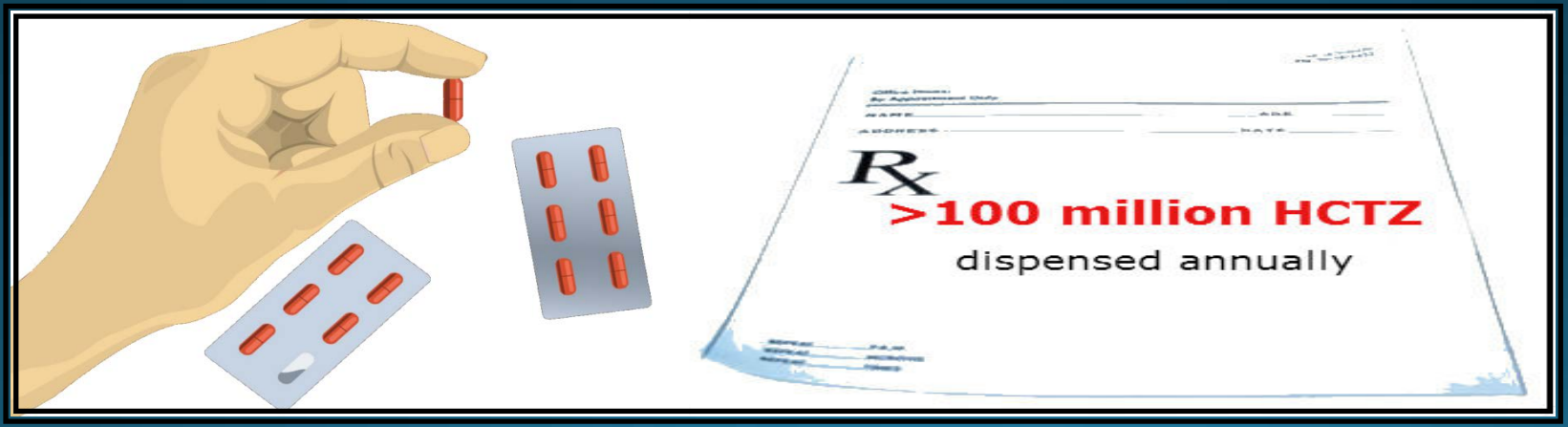


http://www.cdc.gov/bloodpressure/infographic.htm. Accessed November 19th 2016
Mozaffarian D, Benjamin EJ, Go AS, et al. Circulation 2015;131:e29-322.

Hypertension Control and Hydrochlorothiazide (HCTZ)

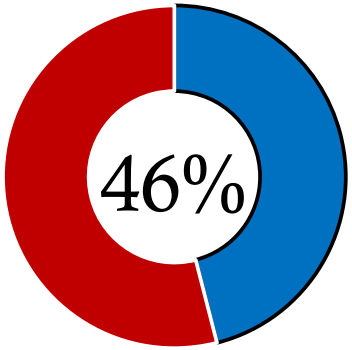
1st Line Treatment

Uncomplicated Essential Hypertension



http://drugtopics.modernmedicine.com. Accessed November 19th 2016.
James PA, et al.. JAMA 2014;311:507-20. Weber MA, et al.. Journal of hypertension 2014;32:3-15.

Hydrochlorothiazide Response



ONLY ABOUT HALF

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

Current Approach for therapy selection and BP control is

Suboptimal

Materson BJ. The Am J Med. 2007;120:S10-20.
Materson BJ, et al.. NEJM 1993;328:914-21.

Genome Wide Association of European American HCTZ Treated Patients

Genomic Association Analysis of Common Variants Influencing Antihypertensive Response to Hydrochlorothiazide

Stephen T. Turner, Eric Boerwinkle, Jeffrey R. O'Connell, Kent R. Bailey, Yan Gong, Arlene B. Chapman, Caitrin W. McDonough, Amber L. Beitelshees, Gary L. Schwartz, John G. Gums, Sandosh Padmanabhan, Timo P. Hiltunen, Lorena Citterio, Kati M. Donner, Thomas Hedner, Chiara Lanzani, Olle Melander, Janna Saarela, Samuli Ripatti, Björn Wahlstrand, Paolo Manunta, Kimmo Kontula, Anna F. Dominiczak, Rhonda M. Cooper-DeHoff and Julie A. Johnson

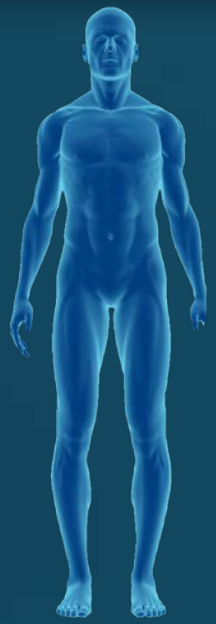
Hypertension. 2013;62:391-397; originally published online June 10, 2013;
doi: 10.1161/HYPERTENSIONAHA.111.00436

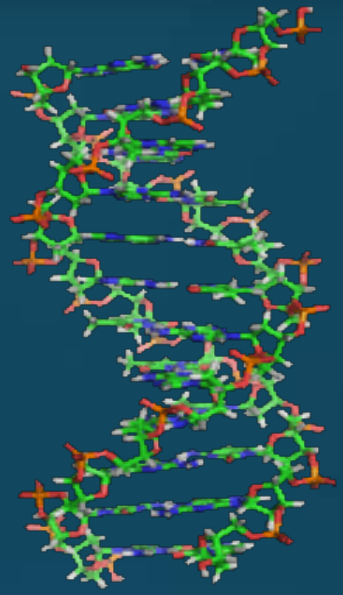
Many other genetic variants with sub-genome wide p-values ($p < 5x10^{-8}$) - might be true positive

But

Difficult to ascertain statistically with genomics data alone

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





DNA

Genomics

RNA

Transcript-
omics

Protein

Proteomics

Metabolites

Metabolomics

Phenotype

Basic Flow of Genetic Information in a Cell

Pharmacometabolomics

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

Laura M. Bozzi[1], Braxton D. Mitchell[1,2], Joshua P. Lewis[1], Kathy A. Ryan[1], William R. Herzog[3], Jeffrey R. O'Connell[1], Richard B. Horenstein[1], Alan R. Shuldiner[1,2], and Laura M. Yerges-Armstrong[1,*]

[1]Program
Endocrino
21201, US

[2]Geriatric
Center, Ba

[3]Sinai Hos

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

R Kaddurah-Daouk[1], SH Boyle[1], W Matson[2], S Sharma[2], S Matson[2,3], H Zhu[4], MB Bogdanov[5], E Churchill[1], RR Krishnan[1,6], AJ Rush[6], E Pickering[7] and M Delnomdedieu[8]

The purpose
depressive d
assigned to s
were profiled
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Hamilton Rat
analyses sho
sertraline mo

OPEN ACCESS Freely available online

PLoS one

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

Miles Trup
Peter D. K

1 Bioinformatics
United States of
of America, 5 Bi
Oakland, Califor

OPEN ACCESS Freely available online

PLOS | ONE

Pharmacometabolomics Reveals Racial Differences in Response to Atenolol Treatment

William R. Wikoff[1]*, Reginald F. Frye[2], Hongjie Zhu[3], Yan Gong[2], Stephen Boyle[3], Erik Churchill[3], Rhonda M. Cooper-Dehoff[2], Amber L. Beitelshees[4], Arlene B. Chapman[5], Oliver Fiehn[1], Julie A. Johnson[2], Rima Kaddurah-Daouk[3,6]*, Pharmacometabolomics Research Network

1 UC Davis Genome Center, University of California Davis, Davis, California, United States of America, 2 Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics, University of Florida, Gainesville, Florida, United States of America, 3 Department of Psychiatry, Duke University Medical Center, Durham, North Carolina, United States of America, 4 Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, 5 Department of Medicine, Emory University, Atlanta, Georgia, United States of America, 6 Duke Institute for Brain Sciences, Duke University, Durham, North Carolina, United States of America

Pharmacometabolomics-Pharmacogenomics Integration

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

Yuan Ji[1,*], Scott Hebbring[1,*], Hongjie Zhu[2,*], Gregory D Jenkins[3], Joanna Biernacka[3], Karen Snyder[4], Maureen Drews[4], Oliver Fiehn[5], Zhaobang Zeng[2], Daniel Schaid[3], David A. Mrazek[4], Rima Kaddurah-Daouk[6,**], and Richard M. Weinshilboum[1,**]

[1] Division of Clinical Pha
Therapeutics, Mayo Clin

[2] Bioinformatics Resear

[3] Department of Health

[4] Department of Psychia

[5] Metabolomic Center, U

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

Meenal Gupta, PhD[#1], Drew Neavin, BSc[#1], Duan Liu, PhD[#1], Joanna Biernacka, PhD[2], Daniel Hall-Flavin, MD[3], William V. Bobo, MD[3], Mark A. Frye, MD[3], Michelle Skime, MSc, CCRP[3], Gregory D. Jenkins, MSc[2], Anthony Batzler, BSc[2], Krishna Kalari, PhD[2], Wayne Matson, PhD[4], Swati S. Bhasin, BSc[4], Hongjie Zhu, PhD[5], Taisei Mushiroda, PhD[6], Yusuke Nakamura, MD, PhD[7], Mic
Daouk, PhD[5], and Richard

[1]Department of Molecular P
MN

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

Laura M. Yerges-Armstrong[1,*], Sandrine Ellero-Simatos[2,3,*], Anastasia Georgiades[4,*], Hongjie Zhu[4], Joshua Lewis[1], Richard B. Horenstein[1], Amber L. Beitelshees[1], Adrie Dane[2,3], Theo Reijmers[2,3], Thomas Hankemeier[2,3], Oliver Fiehn[5], Alan R. Shuldiner[1,#], Rima Kaddurah-Daouk[6,7,#], and Pharmacometabolomics Research Network

[1]Program in Personalized and Genomic Medicine, Division of Endocrinology, Diabetes, and Nutrition, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, United States of America [2]Division Analytical Biosciences, Leiden Academic Centre for Drug Research, Einsteinweg 55, 2333CC Leiden, The Netherlands [3]Netherlands Metabolomics

Hypothesis

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

Aims of Study

1 Aim

Identify metabolites significantly associated with the BP response to HCTZ

2 Aim

Integrate metabolomics with genomics to identify novel pathways and biomarkers associated with HCTZ BP response

II. Materials & Methods

Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR)

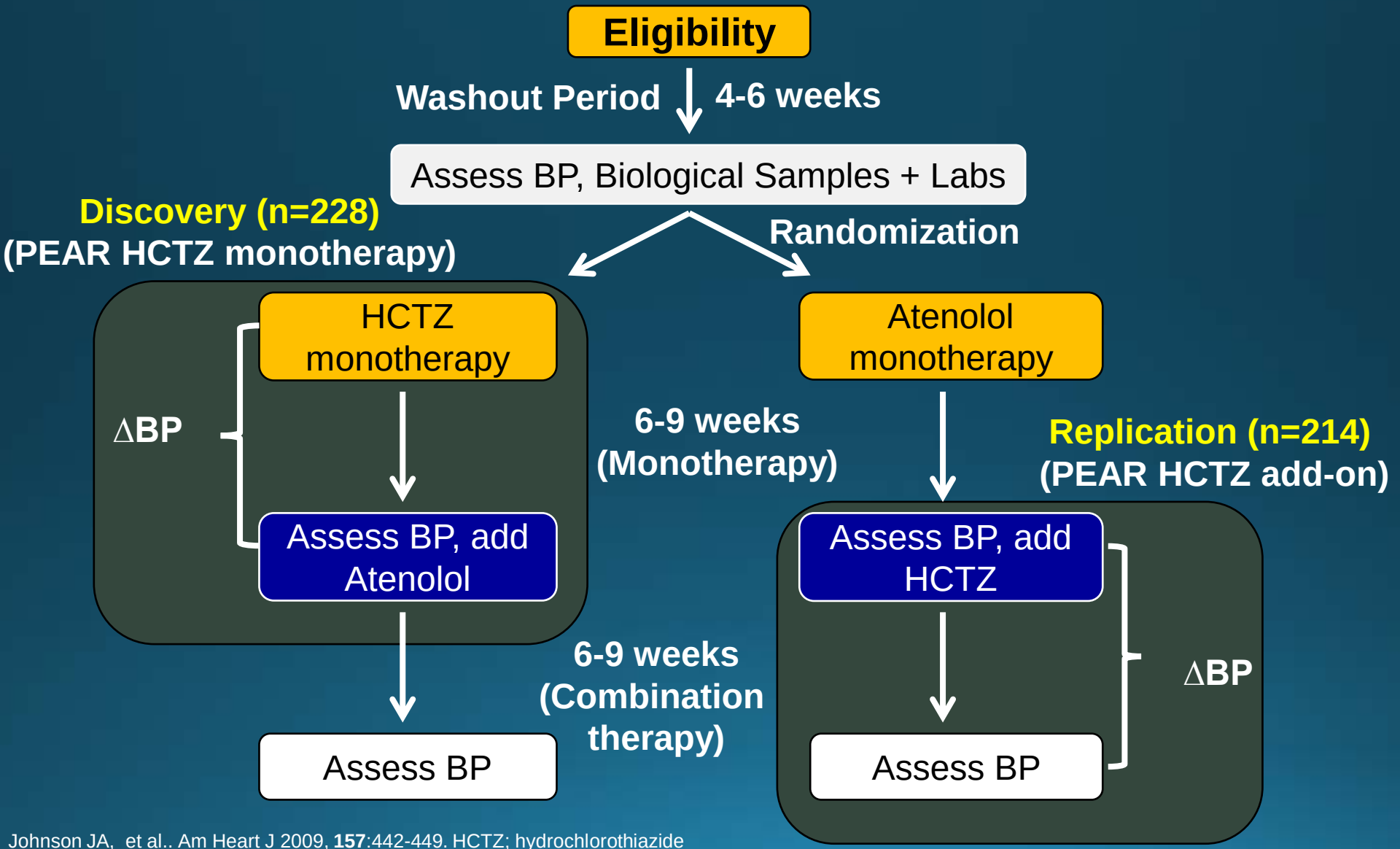
Clinical trials.gov # NCT00246519

- 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)



Johnson JA, et al.. Am Heart J 2009, **157**:442-449. HCTZ; hydrochlorothiazide

Metabolomics Experimental Setup

- 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

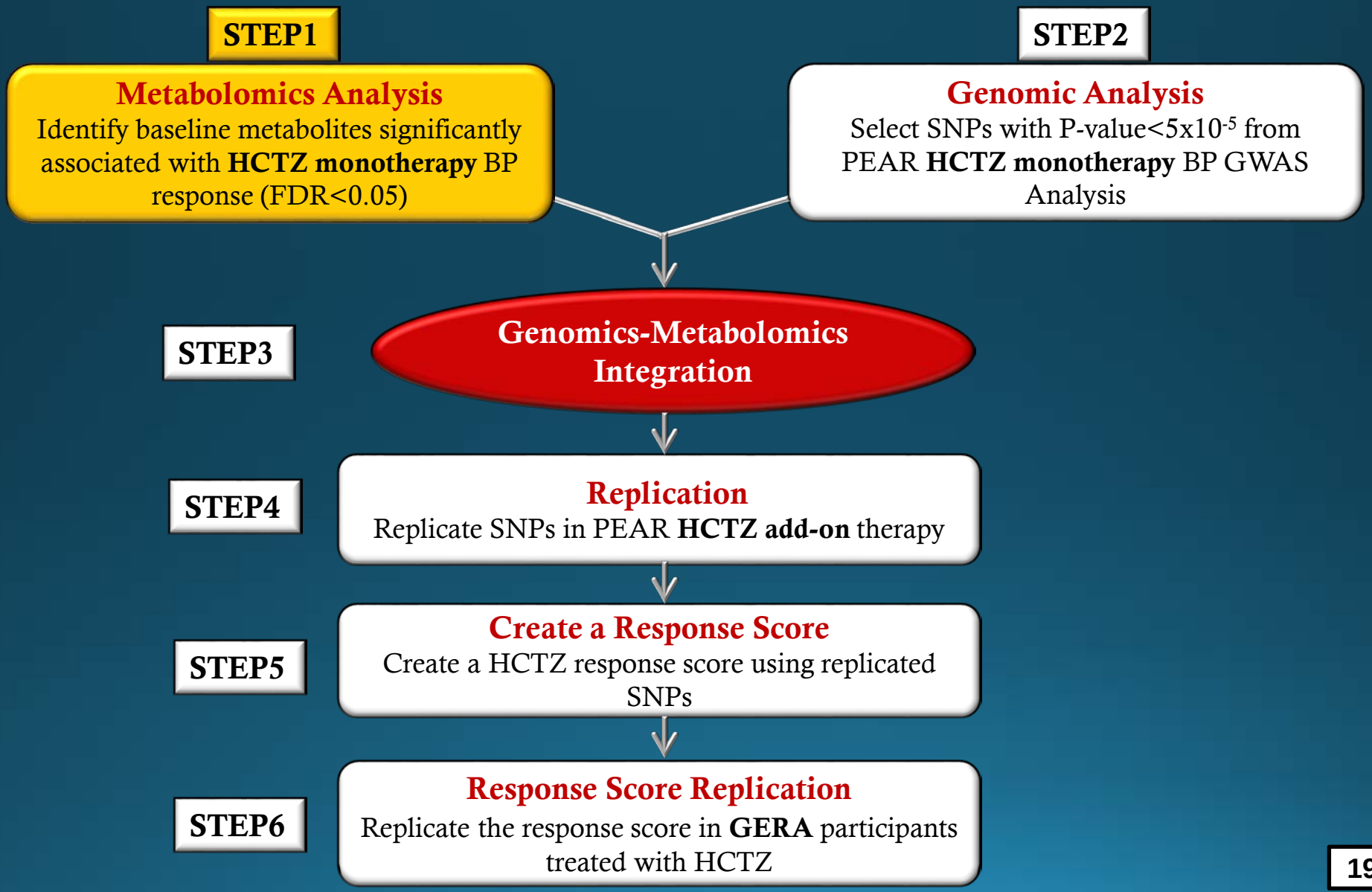
HCTZ; hydrochlorothiazide. GC-TOF MS; Gas Chromatography-Time of Flight Mass Spectroscopy.

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 r^2<0.3

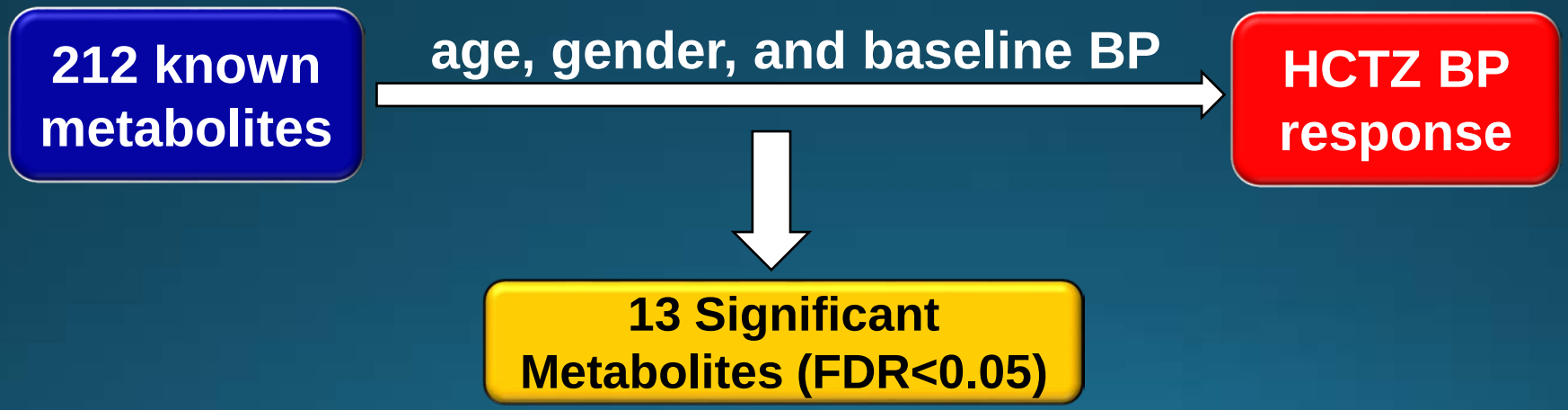
III. Approach & Results

Analyses Framework



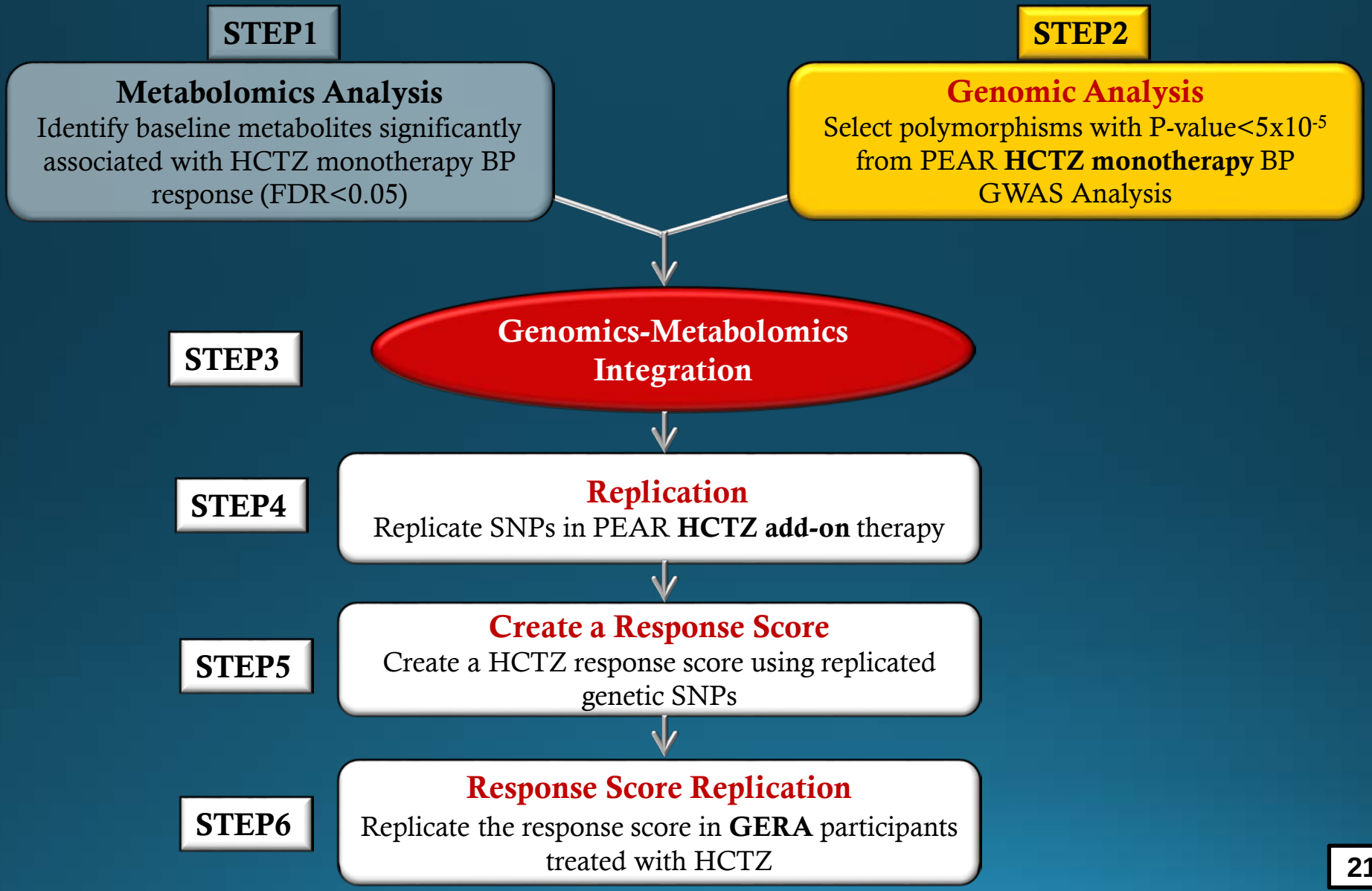
Step 1: Metabolomics Analysis

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

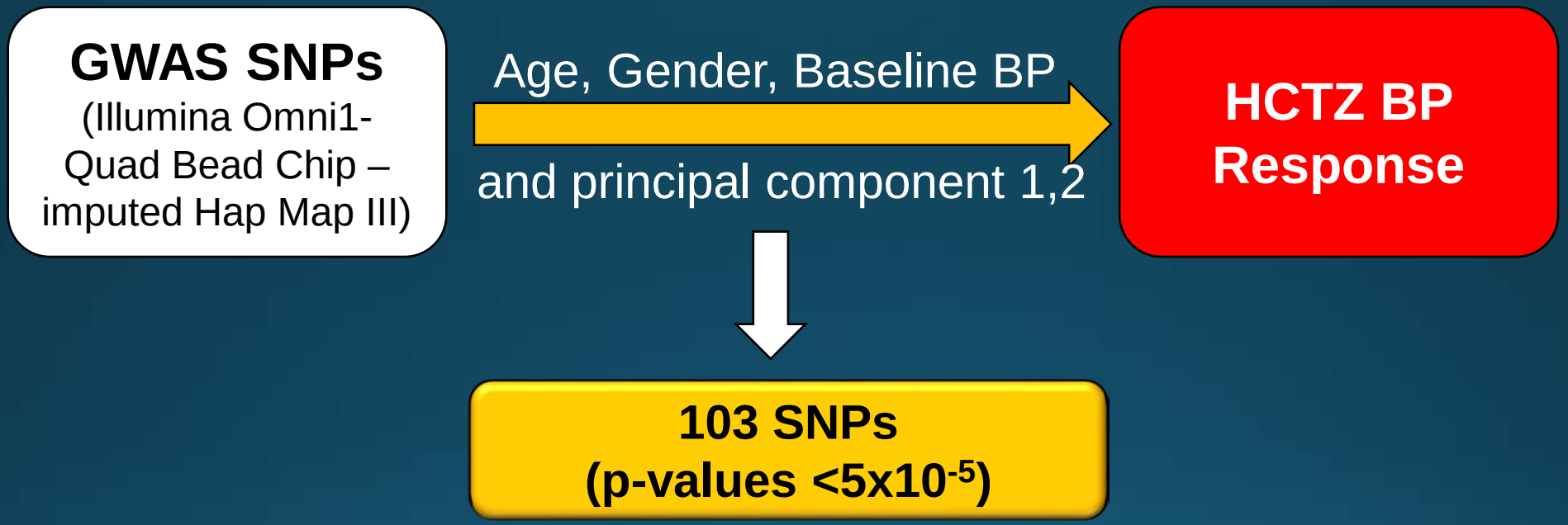


HCTZ; hydrochlorothiazide, FDR; false discovery rate, BP; blood pressure

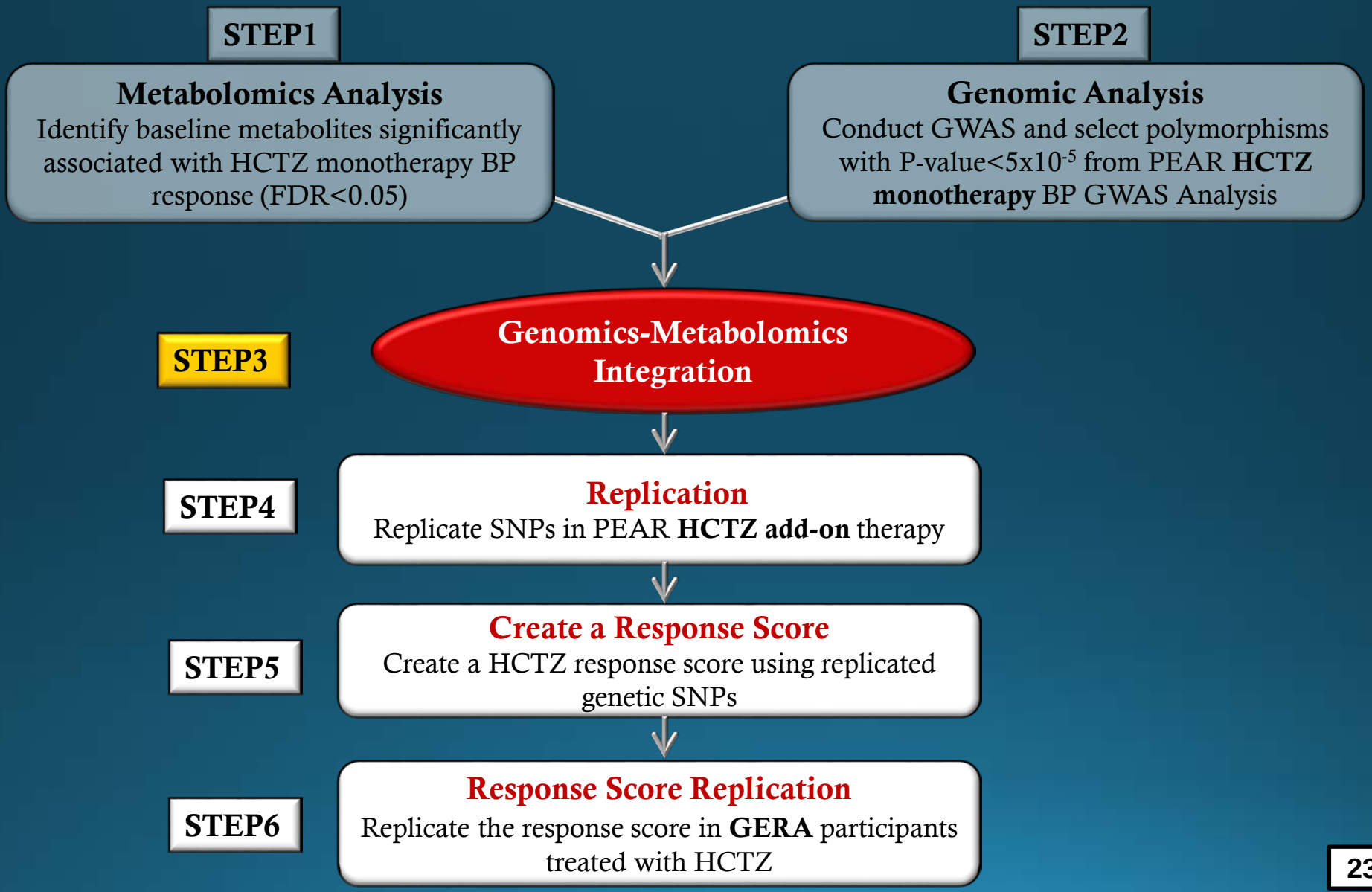
Analyses Framework



Step2: Genomics Analysis



Analyses Framework



Step 3: Metabolomics-Genomics Pathway Integration

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

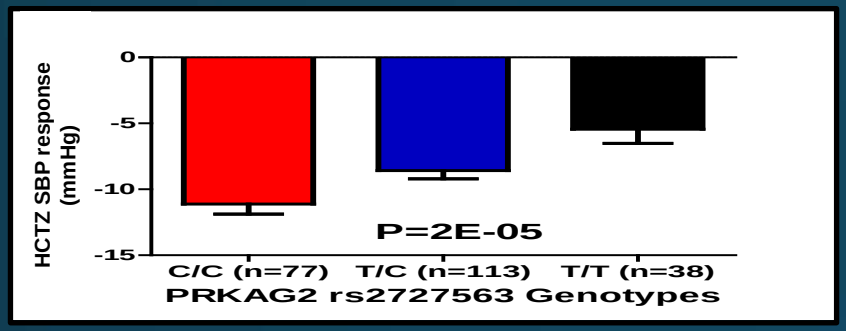
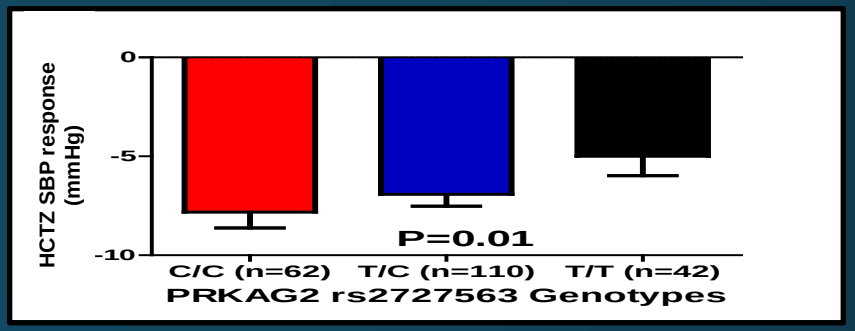
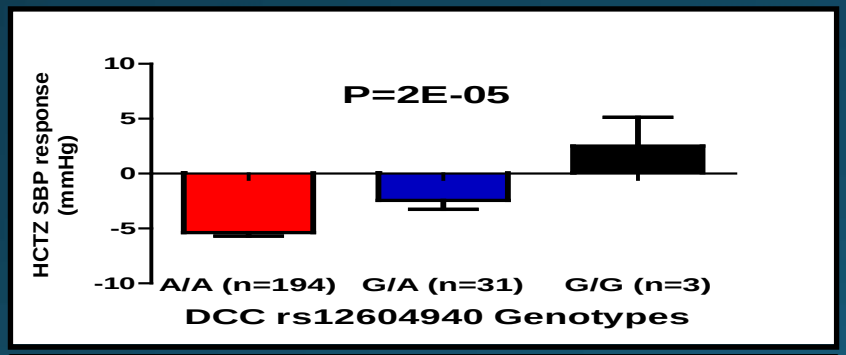
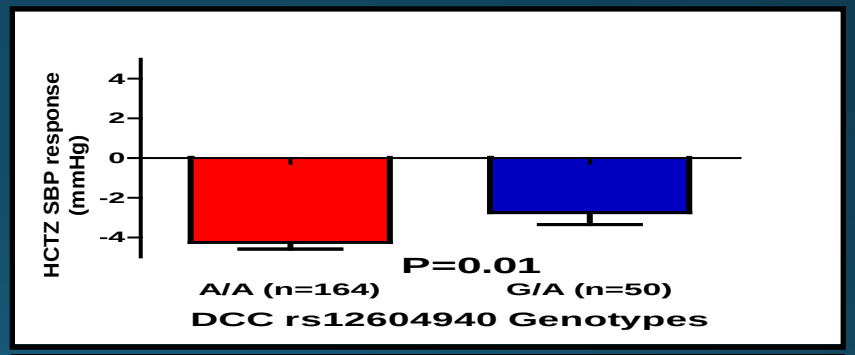
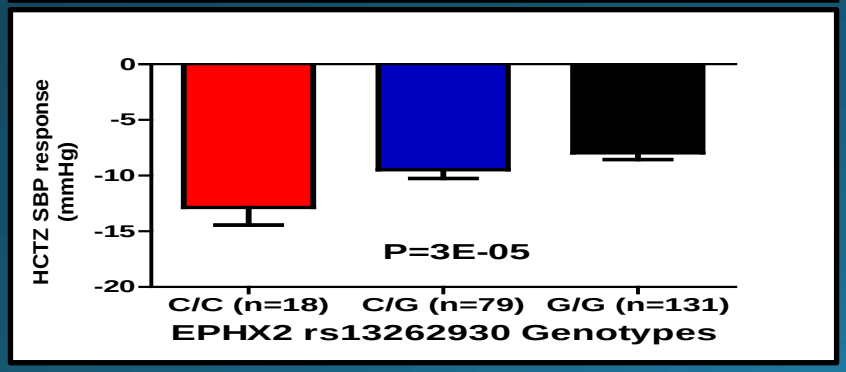
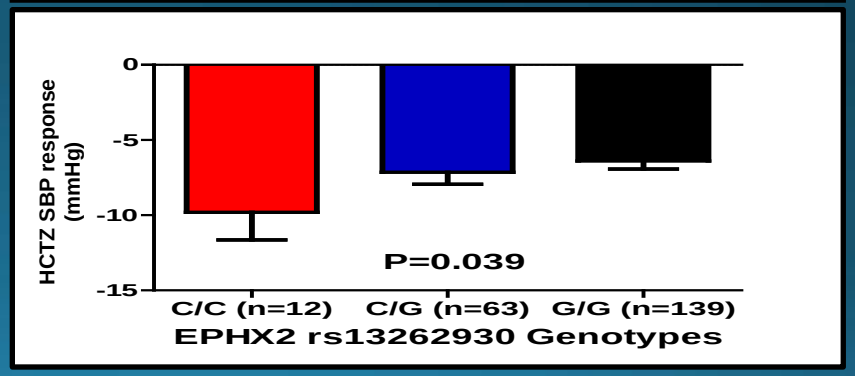
13 significant metabolites (FDR<0.05)

Pathway Analysis

Pathway Name	Genomics	Metabolomics	Pathway P-value
Netrin Signaling Pathway	*PRKAG2* rs2727563 *DCC* rs12604940 *EPHX2* rs13262930	Arachidonic Acid	$1.54x10^{-5}$

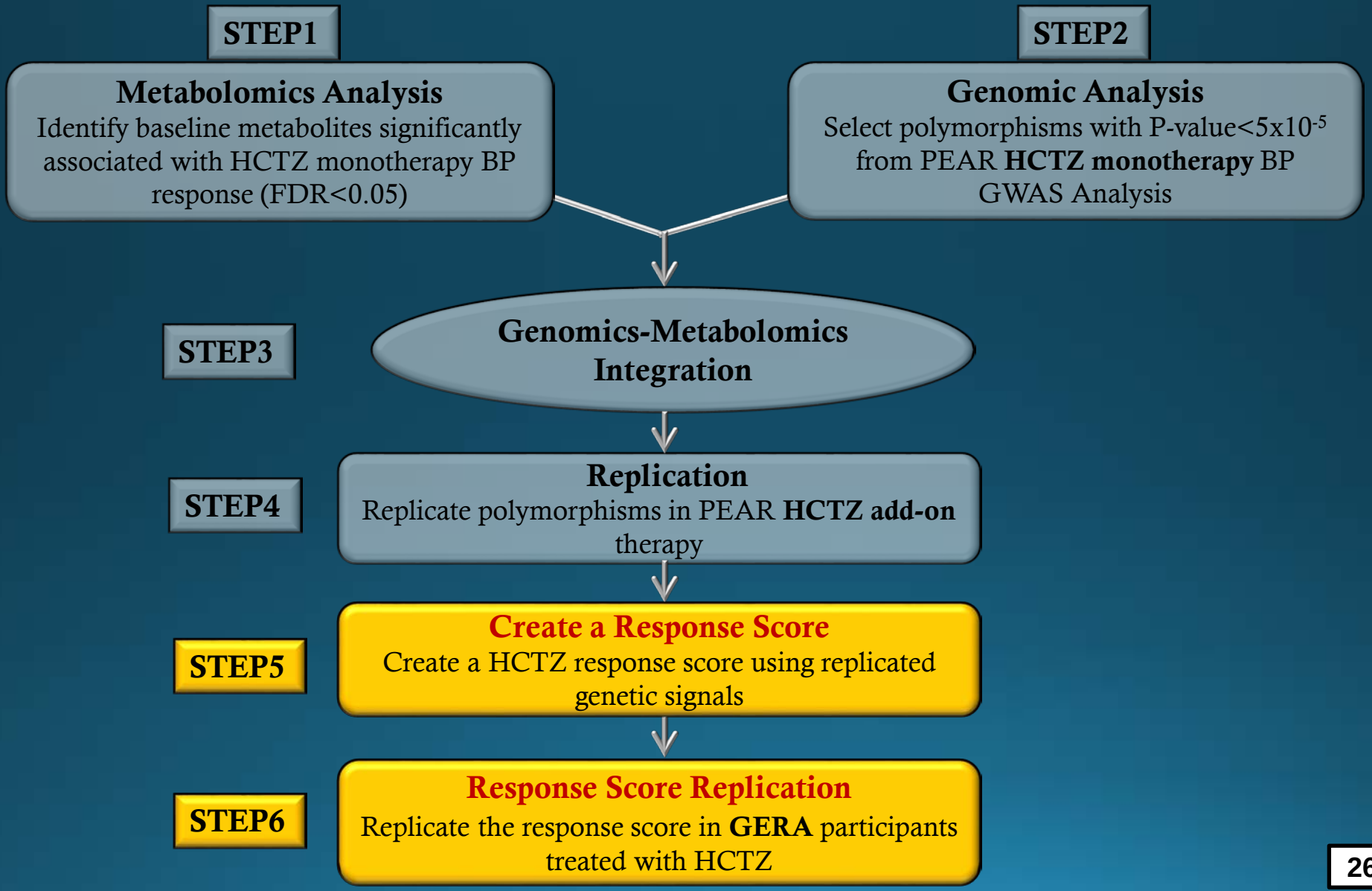
PRKAG2: Protein kinase, AMP-activated, gamma 2 non-catalytic subunit. DCC: Deleted in Colorectal Cancer. EPHX2; Epoxide hydrolase 2

Replication of the 3 identified SNPs with HCTZ BP response

	PEAR HCTZ monotherapy (n=228)	PEAR HCTZ add-on (n=214)
PRKAG2		
DCC		
EPHX2		

PRKAG2: Protein kinase, AMP-activated, gamma 2 non-catalytic subunit. BP; blood pressure.

Analyses Framework



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**

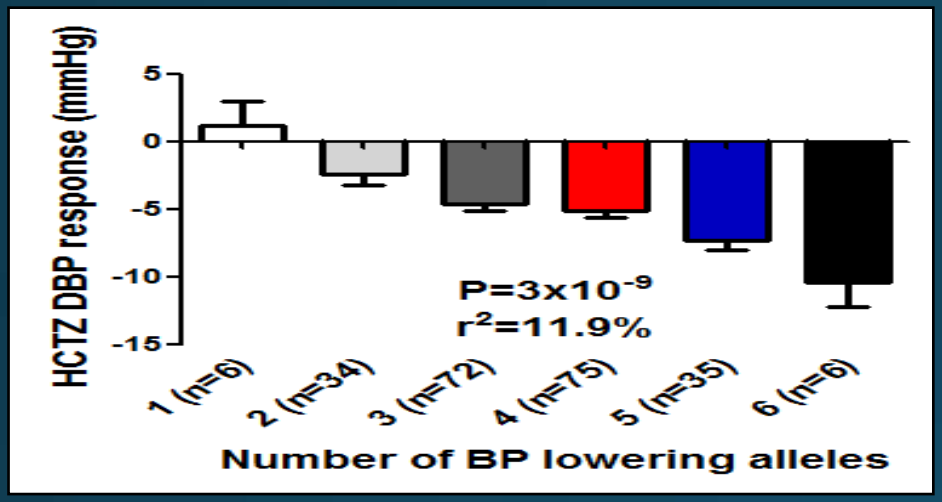
  Homozygous genotype with the worst BP lowering effect = **zero**

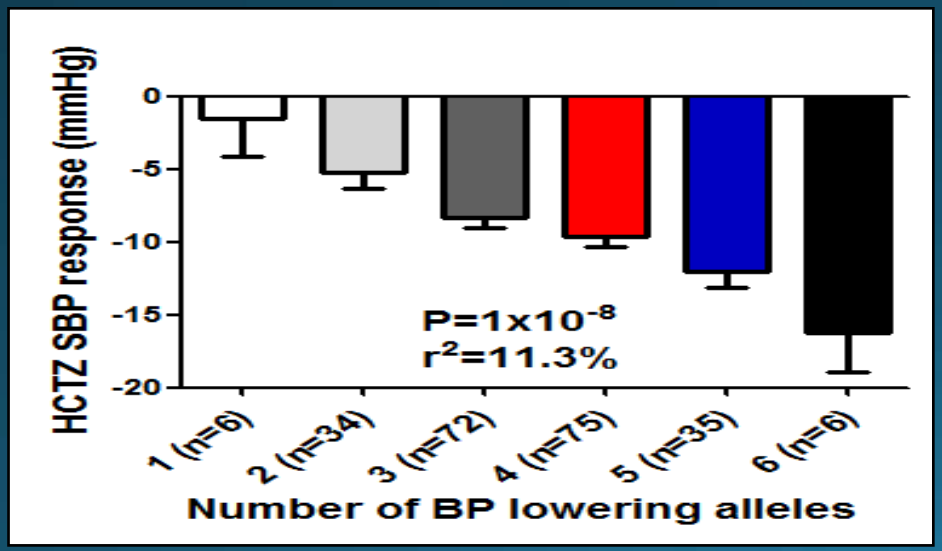
- Alleles with BP lowering effect were then summed up for inclusion in a regression model

- Adjusted for age, gender, baseline BP, and PC1,2

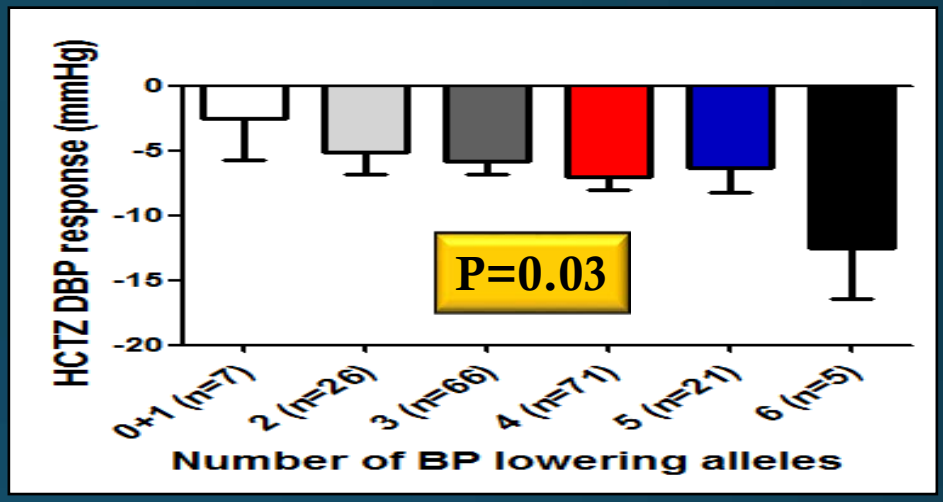
Thiazide Diuretics Genetics Response Score

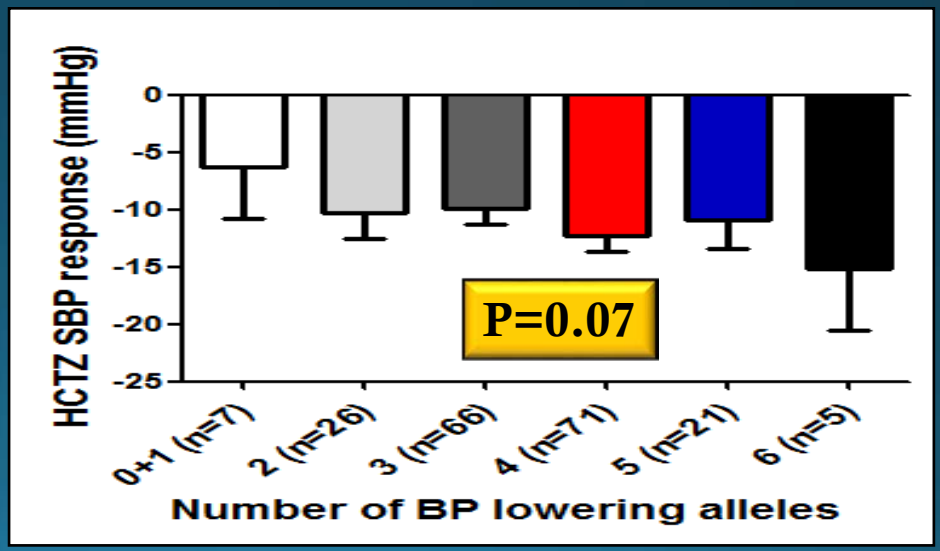
PEAR





GERA





PEAR: Pharmacpgenomic Evaluation of Antihypertensive Responses.
GERA: Genetic Epidemiology of Responses to Antihypertensives

In Summary

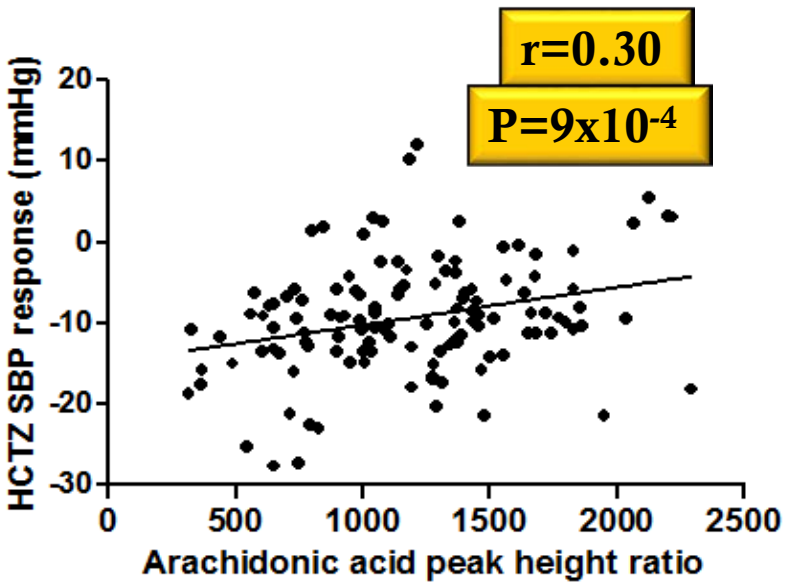
- Identified 13 metabolites significantly associated with HCTZ BP response
- Identified novel signals *PRKAG2* rs2727563, *DCC* rs12604940 and *EPHX2* rs13262930 associated with HCTZ BP response
- Replicated in another independent group of patients treated with HCTZ therapy
- Using the 3 replicated SNPs, 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

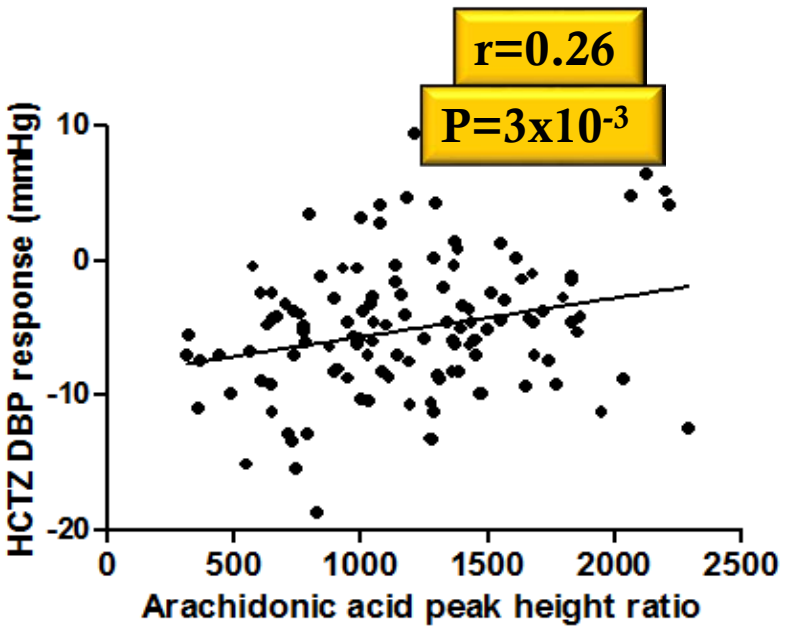
PRKAG2: protein kinase, AMP-activated, gamma 2 non-catalytic subunit. DCC: Deleted in Colorectal Cancer.

Follow-up to the genomics-metabolomics findings

Pilot Metabolomics Study (Research in Progress)

Genomics-Metabolomics Integration Findings





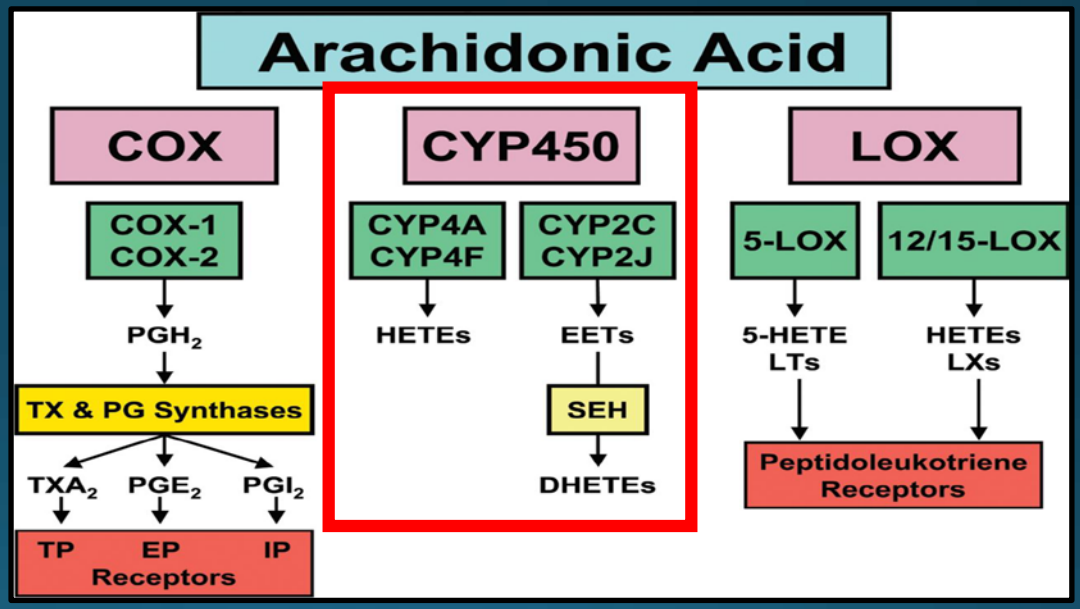
PRKAG2: Protein kinase, AMP-activated, gamma 2 non-catalytic subunit. DCC: Deleted in Colorectal Cancer. EPHX2; Epoxide hydrolase 2

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

Nasjletti A. Arthur C. *Hypertension*. 1998;31:194-200.
Sassard J and Benzoni D. *J Biomed Sci*. 1994;1:201-203.

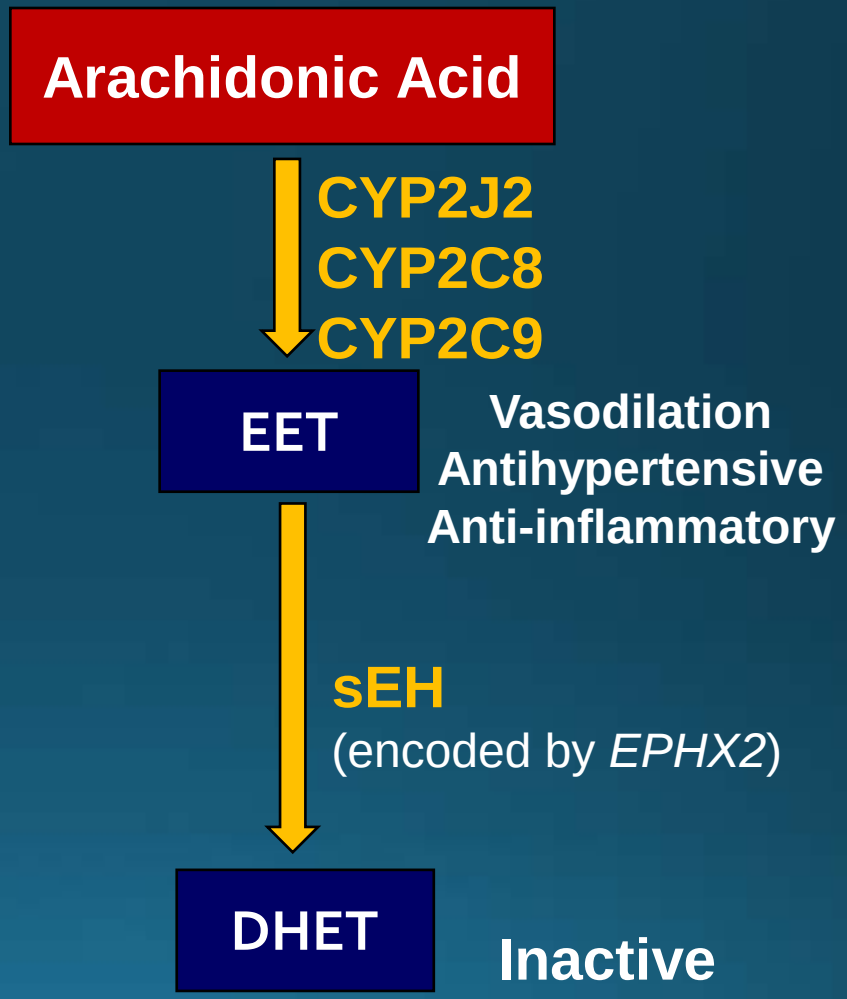
Genomics-Metabolomics Integration Findings

Pathway Name	Genomics	Metabolomics	Pathway P-value
Netrin Signaling Pathway	*PRKAG2* rs2727563 *DCC* rs12604940 *EPHX2* rs13262930	Arachidonic Acid	1.54×10^{-5}

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

EPHX2 (Epoxide Hydrolase 2)

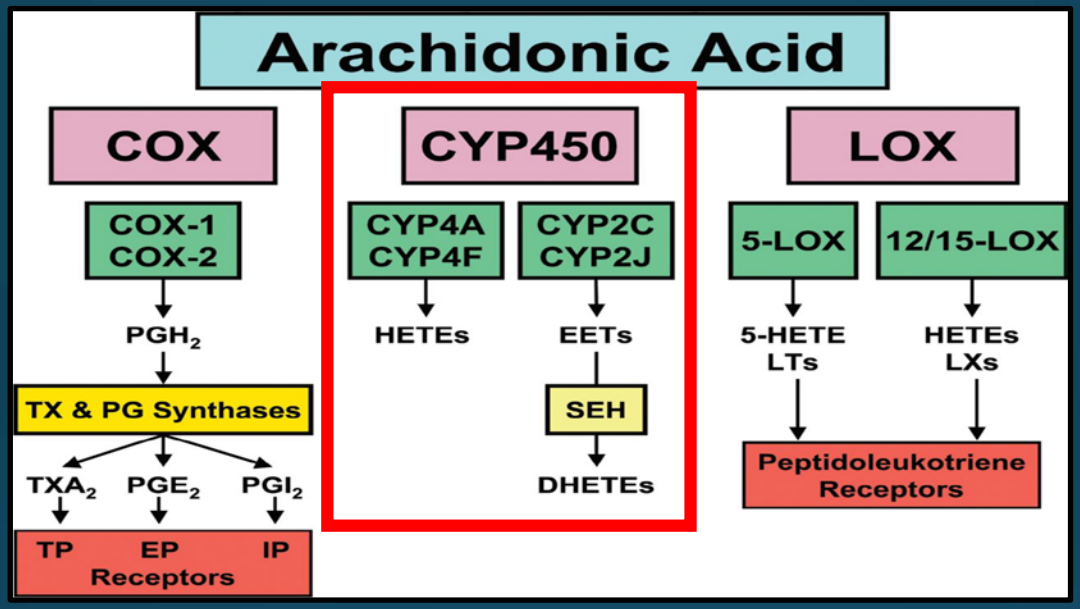
- HCTZ decreases the protein expression of sEH in spontaneously hypertensive rats
- HCTZ might be mediating its antihypertensive BP response via the inhibition of the sEH



EET: **Epoxyeicosatrienoic acid**
DHET: **Dihydroxyeicosatrienoic acid**
sEH**: Soluble Epoxide Hydrolase 2**

Fei Ma, et al. Mol Pharmacol. 2013 Aug; 84(2): 286–295.
Todd R. Harris and Bruce D. Hammock. Gene. 2013 Sep 10; 526(2): 61–74.
Roman RJ. Physiol Rev..2002 Jan;82(1):131-85.

Research in Progress



Pilot Study

"Investigating the Implications of Eicosanoids on the Blood Pressure Response to Thiazide Diuretics"

UF Southeast Center for Integrated Metabolomics
Clinical and Translational Science Institute
UNIVERSITY *of* FLORIDA

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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UNIVERSITY OF MARYLAND
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NIGMS Pharmacometabolomic Research Network

DUKE UNIVERSITY
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UC DAVIS UNIVERSITY
- Oliver Fiehn, PhD

Southeast Center for Integrated Metabolomics

UNIVERSITY OF FLORIDA
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