Announcing
Doctoral Dissertation Defense

Michelle L. Roberts

Candidate for Doctor of Philosophy in Physiology
with a Concentration in Basic and Translational Sciences
Graduate School of Biomedical Sciences
Medical College of Wisconsin

Tuesday, November 30th, 2021 at 11am (CST)

Join Zoom Meeting:
https://mcw-edu.zoom.us/j/91398103851?pwd=WDZOTVI1Wm9PL1huSIIRZdG4vRWxpQT09
Meeting ID: 913 9810 3851, Passcode: fti0GQHK

Dissertation Committee:
Mingyu Liang, MB, PhD (Advisor)
Aron Geurts, PhD
Yong Liu, MS, PhD
Srividya Kidambi, MD, MS
Myriam Fornage, MS, PhD
GRADUATE STUDIES

Graduate Human Physiology (M1 course)
Molecules to Cells (M1 course)
Complement to General Human Physiology
Classical and Molecular Genetics
Mechanisms of Cellular Signaling
Fundamental Practice in Experimental Grant Writing
Physiological Genomics
Critical Reading in Respiratory Physiology
Translational Genomics
Boundaries of Science and Medical Practice
Research Ethics Discussion Series
Ethics and Integrity in Science
Special Problems in Physiology: Statistics
Special Problems in Physiology
Current Topics in Physiology
Reading and Research
Physiology Department Seminar Series (one-hour presentation)
Seminar
Dissertation Title:
Unique Associations of DNA Methylation Regions with 24-hour Blood Pressure Phenotypes in African Americans

Abstract:
Our DNA, environment, lifestyle, and behavior can all interact with one another resulting in dynamic changes in epigenetic features of DNA, such as DNA methylation. This epigenetic mark has been implicated in the development and progression of hypertension, a major risk factor for cardiovascular disease. Blood pressure (BP) is generally measured at a single point in time in everyday patient clinics or population studies, however, this may not be representative of an individual’s 24-hour BP phenotype or diurnal variation. We hypothesized that regions of DNA methylation in blood cells are associated with 24-hour BP phenotypes in African Americans.

From a local and established cohort of African Americans, the DNA of 281 participants was processed by a genome-wide and DNA methylation-dense sequencing workflow, Reduced Representation Bisulfite Sequencing (RRBS). We found several DNA methylation regions (MRs) that were significantly associated with continuously monitored 24-hour average, daytime, or nighttime systolic BP (SBP), diastolic BP (DBP), pulse pressure (PP), and mean arterial pressure (MAP) after adjusting for covariates age, sex, and body mass index (BMI) (False Discovery Rate (FDR) = 0.013 – 0.050). Large portions of 24-h BP variance were explained by these MRs, ranging from 6.5% - 9.4%. However, after FDR adjustment, no MRs were significantly associated with the clinic BPs (FDR > 0.1374) which were calculated from the average of 4 resting measurements (2 per arm) by a sphygmomanometer.

To interrogate specific DNA MRs, we developed a deep and targeted methylation sequencing method termed Bisulfite-Specific PCR ULtrapLEx Targeted Sequencing (BULLET-Seq). We technically assessed this method with use of reference samples for three MRs of interest using a dilution series comprised of six points representing ratios of methylated DNA to unmethylated DNA. BULLET-Seq can accurately quantify 10% changes in the dilution series when the methylation rate ranged from ~40% - 90% (a chr19 MR; $R^2 = 0.95 - 0.97$), can modestly measure these changes when rates range from ~2% - 4% (a chr5 MR; $R^2 = 0.82$), and is questionable when methylation rates are < 2% (a chr13 MR; $R^2 = 0.03 - 0.27$).

In an independent cohort of 117 participants, we validated the chr19 MR in a single BULLET-Seq run. After covariate adjustments, the chr19 region was significantly associated with 24-h BPs (SBP, DBP, and MAP; FDR < 0.05), confirming our findings from the discovery cohort. This MR accounted for up to 1.75% of the variance of the 24-h BP phenotypes.

To conclude, our findings show that several MRs are associated with 24-h BPs in African Americans which may reflect interactions between multiple genetic and environmental factors and can explain a substantial portion of BP variance. The BULLET-Seq workflow is suitable for clinical applications or in population settings for large-scale screenings for the MR markers.
EDUCATION

Medical College of Wisconsin, Milwaukee, WI

- Ph.D., Physiology December 2021
- Concentration, Clinical and Translational Science Institute, Basic and Translational Sciences

University of Wisconsin – Parkside, Kenosha, WI

- M.S., Applied Molecular Biology August 2015
- B.S., Biological Sciences, Psychology Minor December 2008

PREVIOUS EMPLOYMENT AND RESEARCH

- Medical College of Wisconsin, Department of Physiology

  **Laboratory Supervisor/Research Technologist II** December 2015 – July 2017
  **Research Assistant/PhD Student** July 2017 – Present

  Relevant Projects: Investigating the Epigenomics of Hypertension

  - Scope of Projects: American Heart Association (AHA) Strategically Focused Research Network, Hypertension Center
    - This Center revolved around three distinct, but inter-related, areas: Basic Science, Clinical Science, and Population Science. The Basic Science projects investigated the effects of maternal diet on phenotype and DNA methylation of immune cells in the Dahl Salt-Sensitive rat. The Clinical Science projects aimed to discover blood pressure differences and differentially methylated regions of DNA of immune cells among discordant human monozygotic twins as well as in subjects who have undergone a reduction in dietary salt intake for a period of two weeks with tissue collections before and after. The Population Science projects utilized a large human cohort of African American normotensive and hypertensive subjects to examine association with 24-hour blood pressure phenotypes and DNA methylation as well as determine whether the methylation patterns are predictive of hypertension-related cardiovascular disease endpoints.

  Other Projects:

  - Investigating the role of epigenetics in hypertension using CRISPR/dCas9:Effectors for locus-specific, targeted DNA (de-) methylation.
Creation of kidney-specific poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles for the delivery of i) spectral quantum dots (semiconductive particles) to image locations of nanoparticles in rats, ii) plasmids for delivery of CRISPR/dCas9-effectors to alter DNA methylation status of a target, or iii) siRNAs to alter mRNA expression of a target.

PREVIOUS EMPLOYMENT AND RESEARCH (CONT’D)

- City of Racine Health Department Laboratory
  **Graduate Research Assistant I – Intern/ Master’s Student**
  June 2009 – November 2010

  Relevant Project: Assessment of Quantitative Real-Time PCR (QPCR) versus Culture-Based Methods for the Enumeration of Fecal Indicator Bacteria *Escherichia coli* and Enterococci in Freshwater Environments

  **Scope of Project:** Conducted parallel testing on surface water samples from three fixed points along the Root River as well as seven Lake Michigan monitoring stations at North and Zoo Beaches (as both individual samples and composite samples within each beach) using culture-based (18-24 hours for results) and rapid, real-time molecular methods (QPCR; 2-4 hours for results) in order to further the protection of public health by significantly reducing the experimental sample processing time and lessening occurrence of Type I (prohibiting swimming unnecessarily) or Type II (allowing swimming during elevated risk) errors.

  **Project Accomplishments:**
  - The City of Racine Health Department Laboratory posted its first water quality advisory management decision using QPCR in August 2010 as a part of a pilot study.
  - In May 2012, the laboratory received approval for the first time from the U.S. Environmental Protection Agency (USEPA) to use QPCR for the daily assessment of *E. coli* in composite samples and have used it as a primary support tool for the last several summers.
  - The USEPA finalized ongoing studies and was able to publish new Recreational Water Quality Criteria in 2012 (EPA 820-F-12-058) with the information provided by this research project, among others, which was implemented nationwide under the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 (Public Law 106-284).

- University of Wisconsin – Parkside
  **Graduate Research: Review of Environmental Archaeal Roles** 2015
  **Graduate Research: Insect Genetic Mapping** 2009
  **Undergraduate Research: Zoology Field Research** 2008
PEER-REVIEWED PUBLICATIONS

ORCID iD: https://orcid.org/0000-0001-8920-930X

NCBI My Bibliography URL:
https://www.ncbi.nlm.nih.gov/myncbi/1tU0ohz0rt5Uuh/bibliography/public/


- Kinzelman JL, **Leittl ML**. Validity of composite sampling for enumerating E. coli from recreational waters by molecular methods (qPCR). In: Kay D, Fricker C, editors. The


PRESENTATIONS


- “Unique Associations of DNA Methylation with 24-hour Blood Pressure Phenotypes in African Americans.” Awarded for being a Top 10 Finalist for the Biohealth Communications Competition, 2021 Wisconsin Biohealth Summit, Madison, WI. 2021.


- Pan, X and Roberts, ML. “Stability of whole-blood DNA methylation profiles under different storage durations and conditions.” Midwest Chromatin and Epigenetics Meeting at Purdue University, West Lafayette, Indiana. 2018. Poster Presentation.


LEADERSHIP ACTIVITIES

- **Precision Cardiovascular Medicine course moderator.** “Use of Stem Cells for Disease Modeling and Therapies in Cardiovascular Diseases.” Fall 2021.
• **M1 Physiology Case-Based Discussion Teaching Assistant.** Medical College of Wisconsin. Fall 2021.

• **Mentor to medical & graduate students.** Medical College of Wisconsin. 2015 – present.


• **Molecular Biology and Bioinformatics Club.** University of Wisconsin – Parkside, member from Fall 2014 (club start-up) – August 2015.

**RESEARCH COMPETENCIES**

• Assisted in the review of multiple manuscripts for Hypertension under guidance of Mingyu Liang, MB, PhD

• Assisted in the review of a project proposal for the Czech Science Foundation under guidance of Sherry-Ann Brown, MD, PhD, FACC, FAHA.

• Ability to utilize multiple internet-based databases and bioinformatics tools (i.e. Qiagen Ingenuity Pathway Analysis, UCSC Genome Browser, NCBI PubMed, GenBank, and BLAST, ORF Finder, EMBL-EBI EMBOSS tools, R language, etc.

• Successfully analyzed a publicly available microarray dataset by coding in R after a learning bootcamp in the Physiological Genomics course.

• CITI-trained with training and competencies in Clinical and Translational Science Institute (CTSI) tools, including: Clinical Research Data Warehouse (CRDW) i2b2 Cohort Discovery Tool, TriNetX, Honest Broker data extraction tool, Epic, REDCap Secure Data Collection and Storage, MCW Tissue Bank biospecimens, etc.


• Grant writing capabilities
  
o  Example of grant proposal entitled, “Investigating the role of epigenetics in hypertension using CRISPR/dCas9:effectors for targeted DNA (de-) methylation” for the Pre-Doctoral Fellowship of the American Heart Association (AHA), unfunded; resubmitted the following year/cycle, unfunded.

• Attended AI and machine learning workshops.

• CRISPR Workshop Certification. Sponsored by Medical College of Wisconsin Dean’s office, Clinical and Translational Science Institute, Cancer Center, Cardiovascular Center. 2016.
PROFESSIONAL MEMBERSHIPS

• 2021-present: American Physiological Society (APS)
• 2019-present: American Heart Association (AHA)
• 2019-present: Medicine and Artificial Intelligence in Research (MARs)
• 2018-present: Genome Writer’s Guild (GWG)
• 2015-2019: American Heart Association Strategically Focused Hypertension Research Center at MCW
• 2015-present: MCW Center of Systems Molecular Medicine (CoSMM)

SCHOLARSHIPS AND HONORS

• 2017-2021: Full Scholarship, Medical College of Wisconsin, Physiology PhD program
• 2019-2020: Excellence in Science Program, Medical College of Wisconsin, AAAS/Science Membership
• 2019: Advancement to PhD candidacy with Highest Rating (Excellence) in Qualifying Examination