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Short communication

# Gut microbiota and serum metabolite differences in African Americans and White Americans with high blood pressure☆

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ABSTRACT

*Background:* Black Americans have greater rates, severity and resistance to treatment of hypertension than White Americans. The gut microbiota and its metabolites may contribute to this. This concept was tested in a pilot study.
*Methods:* Subjects with high (HBP, >140/80 mm Hg) and normal (NBP, <120/80 mm Hg) blood pressure (BP) provided stool and blood samples for whole genome sequencing (WGS) of gut microbiota and global untargeted metabolomics of serum. Patients were either black (B) with NBP (n = 10 for WGS, 5 for metabolomics) and HBP (n = 10 and 9, BHBP) or white (W) with NBP (n = 20 and 13, WNBP) and HBP (n = 12 and 8, WHBP).
*Results:* All four subject groups had distinct gut microbiota taxonomy by partial least squares discriminant analysis (PLS-DA). More importantly, linear discriminant analysis effect size showed marked differences in function of the microbiota of BHBP and WHBP (PLS-DA) with LDA scores <1. This included pathways for synthesis and interconversion of amino acids and inflammatory antigens. Similarly, metabolites differed (PLS-DA) with BHBP having significantly higher sulfacetaldehyde, quinolinic acid, 5-aminolevulinic acid, leucine and phenylalanine and lower 4-oxoproline and L-anserine.
*Discussion:* Combination analyses of functional gut metabolic pathways and metabolomics data in this small pilot study suggest that BHBP may have greater oxidative stress markers in plasma, greater inflammatory potential of the gut microbiome and altered metabolites with gut microbial functions implying insulin resistance. A fuller understanding of these potential differences could lead to race-based treatments for hypertension.


## 1. Introduction

Black and White Americans have different incidence, severity and effective treatment of high blood pressure (HBP) [1]. Recent evidence highlighting gut microbiota alterations in HBP [2] suggests potential racial differences in gut microbiomes may contribute to HBP disparities. We performed a pilot investigation of this relationship in Black (African descent) and White (European descent) Americans.

## 2. Methods

After IRB protocol approval and written informed consent, subjects provided fecal samples for whole genome sequencing (WGS) analysis of functional differences in gut microbiota, and blood for analysis of differential metabolites and gut ischemia markers. Black and white subjects were grouped by blood pressure (high blood pressure, HBP ≥140/80; normal blood pressure, NBP ≤120/80) at sampling. Systolic blood pressures between BHBP (151 ± 4 mm Hg) and WHBP (147 ± 5 mm Hg) were comparable: blood glucose was in normal range for all but 1 BHBP and 2 WHBP. Sample sizes were different between analyses as not every subject provided a blood sample. No differences by sex or age occurred but blacks had higher ($p < 0.05$) body mass indexes.

### 2.1. WGS and analysis

Stool was preserved (Quick-DNA™ Fecal/Soil Microbe Miniprep Kit; Zymo Research, Irvine, CA) and stored (−80 °C ≤ 12 months). DNA was extracted, sequenced and analyzed in one batch (Wright Labs, LLC; Huntingdon, PA). DNA (1 ng) underwent Nextera XT (Illumina, CA) tagmentation and library preparation with dual index barcoding. After quality assessment (high sensitivity bioanalyzer chip; Agilent, CA), equimolar library amounts were pooled and purified (QIAquick gel purification kit; Qiagen, CA) and sequenced (Illumina HiSeq4000) with a 2 × 150 index run. Raw read quality was assessed with FastQC, trimmed with Trimmomatic, using sliding window quality filtration at a 4-base average Q score of ≤28, and reads ≤120 basepairs discarded. Filtered reads were paired, concatenated and human DNA sequences removed (Kneaddata pipeline); unpaired reads were discarded.

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[2] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. This author declares no conflicts of interest.

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MetaPhlan quantified the samples' bacterial taxonomic profiles, outputs were merged into a tsv table, then converted to a biom file for analyses. For functional gene profiles, filtered data were annotated (Uniref90 database within Humann2), regrouped (as KEGG orthology, KO) terms and counts per million normalized within Humann2. Partial least squares discriminant analysis (PLS-DA) of functional counts (METAGENassist) with interquartile range filtering. Variables with >85% zeros were removed and abundances normalized by autoscaling within METAGENassist. KOs were summarized in functional pathways using the KEGG database, relative abundances multiplied by 1 million and formatted as described [3]. LEfSe comparisons were made considering metacyc pathway data with "Race" as the main categorical variable ("Class"). Alpha levels of 0.05 were used for both Kruskal-Wallis and pairwise Wilcoxon tests. Linear Discriminant Analysis (LDA) scores >1 were displayed.

### 2.2. Metabolomics

Plasma samples underwent cellular extraction (Southeast Center for Integrated Metabolomics, SECIM). Global metabolomics profiling was performed (Thermo Q-Exactive Orbitrap mass spectrometer with Dionex UHPLC and autosampler). Samples were analyzed in positive and negative heated electrospray ionization with mass resolution of 35,000 at $m/z$ 200 as separate injections and separated on an ACE 18-pfp 100 × 2.1 mm, 2 μm column with mobile phase A, 0.1% formic acid in water and mobile phase B, acetonitrile. This polar embedded stationary phase provides comprehensive coverage, but misses very polar species. Flow rate was 350 μL/min with 25 °C column temperature. 4 μL was injected for negative and 2 μL for positive ions. MZmine (freeware) identified and aligned features, deisotoped, and performed gap filling of features missed in first alignment. Adducts and complexes were removed. Data were searched against SECIM internal retention time metabolite library. Values absent in 80% of data were removed. Missing values were imputed using k-nearest neighbor, quantile normalized, log2 transformed and autoscaled. Analyses were performed on combined positive and negative ions' data using Metaboanalyst 3.0, an open source R-based program for metabolomics [4]. PLS-DA determined differential metabolites between groups and Student's $t$-tests for differences between BHBP and WHBP, with $p < 0.05$ considered significant.

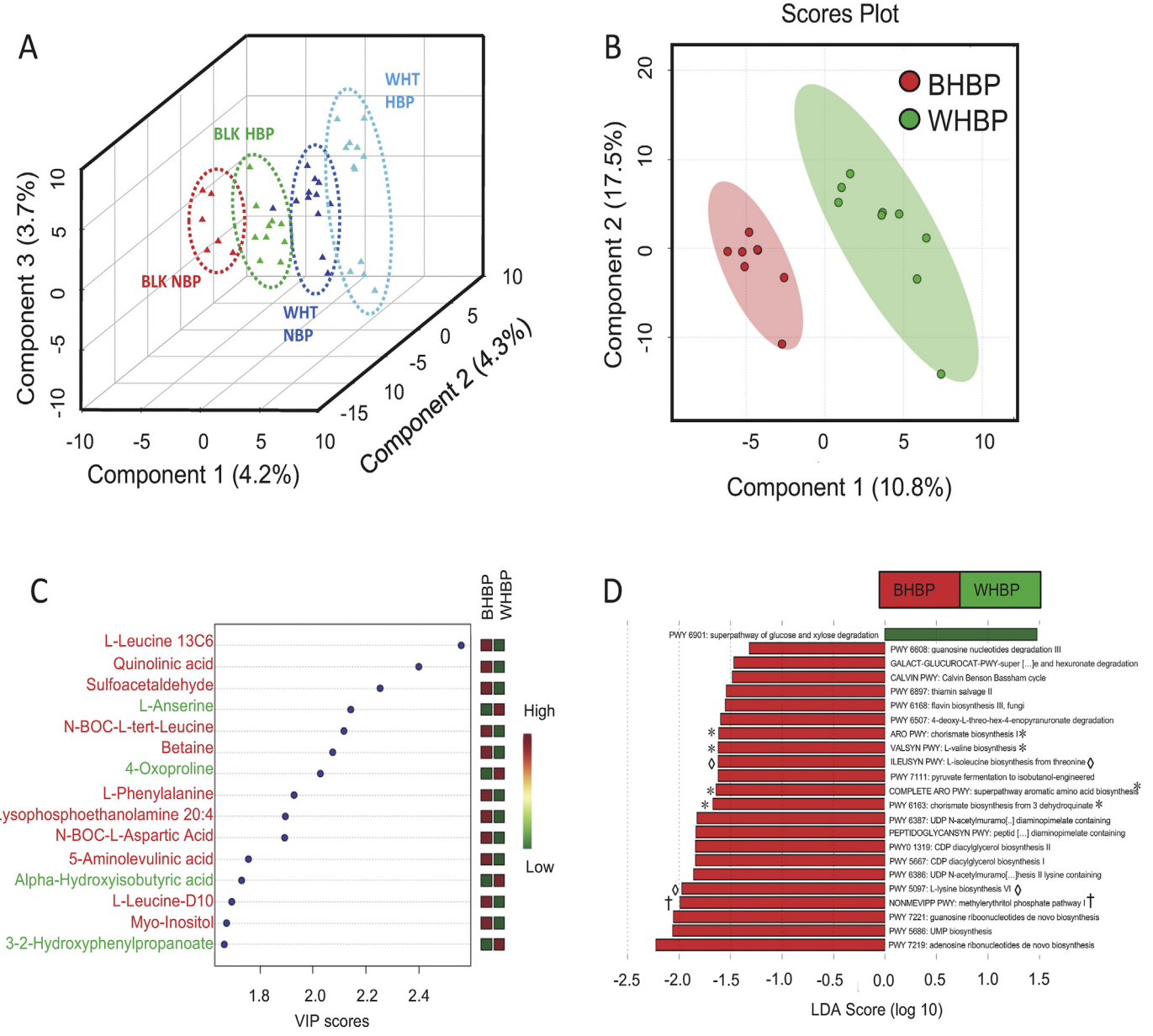


**Fig. 1. A**. Partial least squares discriminant analysis (PLS-DA) of shotgun metagenomics determined bacterial populations in gut microbiota of blacks and whites with and without hypertension in this small cohort. Colored triangles represent subjects; black normal blood pressure (BLK NBP; red, n = 6), black high blood pressure (BLK HBP; green, n = 10), white normal blood pressure (WHT NBP; blue n = 12), white high blood pressure (WHT HBP; aqua n = 12). Dotted ovals are drawn as visual aids. **B**. PLS-DA scores plot representing known metabolites altered between black high blood pressure (BHBP; red, n = 8) and white high blood pressure (WHBP; green, n = 9) subjects. Pink and green ovals are 95% confidence interval ranges for BHBP and WHBP subjects, respectively. **C**. PLS-DA variable importance plot. A higher value indicates the importance of that metabolite in separating the groups shown in scores plot B. Metabolites named in red font are important variables in the African American (BHBP) group, and in green in the White American group (WHBP) **D**. Linear Discriminant Analysis (LDA) scores plot (log 10) of functional gene pathways overrepresented in gut microbiota of Black Americans with high blood pressure (BHBP; red bars) or White Americans with high blood pressure (WHBP; green bars) obtained from microbial whole genome sequence analysis of stool. * denotes pathways of de novo synthesis of amino acids, ◊ denotes pathways of interconversion of amino acids, † denotes microbial pathway for synthesis of the inflammatory antigens, isoprenoids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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