Depressive hypertension: A proposed human endotype of brain/gut microbiome dysbiosis

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Background Hypertension (HTN) is frequently linked with depression (DEP) in adults with cardiovascular disease (CVD), yet the underlying mechanism and successful management remain elusive. We approached this knowledge gap through the lens that humans are eukaryote-prokaryote "meta-organisms," such that cardiovascular disease dysregulation is a mosaic disorder involving dysbiosis of the gut. We hypothesized that patients diagnosed with hypertension plus depression harbor a unique gut microbial ecology with attending functional genomics engaged with their hosts' gut/brain axis physiology.

Methods Stool microbiome DNA was analyzed by whole metagenome shotgun sequencing in 54 subjects parsed into cohorts diagnosed with HTN only (N = 18), DEP only (N = 7), DEP plus HTN (DEP-HTN) (N = 8), or reference subjects with neither HTN nor DEP (N = 21). A novel battery of machine-learning multivariate analyses of de-noised data yielded effect sizes and permutational covariance-based dissimilarities that significantly differentiated the cohorts (false discovery rate (FDR)-adjusted $P \leq .05$); data clustering within 95% confidence interval).

Results Metagenomic significant differences extricated the four cohorts. Data of the cohort exhibiting DEP-HTN were germane to the interplay of central control of blood pressure concomitant with the neuropathology of depressive disorders. DEP-HTN gut bacterial community ecology was defined by co-occurrence of *Eubacterium siraeum, Alistipes obesi, Holdemania filiformis*, and *Lachnospiraceae bacterium 1.1.57FAA* with *Streptococcus salivariu*. The corresponding microbial functional genomics of DEP-HTN engaged pathways degrading GABA and beneficial short chain fatty acids (SCFA), and are associated with enhanced sodium absorption and inflammasome induction.

Conclusions These data suggest a new putative endotype of hypertension, which we denote "depressive-hypertension" (DEP-HTN), for which we posit a model that is distinctive from either HTN alone or DEP alone. An "endotype" is a subtype of a heterogeneous pathophysiological mechanism. The DEP-HTN model incorporates a unique signature of microbial taxa and functional genomics with crosstalk that putatively intertwines host pathophysiology involving the gastrointestinal tract with disruptions in central control of blood pressure and mood. The DEP-HTN endotype model engages cardiology with gastroenterology and psychiatry, providing a proof-of-concept foundation to explore future treatments, diagnosis, and prevention of HTN-coupled mood disorders. (Am Heart J 2021;239:27–37.)

Background

Hypertension (HTN) is the most prevalent modifiable risk factor for cardiovascular disease (CVD) impacting almost half of adult Americans and is a major problem world-wide. Depression (DEP) is the leading medical cause of disability-adjusted-life-years. High blood pressure (BP) coupled with DEP represents a major comorbid dyad (DEP-HTN), affecting ~25% of adults with CVD worldwide.[1] Despite advances in management of these disorders as independent entities, successful control of HTN with comorbid DEP remains elusive. A fun-

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Abbreviations: ACE, angiotensin converting enzyme; BP, blood pressure; CVD, cardiovascular disease; dapE, N-succinyl-L,L-diaminopimelate desuccinylase, EC:3.5.1.18; DEP, patient cohort endotype with depression; DEP-HTN, patient cohort endotype with depressive-hypertension; ENS, enteric nervous system; fabZ, 3-hydroxyacyl- [acyl-carrier-protein] dehydratase, EC:4.2.1.59; FDR, False Discovery Rate-adjusted P value; GABA, γ−aminobutyric acid; HPA, hypothalamic-pituitary-adrenal axis; HTN, patient cohort endotype with hypertension; LPS, lipopolysaccharide; lpxC, UDP-3-O-acyl-N-acetylglucosamine deacetylase, EC:3.5.1.108; PCA, principal component analysis; PCoA, principal coordinates analysis; PVN, paraventricular nucleus of the hypothalamus; RAS, renin-angiotensin-system; RAAS, renin-angiotensin-aldosterone-system; REF, reference control cohort (no hypertension and no depression); SCFA, short chain fatty acid; SNS, sympathetic nervous system; WMGS, whole metagenome shotgun sequencing.

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damental understanding of the pathophysiology of this dyad is essential to develop innovative strategies for management.

We postulate that the gut microbiome is a central player in this dyad. The rationale is based upon emerging evidence from our group[2-9] and others (references articulated in results and discussion) that includes the following: (1) gut microbial dysbiosis, pathology, and compromised barrier permeability with blood brain barrier (BBB) dysfunction are all associated with HTN or with DEP in preclinical models as well as in patients, (2) impaired gut-brain communication associated with neuroinflammation is crucial in the establishment of HTN, (3) both gut dysbiosis and neuroinflammation-linked altered gut-brain communication are implicated in many mental and neurobehavioral disorders, and (4) brain circuits intertwine emotion and mood centers with central BP control. Collectively these observations led us to propose the existence of a unique DEP-HTN gut microbiome community, with its attending functional genomics as pathways responsible for coalescing BP- and DEP-relevant pathophysiologic mechanisms in patients manifesting both DEP and HTN.

Methods

Subjects

Stool samples were obtained from 54 volunteer subjects without inflammatory bowel disorders and without antibiotic use for at least 30 days prior to stool donation. Four cohorts were grouped as shown with clinical characteristics in Table I: hypertensive systolic BP $\geq$ 140 mm Hg without a depressive disorder ("HTN"); normotensive systolic BP $\leq$ 120 mm Hg and with an outpatient chart meeting DSM-5[10] criteria for an ongoing depressive disorder ranging from moderate DEP to major depressive disorder excluding psychosis ("DEP"); hypertensive (systolic BP $\geq$ 140 mm Hg) and also with an outpatient chart meeting DSM-5 criteria for an ongoing depressive disorder ranging from moderate DEP to major depressive disorder excluding psychosis ("DEP-HTN"); and reference patients exhibiting systolic BP $\leq$ 120 mm Hg without a depressive disorder ("REF"). Use of antidepressants was not a non-exclusion criterion in any cohort. The University of Florida Institutional Review Board approved the protocols and all subjects provided written informed consent. This study complies with the Declaration of Helsinki. This work was supported by National Institutes of Health grants HL132448, HL33610 (MKR, CJP); University of Florida (UF) Clinical and Translational Science Institute grant (BRS, CJP) from National Institutes of Health award UL1TR001427; Gatorade Trust through the UF Department of Medicine (CJP); Patient-Centered Outcomes Research Institute One Florida Clinical Research Consortium 1501-26692 (CJP); and UF Department of Physiology & Functional Genomics (BRS, MKR).

Metagenome sequencing and profiling

Stool samples were collected with OMNIgene GUT fecal collection kits (DNAGenotek, Ottawa, Ontario, Canada) and stored at -80°C until DNA extraction. Gut microbiome DNA whole metagenome shotgun sequencing (WMGS), Metaphlan, Uniref90 taxa, and KEGG ortholog assignments were performed as described by us elsewhere[4].

Machine-learning analyses and multivariate statistics

A machine-learning pipeline incorporating R package PIME coupled with differential abundance analysis R package ALDEx2 was developed by us[9,11] to derive denoised prevalence-based datasets that were subjected to a battery of multivariate analyses. For both taxa and functional genomic pathways, the datasets were based on selecting the most prevalent differentially abundant data for metadata comparisons by removing the within-group variations and capturing only the significant differences having the highest prevalence by a machine-learning algorithm.[9,11] Briefly, based on 100 bootstraps of 5% prevalence bins over the range 5% to 95%, the pipeline yielded Mann-Whitney plots with data assigned as either significantly differentially abundant at $q < 0.1$, non-differentially abundant, or noisy discardable nonabundant.[9] Pipeline results were confirmed by Dirichlet Monte-Carlo simulation of random forest classifications running 100 bootstrap aggregations on each prevalence interval, with lack of bias verified by random data scrambling. Robust effect sizes were derived based on 71% the size of Cohen's d on a normal distribution. We have previously demonstrated[9] that this pipeline identified human DEP cohort as significantly different from REF, based on gut microbiomes.

Biomarker discovery characterization was examined by multivariate covariance clustering relationships among cohort metadata employing sparse partial least squares discriminant analysis supervised principal component analysis (PCA) with least absolute shrinkage and selection operator (LASSO) variable selection that classified the group clusters with 95% confidence interval (CI) ellipses, utilizing the R package mixOmics splsda. Pearson correlation heatmaps were generated based on relative Rho magnitudes. Network analyses were examined using positive taxa nodes placed with Bray-Curtis dissimilarity ordination distances connected by PCoA edge placement (false discovery rate-adjusted $P \leq .05$), with similarity cutoff at 0.25. Forest plots comparing metadata were generated using odds ratios calculated by logistic regressions with 95% CI. Software analyses included R packages run locally with Mac OSx, or online with Calypso[12] or Galaxy.[13]

Table I. Patient characteristics

Characteristic	Cohort code	Systolic BP(mm Hg)	Depression*	N	Age	Gender	BMI	Weight(Kg)	Diabetes	CKD	PAD	Stroke/TIA	AF	HF
Reference (not hypertensive and no depression)	*REF*	≤ 120	No	21	53.0 ± 14.8	62% F; 38% M	30.7 ±7.0	84.9 ±20.7	57% No; 10% Pre; 5% T1D; 28% T2D	90% No; 10% Yes	100 % No; 0 % Yes	100 % No; 0 % Yes	100 % No; 0 % Yes	90% No; 10% Yes
Hypertensive only	*HTN*	≥ 140	No	18	59.9 ± 17.6	56% F; 44% M	37.5 ±13.4	107.1 ±33.1	56% No; 0% Pre; 11% T1D; 33% T2D	61% No; 39% Yes	89% No; 11% Yes	94% No; 6% Yes	83% No; 17% Yes	94% No; 6% Yes
Depressive disorder only	*DEP*	≤ 120	Yes	7	63.8 ± 6.2	57% F; 43% M	27.3 ±5.9	75.7 ±16.5	71% No; 0% Pre; 0% T1D; 29% T2D	100% No; 0% Yes	100% No; 0% Yes	71% No; 29% Yes	100% No; 0% Yes	100% No; 0% Yes
Hypertensive plus depressive disorder	*DEP-HTN*	≥ 140	Yes	8	67.0 ± 10.7	63% F; 37% M	34.2 ± 10.5	96.3 ± 29.9	75% No; 0% Pre; 0% T1D; 25% T2D	62.5% No; 37.5% Yes	100% No; 0% Yes	100% No; 0% Yes	100% No; 0% Yes	100% No; 0% Yes

Data are mean ± SD.

*Any DSM-IV or DSM-5 depressive disorder ranging from moderate depression to major depressive disorder, excluding psychosis.AF, atrial fibrillation; BMI, body mass index; BP, blood pressure; CKD, chronic kidney disease; HF, heart failure; PAD, peripheral artery disease; TIA, transient ischemia attack; T1D, type 1 diabetes; T2D, type 2 diabetes.

Table II. Dominant prevalent taxa of each cohort

Patient cohort	Dominant prevalent taxa	Effect size
REF	*Bifidobacterium_longum*	1.01
	Coprococcus_catus	1.02
	Escherichia_coli	0.96
	Roseburia_intestinalis	0.93
DEP	*Clostridium_symbiosum*	1.50
	Lachnospiraceae_bacterium_3_1_46FAA	1.74
	Veillonella_unclassified	1.73
HTN	*Akkermansia_muciniphila*	0.98
	Alistipes_senegalensis	0.94
	Bacteroides_massiliensis	0.96
	Bilophila_wadsworthia	0.65
	Odoribacter_splanchnicus	1.17
DEP-HTN	*Alistipes_obesi, (Bacteroidales_bacteriium_ph8*	1.07
	Eubacterium_siraeum	1.12
	Holdemania_filiformis	1.13
	Lachnospiraceae_bacterium_1_1_57FAA	1.14
	Streptococcus_salivarius	3.78

Based on the data of Figure 1 and Supplementary Figures S1 and S2, prevalent taxa effect sizes with absolute values > 0.5 were first ranked based on all permutations of the 4 Table I cohort metadata pairwise comparisons, then the pairings were parsed by a consensus/elimination process that removed overlap of a given taxon appearing more than once in the cohort comparisons. This identified the dominant prevalent taxa gut ecology community members specific to each metadata cohort.

Results

Gut microbial taxa ecology differences

Multivariate covariance clustering of cohort metadata was explored for initial biomarker characterization of all pooled taxonomic data which were fit by four discrete metadata cohort clinical characterizations (Table I; Figure 1A), namely reference subjects (REF), DEP, HTN, and DEP-HTN. Taxonomic differentiation responsible for this metadata clustering was indicated by a correlation heatmap (Figure 1B), for which the four separate cohorts were readily distinguishable based on the color pattern of distinct patches of taxa relative magnitudes showing positive Spearman correlations (red; Rho $\geq$ 0.5) or negative (green; Rho $\leq$-0.5), with shared commonality denoted as black (Rho = 0). The dendrogram of Figure 1B further indicates that the four cohorts were isolated based on hierarchical cluster dissimilarities.

Our denoising machine-learning pipeline for multivariate analyses[9] assigned prevalent taxa to each Table I metadata cohorts (REF, DEP, HTN, DEP-HTN), for which statistical significances were validated by Mann-Whitney plots of differential abundances, Bray-Curtis dissimilarity network analyses, Spearman's Rho correlation heatmaps, and effect sizes, and further confirmed by running control null-hypothesis Dirichlet Monte-Carlo simulations using randomly scrambled datasets; these differences are articulated in Supplementary Figures S1 and S2. Subsequently, the dominating prevalent taxa specifically attributable to each of the four clinical cohorts were identified by generating consensus groupings (Table II), based on parsing effect size data collectively pooled from all metadata pair-wise group comparisons of the data represented in Supplementary Figures S1 and S2.

Gut microbiome functional genomics differences

Prevalent microbiome functional KEGG metabolic pathways and genes associated with each clinical cohort of Table I was evaluated by multivariate analyses of 4,744 total functions derived from gut microbiome WMGS. Machine-learning metadata comparisons of denoised prevalence datasets[9] were fit to the four metadata clinical cohorts (Supplementary Figure S3). A supervised 389-item subset of microbiome metabolic functions was identified as relevant to potential interplay and crosstalk with host eukaryotic physiology: amino acids, biogenic amines, short chain free fatty acids, proinflammatory molecules, and vitamins. A Spearman correlation heatmap of these 389 metabolic functions distinguished the four host cohort groups (Supplementary Figure S4). Subsequently, the most salient function differences amongst the data of Supplementary Figures S3 and S4 were then assembled as shown in Figure 2 which displays Bray-Curtis dissimilarity network analyses, sparse partial least squares discriminant analysis supervised PCA with LASSO variable selection, and Forest plot odds ratios differences.

Discussion

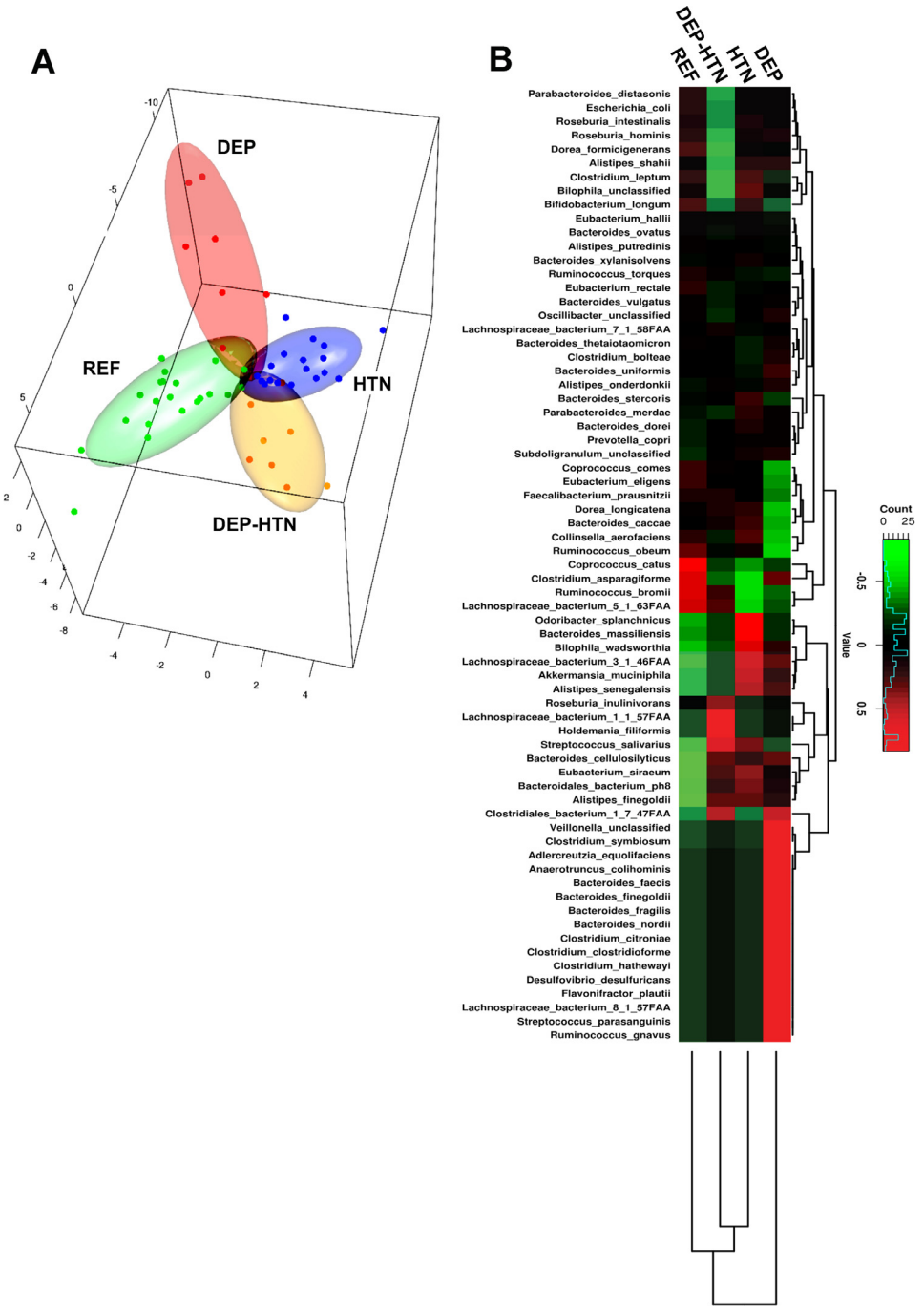
Discrete cohort endotypes

The main finding of this study is that machine learning-enhanced denoised multivariate analyses of human gut microbiome WMGS metagenomics data collectively extricated 4 discrete clinical cohorts which were discernable by differences in bacterial taxa and their functional pathways relating to host pathophysiology of HTN and DEP. Notably, DEP-HTN was significantly differentiated from HTN and from DEP, with each of those being distinctive from REF. Therefore, this study provides proof-of-concept that DEP-HTN may be considered to be a unique CVD "endotype." An "endotype" is defined[14] as a subtype classification of a heterogeneous medical disorder and/or behavioral condition identifiable by discrete biomarkers that reflect a distinct pathophysiological mechanism. Below we first discuss key microbiome factors that differentiate the cohorts, and then we integrate the Results into a pathophysiological model of DEP-HTN.

Amines

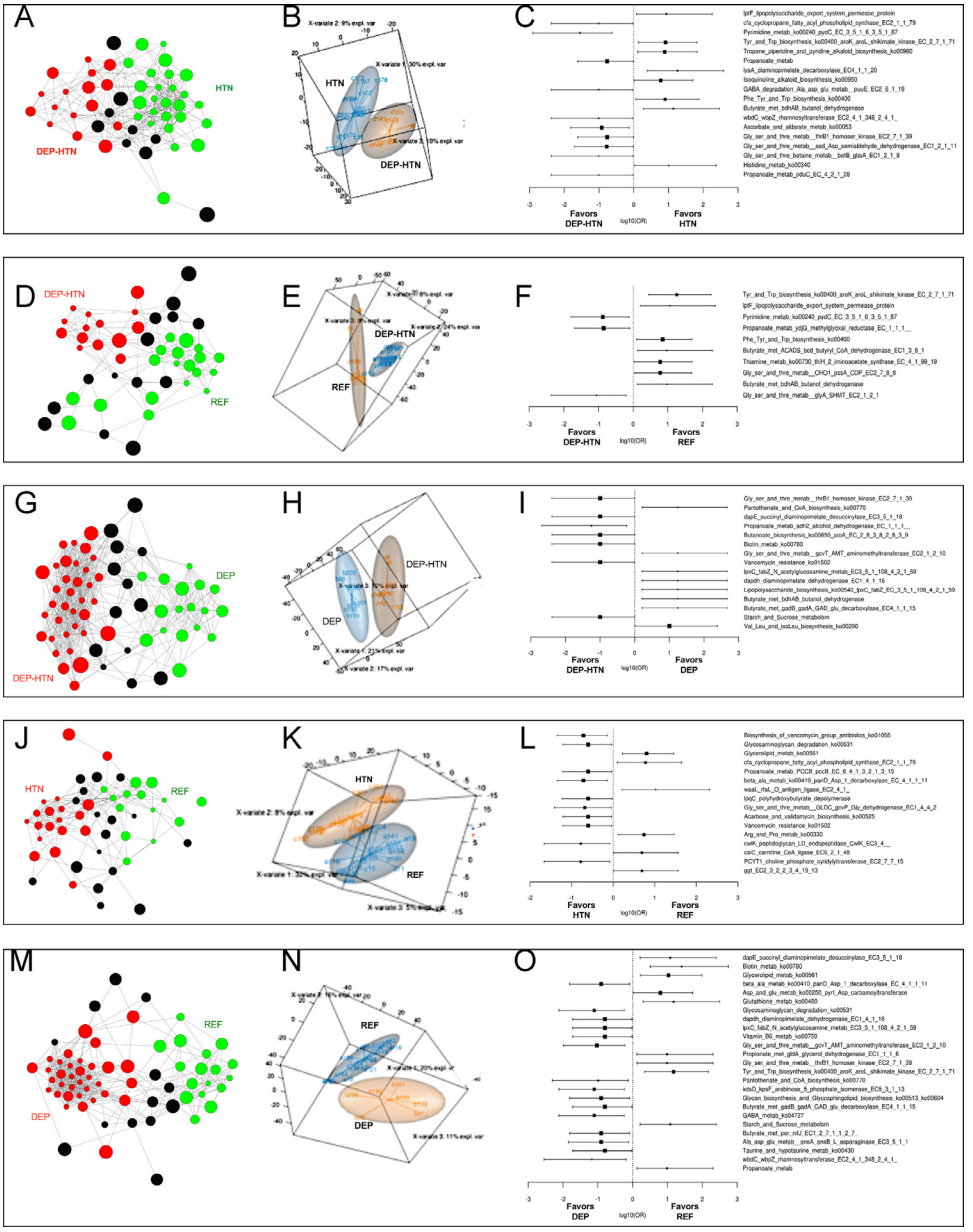
Amino acids, bioactive amines, and γ-aminobutyrate (GABA) metabolism pathways of DEP-HTN (Figures 2, S3, S4) are consistent with metabolomic markers of both DEP[15,16] and elevated BP.[3-6] Conversely, the patterns of REF and DEP amino acid pathways are inversely correlated with BP via antiischemic vasodilatory effects and upregulation of cardioprotective β-arrestin-2.[17,18] Absent in DEP-HTN but present in REF (Figures 2, S3,

Figure 1



Taxa discrimination classified by metadata cohorts. A, Multivariate metadata variable selection and classification of taxa group clusters with 95% CI ellipses obtained by sparse partial least squares discriminant analysis (sPLS-DA) supervised PCA with LASSO variable selection. B, Multivariate analysis correlation heatmap. Four separate cohorts (Table I ; REF, DEP, HTN, and DEP HTN) were readily distinguished based on the patterns of color from distinct patches of relative magnitudes of taxa shown with positive (red; Rho $\geq$ 0.5) or negative (green; Rho $<$ -0.5) Spearman Rho correlations, with commonality denoted in black (Rho = 0). The dendrogram further indicates that the four cohorts were isolated based on hierarchical cluster dissimilarities. These data represent the top 75 relatively abundant taxa. (Color version of figure is available online.)

Figure 2



Prevalent KEGG gene and pathways multivariate analyses. A 389-item subset of the pool of all functional KEGG pathways and genes in the microbiome (Supplementary Figures S3, S4) was identified as relevant to potential interplay and crosstalk with host eukaryotic physiology; these included biosynthesis or degradation of amino acids, biogenic amines and methylamines, short chain free fatty acids, proinflammatory molecules, and vitamins. These functions were simultaneously compared among the Table I metadata cohorts, DEP-HTN vs. HTN, DEP-HTN vs. REF, DEP-HTN vs. DEP, HTN vs. REF, DEP vs. REF. A, D, G, J, M, Bray-Curtis dissimilarity network analyses showing node (KEGG pathway and genes) color clustering, distances, relative diameters, and edge interconnections. Metadata nodes sizes are proportional to relative magnitudes of each KEGG ontology within each green vs. red dataset, with black representing shared pathways of each pairing. B, E, H, K, N, Multivariate metadata variable selection and classification of taxa group clusters with 95% CI ellipses obtained by sPLS-DA supervised PCA with LASSO variable selection. C, F, I, L, O, Forest plot odds ratios for KEGG pathways and genes. Microbiome KEGG pathways or genes were derived from machine-learning pipeline prevalence datasets relevant to human host physiology. Logistic regressions of odds ratios with 95% upper and lower CI were selected where no CI bars touch the zero axis.

S4), are the anti-inflammatory effects of tryptophan and other nutrient-signaled amino acid ligands transported by B^0AT1[19] that suppress the peripheral, gut, and brain inflammasome, and enhance gut barrier integrity.[20-26] Microbial degradation of GABA is favored in DEP-HTN over HTN alone, as well as favored in DEP over REF (Figure 2). This is consistent with GABA deficits that destabilize crosstalk between mood centers and hypothalamic paraventricular nucleus control of baroreceptor reflex and sympathetic nervous system control of peripheral vasomotor tone and renal salt and water homeostasis, and augmentation of hypothalamic-pituitary-adrenal axis (HPA) axis activity.[27,28] GABA-consuming taxa in DEP-HTN and DEP (Figures 2C, S1, S2) include *Eubacterium siraeum, Alistipes spp*., and *Veillonella spp.* in contrast to *Bifidobacterium spp,* in REF, which produces GABA.[29]

Sodium and gut renin-angiotensin-aldosterone system

Gut microbial communities modulate local intestinal renin angiotensin aldosterone system that control mucosal water and Na^+ absorption and modulate gut wall hemodynamics/oxygen tensions that governs the relative compositions of anaerobic and facultative bacterial gut communities.[6,8,19,30-33] In Table II, prominence of DEP-HTN taxa *Eubacterium siraeum, Holdemania spp., Streptococcus spp.*, and *Alistipes spp*. are directly correlated with elevated BP due to their inverse association with production of food-derived bioactive antihypertensive peptides fragments that antagonize gut tissue renin angiotensin aldosterone system.[34-36] Gut taxa composition is shaped by dietary sodium intake; notably (Figures 1, S1, S2) low dietary intake of sodium promotes viability and abundances of beneficial REF taxa *Roseburia spp., Bifidobacterium spp., and Coprococcus spp.*, while elevated intestinal lumen sodium diminishes these taxa in HTN,[34] as reflected in their decreases in DEP-HTN and HTN. Consistent with DEP-HTN and HTN (Table II), high-salt diets induce relative abundances of *Alistipes spp*. and *Bacteroides spp*. in experimental HTN.[24]

Short chain fatty acids

Degradation/metabolism of short chain fatty acids was favored in DEP-HTN over other endotypes (Figures 2, S3, S4), consistent with gut dysbiosis-blunted butyrate and propionate levels implicated in CVD, DEP and anxiety, and attending pathophysiology of disrupted catecholamine neurotransmitter biosynthesis, RAS, satiety, and biological clock gene circadian oscillations.[37-40] Conversely, elevated short chain fatty acids in REF (Figures 2,S3, S4) beneficially regulate mood and BP.[2] SFCAs modulate central control of BP via sympathetic control from the paraventricular nucleus and HPA axis, and crosstalk between hypothalamic nuclei and various brain mood centers.[3]

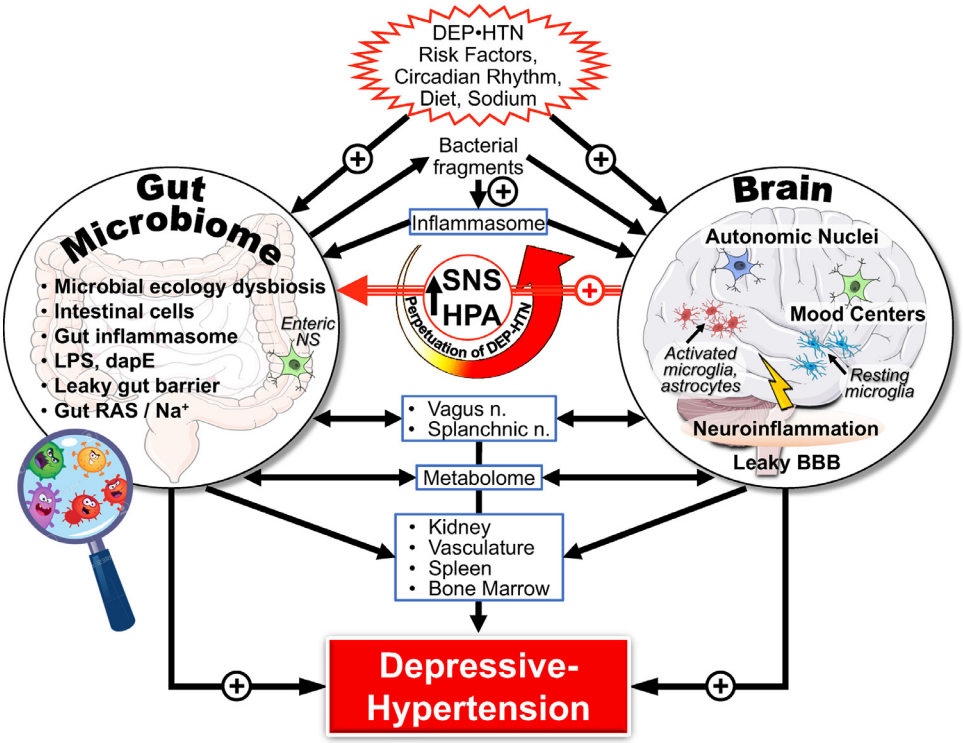
Lipopolysaccharide and glycans

DEP-HTN is directly correlated with enhanced proinflammatory bacterial cell wall peptidoglycan/lipopolysaccharide (LPS) biosynthesis activity (Figures 2, S3, S4), which is implicated in elevated BP and mood disorders.[2,4] The putative DEP-HTN endotype is in concert with our prior studies, in which plasma LPS levels were positively correlated with zonulin and FABP2 tight junction components of leaky gut and BBB, as directly associated with DEP[2] and with high BP.[4] The enzymes N-succinyl-L,L-diaminopimelate desuccinylase ("dapE"), 3-hydroxyacyl- [acyl-carrier-protein] dehydratase ("fabZ"), and UDP-3-O-acyl-N-acetylglucosamine deacetylase ("lpxC") expressed by multidrug-resistant pathogens, are drug targets because these glycan steps are compulsory for their overall viability, formation of cell walls, and host inflammasome induction.[41-44] Notably (Figures 2, S4), dapE is prominent in DEP-HTN over DEP, while fabZ and lpxC are favored in DEP over DEP-HTN. Of significance, the zinc metallo-binding site of dapE is competitively inhibited by captopril and other ACE inhibitors, and also blocked by food-derived dipeptides such as asp-leu.[41] We posit that in DEP-HTN patients, the antihypertensive impact of ACE inhibitors and certain functional food proteolysis fragments[32,33] may impart at least a portion of their efficacy through an antibiotic mechanism. Extending this concept, our findings collectively provide an impetus to repurpose or develop new drugs which are lpxC-, fabZ-, and dapE-based antibiotics targeting resistant DEP-HTN, HTN, and/or DEP.

Bacterial taxa

Cluster patterns of taxa differentiating the cohorts (Figures 1, S1, S2) are consistent with large scale studies[22,45-51] reflecting the complexity of microbial community interactions within niche compositions among themselves and bidirectionally with host physiology. In preclinical and human studies, *Bifidobacterium longum*, *Coprococcus catus,* and *Roseburia intestinalis* were positively correlated with REF status,[32,35,52-56] while *Holermania spp., Bacteroides spp*., and *Eubacterium spp.* were positively correlated with increased systolic and diastolic BP.[34,39,54] *Holdemania spp*. is concomitantly associated with mood disorders.[57,58] *Veillonella spp*. and *Strepococcus spp*. are positively correlated with HTN and endocarditis in rodents and DEP in humans.[5,35,36,59,60] *Akkermansia spp., Bacteroides spp., or Lachnospiraceae spp*. have been correlated with DEP or HTN consistent with Figures 1, S1, S2, but this can be blunted by modifying dietary fat and fiber.[9,58,61] *Bilophila wadsworthia* promotes HTN inflammation and intestinal barrier dysfunction with a high fat diet, but its untoward effects are muted in the presence of *Lactobacillus rhamnosus*.[62]

Figure 3



Depressive-hypertension endotype (DEP-HTN) proposed mechanism model. The proposed DEP-HTN endotype is putatively a whole-body engaged impairment in gut-brain communications that intertwines the mechanisms of mood and blood pressure homeostasis, as governed by the gut microbiome. DEP-HTN pathophysiology involves disrupted crosstalk within and between mood centers and brain autonomic nuclei. This results in enhanced activities of sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal axis (HPA), heightened central, peripheral and local gut inflammasome activities, and dampened parasympathetic responses. Bottom-up and/or top-down pathophysiological events may occur dynamically and interactively involving systemic and central events. BBB, blood brain barrier; dapE, N-succinyl-L,L-diaminopimelate desuccinylase, EC:3.5.1.18; HPA, hypothalamic-pituitary-adrenal axis; LPS, lipopolysaccharide; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system.

Depressive-hypertension model

The DEP-HTN cohort was differentiated based on bacterial genes and functional pathways (Results Section 3.2) that can interact with host pathophysiology, as correlated with the attending taxa community ecology (Results Section 3.1). Based on the results and above discussion, we posit a model for DEP-HTN which is articulated in Figure 3. Here, the proposed DEP-HTN endotype is a whole-body engaged impairment in gut-brain communications that intertwines the mechanisms of mood and central control of BP homeostasis, as governed by the gut microbiome. Risk factors include: (1) psychosocial stress, (2) diurnal biological clock-related metabolomics and behaviors, including dietary intake of sodium, fiber, saturated fat, reduced exercise, and sleep cycles, and (3) predisposed endogenous brain neural circuitry wiring.

We propose that DEP-HTN pathophysiology involves disrupted crosstalk within and between mood centers and brain autonomic nuclei. This results in enhanced activities of sympathetic nervous system and HPA, heightened central, peripheral and local gut inflammasome activities, and dampened parasympathetic responses. Bottom-up and/or top-down pathophysiological events may occur dynamically involving systemic and central events. A coordinated mechanism involving both top-down and bottom-up interactions cannot be ruled out.

Bottom-up events may include influences on: (1) unfavorable imbalance of the plasma metabolomics profiles and autonomic tracts that adversely affect key organs including bone marrow, kidney, spleen, heart, and vasculature, (2) compromised integrities of gut barrier and BBB, (3) untoward systemic and gut wall immunome and neuroinflammatory status, (4) altered central balancing of

sympathetic/vagal/splanchnic activities, and (5) dysfunctional interactions among central BP-control nuclei and mood regions as the consequence of activated microglia and astrocytes, thereby perpetuating the neural dysregulation of DEP-HTN. Bottom-up regulations may be steered by top-down risk factors that directly influence gut microbiome community taxa composition and functional genomics.

Top-down influences may include autonomic efferent tracts and host metabolomics that steer gut interactions amongst microbiome/epithelium/mucosal blood flow/enteric nervous system. These interactions: (1) shape gut microbiome community composition that is based on fiber- and salt-dependent species, (2) disrupt microbial community biological clock circadian rhythms, (3) increase gut barrier permeability, alter gut wall immunome activity, release microbiome-derived bioactive molecules into the circulation, and (4) enhance intestinal sodium transporter absorption activities. Further top-down influences of DEP-HTN include untoward systemic effects on bone marrow, kidney, spleen, heart, and vasculature.

The DEP-HTN model is not simply two parallel comorbid syndromes—rather, it synthesizes both DEP and HTN into an integrated physiological mechanism. This premise is based on: (1) CVD dysregulation as a mosaic metabolic disorder involving the gut, (2) DEP as a whole-body phenomenon, and (3) the gut microbiome as a player shaping human "metaorganism" pathophysiology. DEP-HTN involves a whole-body ecosystem that dynamically interweaves cross-talk communications between and within the gut bacterial community and its human host's functional genomics that relate to both mood and BP.

Study limitaions

A limitation of this study is the small sample size of the cohorts which precluded in-depth detailed comparisons of differences amongst age groups, sex, ethnic diversities, and diet. However, in spite of small sample sizes, the permutational multivariate analyses of covariance-based dissimilarities and effect sizes (Methods 2.3; Results 3.1 and 3.2) nevertheless identified valid statistically significant differences and effect sizes between the cohorts as grouped by Table I clinical characteristics. Another limitation is that while the study provides a valuable testable model as the gateway for outside research groups to execute future experiments and translational applications, it is currently premature to report results of such experiments that are underway by our group.

Conclusions

In conclusion, we identified a DEP-HTN endotype as a putatively novel clinical disorder in human patients. This endotype model suggests a unique signature of microbial taxa and functional genomics that intertwines pathophysiology involving the gastrointestinal tract with disruptions in central control of BP and mood. DEP-HTN provides insight into gut-host pathophysiology placing emphasis on functional genomics, rather than emphasizing particular taxa *per se*.[45,46] Using Figure 3 as a testable model, our future studies shall include preclinical experiments designed to measure effects of isolated cultures of the various bacterial taxa represented in Table II, as accompanied by metabolomic analyses of the corresponding microbiota and their human hosts. The proposed DEP-HTN mechanism shifts HTN-coupled mood disorders toward engaging the gastrointestinal tract as a template model for future translational studies leading to improved management of CVD and/or DEPDEP.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ahj.2021.05.002.

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