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Alteration of gut microbiota associated with hypertension in children

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Abstract

Background The association of disturbance in gut microbiota with hypertension (HTN) defined on three separate occasions among children and adolescents remains unclear. In this study, we aimed to compare the differences in gut microbiota composition and diversity between children with HTN and those with normal blood pressure (BP).

Methods Data and stool samples were collected from the second follow-up of a childhood cardiovascular health cohort study in 2021. 16 S ribosomal RNA gene sequencing was conducted to determine the relative abundance of microbial taxa in 51 children aged 10–14 years with HTN and 51 children with normal BP.

Results Compared with children with normal BP, those with HTN had decreased gut microbiome diversity. At the genus level, after adjusting for the false discovery rate (FDR), the proportions of several gut microbiota such as *Blautia* (P_{FDR}=0.042), *Coprococcus* (P_{FDR}=0.042), *Eubacterium_ventriosum_group* (P_{FDR}=0.027), *Christensenellaceae_R-7_group* (P_{FDR}=0.027), and *norank_f__Lachnospiraceae* (P_{FDR}=0.015) significantly decreased in children with HTN compared to those with normal BP. Receiver operating characteristic analysis, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were performed and showed that the genera *norank_f__Lachnospiraceae* and *Dorea* significantly enhanced the ability of body mass index to differentiate between children with HTN and those with normal BP (area under the receiver operating characteristic curve: 0.95, 95% confidence interval 0.91–0.99; NRI > 0; IDI = 0.12, $P < 0.05$). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States showed that the mean proportions of cofactors and vitamins metabolism pathway and the glycan anabolism pathway were higher in children with HTN.

Conclusions Disturbances in the abundance and diversity of gut microbiota may contribute to the development of HTN in children. Gut microbiota biomarkers may be of significant importance in the early identification and diagnosis of childhood HTN.

Clinical trial number Not applicable.

Keywords Blood pressure, Child, Intestinal microbiome

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Introduction

Cardiovascular disease (CVD), the leading cause of mortality worldwide, accounted for nearly one-third (i.e., 17.9 million) of total deaths in 2019 [1]. Hypertension (HTN), a major risk factor for CVD, affects 1.28 billion adults aged 30 to 79 years worldwide, but only 42% are diagnosed and treated [2]. Moreover, an estimated 16 million people die from HTN-related complications each year [3]. Therefore, early prevention of HTN is likely to lead to a significant reduction in the burden of CVD and mortality later in life.

HTN can develop in childhood and track into adulthood [4–6]. Moreover, elevated blood pressure (BP) and HTN in childhood are associated with subclinical markers of CVD [7, 8] and fatal and non-fatal CVD events [9]. A diagnosis of HTN in children relies on measurements from at least three separate occasions followed by the application of complex tables that stratify individuals' systolic and diastolic BP levels by age, sex, and height percentile [10–12]. This approach poses numerous challenges to implementation [13] in community- and clinic-based settings, which leads to the underdiagnosis of pediatric HTN [14, 15]. Therefore, exploring alternatives to detect HTN could facilitate diagnosis and lead to the development of new therapeutic strategies to reduce the short- and long-term CVD burden associated with HTN.

With only 5.7% of BP variability accounted for by genetic variants [16], environmental factors, such as diet [17], physical activity [18], and sleep [19], are important modifiable factors in the development of HTN. Recently, it has been reported that disturbance of gut microbial community diversity, taxonomic composition, and related pathways was associated with high BP in animal models [20, 21] and human adults [22–24]. Despite these associations observed in adult populations and animals, the association between gut microbiota disturbances and HTN, as determined through three separate occasions among children and adolescents, remains poorly understood.

In this study, we aimed to compare the diversity, composition, and abundance of gut microbiota by sequencing the 16 S ribosomal RNA (16 S rRNA) of stool samples from 51 children with HTN, as determined through three separate occasions, and 51 children with normal BP from the second follow-up of the "Huantai Childhood Cardiovascular Health Cohort Study". Furthermore, we selected gut microbiota biomarkers to identify HTN in children.

Methods

Participants and sample collection

Children were recruited from the second follow-up of the "Huantai Childhood Cardiovascular Health Cohort Study", which was conducted in Zibo, Shandong, China, from November to December 2021. A total of 51 children aged 10–14 years with HTN (i.e., HTN group) and 51 randomly selected children with normal BP (i.e., NBP group) were included in this study. All participants had no history of antibiotic use, vomiting, diarrhea, and gastrointestinal diseases within the past three months. Informed consent was obtained from all participants and their guardians. This study was approved by the Ethics Committee of Shandong University (Number: 20160308), China.

Measurement and definition

After a rest of at least 10 mins, seated BP values were measured by trained staff using a verified electronic sphygmomanometer (Omron HBP-1300) [25] in a quiet room using a suitable cuff according to the individual's right upper arm circumference. Three consecutive BP measurements were recorded and subsequent measurements were performed after at least 1 min. If the difference between any two of the three BP readings exceeded 10 mmHg, an additional BP measurement was performed. The mean value of the last two readings was used for analysis.

Elevated BP was defined using sex-, age-, and height-specific 95th percentile cutoffs of systolic BP (SBP) and/or diastolic BP (DBP) for Chinese children [10]. Participants with elevated BP at the first visit underwent a second visit at least 2 weeks later. A third visit was conducted at least another 2 weeks later if elevated BP persisted at the second visit, according to the same procedures. Children who had elevated BP on all three separate occasions were identified as HTN [11, 12].

Covariates

A self-designed structured questionnaire, as used previously [26], was completed by participants to collect information on demographic characteristics (e.g., sex, age), diet (frequency of breakfast consumption, fruit, vegetables, and carbonated beverages during the past 30 days, intake of western food during the past 7 days, intake of dairy products, yogurt, aquatic products, nuts, spicy foods, and beans/bean products during the past 12 months, numbers of eggs consumed per day, and the salty taste of participants), and lifestyle variables (e.g., the average frequency of exercise per week [more than 10 mins each time], screen time per day, sleep duration per day, and secondhand smoke exposure at home during the past 7 days).

Weight and height were measured twice using an electronic ultrasonic height and weight scale (HGM-300) with participants in light clothing after removing hats and shoes. Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. The mean of the two measurements was used for data analysis. Body mass index (BMI, kg/m^2) was calculated as weight divided by

the square of height. Waist circumference (WC) was measured twice using a standard non-elastic tape positioned horizontally 1 cm above the umbilicus at the end of expiration. WC was measured to the nearest 0.1 cm, with the mean of replicate measures used for analysis.

Blood samples were collected after fasting for at least 10 h. Blood biochemistry (e.g., fasting plasma glucose [FPG], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], total cholesterol [TC], and triglyceride [TG]) was determined using an automatic electrochemiluminescence immunoanalyzer (Cobas e 601) according to the kit instructions.

Fecal sample collection and 16 S rRNA gene sequencing

A sampling spoon was used to pry off the surface of the stool, and fresh internal fecal samples were collected from each participant using a 2 ml enzyme-free cryopreservation tube without enzymatic substances that can catalyze specific chemical reactions, thus effectively preventing contamination of samples with enzymes and protecting the integrity and activity of the samples. During the entire operation process, avoid direct contact between the sampling spoon and hands or other objects. Fecal samples were then flash-frozen with liquid nitrogen and stored at -80 °C until microbiome sequencing and analysis.

Microbial DNA was extracted from each homogenized fecal sample using DNA Stool Kit and then detected using 1% agarose gel electrophoresis. The primers 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 region of the bacterial 16 S rRNA gene [27, 28]. The designated hypervariable sequencing region was amplified according to the protocol of TransStart FastPfu DNA Polymerase (TransGen AP221-02). Each sample was carried out with three replicates. After recovering with AxyPrep DNA Gel Recovery Kit (Axygen Corp., USA) and eluting with Tris-HCl, the PCR products of the same sample were mixed and then detected by 2% agarose gel electrophoresis [29].

According to the preliminary electrophoretic quantitative results, the PCR products were detected and quantified utilizing the QuantiFluor™ - St Blue Fluorescence Quantitative System (Promega Corp., USA). The resulting PCR amplicons were prepared using TruSeq™ DNA Sample Prep Kit (Illumina, USA), and the library was then sequenced on an Illumina MiSeq platform.

Bioinformatics analysis

After quality control, adapter trimming, quality filtering, and data filtering of the sequencing data using the Fastp tool (version 0.19.6, https://github.com/OpenGene/fastp) [30], Fast Length Adjustment of Short Reads (FLASH, version 1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml) was used to paired-end reads merging [31]. The subsequent microbial community and diversity analysis was conducted using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1, http://qiime.org/install/index.html).

First, during preprocessing, we conducted Operational Taxonomic Units (OTU) clustering to assign sequences to different microbial taxa. Second, at a similarity level of 97%, non-repeating sequences (excluding single sequences) were clustered into OTU using Uparse software (version 7.0.1090, http://drive5.com/uparse/). After removing the chimera during clustering, we obtained representative sequences of OTUs. Third, we calculated the relative abundance of each OTU within each sample, which was the number of sequences assigned to that OTU divided by the total number of sequences in the sample. This approach eliminates the influence of different sequencing depths on comparisons across samples, resulting in the normalization of the microbial abundances. Based on the RDP classifier Bayesian algorithm Classifier (version 2.11, http://sourceforge.net/projects/rdp-classifier/) at a confidence threshold of 0.7, species classification annotation of representative OTU sequences was conducted according to the SILVA database (Release138, http://www.arb-silva.de).

Statistical analyses

Continuous variables including age, height, weight, BMI, WC, SBP, DBP, screen time, sleep duration, FPG, HDL-C, LDL-C, TC, and TG were presented as mean ± standard deviation or median (P25, P75), and the differences between HTN and NBP were examined using *t*-test (for data normally distributed or nearly normally distributed) or rank sum test (for data non-normally distributed). Categorical variables, such as sex, were presented as *n* (%) and the difference between the two groups was examined using the chi-square test. SAS v9.4 (SAS Institute, Cary, NC, USA) was used for data analyses and a two-sided $P < 0.05$ was considered statistically significant.

16 S rRNA microbial analysis was conducted by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Alpha diversity indexes were calculated to assess the community richness (e.g., Chao and Ace) and diversity (e.g., Shannon) at the OTU level based on Mothur v1.30.2 (https://www.mothur.org/wiki/Download_mothur) [32]. Rarefaction curves were constructed by randomly subsampling a certain number of sequences from each sample and plotting the alpha diversity index (not limited to the number of OTUs) against the number of sampled sequences, providing an overview of the diversity trends across different groups. To minimize the effects of sequencing depth on alpha and beta diversity measures, the numbers of 16 S rRNA gene sequences from each sample were rarefied to 26,837.

Species Venn diagram analysis (R package "*VennDiagram*") [33] was used to count the number of shared and unique OTUs between HTN and NBP groups. The composition and relative abundance of gut microbiota at the phylum level and genus level were visualized based on the community pie plots. The Principal Co-ordinate Analysis (PCoA, R package "*Vegan*") [34] was performed to visualize the beta diversity of bacterial communities based on weighted-unifrac distance matrices (FastUniFrac, http://UniFrac.colorado.edu/). Adonis analysis (QIIME, http://qiime.org/install/index.html) was used to compare the differences between groups.

After adjusting for false discovery rate (FDR), we used the non-parametric Wilcoxon rank-sum test to compare the relative abundance of gut microbiota at the genus, phylum, and OTU levels, respectively, between NBP and HTN groups. The redundancy analysis (RDA, R package "*Vegan*") [35] was used to reflect the association of gut microbiota with multiple environmental factors, including WC, BMI, intake frequency of breakfast, fruit, vegetables, carbonated beverages, western food, dairy products, yogurt, aquatic product, nuts, spicy foods, and beans/bean products, numbers of eggs consumed per day, the salty taste of participants, the frequency of exercise, secondhand smoke exposure, screen time, sleep duration, and levels of FPG, HDL-C, LDL-C, TC, and TG. The Random Forest algorithm was used, via the R package "*randomForest*" [36], to screen out predominant biomarkers that could distinguish HTN from NBP based on the area under the curve (AUC) validation method. We used different combinations of top-ranked species to construct random forest models and evaluated their ability to discriminate between the two groups. The AUC values indicate the predictive power of these models. By varying the number of species included, we found that certain combinations resulted in higher AUC values, suggesting better discrimination between groups. Among the top n important biomarkers in the barplot of variable importance, logistic regression models were used to evaluate the odds ratio (OR) and its 95% confidence intervals (CI) between the biomarkers and childhood HTN, after adjusting for key environmental factors. Those significant biomarkers were selected to perform receiver operating characteristic (ROC) curve analyses to evaluate their performance in discriminating HTN from NBP children. In addition, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) [37] were calculated to quantitatively evaluate the improvement in discriminating HTN from NBP children using significant intestinal biomarkers, based solely on BMI, which is an important predictor of childhood HTN. The aforementioned analyses were conducted using SAS v9.4 software. To illustrate the metabolic functions of the gut microbiota, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) 2 v2.2.0 (https://github.com/picrust/picrust2/) [38].

Results

Basic characteristics

Compared with children with NBP, those with HTN had higher levels of BMI, WC, and TG ($P<0.05$), shorter sleep duration, and lower HDL-C ($P<0.05$). No significant differences were found in age, sex, FPG, LDL-C, TC, screen time, diet variables, physical activity, and exposure to secondhand smoke at home (Table 1).

Rarefaction curve analysis

A total of 5,160,048 optimization sequences and 2,125,606,197 bases with an average length of 412 (min length: 404; max length: 426) were generated. The rarefaction curve (Ace, Chao, and Shannon indices) tended to be flat, indicating that the amount of sequencing data in this study was sufficient (Fig. 1A-C).

Gut microbial diversity, richness, and composition

We evaluated alpha diversity based on measurements of the community richness (Chao and Ace) and diversity (Shannon) of the gut microbiota in two groups. The values of Chao ($P=0.006$) and Ace ($P=0.022$) showed that children with NBP had a higher community richness than those with HTN (Fig. 1D-E). Shannon index also indicated a higher diversity in children with NBP compared with those with HTN ($P=0.005$, Fig. 1F). Higher indices of alpha diversity were observed in boys and older children (>12 years) who had NBP, similar to the main results. However, no significant differences in alpha diversity were observed between HTN and NBP groups in girls and younger children (≤12 years) (Table S1). Microbial community analysis identified a total of 874 OTUs from 102 fecal samples. Of these, 620 (70.94%) were shared by all samples, while 153 (17.51%) and 101 (11.56%) OTUs, respectively, were uniquely identified in children with NBP and HTN (Fig. 1G).

Community analysis showed that at the phylum level, the proportion of *Bacteroidota* (17.18% vs. 6.69%, $P=0.006$) increased, but the proportion of *Firmicutes* (62.88% vs. 71.82%, $P=0.030$) decreased in children with HTN compared with those with NBP, and the differences disappeared after adjustment of FDR (Fig. 2A, B, C). The proportions of *Bacteroidota* and *Firmicutes*, along with the *P*-values after adjustment of FDR, are shown in Table S2. The proportion of gut microbiota at the genus level was also different in the HTN group compared to the NBP group (Fig. 2D, E). For example, the abundance of *Blautia* ($P_{FDR}=0.042$), *Coprococcus* ($P_{FDR}=0.042$), *Eubacterium_ventriosum_group* ($P_{FDR}=0.027$), *Christensenellaceae_*

Table 1 Characteristics of children with hypertension and children with normal blood pressure

Characteristics	Total ($n=102$)	HTN ($n=51$)	NBP ($n=51$)	*P* value
Age, years	12.8 ± 1.5	12.9 ± 1.5	12.7 ± 1.5	-
Boys, n (%)	66 (64.7)	33 (64.7)	33 (64.7)	-
BMI, kg/m^{2*}	23.4 ± 5.4	27.1 ± 4.4	19.6 ± 3.3	< 0.001
WC, cm*	79.7 ± 14.5	89.2 ± 12.4	70.1 ± 9.3	< 0.001
SBP, mmHg*	121.9 ± 14.2	134.0 ± 8.1	109.8 ± 6.4	< 0.001
DBP, mmHg*	63.2 ± 7.6	66.5 ± 6.8	59.9 ± 6.9	< 0.001
FPG, mmol/L	4.9 (4.6, 5.2)	4.9 (4.6, 5.3)	4.9 (4.5, 5.2)	0.187
HDL-C, mmol/L	1.5 (1.3, 1.8)	1.3 (1.1, 1.5)	1.7 (1.4, 1.9)	< 0.001
LDL-C, mmol/L	2.2 (1.8, 2.7)	2.2 (1.8, 2.7)	2.2 (1.8, 2.8)	0.817
TC, mmol/L	4.2 (3.7, 4.8)	4.0 (3.6, 4.7)	4.3 (3.7, 4.9)	0.165
TG, mmol/L	0.9 (0.7, 1.2)	1.1 (0.9, 1.4)	0.8 (0.6, 0.9)	< 0.001
Breakfast, n (%)				0.373
Often/every day	89 (87.3)	43 (84.3)	46 (90.2)	
Never/seldom/sometimes	13 (12.8)	8 (15.7)	5 (9.8)	
Vegetable & fruit, n (%)				0.236
≥ 5 times/day	23 (22.6)	9 (17.7)	14 (27.5)	
< 5 times/day	79 (77.4)	42 (82.4)	37 (72.5)	
Carbonated drinks, n (%)				0.813
< 1 time/week	79 (77.5)	39 (76.5)	40 (78.4)	
≥ 1 time/week	23 (22.5)	12 (23.5)	11 (21.6)	
Western-style food, n (%)				0.813
≤ 1 time/week	79 (77.5)	39 (76.5)	40 (78.4)	
> 1 time/week	23 (22.5)	12 (23.5)	11 (21.6)	
Dairy, n (%)				0.219
≥ 1 time/week	90 (88.2)	43 (84.3)	47 (92.2)	
< 1time/week	12 (11.8)	8 (15.7)	4 (7.8)	
Yogurt, n (%)				0.154
≥ 1time/week	63 (61.8)	28 (54.9)	35 (68.6)	
< 1time/week	39 (38.2)	23 (45.1)	16 (31.4)	
Aquatic product, n (%)				0.532
≥ 1time/week	35 (34.3)	16 (31.4)	19 (37.3)	
< 1time/week	67 (65.7)	35 (33.5)	32 (62.8)	
Nut, n (%)				0.839
≥ 1time/week	39 (38.2)	20 (39.2)	19 (37.3)	
< 1time/week	63 (61.8)	31 (60.8)	32 (62.8)	
Spicy, n (%)				0.842
< 1time/week	57 (55.9)	28 (54.9)	29 (56.9)	
≥ 1time/week	45 (44.1)	23 (45.1)	22 (43.1)	
Bean products, n (%)				0.316
≥ 1time/week	43 (42.2)	24 (47.1)	19 (37.3)	
< 1time/week	59 (57.8)	27 (52.9)	32 (62.8)	
Eggs, n (%)				0.480
< 1/day	30 (29.4)	13 (25.5)	17 (33.3)	
1–2/day	68 (66.7)	35 (68.6)	33 (64.7)	
> 2/day	4 (3.9)	3 (5.9)	1 (2.0)	
Taste, n (%)				0.477
Normal	79 (77.5)	41 (80.4)	38 (74.5)	
Salty	23 (22.5)	10 (19.6)	13 (25.5)	
Physical activity, n (%)				0.843
≥ 5 times/week	49 (48.0)	24 (47.1)	25 (49.0)	
< 5 times/week	53 (52.0)	27 (52.9)	26 (51.0)	
Passive smoking at home, n (%)				0.418
0	87 (85.3)	41 (80.4)	46 (90.2)	

Table 1 (continued)

Characteristics	Total ($n=102$)	HTN ($n=51$)	NBP ($n=51$)	*P* value
1–4 days/week	8 (7.8)	5 (9.8)	3 (5.9)	
5–7 days/week	7 (6.9)	5 (9.8)	2 (3.9)	
History of parental hypertension, n(%)				0.257
None	90 (89.1)	43 (84.3)	47 (94.0)	
Father or mother	10 (9.9)	7 (13.7)	3 (6.0)	
Both father and mother	1 (1.0)	1 (2.0)	0 (0.0)	
Screen time, hours/day	0.6 (0.3, 1.1)	0.6 (0.3, 1.1)	0.6 (0.3, 1.2)	0.607
Sleep duration, hours/day	8.5 (7.8, 9.1)	8.3 (7.6, 9.0)	8.7 (8.0, 9.3)	0.036

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG: fasting plasma glucose; TC, total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HTN: hypertension; NBP: normal blood pressure."*" indicates the continuous variables were normally distributed

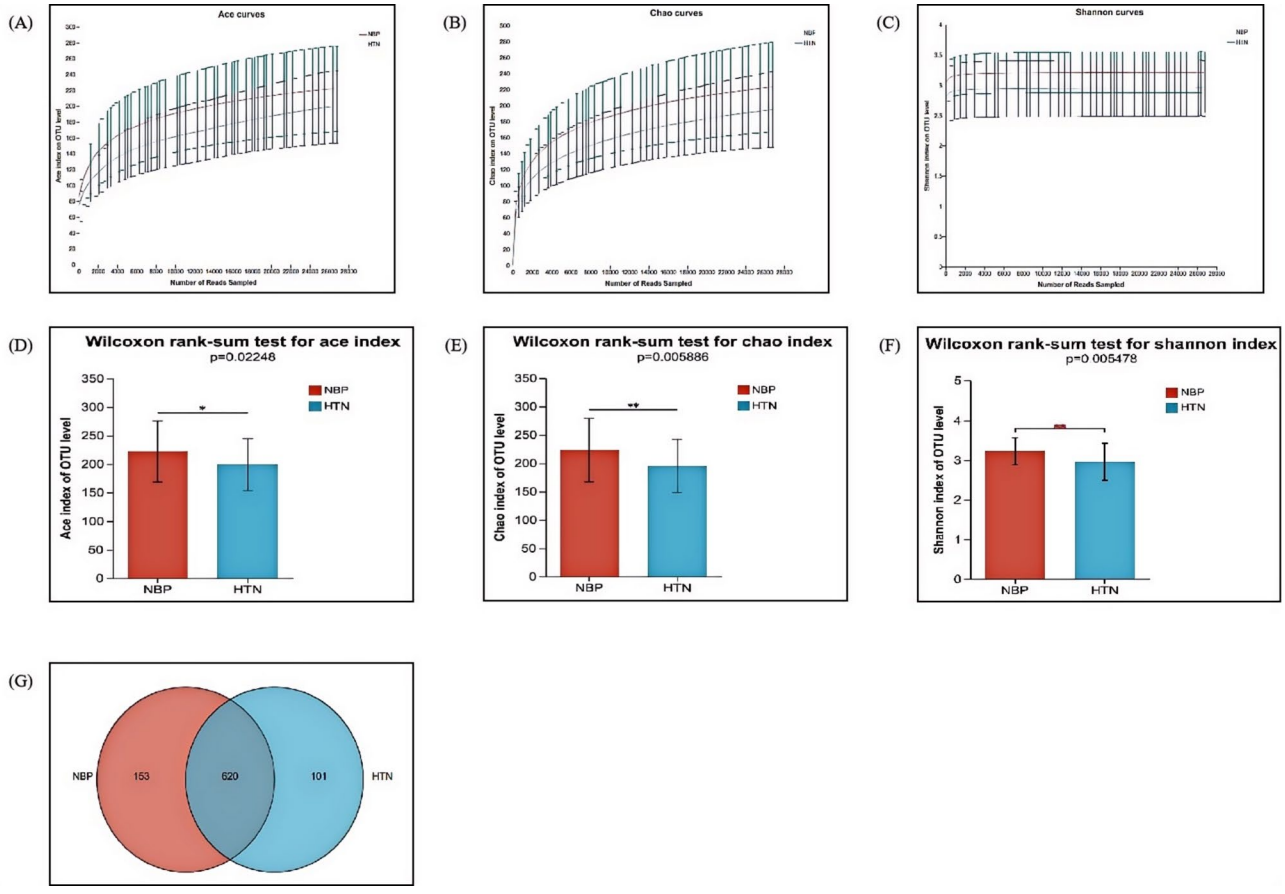

Fig. 1 Differences in essential features and *a* diversity of gut microbiota between children with HTN and those with NBP. The rarefaction curves based on the (**A**) Ace index, (**B**) Chao index, and (**C**) Shannon index were used to demonstrate the amount of sequencing data in this study; Alpha diversity was evaluated using (**D**) Ace; (**E**) Chao; and (**F**) Shannon indexes. (**G**) Venn diagram illustrating the distribution of unique or common OTUs. * $P<0.05$; ** $P<0.01$

R-7_group (P_{FDR}=0.027), *norank_f__Lachnospiraceae* (P_{FDR}=0.015) decreased in the HTN group (Fig. 2F). Figure 2G presents the differences in the relative abundance of OTUs between groups. The PCoA analysis also revealed that the gut microbiota based on the OTU profile in the HTN group could be significantly distinguished from the NBP group ($R^2=0.052$, $P=0.002$; Fig. 2H).

Identification of children with hypertension based on gut microbiota

The RDA analysis revealed that the 7 environmental factors, including frequency of breakfast consumption ($r^2=0.106$, $P=0.007$), sleep duration ($r^2=0.087$, $P=0.011$), secondhand smoke exposure ($r^2=0.095$, $P=0.014$), BMI ($r^2=0.078$, $P=0.022$), LDL-C ($r^2=0.070$, $P=0.031$), WC ($r^2=0.074$, $P=0.035$), and fruit intake ($r^2=0.070$,

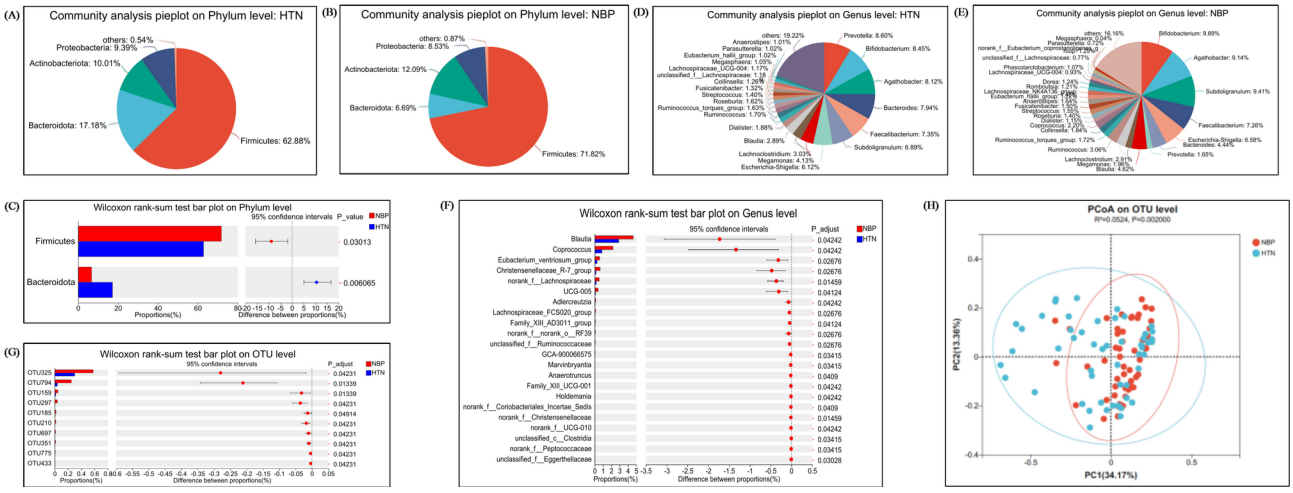

Fig. 2 Differences in the composition of gut microbiota and *β* diversity between children with HTN and those with NBP. Composition of gut microbiota in children with HTN (**A**, **D**) and those with NBP (**B**, **E**) at the phylum and genus levels; Wilcoxon rank-sum test for examining the differences in gut microbiota at phylum (**C**), genus (**F**), and OTU (**G**) levels, respectively; PCoA analysis for evaluating *β* diversity based on the Bray-Curtis distance algorithm on the OTU level (**H**). Original *P*-values are shown in (**C**) since the differences disappeared after the adjustment of FDR.* $P < 0.05$, ** $P < 0.01$

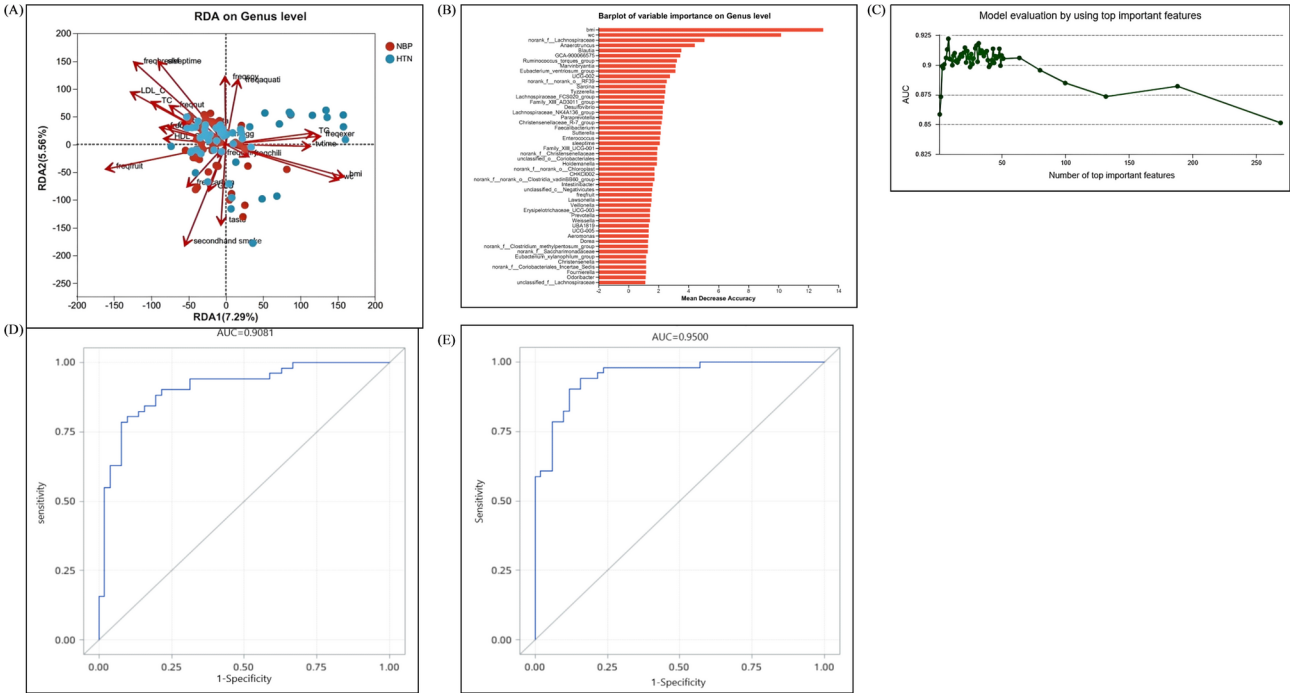

Fig. 3 Screening for biomarkers of gut microbiota after adjustment for environmental factors, and evaluation of the utility of gut microbiota in distinguishing children with HTN from those with NBP. (**A**) The RDA analysis examines the association between environmental factors and the distribution of gut microbiota. (**B**) The random forest algorithm for screening intestinal microbial markers at the genus level. (**C**) The AUC of models developed using top-ranked important features sequentially. (**D**) The utility of BMI in distinguishing children with HTN from those with NBP (**E**) The utility of the combination of BMI with genera *norank_f__Lachnospiraceae* and *Dorea* in distinguishing children with HTN from those with NBP

$P = 0.038$), were significantly associated with the community distribution of gut microbiota (Fig. 3A). Forty-six important features at the genus level were selected using random forest analyses (Fig. 3B) and the models for distinguishing hypertensive children achieved a maximum AUC of 0.92 when the top 8 important features were included sequentially (Fig. 3C). After adjusting for these 7 environmental factors, the logistic regression analysis showed that genus *norank_f__Lachnospiraceae* (OR = 0.984, 95% CI: 0.972–0.997, $P = 0.015$) and genus *Dorea* (OR = 0.997, 95% CI: 0.994–0.999, $P = 0.019$) were inversely associated with childhood HTN after the

further adjustment of lifestyle factors and BMI (Table S3). Our ROC analysis showed that BMI had high utility in distinguishing children with HTN from those with NBP (AUC = 0.91, 95% CI: 0.85–0.97, Fig. 3D). When genus *norank_f__Lachnospiraceae* and genus *Dorea* were combined, the AUC increased to 0.95 (95% CI = 0.91–0.99, Fig. 3E). We further calculated NRI and IDI and found that genera *norank_f__Lachnospiraceae* and *Dorea* could significantly enhance the ability of BMI to distinguish between children with HTN and children with NBP (category NRI = 0.49, 95% CI: 0.32–0.66; category-free NRI = 0.75, 95% CI: 0.39–1.10). In addition, the combination of BMI with genera *norank_f__Lachnospiraceae* and *Dorea* significantly enhanced the comprehensive discriminative ability by 12% (IDI = 0.12, $P < 0.05$) (Table 2).

Metabolic pathway analyses by KEGG

PICRUSt2 showed that cofactors and vitamins metabolism (such as riboflavin metabolism, vitamin B6 metabolism, etc.), as well as glycan biosynthesis and metabolism (such as lipopolysaccharide biosynthesis, glycosphingolipid biosynthesis, etc.), were enriched in the HTN group, while terpenoids and polyketides metabolism (such as limonene and pinene degradation) were enriched in the NBP group (Fig. 4A, B).

Discussion

In this study, we found that alterations in gut microbiota composition and diversity were associated with HTN in children, varied by age and sex. In addition, the combination of BMI, genus *norank_f__Lachnospiraceae*, and genus *Dorea* had a high ability to distinguish children with HTN from those with NBP, with an AUC of 0.95. Our study suggests potential associations between the cofactors and vitamins metabolism pathway, glycan anabolism pathway, and terpenoids and polyketides metabolism pathway and HTN in children, which require further validation to establish causal associations.

It has been demonstrated that gut microbial diversity has decreased in hypertensive animal models and adult humans [21–24]. For example, a microbiome study of the Coronary Artery Risk Development in Young Adults Study found that microbial diversity and richness were inversely associated with SBP and HTN [23]. Another cross-sectional observational study including 315 "metabolically healthy" and "metabolically unhealthy" children aged 8–14 years showed a significantly lower alpha diversity in children with elevated SBP than children with normal SBP [39]. However, elevated SBP mentioned above was defined as ≥ 1.5SD above the mean SBP z-score based on BP measurements taken on only one occasion, which might overestimate the true prevalence of HTN [40, 41]. In this study, we first found that, among children with HTN defined by BP measurement on three separate occasions, there was a significantly lower diversity and richness in gut microbiota compared with children with NBP. Our findings indicate an association between lower diversity and richness in children with HTN, which warrants further investigation as a potential target for intervention.

When we conducted an analysis stratified by sex, only boys with HTN exhibited a lower alpha diversity in gut microbiota, while girls did not. Reduction in diversity of gut microbiota was found among older children with HTN at puberty (> 12 years), but not among prepubertal children. This finding may be attributed to the role of gut microbiota in regulating pubertal timing and sex hormone levels [42]. Several studies have reported that sex may influence gut microbiota differences, however, findings have been inconsistent [28, 43, 44]. A large cohort study including 551 Chinese adults reported that females with obesity exhibited increased alpha diversity, whereas this was not observed among males [28]. However, another study involving 75 coronary heart disease patients from the CORDIOPREV study (39 men and 36 post-menopausal women) revealed that when BMI was ≤ 33 kg/m^2, women exhibited lower alpha diversity, whereas males did not. The opposite was observed when BMI was > 33 kg/m^2 [43]. Given the differences in sample size, disease outcomes, and race/ethnicity, the previous findings related to sex differences should be interpreted with caution. Further studies are warranted to elucidate the role of sex in the association of gut microbiota with HTN among children.

Similar to previous studies conducted in animals [21, 45] and humans [22, 46, 47], we found that *Coprococcus*, a fiber-fermenting bacterium that produces butyrate and propionate, was decreased in children with HTN

Table 2 NRI and IDI analysis to assess the predictive performance of BMI alone and BMI combined gut microbiota in identifying children with hypertension

Models	NRI						IDI		
	Category NRI (95% CI)	*z*	*P*	Category-Free NRI (95% CI)	*z*	*P*	IDI (95% CI)	*z*	*P*
BMI	Reference	-	-	Reference	-	-	Reference	-	-
BMI + *norank_f__Lachnospiraceae* + *Dorea*	0.49 (0.32–0.66)	4.490	< 0.011	0.75 (0.39–1.10)	3.763	< 0.001	0.12 (0.05–0.19)	3.589	< 0.001

BMI, body mass index; IDI, integrated discrimination improvement; NRI, net reclassification improvement

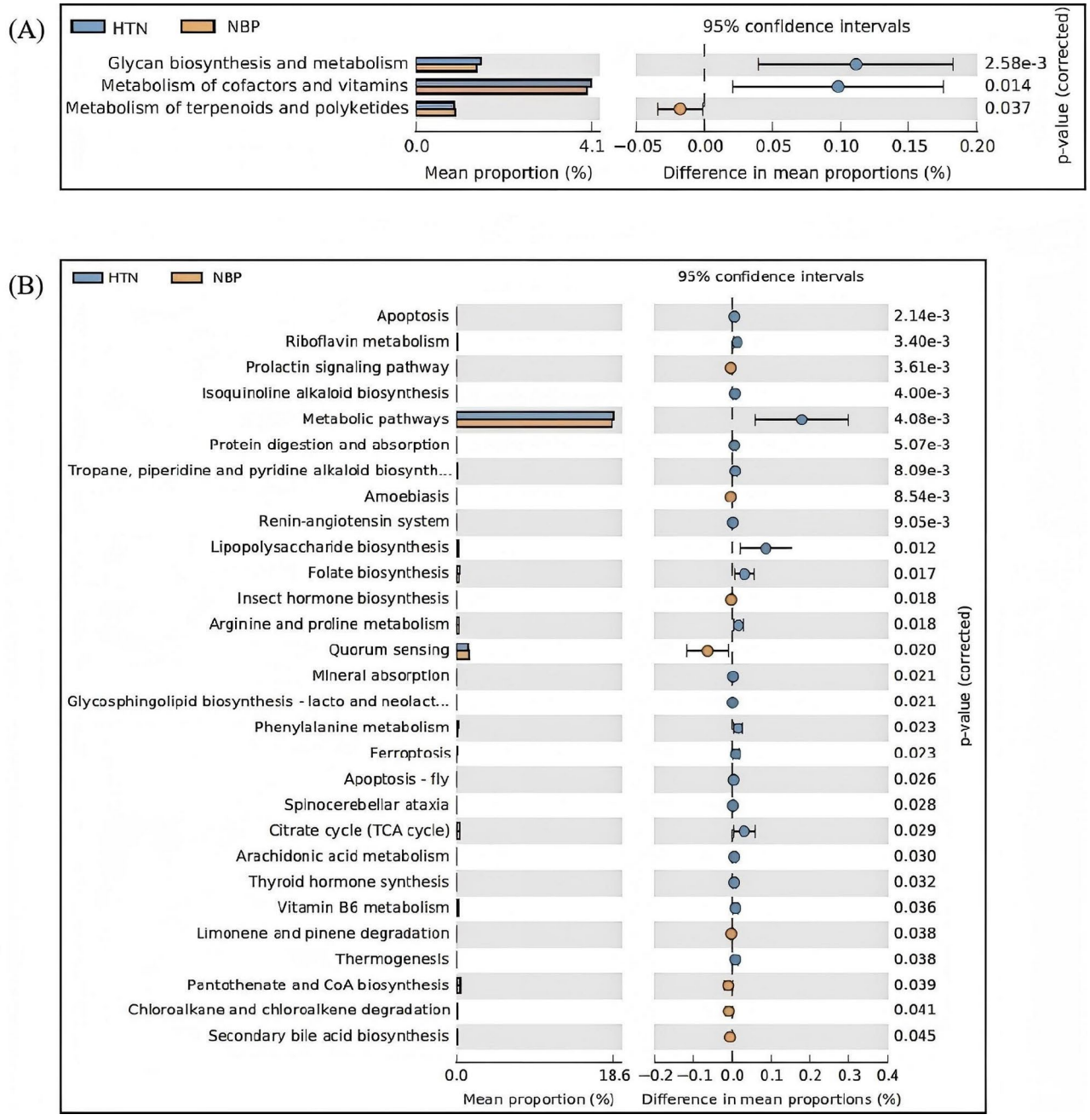

Fig. 4 Difference in metabolic pathway analyses between children with HTN and those with NBP by KEGG. KEGG pathway analysis to illustrate the metabolic function differences of the gut microbiota between HTN and NBP in the (**A**) primary pathway and the (**B**) secondary pathway

compared with those with NBP, as well as another butyric acid-producing bacterium *Blautia*. Although a study of 92 Caucasian participants in Moscow aged 25–76 years reported that glucose metabolism disturbance and arterial HTN were associated with a higher abundance of *Blautia* [48], other studies have reported decreased abundance among Chinese patients with preeclampsia [49], increased abundance among Chinese young adults on a lower-fat diet [50], and decreased abundance among adults with higher TC, LDL-C, and non-HDL-C levels [50]. These findings might help explain the association between decreased abundance of *Blautia* and HTN in children. In addition, two previous Mendelian randomization studies found an association between the decreased abundance of *Eubacterium_ventriosum_group* and elevated SBP level [51] and eclampsia [52]. A cross-sectional study of 29 hypertensive adults and 32 adults with normal BP from Spain showed that the abundance of *Christensenellaceae R-7 group* was negatively correlated with levels of SBP and DBP [53]. We

similarly found that Chinese children with NBP had a higher abundance of *Eubacterium_ventriosum_group* and *Christensenellaceae_R-7_group*, suggesting that these gut microbes might have protective effects on cardiovascular health.

To the best of our knowledge, no prior study has examined the association of *norank_f_Lachnospiraceae* and *Dorea* with HTN in both adults and children. Experimental studies conducted on mice have demonstrated that the abundance of *norank_f_Lachnospiraceae* decreased in immunosuppressive or high-fat-fed mice [54, 55] as well as in mice with elevated levels of inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β) [56]. *Dorea* belongs to the class *Clostridia* within the phylum *Firmicutes* whose abundance positively associates with energy harvesting and obesity [57]. In this study, we found that after adjusting for environmental factors and BMI, genus *norank_f_Lachnospiraceae* and *Dorea* exhibited an independent negative association with childhood HTN. Furthermore, we found that the combination of BMI, genera *norank_f_Lachnospiraceae* and *Dorea*, could accurately differentiate children with HTN from those with NBP. Our findings indicate that the combination of BMI with *norank_f_Lachnospiraceae* and *Dorea* offers high diagnostic utility in identifying children with HTN. To compensate for the limited sensitivity of AUC to model changes, we further conducted NRI and IDI analysis [58, 59] to quantitatively evaluate the ability of genera *norank_f_Lachnospiraceae* and *Dorea* to enhance the ability of BMI in distinguishing between children with HTN and those with NBP. Consistent with the results of ROC analysis, the combination of BMI with genera *norank_f_Lachnospiraceae* and *Dorea* increased the discriminatory ability between children with HTN and those with NBP by 12%, suggesting that these genera significantly enhance the screening ability of BMI for children with HTN. This indirectly suggests an association of *norank_f_Lachnospiraceae* and *Dorea* with the development of childhood HTN.

We identified potential associations between HTN in children and the metabolic pathways of cofactors and vitamins metabolism pathway, glycan anabolism pathway, and terpenoids and polyketides metabolism pathway. Studies have demonstrated that glycans play multiple roles in the initiation and progression of stroke, coronary artery disease, as well as peripheral vascular disease [60]. Several glycans exhibited differences between hypertensive individuals and healthy controls [61]. Specifically, a positive correlation was observed between triantennary glycans and BP levels in adults from China and Croatia, while a negative correlation was observed in glycans containing core-fucose [62]. Cofactors and vitamins play pivotal roles in modulating enzyme activity. It is increasingly recognized that deficiencies and disruptions in cofactors (e.g., magnesium [63], coenzyme Q10 [64]) and vitamins (e.g., vitamin D [65, 66]) are associated with an elevated risk of HTN. However, most of the abovementioned findings were derived from studies conducted on adults with HTN. To the best of our knowledge, our study is the first to demonstrate that the cofactors and vitamins metabolism pathway is enriched in children with HTN compared to children with NBP. However, these findings require further validation. Our findings simply a potential link between HTN and gut microbiota via the abovementioned pathways.

To the best of our knowledge, we are the first to evaluate the gut microbiota associated with HTN in children defined by BP measurements taken on three separate occasions. In addition, we found sex- and age-related differences in gut microbiota among children with HTN. However, several limitations should be considered. First, this study was conducted in one city in China. Given the variation in the prevalence of HTN in different race/ethnicity [67] and age [68] groups, our results should be cautiously extended to populations from other regions or races/ethnicities. Second, as fecal sample collection occurred simultaneously with anthropometric and laboratory measurements, we could not investigate the temporal association between gut microbiota and HTN. Prospective studies are necessary to confirm if the identified microbiota alterations occur before the onset and progression of HTN in childhood. Third, the metabolic pathway analyses were based on PICRUSt, which provides speculative functional inferences at the species level, and thus these findings should be interpreted with caution. Finally, this study, employing 16 S rRNA gene sequencing technology, was limited to the sequencing resolution at the genus level and prevented us from performing in-depth analyses of the associated genetic, functional, and metabolic pathways.

Conclusion

In conclusion, disturbance in gut microbiota was associated with HTN in children, varied by age and sex. In addition, the combination of BMI and specific gut microbes, including genera *norank_f__Lachnospiraceae* and *Dorea* provided high diagnostic utility in distinguishing HTN from NBP children. Further research could explore the underlying mechanisms of this association and its long-term implications for HTN management in children.

Abbreviations

16 S rRNA	16 S ribosomal RNA
AUC	Area under the curve
BMI	Body mass index
BP	Blood pressure
CI	Confidence intervals
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
FDR	False discovery rate
FPG	Fasting plasma glucose
HDL-C	High-density lipoprotein cholesterol

HTN	Hypertension
IDI	Integrated discrimination improvement
KEGG	Kyoto encyclopedia of genes and genomes
LDL-C	Low-density lipoprotein cholesterol
NRI	Net reclassification improvement
OR	Odds ratio
OTU	Operational taxonomic units
PCoA	Principal co-ordinate analysis
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
RDA	Redundancy analysis
ROC	Receiver operating characteristic
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triglyceride
WC	Waist circumference

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03999-1.

Supplementary Material 1: **Supplementary material 1.doc**: **Table S1** shows the comparison of alpha diversity between children with hypertension and children with normal blood pressure by sex and age. **Table S2** shows the comparison of community proportion in gut microbiota between children with hypertension and children with normal blood pressure at the phylum level. **Table S3** shows the association between the genera identified by the Random Forest analysis and childhood hypertension.

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Author contributions

LY drafted the manuscript. JS and BX contributed to the study design. JS analyzed, accessed and verified the data and had full access to all the data in the study. All authors contributed to the interpretation of the data, revised the manuscript, and approved the final version of the manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available in the [National Library of Medicine; National Center for Biotechnology Information; BioProject] repository, [Persistent web link: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA939951 OR Accession number: PRJNA939951].

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shandong University (Number: 20160308), China. Informed consent was obtained from all participants and their guardians. This study was conducted in compliance with the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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