Pharmacometabolomic study of drug response to antihypertensive medications for hypertension marker identification in Han Chinese individuals in Taiwan

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Abstract
Various groups of antihypertensive drugs targeting different pathways have been developed; however, the pharmacometabolic responses to these drugs have rarely been compared to elucidate the common pathway of blood pressure regulation. Here, we performed a comparative multi-dimensional pharmacometabolic study on the four major lines of antihypertensive drugs, namely angiotensin-converting enzyme inhibitors (ACEis), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), and diuretics (DIURs), through ultra-performance liquid chromatography coupled to quantum time-of-flight mass spectrometry. Two hundred fifty patients with young-onset hypertension, who were equally divided among five study groups: non-medicated, ACEi, ARB, CCB, and DIUR groups, were recruited. In a metabolome-wide association study conducted through analysis of covariance, 37 molecular features significantly associated with pharmacometabolic responses to antihypertensive drugs were identified. One-third of these features were shared by multiple medications. ACEis, ARBs, and DIURs shared more features than CCB, partially reflecting that ACEis, ARBs, and DIURs affect the renin-angiotensin-aldosterone system. Thirteen molecular features were consistently identified by all four models of the analysis of covariance. A tandem mass spectrometry (or MS/MS) experiment was performed to decipher the chemical structure of these 13 molecular features, including ARB-associated lysoosphatidylcholine (P4135), CCB-associated diacylglycerol (15:0/18:2) (P1175), and DIUR-associated oleamide (P1516). In addition, diacylglycerol (15:0/14:2) (P408) was significantly associated with the pharmacometabolic response to all four antihypertensive drugs. The identified metabolites provide insights into the mechanisms of blood pressure regulation and potential predictive markers of pharmacometabolic responses to antihypertensive drugs.

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Abbreviations: ACEi, Angiotensin-Converting Enzyme inhibitor; ARB, Angiotensin Receptor Blocker; CCB, Calcium Channel Blocker; DIUR, Diuretics; LC-MS, Liquid Chromatography-Mass Spectrometry; RT, Retention Time; SMART, Statistical Metabolomics Analysis – An R Tool; ANCOVA, Analysis of Covariance; BMI, Body Mass Index; FDR, False Discovery Rate; HMDB, Human Metabolome Database; DG, Diglycerides; LysoPC, Lysoosphatidylcholine; ADME, Absorption, Distribution, Metabolism, and Excretion.
1. Introduction

Hypertension is a highly prevalent risk factor for cardiovascular disease, stroke, heart failure, atrial fibrillation, peripheral arterial disease, renal insufficiency, and major neurocognitive disorder; thus, it is a major public health concern in global communities [1–4]. Several lines of antihypertensive treatments are currently used to lower the blood pressure of hypertensive patients, and they target different pathways and mechanisms underlying hypertension etiology [5,6]. According to the 2021 World Health Organization (WHO) Guideline for the Pharmacological Treatment of Hypertension in Adults [7], the recommended initial antihypertensive medications include 1) thiazide and thiazide-like agents; 2) angiotensin-converting enzyme inhibitors (ACEis) / angiotensin-receptor blockers (ARBs), and 3) long-acting dihydropyridine calcium channel blockers (CCBs). The hypertension treatment guidelines in Taiwan are very similar to that of WHO [8]. In addition to pharmaceutical management, traditional treatment methods, such as acupuncture, Tai Chi, Qi-Gong, and traditional Chinese herbal medicine, are available to control blood pressure. Many relevant studies have investigated the effects of these non-pharmacological treatments on blood pressure control, but the results have been inconsistent. Nevertheless, previous studies have shown that these approaches, in combination with antihypertensive medications, can significantly further reduce blood pressure compared to using drugs alone [9].

Blood pressure, a downstream physiological outcome regulated by multiple biological pathways, is a frequently used clinical endpoint in studies on hypertension-targeting pharmaceuticals [10,11]. However, blood pressure is highly variable due not only to heterogeneity in genetic makeup but also to environmental influences such as foods, stress, and ambient temperature as well as variations in measurement devices and circumstances [12,13].

Metabolic activities, which are upstream of blood pressure, may be used as intermediate phenotypes of blood pressure to understand the mechanisms underlying blood pressure regulation [14,15]. Therefore, pharmacometabolic responses to antihypertensive drugs have been extensively profiled to clarify hypertension etiology [16–22]. Metabolomics technology has advanced rapidly, and high-throughput and high-resolution gas–liquid chromatography-mass spectrometry has been developed to quantify low-molecular-weight metabolites in biological systems [23]. These technologies may aid in elucidating the complex etiology of common complex diseases such as hypertension [14,16].

The present study uses liquid chromatography-mass spectrometry (LC-MS) based on untargeted pharmacometabolomics to evaluate multi-dimensional metabolic responses to hypertensive drugs among Han Chinese patients with young-onset hypertension in Taiwan. This study aimed to compare complete profiles of thousands of molecular features among four antihypertensive drug groups and one non-medicated (NonMed) group to identify crucial pharmacometabolic response-associated features. The four lines of antihypertensive drugs were angiotensin-converting enzyme inhibitors (ACEis), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), and diuretics (DIURs). They have been designed to target the renin-angiotensin-aldosterone system, calcium channels, and sodium excretion-related ion channels [24,25].

Our results indicated that some of the identified abundance features were specific to a medication group (i.e., drug-specific features), and some were shared in multiple drug classes (i.e., drug-shared features) in comparisons of the treatment and NonMed groups. After the molecular features were found to be associated with the pharmacometabolic response to antihypertensive drugs, the chemical structures of these features were predicted by performing tandem MS (MS/MS). This is the first study to systematically examine pharmacometabolic responses to ACEi, ARB, CCB, and DIUR, particularly in the Han Chinese population. The results revealed the potential effects of diacylglycerols, phosphatidylcholines (PCs), lysoPCs, and oleamide on the pharmacometabolic response to the studied antihypertensive drugs. The results also provide insights into blood pressure regulation and pharmacometabolic pathways. Remarkably, the identified pharmacometabolomic markers, profiles, and pathways may serve as actionable biomarkers for hypertension precision medicine, particularly for East Asian populations.

2. Materials and methods

2.1. Patients and serum samples

We recruited 250 patients with young-onset hypertension in the Academia Sinica Multi-Center Young-Onset Hypertension Study from five study groups (n = 50 each): the NonMed, ACEi, ARB, CCB, and DIUR groups. In the NonMed group, the patient’s systolic blood pressure (SBP) was ≥ 140 mmHg, and/or diastolic blood pressure (DBP) was ≥ 90 mmHg over 2 months. In the four medication (ACEi, ARB, CCB, and DIUR) groups, patient SBP was ≥ 120 mmHg and/or DBP was ≥ 80 mmHg at two consecutive visits over 2 months. All 250 patients had young-onset essential hypertension and were residents of Taiwan; their body mass index (BMI), triglyceride level, and high-density lipoprotein cholesterol level were normal. Their age at hypertension diagnosis ranged between 20 and 51 years, and all the four grandparents of these patients were Han Chinese. The detailed inclusion and exclusion criteria for patients with hypertension and blood pressure measures have been described in Yang et al. [26] and in Supplemental Text 1. Written informed consent was obtained from each patient with hypertension at their initial clinic visit. This study was approved by the Internal Review Board of Academia Sinica (Permit Number: AS-IRB01-08012).

Serum samples were collected from all patients who had fasted for > 8 h and stored at ~80 °C until use. The samples were thawed on ice. Each 100-μL aliquot of the serum sample was placed in a 1.5-ml tube, and 400 μL of acetonitrile containing 0.1 % formic acid (FA) was added. The mixture was stirred using a vortex mixer for 30 s and centrifuged at 12,000 rpm for 30 min at 4 °C. Next, 400-μL of the supernatant was transferred to a 500-μL glass tube and dried under vacuum. The dried analyte was suspended in 400 μL of 50 % methoxylamine hydrochloride containing 0.1 % formic acid (FA) was added. The mixture was stirred using a vortex mixer for 30 s and centrifuged at 12,000 rpm for 30 min at 4 °C. The sample was then stirred for 1 min and kept at room temperature for 1 h, a 1-μL aliquot of the solution was then injected into an LC-MS instrument (Waters, Milford, MA, USA).

The serum samples of all 250 patients were equally divided into five batches, each containing samples from 50 patients: 10 patients from each of the five study groups (NonMed, ACEi, ARB, CCB, and DIUR groups); six technical replicates were performed for each sample.

Ten standard compounds — namely carnitine, acetyl carnitine, phenylalanine, 4-aminooantipyrine, debrisoquine, colchicine, prednisone, lysoPC(14:0), lysoPC(18:0), and lysoPC(20:0) were mixed and used for instrument adjustment in our LC-MS experiment. Debrisoquine (an endogenous substrate) was used as the internal standard to normalize the metabolite features.

2.2. Ultra-performance liquid chromatography separation

LC separation was achieved on a 100 mm × 2.1 mm Acquity 1.7-μm C8 column (Waters) using an ACQUITY Ultra Performance Liquid Chromatography system (Waters). The column was maintained at
45 °C and eluted using the following linear gradient: 0–1.25 min, 1 %-50 % B; 1.25–2.5 min, 50 %-99 % B; 2.5–5 min, 99 % B; and 5.1–7 min, 1 % B for re-equilibration. Solvent A was water, and solvent B was acetonitrile; both solvents contained 0.1 % FA. Elution was performed at a constant flow rate of 0.5 mL/min.

2.3. Electrospray ionization quantum time-of-flight MS analysis

MS was performed using a quantum time-of-flight (QTOF) MS system (SYNAPT HDMS; Waters) operated in the electrospray ionization (ESI)-positive ion mode. The scan range was 20–990 m/z. The desolvation gas flow was set at 700 L/h at 300 °C, the cone gas flow at 25 L/h, and the source temperature at 80 °C. The capillary voltage and cone voltage were set at 3,000 and 35 V, respectively. The microchannel plate detector voltage was set at 1,650 V. The QTOF MS acquisition rate was 0.1 s with a 0.02-s interscan delay. Data were collected in the centroid mode.

All analyses were performed using the lock spray to ensure accuracy and reproducibility; sulfadimethoxine was used as the lock mass at a concentration of 60 ng/mL and a flow rate of 6.5 μL/min (an [M + H]+ ion at 311.0814 Da in ESI + mode). The lock spray frequency was 10 s.

2.4. Peak alignment of LC-MS data

The raw LC-MS data were extracted using MarkerLynx (Waters). We focused on the compounds with a retention time (RT) of 0.34–3.24 min and a mass of > 50 Da. Data were aligned for RT and mass using the following conditions: a mass tolerance of 0.03 Da, RT window of 0.1 min, noise elimination level of 6 standard deviations above the background intensity, and intensity threshold of 50 cps.

2.5. LC-MS data preprocessing

Statistical Metabolomics Analysis – An R Tool (SMART), a comprehensive tool for analyzing metabolomic data [27], was used for data preprocessing. For each individual, metabolite abundance was scaled by an internal standard, debrisoquine (m/z = 176.1186, RT = 69.6840 s). The scaled abundance values were transformed using a generalized log₂ function. Finally, the log-scale abundance values of different replicate samples were determined using a quantile normalization procedure.

2.6. Quality control

Metabolite abundance data quality was evaluated using SMART [27]; peak- and sample-based quality control was performed. For peak-based quality control, the peaks with zero rates equal to 1 were filtered out. For sample-based quality control, the steps of SMART were followed. Poor-quality individuals or replicate samples of an individual were removed through hierarchical clustering analysis in SMART. The average-linkage, complete-linkage, and Ward’s methods were employed to evaluate the quality of replicate samples. After removing poor-quality individuals and their replicate samples, all study replicate samples’ molecular feature abundance data were realigned using MarkerLynx.

2.7. Batch effect detection

Principal component analysis and clustering analysis in SMART were performed to explore hidden structures and latent groups within the metabolite abundance data. A few principal components that could explain the observed patterns were extracted. The two-group structure was determined using a clustering analysis, which may have resulted from experiment batches or other uncontrolled experimental conditions. This structure was identified using a binary group indicator. In the association analysis mentioned below, the binary group indicator or major principal components were included in statistical models to adjust for potential confounding effects.

2.8. Metabolome-wide association study

Nested analysis of covariance (ANCOVA) was used to identify the molecular features with significantly higher or lower abundance means in each medication group (ACEi, ARB, CCB, or DIUR) compared with those in the NonMed group. The dependent variable was the normalized abundance value. The independent variables were the medication and non-medication indicators, experimental time (in months), and replicate sample ID nested within each of the NonMed and medication groups. Moreover, additional variables in the nested ANCOVA model were adjusted for uncontrollable experimental effects that could not be fully explained by the experimental time (month). These variables were the binary group indicator (in the ANCOVA-2LG model), the most crucial principal component (in the ANCOVA-1PC model), the first three principal components (in the ANCOVA-3PC model), and the first 12 principal components (in the ANCOVA-12PC model). Analyses were performed without any adjustment and with a simultaneous adjustment for age, sex, and BMI. These models were also constructed using the association analysis module of SMART. The false discovery rate (FDR) [28] was calculated to adjust for multiple tests.

In addition, permutations were performed to calculate empirical p-values to verify further the significance of the molecular features identified in the initial association tests (p < 0.05). The status of the medication and NonMed groups was randomly permuted 100,000 times, and the permutation data were analyzed using nested ANCOVA. Empirical p-values after FDR (epFDR) multiple testing correction were also calculated. A volcano plot of epFDR versus the magnitude of change (i.e., fold change) were drawn. The beta coefficient in ANCOVA was used as an indicator of fold change. A molecular feature was considered to be a crucial feature associated with the pharmacometabolic response and a potential predictor marker if it satisfied the following two criteria: (1) epFDR < 0.05 and (2) the absolute fold change > 0.3. A feature with epFDR < 0.05 but absolute fold change of 0.1–0.3 was regarded as a feature with suggestive evidence. Finally, the most significant metabolite in terms of statistical significance (epFDR) and biological significance (the absolute fold change) was evaluated in each treatment group. The metabolites that reached a significant level in most ANCOVA models were identified as the most critical metabolites.

2.9. Peak identification

Peak identification was performed for the significantly differentially expressed peaks between the medication and NonMed groups. First, the identified molecular features were compared with the mass information in the Human Metabolome Database (HMDB) [29,30] to identify metabolites according to the criteria: ESI in the positive ion mode and absolute mass tolerance of < 1 ppm. Second, an MS/MS experiment was performed to identify the molecular features’ chemical structure through QTOF-MS in the positive mode. The features were set for MS/MS fraction with collision energy from 20 to 45 eV. The RT was 7 min, and the MS range was set at 20–990. Finally, the peaks were compared with the MS/MS spectra in the HMDB.

3. Results

In this study, we collected the demographic data, including age, sex, and BMI of the patients who received and did not receive a hypertensive drug (Table 1). Patients in the medication groups...
were significantly older and had a significantly higher BMI than the NonMed group. No significant sex differences were noted between the medication and NonMed groups. For the clinical data, in addition to blood pressure (Table 1), the self-reported drug name (Supplemental Table 1) and treatment dosage were collected from the questionnaires. The relevant results are not shown because of a high proportion of missing data. Fig. 1 displays the flow and main results of all the analyses, including peak alignment, data preprocessing, quality control, batch effect detection, association analysis, and peak identification. Supplemental Fig. 1 summarizes the three detailed models and results of association analysis: 1) without any multiple testing correction, 2) with a multiple testing correction but no fold change constraint, and 3) with a multiple testing correction and a fold change constraint.

### 3.1. Peak alignment, data preprocessing, and quality control

Peak alignment was performed using MarkerLynx, and the analysis of total ion chromatograms of 1,499 replicate samples from 250 individuals provided 4,217 molecular features in the positive mode. Hierarchical cluster analysis revealed that the numbers of poorly clustered, well clustered, and perfectly clustered individuals were 15, 79, and 156, respectively. After data preprocessing, the numbers of poorly clustered, well clustered, and perfectly clustered individuals changed to 0, 11, and 239, respectively. All replicate samples from 239 perfectly clustered individuals were included in the subsequent analysis. Moreover, 14 replicate samples from the 11 well-clustered individuals that their replicate samples were not wholly clustered for the same individual; however, only 7 replicate samples (1, 1, 4, and 1 in the ACEi, ARB, CCB, and NonMed groups, respectively) had a significantly longer distance from the major group of replicate samples of the same individual. After removing the seven distant replicate samples, the data of the remaining replicate samples were realigned using MarkerLynx. Next, debrisoquine, the internal standard for normalization, was removed. Finally, 4,158 molecular features were discovered for 1,492 replicate samples (Fig. 1).

### 3.2. Abundance data substructure

The abundance data were re-normalized using SMART and by adopting the mean abundance of the internal standard of 1,492 individuals (mean = 4.6785). An identical data pre-processing process for the re-normalized data was performed. The principal component plots of the normalized abundance data were generated to detect batch effects. The results revealed more than two substructures (right-hand, upper left-hand, and lower left-hand sides) and demonstrated the subgroups in experimental months on the left-hand-side replicate samples (Fig. 2A). In addition, clustering analysis revealed a substructure of two latent groups and a potential batch effect of the experimental month (Fig. 2B).

### 3.3. Metabolome-wide association study

In association analyses, first, molecular features were selected if they met the following criteria: epFDR < 0.05 and absolute fold change > 0.3. Moreover, p-values, fold changes, and numbers of significant features in the four ANCOVAs, including ANCOVA-2LG (Supplemental Figs. 2A–2B), ANCOVA-1PC (Supplemental Figs. 3A–3B), ANCOVA-3PC (Supplemental Figure 4A–4B), and ANCOVA-12PC (Supplemental Figure 5A–5B) are provided. In total, 37 distinct molecular features were identified in at least one of the four ANCOVA models (Fig. 3A); moreover, 13 molecular features were identified in all four models (Fig. 3B and Table 2). Two-thirds of the 37 distinct molecular features were drug-specific, whereas the remaining were shared across multiple drugs. ACEis, ARBs, and DIURs shared more features identified in association analyses than CCB, partially reflecting that ACEi, ARB, and DIUR affect the same pathway: renin-angiotensin-aldosterone system. Furthermore, P2022, P716, P2022, and P408 were the most significant metabolites associated with the ACEi, ARB, CCB, and DIUR groups, respectively.

After simultaneous adjustment for age, gender, and BMI, four significant metabolites (P408, P1150, P1866, and P2022; Supplemental Table 2) with epFDR < 0.05 and absolute fold change > 0.3 were identified. P408 and P1150 were predictive markers of the response to DIURs and ACEis, respectively; P1866 of the response to ACEis and CCBs; P2022 of the response to ACEis, CCBs, and DIURs. Fold changes are provided in Supplemental Table 3. When the absolute fold change criterion was relaxed to 0.1–0.3, 96 metabolites reached the statistically significant threshold (i.e., epFDR < 0.05; Supplemental Table 4). Note that all the 13 molecular features in Table 2 are also included in this list (Supplemental Table 5). Therefore, in addition to the four significant metabolites (P408, P1150, P1866, and P2022), the remaining nine metabolites have potential roles in blood pressure regulation.

### 3.4. Peak identification

Of the 13 identified molecular features (Table 2), six were found in the HMDB, of which five were one-to-one matched, and one was one-to-multiple matched. P716 (m/z = 181.0875, RT = 1.9024) matched with propyl 2-furanacrylate (HMDB0037733), P4135 (m/z = 542.3240, RT = 2.3432) with lysoPC(20:5(5Z,8Z,11Z,14Z,17Z)/0:0) (HMDB0010397), and P1516 (m/z = 282.2792, RT = 2.9025) with oleamide (HMDB0002117); moreover, P1252 and P1382 were found to be fragments of P1516. P4141 (m/z = 543.4025, RT = 3.1376) matched with 3beta-acetoxy-11alpha-methoxy-12-ursen-28-oic acid (HMDB0031675), and P2022 (m/z = 338.3406, RT = 3.1729) with erucic acid (HMDB0002068); P1866 was found to be a fragment of P2022. P3469 (m/z = 491.3687, RT = 2.9942) one-to-multiple matched with Cillingolosin E (HMDB0034523), Barringtogenol E (HMDB0034523), and Ganoderol H (HMDB00037783).

For the remaining four molecular features, no matches were noted in the HMDB, and they were further identified by MS/MS experiment. The MS/MS data showed that P408 and P1175 exhibited fragments (m/z = 339.2516) matching with MG(15:0) + Na. The differences in the mass between molecular features and the fragments were 224.0814 and 280.2398, suggesting the possible loss of C(14:2) + NH3 and C(18:2) + NH3. P1141 and P1150 exhibited...
fragment \((m/z = 337.2352)\) matching with MG(15:1) + Na. The mass losses between these molecular features and this fragment were 278.2252 and 269.7596, suggesting that they matched with C(18:3) + NH3 and C(18:2) + NH3, respectively. Except for P3469 which demonstrated a match with multiple identities, all other metabolites had only one identity. The MS/MS spectra of the 13 molecular features are presented in Supplemental Figure 6.

4. Conclusions and discussion

This study identified 13 metabolites significantly associated with pharmacometabolic responses in all four ANCOVA models. Except for P3469, all metabolites had only one match in the HMDB. Of these 13 metabolites, P408 was significantly associated with pharmacometabolic responses to all four antihypertensive drugs. P408 was matched with diglycerides (DGs), glycerides comprising two fatty acid chains covalently bonded to a glycerol molecule through ester linkages [31]. DG levels have been noted to be higher in patients with hypertension and spontaneously hypertensive rats than in normotensive controls and the Wistar-Kyoto rats, respectively [32,33]. Vasoconstrictors such as angiotensin II, endothelin, and noradrenaline are critical in hypertension development [34–36]. These vasoconstrictors bind to G-protein-coupled receptors, stimulate the intracellular phospholipase C-mediated hydrolysis of phosphoinositol 4,5-bisphosphate, and produce inositol1,4,5-trisphosphate and DGs [37]. Therefore, this explains our result that DG levels were lower in hypertension patients taking blood pressure–lowering drugs than in those not taking any medication (NonMed) group. The metabolites P1141, P1150, and P1175 were also noted as DGs but with different fatty acids attached to the glycerol [38]. Our analysis showed that DG levels were lower in hypertension patients taking blood pressure–lowering drugs than in those not taking any medication (NonMed) group. The metabolites P1141, P1150, and P1175 were also noted as DGs but with different fatty acids attached to the glycerol [38]. Our analysis showed that DG levels were lower in hypertension patients taking blood pressure–lowering drugs than in those not taking any medication (NonMed) group. The metabolites P1141, P1150, and P1175 were also noted as DGs but with different fatty acids attached to the glycerol [38]. Our analysis showed that DG levels were lower in hypertension patients taking blood pressure–lowering drugs than in those not taking any medication (NonMed) group.
Lysophosphatidylcholine (lysoPC) (20:5(5Z,8Z,11Z,14Z,17Z)/0:0) in HMDB. LysoPC is a major lysophospholipid generated during the breakdown of lipoprotein phosphatidylcholines (PCs) through endogenous phospholipase A$_2$ activation or from the hydrolysis of oxidized PCs by the platelet-activating factor acetylhydrolase [39,40]. LysoPC plays crucial physiological and pathophysiological roles in vascular development, reproduction, myelination, neurological diseases, and cancers in humans and animals [39]. Few studies have reported that LysoPC is directly associated with blood pressure regulation [41]. Kim et al. [40] identified prehypertension-associated changes in amino acid metabolism and lysoPCs, including lysoPC(14:0), lysoPC(16:1), lysoPC(16:0), lysoPC(18:2), lysoPC(18:1), lysoPC(18:0), lysoPC(20:5), lysoPC(20:4), lysoPC(20:3), and lysoPC(22:6).
Although the mechanism of action of oleamide remains unclear, such as canola, rapeseed, mustard seed, and broccoli [49]. As mentioned, erucic acid is a 22-carbon monounsaturated omega-9 fatty acid found mainly in the Brassica family, which includes plants such as canola, rapeseed, mustard seed, and broccoli [49].

P1516, P1252, and P1382 were identified as oleamide, and their fragments were noted to be major diuretics, facilitating sodium excretion. Oleamide is a fatty acid amide primarily found in the mammalian nervous system, and it accumulates in the cerebrospinal fluid of rats after sleep deprivation [42]. The fatty acid derivatives may act as neuronal function modulators [43]. Both 5-hydroxytryptamine and gamma-aminobutyric acid (5-hydroxytryptamine) and gamma-aminobutyric acid receptors [44]. Sodium homeostasis is a major determinant of blood pressure [45]. Both 5-hydroxytryptamine and gamma-aminobutyric acid are involved in blood pressure regulation [46,47]. However, evidence of the direct relationship between oleamide provision and blood pressure change is scant [48].

P2022 and P1866 were identified as erucic acid and its fragment. Erucic acid is a 22-carbon monounsaturated omega-9 fatty acid found mainly in the Brassica family, which includes plants such as canola, rapeseed, mustard seed, and broccoli [49]. As mentioned in HMDB, erucic acid is broken down into shorter-chain fatty acids in the liver by long-chain acyl CoA dehydrogenase [50]. The association of dietary erucic acid with myocardial lipidosis has been observed in many species [51]; for instance, the association of dietary erucic acid with heart lesions has been reported in rats [51]. However, thus far, evidence on erucic acid’s association with human hypertension is limited [52]. In this study, the erucic acid levels in the medication groups (especially for ACEi and CCB groups) were lower than those in the NonMed group (with absolute fold change > 0.3), suggesting a potential relationship between erucic acid and blood pressure.

P4141 was observed to match with 3beta-acetoxy-11alpha-methoxy-12-ursen-28-oic acid, a triterpene comprising six isoprene units. Triterpenoids have been reported to have in vitro ACE inhibitory activity and in vivo antihypertensive effects in rats [53]. In the current study, P4141 was significantly associated with the ACE-related drugs ACEis and ARBs (epFDR < 0.05). Although the absolute fold change of P4141 in the ACEi group was < 0.3, the directions of the effect in both the ACEi and ARB groups were the same. P4141 abundance levels in both the ACEi and ARB groups were lower than those in the NonMed group. This may be attributed to the antihypertensive drugs’ suppression of ACE activity.

Finally, we also found that the abundance levels of propyl 2-furanacrylate (P716) were elevated in all four medication groups. Propyl 2-furanacrylate is a flavoring agent. Although the differences in P716 levels between the four medication groups and the NonMed group were significant, no report has indicated the association of this metabolite with blood pressure or hypertension. We speculate that this metabolite may be an additive product involved in pharmaceutical and medication processes. However, because of the data limitation in this study, the current study could not investigate whether this metabolite originated from drug metabolism or was contributed by other external factors such as the environment and diet. This warrants further research.

Another limitation of this study is the lack of clinical information. Further investigations are warranted to understand the potential effects of clinical factors such as medication doses and duration on pharmacometabolomic responses. This study did not collect the pretreatment data for the patients in the drug groups. The baseline in the medication and NonMed groups could not be evaluated. However, this study enrolled only Han Chinese patients using strict criteria (normal BMI, normal triglyceride level, normal high-density lipoprotein cholesterol level, and young-onset hypertension) to reduce patient heterogeneity related to ethnicity and clinical conditions. Age, sex, BMI, and potential batch effects were adjusted for to further mitigate potential confounding in the comparative analysis of the medication and NonMed groups.

In summary, the current results provide new insights into the impact of hypertensive treatment on fatty acid and other metabolic pathways related to the blood pressure response. Moreover, they shorten the knowledge gap caused by the lack of antihypertensive pharmacometabolomic studies, particularly in East Asian populations. The identified metabolites and pathways can enrich the catalog of biomarkers applied in hypertension treatment response, risk evaluation, and precision medicine.

Several antihypertensive pharmacometabolomic studies have been reported thus far. However, the findings of these studies are not reproducible [16,19–22] for various reasons, including differences in study populations, antihypertension drug types, study designs, experiment platforms, sample preparation, and follow-up durations [16]. Differential genetic background is also a crucial factor in this type of study. Most previous studies have focused on White and Black populations [19,20]; relevant research in Asian populations is lacking. This study aimed to fill this gap. The current study included only Han Chinese people with East Asian ancestry and studied additional drug classes. Our findings revealed the
potential effects of diacylglycerols, lysoPCs, oleamide, and PCs on the pharmacometabolic response to the four studied antihypertensive drugs. Our results provide insights into blood pressure regulation and potential predictive markers of pharmacometabolic responses to antihypertensive drugs, particularly for East Asian populations, especially Han Chinese individuals.

Absorption, distribution, metabolism, and excretion (ADME) of drugs are highly variable and significantly affected by an individual's characteristics. ADME varies with many factors, including ethnicity, demographic and physiological variables, food preference, social behavior, and genetic makeup. The metabolome has a major role in ADME; therefore, metabolite markers can aid in predicting disease risk and monitoring efficacy and side effects of medical treatment [54]. Here, several crucial metabolites and pathways strongly associated with the treatment response to antihypertensive drugs were identified. These pharmacometabolomic markers and profiles may serve as biomarkers of the treatment response to different antihypertensive drug types. Our results thus enrich the catalog of biomarkers of the pharmacometabolic response and precision medicine for hypertension. In particular, our results can provide supplemental information to the current clinical practice in the Hypertension Treatment Guideline, which focuses on only controlling highly variable blood pressure [55]. In general, metabolome-based precision medicine may improve clinical pharmacology and translational hypertension research further.

At present, the mechanisms underlying pharmacometabolic responses to antihypertensive medications remain unknown. Individual heterogeneity (e.g., genetic makeup) and disease heterogeneity (e.g., hypertension etiology) among patients considerably complicate this investigation. Further research with large sample sizes and multi-omics information, such as pharmacogenomics and pharmacoepigenomics is warranted to close this knowledge gap.

5. Author statement

Y.-J. Liang, K.-M. Chiang, Li-li Xiu et al. prepared the original manuscript and revision; H.C.Y. and M.L.C. performed metabolomics experiments. Y.J.L., K.M.C. H. C.Y., W.H.P. conceived the study and supervised the project. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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