Research Article

Exploration of Macro-Micro Biomarkers for Dampness-Heat Syndrome Differentiation in Different Diseases

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Increased attention is being paid to traditional Chinese medicine (TCM) as a complementary and alternative medicine to provide an effective approach for personalized diagnosis and clinical treatment. TMC performs treatment based on differentiation of TCM syndrome (ZHENG), which may identify special phenotypes by symptoms and signs of patients even if they are in different diseases. There has, however, been skepticism and criticism because syndrome classification only depends on observation, knowledge, and clinical experience of TCM practitioners, which lacks objectivity and repeatability. In order to transform syndrome classification into mainstream medicine, we introduce a macro-micro approach that combines symptoms, clinical indicators, and metabolites. The present paper explores the macro-micro biomarkers of dampness-heat syndrome in chronic hepatitis B and nonalcoholic fatty liver patients, which could provide the basis for developing a possible population-screening tool for selecting target individuals and creating an evaluation index for personalized treatment.

1. Introduction

Chronic hepatitis B (CHB) and nonalcoholic fatty liver disease (NFL) are two common diseases occurring throughout the world that have continuously increasing morbidity [1]. It is worth noting that 12.1% [2] and 37.1% [3] of patients with CHB and NFL, respectively, exhibit the same symptoms (e.g., yellow and slimy fur), which are characteristics of dampnessheat syndrome (DH) in traditional Chinese medicine (TCM). Although CHB and NFL have different etiologies in Mainstream Medicine, TCM practitioners may perform the same treatment for these patients.

Actually, different diseases may be treated similarly in TCM particularly when the same syndrome appears in these diseases [4]. In this respect, syndrome differentiation and treatment (bian zheng lun zhi) may provide some new revelations to modern personalized medicine [5–7]. Syndrome differentiation is still debated, because it depends on clinical observation and TCM practitioners' experiences, which are

thought to be subjective and unrepeatable. The success of personalized medicine relies on having accurate diagnostic tests that identify those patients who can benefit from targeted therapies [8]; thus, the ability to achieve objectivity and repeatability in TCM diagnosis would provide a greatly needed breakthrough.

Recently, researchers and scientists of TCM have explored incorporating several potentially beneficial methods, including, for example, physiology and biochemistry [9], molecular biology [10], and tongue image digitization [11, 12]. However, the classifications have been less than satisfactory. The main reason might be that these methods only focus on one or several indicators and thus cannot generalize the entire state of the syndrome. We therefore conceived the possibility of a macro-micro approach that includes a combination of metabolites, symptoms, and clinical indicators. Clinical manifestations are the macroeconomic performance, and metabolic molecules and indicators are microscopic. To serve in TCM diagnosis and treatment, here we report our findings macro-micro biomarkers of DH in CHB and NFL patients.

2. Experimental

2.1. Subjects and Experiment Design. Twenty healthy volunteers and 115 patients (60 patients for training and another 55 patients for testing) of dampness-heat syndrome chronic hepatitis B (DHHB), nondampness-heat syndrome chronic hepatitis B (NDHHB), and dampness-heat syndrome nonalcoholic fatty liver (DHFL) were enrolled in the study. The clinical study was approved by the local ethics committee and was performed in accordance with the principals contained in the Declaration of Helsinki. All individuals provided informed consent before inclusion into the study. Diagnostic standard of HB and FL patients was referred to "the guideline of prevention and treatment for chronic hepatitis B" [13] and "guidelines for management of nonalcoholic fatty liver disease: an updated and revised edition" [14]. Cases meeting the diagnostic criteria for chronic hepatitis B and nonalcoholic fatty liver, respectively, at 18-65 (39.9 mean \pm 13.5 std. dev.) years of age who signed the informed consent form were included in the study. Individuals were excluded from the study if they met any of the following criteria. (1) Cases complicated with other hepatotropic virus hepatitis and alcoholic fatty liver. (2) Chronic severe hepatitis. (3) HB and FL patients associated with serious primary disease of heart, kidney, lung, endocrine, blood, metabolic and gastrointestinal, or psychotic patients. (4) Pregnant or lactating women. A junior medical physician made the initial diagnosis and recorded the information of four traditional examinations accurately and completely. Three more senior physician (either chief or deputy physicians) subsequently confirmed the initial diagnosis by the records and gave the hierarchical results of typical degree. Only those cases that were identified as classical DH patients by both the junior and the senior physicians were included in the study to guarantee the correctness of ZHENG differentiation.

2.2. Chemicals and Drugs. N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA with 1% TMCS) and urease were purchased from Sigma-Aldrich Co. LLC (USA). Methoxyamine hydrochloride, methanol, ethanol, myristic acid, chloroform and pyridine were purchased from China National Pharmaceutical Group Corporation (Shanghai, China).

2.3. Sample Collection and Preparation. A complete physical examination was given, and the health condition was recorded on a scale including the information obtained through four traditional examinations: looking, listening and smelling, asking, and touching when the patient entered the study. Seventy-one clinical indicators and 115 contents from the four methods of examinations were acquired for the basic information.

Urine samples were collected from all subjects and were stored at -80° C until GC-MS assay. All urine samples were thawed in an ice water bath and vortex-mixed before analysis. Each 1 mL aliquot of standard mixture or urine sample was placed into a screw top tube, samples were centrifuged for



FIGURE 1: Schematic diagram of research approach for selection of DH.

10 min at (12,000 rpm), and 150 μ L supernatants were then transferred into clean screw top tubes. After adding $70 \,\mu\text{L}$ of urease (4 mg/mL) and vortex-mixing for 30 s, samples were conditioned at 37°C for 15 min to remove the urea. After the addition of 800 μ L methanol and 10 μ L of myristic acid in methanol (1mg/mL) and mixing for 1min, the solution was centrifuged at 13,000 rpm for 10 min. A 200 μ L aliquot of supernatant was then transferred into a GC vial and evaporated to dryness under N₂ at 30°C. Fifty μ L of methoxyamine in pyridine (15 mg/mL) was added to the GC vial, and vortex-mixed for 1 min, and the methoximation reaction was carried out for 90 min rocking in a shaker at 30° C, then 50 μ L of BSTFA plus 1% TMCS was added to the samples for trimethylsilylation for another 1 h at 70°C. In the final step, 30 μ L of heptane was added to the GC vial, and the solution was analyzed utilizing GC-MS after vortex for 30 s.

2.4. Data Acquisition. All GC-MS analyses were performed by a mass spectrometer 5975B (Agilent technologies, USA) coupled to an Agilent 6890 (Agilent technologies, USA) gas chromatography instrument. In the gas chromatographic system, a catabletary column (Agilent J&W DB-5 ms Ultra Inert 30 m \times 0.25 mm, film thickness 0.25 μ m) was used. Helium carrier gas was used at a constant flow rate of $1.0 \text{ mL} * \text{min}^{-1}$. One μ L of derivatized samples was injected into the GC/MS instrument, and splitless injection mode was used. A programmed column temperature was optimized to acquire a well separation. The temperatures of the injection port, the interface, and source temperature were set at 280°C, 260°C and 230°C, respectively. The measurements were made with electron impact ionization (70 eV) in the full scan mode $(m/z \ 30-550)$. The solvent post time was set to 5 min. The GC-MS operating condition was the same as the previous experiment [15] except the column temperature program.

2.5. Data Analysis. Due to experimental variation and column aging, shifts in retention time between fingerprints may occur. When the total ion current chromatograms (TICs) were obtained, peak-alignment or warping techniques are commonly applied to compensate for minor shifts in retention times. Thus, in the subsequent data processing, the same variable manifested synchronous information in every



FIGURE 2: OPLS score plot of three syndromes compared to a healthy control group by symptoms and clinical indicators. (a) OPLS score plot of control and DHHB. (b) OPLS score plot of control and DHFL. (c) OPLS score plot of control and NDHHB.

profile. Therefore, all GC-MS raw files were converted to CDF format via the Agilent MSD Workstation software, and were subsequently processed by the XCMS toolbox (http:// metlin.scripps.edu/download/) using XCMS's default settings with the following exceptions: xcmsSet (full width at halfmaximum: fwhm = 5; S/N cutoff value: snthresh = 10, max = 25), group (bw = 5). The resulting table (CSV file) was exported into Microsoft Excel (Microsoft Inc., USA) where normalization was performed prior to multivariate analyses. The resulting three-dimensional matrix involving peak index (RT-m/z pair), sample names (observations), and normalized peak area percent was introduced into Simca-P 11.5 Umetrics software (Umea, Sweden) that was used for analysis of principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares (OPLSs). Differential variables with VIP values [16] exceeding 1.5 between two different groups were generated from OPLS loadings plot. Subsequently, those variables were further analyzed by Mann-Whitney U-test to confirm the changes in metabolites by SPSS 17.0 (SPSS, Chicago, IL, USA) with the threshold P value set at 0.1. Firstly, the variables were identified by searching in NIST 2005 database. Then, standard compounds were used to confirm some of the identified metabolites.

Figure 1 shows a schematic diagram of the steps followed to determine the final list of potential biomarkers. The first step was to remove the differential information of CHB from the DHHB by removing the intersection of NDHHB and DHHB's differential information based on the ideas of the "same disease with different syndrome." The reduced set of first-step biomarkers were further filtered by taking advantage of the "different diseases with same syndrome." The final biomarkers were obtained from the intersection of the first-step DH biomarkers and biomarkers of DHFL.

3. Results

3.1. Establishment of the Potential Biomarkers of Clinical Symptoms and Indicators. All symptoms and clinical indicators were analyzed and utilized to distinguish the three syndrome groups (DHHB, NDHHB, and DHFL) and the healthy control group (control). Orthogonal partial least squares (OPLSs) was used to effectively extract variables responsible for the separation by removing variables unrelated to pathological status. Figures 2(a), 2(b), and 2(c) depict the OPLS score plots, which show that DHHB, NDHHB, and DHFL groups were clearly separated from the control group. The most meaningful characteristics were screened by OPLS loading



FIGURE 3: OPLS score plot of three syndromes compared to healthy control group by metabolites. (a) OPLS score plot of control and DHHB. (b) OPLS score plot of control and DHFL. (c) OPLS score plot of control and NDHHB.

plot analysis and are listed in Table 1. The quality of the model was characterized by two performance statistics, R^2Y (cum) and Q^2Y (cum), indicating the total explanation and predictability of the model [17]. The information of models is summarized in Table 4.

3.2. Establishment of the Potential Biomarkers of Urinary Metabolic Profiles. Urine profiles obtained from GC-MS were analyzed for distinctions among the three syndromes and the control group by OPLS. Figures 3(a), 3(b) and 3(c) indicate the OPLS score plot, which show a clear separation for DHHB, NDHHB, and DHFL groups from the control group. The most important variables for the discriminative models were screened by loading plot analysis. The potential metabolic biomarkers of each syndrome differentiated from control group were identified by the NIST database and are summarized in Table 2. Model information is summarized in Table 4.

3.3. Establishment of Potential Biomarkers of DH in CHB and FL. Because groups of selected markers may contain information of syndrome and disease, the biomarkers of DH were further filtered. Thus the final set of potential biomarkers considered were those that remained after the intersection

of DHHB and NDHHB was removed from DHHB, and were intersected with DHFL. Figure 1 shows a schematic diagram of the steps. As to the former works, the potential macromicro biomarkers were obtained from the integration of differential metabolites, hierarchical corresponding symptoms and clinical indicators. The potential biomarkers are listed in Table 3.

3.4. Preliminary Verification of Identified Biomarkers. The potential biomarkers were verified in 55 blind test cases of CHB with two Syndromes (Dampness-Heat Syndrome (DH) and Non-Dampness-Heat Syndrome (NDH)) for Syndrome classification. Using only the potential biomarkers or only the clinical symptoms and indicators did not differentiate the two syndromes satisfactorily (Figures 4(a) and 4(b)); however, by including metabolites, symptoms, and clinical indicators in the analysis, resulted in a stronger differentiation (Figure 4(c)). It is worth mentioning that former classifications (in Sections 3.1 and 3.2) were performed by supervised OPLS, owing to the complexity of clinical samples. However, the DH could be classified from NDH by unsupervised PCA in this verification with the selected biomarkers, which revealed the strong ability of DH differentiation, though they need further verification in clinical.

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TABLE 1: Significantly	different symptoms	and clinical indicator	rs identified in the three s	vndromes com	pared to a health	v control group.
				/		

Indicators and symptoms	Group	VIPa	$P(M,W)^{b}$	FN ^c
Alkaline phosphatase	Онир	1 69	0.00	+2.03
Anolinoprotein A-1	DHHB	1.09	0.00	+1 91
Aspartate aminotransferase	DHHB	1.62	0.00	+2.77
Glutamyltransferase	DHHB	1.62	0.00	+2.06
Immunoglobulin G	DHHB	2.01	0.00	+2.06
Prealbumin	DHHB	2.01	0.00	-2.56
<i>B</i> -globin	DHHB	2.0	0.00	+2.50
Thick fur	DHHB	1.81	0.00	+1 73
Bitter taste	DHHB	1.01	0.00	+1.89
Slimy and curdy fur	DHHB	1.75	0.00	-1.73
Mean corpuscular hemoglobin concentration	DHHB	1.7.5	0.00	+2.15
Tongue color	DHHB	2 15	0.00	+1.96
Basonhil	DHHB	2.13	0.00	+2.56
Fur color	DHHB	2.68	0.00	+2.58
String-like pulse	DHHB	1.81	0.00	+1 73
Alanine aminotransferase	DHFI	2.08	0.00	+2.16
Aspartate aminotransferase	DHFI	1.78	0.00	+1.82
Total cholesterol	DHFL	2.10	0.00	+1.87
Triglyceride	DHFI	2.10	0.00	+2.60
β-globin	DHFL	2.54	0.00	+2.31
Thick fur	DHFL	1.92	0.00	+1.64
Lack of strength	DHFL	2.11	0.00	+1.64
Dysphoria	DHFL	1.98	0.00	+1.67
Slimy and curdy fur	DHFL	2.58	0.00	-2.03
Uric acid	DHFL	2.45	0.00	+2.31
Glucose	DHFL	1.95	0.00	+1.92
Tongue color	DHFL	2.03	0.00	+1.68
Systolic pressure	DHFL	1.89	0.00	+2.02
Diastolic pressure	DHFL	2.29	0.00	+2.37
Fur color	DHFL	3.08	0.00	+2.58
Weight	DHFL	2.79	0.00	+2.44
String-like pulse	DHFL	1.92	0.00	+1.64
Alkaline phosphatase	NDHHB	1.61	0.00	+1.87
Apolipoprotein A-1	NDHHB	2.07	0.00	+2.39
Activated partial thromboplastin time	NDHHB	1.60	0.00	+1.68
Hepatitis B core antibody	NDHHB	2.85	0.00	+2.90
Hepatitis B core antibody-immunoglobulin M	NDHHB	1.76	0.00	+2.90
Hepatitis B surface antigen	NDHHB	3.04	0.00	+2.90
Immunoglobulin G	NDHHB	1.60	0.00	+1.97
Prealbumin	NDHHB	2.38	0.00	-2.28
Triglyceride	NDHHB	1.77	0.00	+1.85
Total protein	NDHHB	1.85	0.00	+2.01
β -globin	NDHHB	2.54	0.00	+2.79
Teeth-marked tongue	NDHHB	2.11	0.00	+1.92
Gallbladder	NDHHB	2.04	0.00	+1.92
Relaxed pulse	NDHHB	1.69	0.00	+1.56
Lack of strength	NDHHB	1.93	0.00	+1.73
Mean corpuscular hemoglobin concentration	NDHHB	1.79	0.00	+2.11
Pre-S1 antibodies	NDHHB	3.14	0.00	+2.90
Pre-S1 antigen	NDHHB	3.14	0.00	+2.90

0.00

FN^c +1.73 +1.73 +2.41 +1.72

+2.58

	IABLE I.	Continued.		
Indicators and symptoms	Group	VIP ^a	$P(M-W)^{b}$	
Luxuriant or withered tongue	NDHHB	1.73	0.00	
Soggy pulse	NDHHB	1.93	0.00	
Basophil	NDHHB	2.34	0.00	
Diastolic pressure	NDHHB	1.56	0.00	

TABLE 1: Continued.

^aVIP: variable importance in the project.

Mean platelet volume

 ${}^{b}P(M-W)$ value was obtained from Mann-Whitney test (syndromes compared to healthy control).

NDHHB

^cFN is fold change of mean ranks calculated by the Mann-Whitney test (syndromes compared to healthy control). "+" means upregulated and "-" means downregulated.

2.30



FIGURE 4: PCA score plot of DH versus NDH with potential biomarkers. (a) PCA score plot of DH and NDH only by symptoms and clinical indicators. (b) PCA score plot of DH and NDH only by metabolites. (c) PCA score plot of DH and NDH by macro-micro biomarkers from the integration of differential metabolites, hierarchical corresponding symptoms and clinical indicators.

4. Discussion

In this study, we attempted to explore the macro-micro biomarkers of DH, which could provide the feasibility and robustness for syndrome differentiation. The selected metabolites of DH were considered to be related with the pathogenesis. By analysis of KEGG (http://www.genome.jp/kegg/), the 11 metabolic markers are related to biosynthesis of secondary metabolism, microbial metabolism in diverse environments, carbon fixation pathway in prokaryote, proteins digestion and absorption, and carbohydrate digestion and absorption, which could be classified in microbial metabolism and digestive capacity. These may correspond with "the disorder in transportation and transformation of the essence from food and drink" in TCM, which was regarded as one important reason for Dampness-Heat Syndrome [18, 19].

Aspartate transaminase (AST) is the only clinical indicator in our biomarkers. It may suggest that the clinical indicators are limited to classify the syndromes. But AST has been reported to be connected to DH, with odds ratio (OR) value equal to 5.49 [20]. There is thus strong evidence that DH reflects inflammation of the liver damage.

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Actic acid DHHB 1.87 0.00 -2.07 Succinic acid ¹ DHHB 1.85 0.01 -1.61 D-Xylose ¹ DHHB 2.22 0.00 -1.86 Mahose DHHB 1.92 0.00 -1.86 Markose DHHB 1.67 0.00 -1.72 Annio levulinic acid ¹ DHHB 1.67 0.00 -1.73 Sibiol DHHB 1.27 0.01 -1.63 Renzenc ¹ DHHB 1.88 0.01 -1.56 Sibiol DHHB 1.88 0.01 -1.59 Glataconic acid ² DHHB 1.68 0.02 -1.54 Tartronic acid ² DHHB 1.58 0.02 -1.51 Schatocnic acid ² DHHB 1.61 0.02 -1.51 Schatocnic acid ⁴ DHHB 1.53 0.01 -166 Schatocnic acid ⁴ DHHB 1.61 0.02 -151 Schatocnic acid ⁴ DHH 1.58 <t< th=""><th>Compound</th><th>Group</th><th>VIP^a</th><th>$P(M-W)^{b}$</th><th>FN^c</th></t<>	Compound	Group	VIP ^a	$P(M-W)^{b}$	FN ^c
Succhine add'DHHBL850.01-1.60MalkoseDHHB1.920.00-1.96MalkoseDHHB1.870.00-1.22Annolevuline acid'DHHB1.870.00-1.23RibiolDHHB2.220.00-1.93RibiolDHHB2.220.00-1.93Romantine'DHHB1.870.01-1.63Renzen'DHHB1.880.01-1.56Sutencia caid''DHHB1.880.02-1.53RibiolDHHB1.880.02-1.53Gharaomic acid'DHHB1.740.02-1.54Tertonic acidDHHB1.750.00-1.71Annio-L24-trizzoleDHHB1.740.02-1.53PreindineDHHB1.740.02-1.53PreindineDHHB1.740.02-1.53Prindine caid'DHHB1.930.01-1.66Stando extrostic acid'DHH1.580.00-1.71Prazinoic acid'DHH1.580.00-1.73Prazinoic acid'DHFL1.510.01-1.55DefactoseDHFL1.510.01-1.55DefactoseDHFL1.510.01-1.55DefactoseDHFL1.510.00-1.70Succhic acidDHFL1.540.00-1.70Succhic acidDHFL1.540.00-1.70Succhic acidDHFL1.560.00 </td <td>Acetic acid*</td> <td>DHHB</td> <td>1.87</td> <td>0.00</td> <td>-2.07</td>	Acetic acid*	DHHB	1.87	0.00	-2.07
D-Xylos"DHHB2.220.00-1.96MaloseDHHB1.870.00-1.32Aninokevalinic acid"DHHB1.870.00-1.72Aninokevalinic acid"DHHB1.670.00-1.93SchindDHHB1.670.00-1.93Creatinine"DHHB1.880.01-1.63Benzene"DHHB1.880.01-1.562.Nutencic acid"DHHB1.880.02-1.53(R)-Mandelic acid"DHHB1.880.02-1.53(R)-Mandelic acid"DHHB1.880.02-1.53(R)-Mandelic acid"DHHB1.880.02-1.51Stantonic acidDHHB1.740.02-1.51Stantonic acidDHHB1.740.02-1.513-Anino 1.2.4-triazoleDHHB1.730.01-1.663-Indole acento acid"DHHB1.520.00-2.003-Anino 1.2.4-triazoleDHHB1.520.00-2.003-Indole acento acid"DHHB1.520.00-1.703-Anino 1.2.4-triazoleDHHB1.520.00-1.703-Indole acento acid"DHHB1.580.00-1.70Succinca acidDHHL1.520.00-1.70Succinca acid*DHHL1.540.00-1.70Succinca acid*DHHL1.540.00-1.70Succinca acid*DHHL1.540.00-1.70Succinca acid*DHHL	Succinic acid*	DHHB	1.85	0.01	-1.61
MahoseDHHB1920.00+1.89Butyrate"DHHB1.670.00-1.72RibidDHHB1.670.00-1.73RibidDHHB1.220.00-1.93Creatinine"DHHB1.880.01-1.56Subtenci caid"DHHB1.880.02-1.51Gutaconic acid"DHHB1.680.02-1.51Gutaconic acidDHHB1.680.02-1.51Tartronic acidDHHB1.680.02-1.51Jandelic sciat"DHHB1.580.00-1.71Jartronic acidDHHB1.580.00-1.71Janino-1,2,4-triazoleDHHB1.580.00-1.71Jandei butanci caid"DHHB1.580.00-1.70Jandole caid"DHHB1.580.00-1.70Jandole caid"DHHB1.580.00-1.70Succinic acid"DHH1.580.00-1.70Jandole caid"DHHL1.580.00-1.70Jandole caid"DHHL1.580.00-1.70Jandole caid"DHHL1.510.01-1.66Jandole caid"DHHL1.580.00-1.70Jandole caid"DHHL1.580.00-1.70Jandole caid"DHHL1.510.01-1.66Jandole caid"DHHL1.510.00-1.70Jandole caid"DHHL1.580.00-1.70Jandole caid" <td< td=""><td>D-Xylose*</td><td>DHHB</td><td>2.22</td><td>0.00</td><td>-1.96</td></td<>	D-Xylose*	DHHB	2.22	0.00	-1.96
Batyrae"DHHB1870.00-172Aminolevulinic acid"DHHB1670.00-173RibiolDHHB1220.00-133Creatrine"DHHB1770.01-1.65Enzene"DHHB1.810.02-1.53(D)-Mandelic acid"DHHB1.680.02-1.51Glutaconic acidDHHB1.680.02-1.51Glutaconic acidDHHB1.680.02-1.51Fartronic acidDHHB1.680.02-1.51Fartronic acidDHHB1.740.02-1.51Shahoonca caid"DHHB1.750.00-1.71Shahoonca caid"DHHB1.740.02-1.51Shahoonca caid"DHHB1.530.01-1.66Shahoonca caid"DHHB1.530.00-1.73Shahoonca caid"DHH1.520.00-1.70Shanoora caid"DHH1.520.00-1.73Shahoonca caid"DHH1.520.00-1.70Succinci acid"DHH1.520.00-1.70Succinci acid"DHH1.540.00-1.59DefactoseDHH1.540.01-1.61DefactoseDHH1.540.00-1.79Baryate"DHH1.540.00-1.79Succinci acidDHH1.540.00-1.79DefactoseDHH1.540.00-1.70Sutyate"DHH1.54 <td>Maltose</td> <td>DHHB</td> <td>1.92</td> <td>0.00</td> <td>+1.89</td>	Maltose	DHHB	1.92	0.00	+1.89
Aminoleulinic acidDHHBL670.00-1.75RibitolDHHB2.220.01-1.43Creatinine*DHHBL880.01-1.56Suttencic acid*DHHBL880.02-1.51Gluazonic acid*DHHBL880.02-1.51Gluazonic acid*DHHBL880.02-1.51BernophenoneDHHBL580.02-1.51FirdineDHHBL580.02-1.51Jandoi Cacid*DHHBL740.02-1.51Jandoi Cacid*DHHBL750.00-1.71Janino L,2,4 triazoleDHHBL920.00-1.51Jandoi Cacid*DHHBL920.00-1.51Jandoi Cacid*DHHBL920.00-1.51Jandoi Cacid*DHHBL920.00-1.51Jandoi Cacid*DHHBL920.00-1.51Standoi Cacid*DHHBL920.00-1.51Pyrazinoic acid*DHHLL580.00-1.70Succinic acid*DHHLL540.01-1.65D-Gluaconic acidDHFLL540.01-1.65D-Gluaconic acidDHFLL540.01-1.65D-Gluaconic acidDHFLL540.00-1.70Succinic acidDHFLL540.00-1.70Gluaracic acid*DHFLL540.00-1.62D-Gluaconic acidDHFLL560.00-1.72Jandole Cacid*	Butyrate*	DHHB	1.87	0.00	-1.72
RbiolDHHB2.220.00-1.93Creatinine'DHHB1.770.01-1.63Benzere'DHHB1.880.01-1.562.Butenoic acid'DHHB1.880.02-1.51(D)Mandelic acid'DHHB1.680.01-1.59Glutaconic acid'DHHB1.880.02-1.51Tartronic acidDHHB1.780.00-2.10BenzophenoneDHHB1.740.02-1.513.Indole butanoic acid'DHHB1.740.02-1.513.Indole butanoic acid'DHHB1.580.00-1.733.Indole butanoic acid'DHHB1.580.00-1.663.Indole butanoic acid'DHHB1.520.00-1.70Succinic acid'DHHB1.520.00-1.70Succinic acid'DHFL1.510.01-1.59D-GalactoseDHFL1.540.00-1.70Succinic acidDHFL1.540.00-1.70Succinic acidDHFL1.540.00-1.70Succinic acidDHFL1.540.00-1.70Succinic acidDHFL1.540.00-1.70Succinic acidDHFL1.540.00-1.70Succinic acidDHFL1.540.00-1.70Gutaconic acidDHFL1.540.00-1.70Gutaconic acidDHFL1.650.00-1.71Gutaconic acidDHFL1.680.00 <td< td=""><td>Aminolevulinic acid[*]</td><td>DHHB</td><td>1.67</td><td>0.00</td><td>-1.75</td></td<>	Aminolevulinic acid [*]	DHHB	1.67	0.00	-1.75
Creatinine* DHHB 1.77 0.01 -1.63 Benzene* DHHB 1.88 0.01 -1.53 (R)-Mandelic acid* DHHB 1.68 0.01 -1.53 (R)-Mandelic acid* DHHB 1.68 0.02 -1.53 Tartronic acid DHHB 1.68 0.02 -1.53 Tartronic acid DHHB 1.28 0.00 -2.10 Benzophenone DHHB 1.75 0.00 -1.71 3-Anino-1,2,4 triazole DHHB 1.75 0.00 -1.71 3-Anino-1,2,4 triazole DHHB 1.73 0.01 -1.66 3-Indole trainoic acid* DHHB 1.58 0.00 -1.71 3-Indole acetic acid* DHHB 1.58 0.00 -1.70 3-Indole acetic acid* DHHB 1.58 0.00 -1.71 3-Indole acetic acid* DHHE 1.52 0.00 -2.00 Acetic acid* DHFL 1.52 0.00 -1.75 D-Galacotac DHFL 1.54 0.00 -1.59 Benzoic acid <td< td=""><td>Ribitol</td><td>DHHB</td><td>2.22</td><td>0.00</td><td>-1.93</td></td<>	Ribitol	DHHB	2.22	0.00	-1.93
Bernsene"DHHB1.880.01-1.562-Butnoic acid"DHHB1.810.02-1.53(D)-Mandelic acid"DHHB1.680.02-1.54Glutaconic acidDHHB1.680.02-1.54Tartronic acidDHHB1.580.00-2.10BenzophenoneDHHB1.750.00-1.71S-Annico-1.2.4-triazoleDHHB1.750.00-1.713-Indole butanoic acid"DHHB1.740.02-1.533-Indole butanoic acid"DHHB1.610.02-1.503-Indole butanoic acid"DHHB1.580.00-1.70Scacinic acid"DHHB1.620.00-1.70Succinic acid"DHFL1.520.00-1.70Succinic acidDHFL1.580.00-1.75D-GlactoseDHFL1.580.00-1.79Succinic acidDHFL1.540.01-1.59Butyrate"DHFL1.540.01-1.59Butyrate"DHFL1.540.00-1.70Glucarita acidDHFL1.540.00-1.70Glucarita acidDHFL1.540.00-1.70Glucarita acidDHFL1.540.00-1.70Glucarita acidDHFL1.540.00-1.70Glucarita acidDHFL1.540.00-1.70Glucarita acidDHFL1.660.00-1.70Glucarita acidDHFL1.680.00 <td>Creatinine*</td> <td>DHHB</td> <td>1.77</td> <td>0.01</td> <td>-1.63</td>	Creatinine*	DHHB	1.77	0.01	-1.63
2 Butenoic acidDHHB1.810.02-1.53(R)-Mandelic acid*DHHB1.680.01-1.59Tartronic acidDHHB1.680.02-1.54Tartronic acidDHHB1.580.02-1.51BenzophenoneDHHB1.750.00-1.713-Anino-1,2,4-triazoleDHHB1.750.00-1.713-Anino-1,2,4-triazoleDHHB1.610.02-1.501-Cyclobexene carboxylic acidDHHB1.630.01-1.663-Indole butanoic acid*DHHB1.580.00-1.703-Indole carcia caid*DHHB1.580.00-1.73Pyrazinoc acid*DHHE1.580.00-1.70Scacchiz acid*DHFL1.580.00-1.70Scacchiz acid*DHFL1.590.00-1.70D-FructoseDHFL1.590.00-1.59D-GalactoseDHFL1.510.01-1.69D-Guica caidDHFL1.510.01-1.69CaractiaDHFL1.510.00-1.70Amino levulinic acid*DHFL1.570.01-1.61Creatinic*DHFL1.650.00-1.70Amino levulinic acid*DHFL1.630.00-1.70Amino levulinic acid*DHFL1.630.00-1.70Amino levulinic acid*DHFL1.640.00-1.70Glucaric acidDHFL1.650.00-1.71Sindole butanoic	Benzene*	DHHB	1.88	0.01	-1.56
(R)-Mandelic acid" DHHB 1.68 0.01 -1.59 Glutaconic acid" DHHB 1.68 0.02 -1.54 Glutaconic acid" DHHB 1.28 0.00 -2.10 Benzophenone DHHB 1.75 0.00 -1.71 Janito 1, 2,4-triazole DHHB 1.75 0.00 -1.71 Janito 1, 2,4-triazole DHHB 1.74 0.02 -1.51 Janito 1, 2,4-triazole DHHB 1.61 0.02 -1.51 Jandole butanoic acid" DHHB 1.62 0.00 -1.70 Actici acid" DHHB 1.52 0.00 -1.70 Succinic acid" DHFL 1.52 0.00 -1.70 Succinic acid" DHFL 1.58 0.00 -1.70 Succinic acid DHFL 1.58 0.00 -1.70 Succinic acid DHFL 1.58 0.00 -1.70 Succinic acid DHFL 1.54 0.01 -1.55 D-Gluconic acid DHFL 1.54 0.01 -1.62 D-Gluconic acid <td< td=""><td>2-Butenoic acid*</td><td>DHHB</td><td>1.81</td><td>0.02</td><td>-1.53</td></td<>	2-Butenoic acid*	DHHB	1.81	0.02	-1.53
Glutaconic acid DHHB 1.68 0.02 -1.54 Tartronic acid DHHB 2.28 0.00 -2.10 Benzophenone DHHB 1.58 0.02 -1.53 Pteridine DHHB 1.75 0.00 -1.71 3.Amino-1,2.4-triazole DHHB 1.61 0.02 -1.53 3.Indole butanoic acid* DHHB 1.63 0.00 -1.73 3.Indole butanoic acid* DHHB 1.58 0.00 -1.73 Pyrazinoic acid* DHHB 1.58 0.00 -1.73 Synzanico acid* DHHFL 1.52 0.00 -1.73 Dyrazinoic acid* DHFL 1.58 0.00 -1.79 Succinic acid* DHFL 1.58 0.00 -1.79 D.Galactose DHFL 1.51 0.01 -1.65 D.Gluconic acid DHFL 1.54 0.00 -1.70 Amino levulinic acid* DHFL 1.54 0.00 -1.20 Gultaconic acid <td< td=""><td>(R)-Mandelic acid*</td><td>DHHB</td><td>1.68</td><td>0.01</td><td>-1.59</td></td<>	(R)-Mandelic acid*	DHHB	1.68	0.01	-1.59
Tartronic acid DHHB 2.28 0.00 -2.10 Benzophenone DHHB 1.58 0.02 -1.51 3-Indole butanoic acid* DHHB 1.75 0.00 -1.71 3-Indole butanoic acid* DHHB 1.74 0.02 -1.51 3-Indole butanoic acid* DHHB 1.93 0.01 -1.66 3-Indole acetic acid* DHHB 1.58 0.00 -1.71 Pyrazinoic acid* DHHB 1.52 0.00 -1.70 Succinic acid* DHFL 1.90 0.00 -1.80 D-Fouctose DHFL 1.52 0.00 -1.75 D-Galactose DHFL 1.58 0.00 -1.75 D-Galactose DHFL 1.54 0.01 -1.59 Butyrate* DHFL 1.54 0.00 -1.80 Glucanic acid DHFL 1.54 0.00 -1.82 Glucanic acid DHFL 1.54 0.00 -1.20 Succinic acid* DHFL 1.57 0.01 -1.61 Caluarate DHFL 1.	Glutaconic acid*	DHHB	1.68	0.02	-1.54
Intervaluation DHIB L58 0.02 -1.13 Bernzphenone DHHB 1.75 0.00 -1.71 3-Amino-1,24-triazole DHHB 1.74 0.02 -1.51 3-Indole butanoic acid* DHHB 1.61 0.02 -1.50 1-Cyclohexene carboxylic acid DHHB 1.62 0.00 -1.73 Standou cacid* DHHB 1.62 0.00 -1.66 Standou cacid* DHHE 1.52 0.00 -1.73 Succinic acid* DHFL 1.90 0.00 -1.81 D-Fractose DHFL 1.58 0.00 -1.75 D-Galactose DHFL 1.51 0.01 -1.59 Benzoit acid DHFL 1.51 0.00 -1.82 Glutarate DHFL 1.54 0.00 -1.82 Glutarate DHFL 1.65 0.00 -1.63 S-Indole acid* DHFL 1.65 0.00 -1.23 Glutarate DHFL 1.65<	Tartronic acid	DHHB	2.28	0.00	-2.10
Drift Drift Drift Drift Drift 3 Amino-1,2,4-triazole DHHB 1.74 0.02 -1.51 3 Indole burnoic acid" DHHB 1.64 0.02 -1.50 1-Cyclohexene carboxylic acid DHHB 1.93 0.01 -1.66 3-Indole burnoic acid" DHHB 1.52 0.00 -2.20 Acetic acid" DHFL 1.52 0.00 -1.70 Succinic acid" DHFL 1.58 0.00 -1.81 D-Fructose DHFL 1.69 0.00 -1.81 D-Fructose DHFL 1.51 0.01 -1.65 D-Galactose DHFL 1.51 0.01 -1.62 D-Gluconic acid DHFL 1.51 0.01 -1.61 D-Gluconic acid DHFL 1.57 0.01 -1.61 Clutarate DHFL 1.57 0.01 -1.61 Clutarate DHFL 1.63 0.00 -2.09 Glutarate DHFL 1.	Benzophenone	DHHB	1.58	0.02	-1.53
Armitor DHR 1.53 0.00 -1.51 3-Amino-1,2,4-triazole DHHB 1.61 0.02 -1.51 3-Indole butanoic acid" DHHB 1.61 0.02 -1.51 3-Indole acetic acid" DHHB 1.63 0.00 -1.73 Pyrazinois acid" DHHE 1.52 0.00 -2.00 Acetic acid" DHFL 1.52 0.00 -1.70 Succinic acid" DHFL 1.52 0.00 -1.70 Succinic acid" DHFL 1.58 0.00 -1.81 D-Fauctose DHFL 1.51 0.01 -1.69 Batyrate" DHFL 1.51 0.01 -1.69 Butyrate" DHFL 1.54 0.00 -1.70 Amino levulinic acid DHFL 1.54 0.00 -1.81 Calutare DHFL 1.54 0.00 -1.62 Glutarate DHFL 1.65 0.00 -1.62 Glutarate DHFL 1.65	Pteridine	DHHB	1.35	0.00	-1.71
Drinkov, J., P. C. M. (2000) Drink Drink <th< td=""><td>3-Amino-1 2 4-triazole</td><td>DHHB</td><td>1.73</td><td>0.02</td><td>-1.51</td></th<>	3-Amino-1 2 4-triazole	DHHB	1.73	0.02	-1.51
Drive outside Drifter	3-Indole butanoic acid*	DHHB	1.7 1	0.02	-1.50
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	1-Cyclobevene carboxylic acid	DHHB	1.01	0.02	-1.66
Difference Differenc Differenc Differenc	3-Indole acetic acid*	DHHB	1.55	0.00	-1.73
Printing and Acetic acid ^a DHFL 1.52 0.00 -1.50 Succinic acid ^a DHFL 1.90 0.00 -1.81 D-Fructose DHFL 1.58 0.00 -1.75 D-Galactose DHFL 1.58 0.00 -1.99 Benzoic acid DHFL 1.51 0.01 -1.59 Defluconic acid DHFL 1.54 0.00 -1.70 Amino levulinic acid [*] DHFL 1.54 0.00 -1.81 O-Gluconic acid DHFL 1.54 0.00 -1.62 Gultarate DHFL 1.65 0.00 -1.63 Gultarate DHFL 1.65 0.00 -1.63 Gulcaric acid DHFL 1.65 0.00 -1.63 Sindole acetic acid [*] DHFL 1.65 0.00 -1.63 Sindole acetic acid [*] DHFL 1.68 0.00 -1.68 1-Mole batanoic acid [*] DHFL 1.76 0.00 -1.71 Sindole batanoic acid [*]	Pyrazinoje acid*	DHHB	1.50	0.00	-2.00
Active and DFIL 1.22 0.00 -1.70 Succinic acid* DHFL 1.52 0.00 -1.81 D-Fructose DHFL 1.58 0.00 -1.99 Benzoic acid DHFL 1.51 0.01 -1.59 Butyrate* DHFL 1.51 0.01 -1.65 D-Gluconic acid DHFL 1.54 0.00 -1.82 Glutarate DHFL 1.54 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.65 0.00 -1.69 Glucaric acid DHFL 1.65 0.00 -1.61 S-Indole acetic acid* DHFL 1.65 0.00 -2.09 S-Indole butanoic acid* DHFL 1.76 0.00 -1.73 S-Indole butanoic acid* DHFL 1.79 0.00 -2.10 Glutacoci acid* DHFL 1.77 <td>A cetic acid*</td> <td>DHEI</td> <td>1.52</td> <td>0.00</td> <td>_1.70</td>	A cetic acid*	DHEI	1.52	0.00	_1.70
Sutchine actu DHFL 1.50 0.000 -1.35 D-Fructose DHFL 1.58 0.00 -1.75 D-Galactose DHFL 1.51 0.01 -1.59 Butyrate* DHFL 1.51 0.00 -1.70 Amino levulinic acid DHFL 1.51 0.00 -1.70 Amino levulinic acid* DHFL 1.54 0.00 -1.82 Glutarate DHFL 1.57 0.01 -1.61 Creatinine* DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.63 0.00 -2.02 3-Indole acetic acid* DHFL 1.76 0.00 -1.72 Glucaric acid* DHFL 1.76 0.00 -1.73 3-Indole batanoic acid* DHFL 1.77 0.00 -1.71 Chobexenecarboxylic acid DHFL	Succipic acid*	DHEI	1.52	0.00	-1.70
Driftuctose Drift 1.55 0.00 -1.79 De-Galactose DHFL 1.51 0.01 -1.59 Butyrate* DHFL 1.51 0.00 -1.79 Butyrate* DHFL 1.54 0.00 -1.70 Amino levulinic acid* DHFL 1.54 0.00 -1.82 Glutarate DHFL 1.65 0.00 -1.82 Glucaric acid DHFL 1.65 0.00 -1.82 Glucaric acid DHFL 1.65 0.00 -1.82 Glucaric acid DHFL 1.63 0.00 -2.02 3-Indole acetic acid* DHFL 1.63 0.00 -2.02 3-Indole butanoic acid* DHFL 1.68 0.00 -1.81 Pseudouridine DHFL 1.68 0.00 -1.81 Pseudouridine DHFL 1.63 0.01 -1.57 Pdicaconic acid DHFL 1.51 0.01 -1.57 Prexinoic acid* DHFL 1.63 <td>D Eructore</td> <td>DHEI</td> <td>1.50</td> <td>0.00</td> <td>-1.81</td>	D Eructore	DHEI	1.50	0.00	-1.81
Drivitation DHFL 1.59 0.00 -1.59 Benzoic acid DHFL 1.51 0.01 -1.59 Butyrate* DHFL 1.51 0.00 -1.70 Amino levulinic acid DHFL 1.51 0.00 -1.70 Amino levulinic acid* DHFL 1.54 0.00 -1.82 Glutartae DHFL 1.65 0.00 -1.61 Creatinine* DHFL 1.65 0.00 -1.62 Glucaric acid DHFL 1.63 0.00 -2.02 SI-Indole acetic acid* DHFL 1.63 0.00 -2.09 (R)-Mandelic acid* DHFL 1.63 0.00 -1.61 SI-Indole butanoic acid* DHFL 1.68 0.00 -1.61 Pscudouridine DHFL 1.72 0.00 -2.10 Glutaconic acid* DHFL 1.63 0.00 -1.61 Pscudouridine DHFL 1.63 0.00 -1.73 Pyrazinoic acid* DHFL	D Calactore	DHEI	1.50	0.00	-1.73
DHFL 1.11 0.01 -1.57 D-Gluconic acid DHFL 1.54 0.01 -1.65 D-Gluconic acid DHFL 1.54 0.00 -1.82 Glutarate DHFL 1.57 0.01 -1.61 Creatinine* DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.63 0.00 -2.02 3-Indole acetic acid* DHFL 1.76 0.00 -2.02 3-Indole butanoic acid* DHFL 1.76 0.00 -2.02 3-Indole butanoic acid* DHFL 1.68 0.00 -1.61 Pseudouridine DHFL 1.76 0.00 -1.71 Glutaconic acid* DHFL 1.79 0.00 -1.71 Glutaconic acid* DHFL 1.68 0.00 -1.61 Pseudouridine DHFL 1.51 0.01 -1.72 Glutaconic acid DHFL 1.51 0.01 -1.73 Prazinoic acid* DHFL 1.53 0.00<	D-Galactose Bonzoic acid	DHEI	1.09	0.00	-1.99
Duryrae DHRL 1.54 0.01 -1.69 D-Gluconic acid DHFL 1.51 0.00 -1.70 Amino levulinic acid* DHFL 1.57 0.01 -1.61 Glutarate DHFL 1.65 0.00 -1.69 Glucaric acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.63 0.00 -2.02 3-Indole acetic acid* DHFL 1.72 0.00 -2.02 3-Indole acetic acid* DHFL 1.76 0.00 -1.75 3-Indole butanoic acid* DHFL 1.68 0.00 -1.81 Pseudouridine DHFL 1.79 0.00 -2.10 Glutaconic acid* DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.53 0.01 -1.72 Quotic acid DHFL 1.53 0.00 -1.73 Pyrazinoic acid* DHFL </td <td>Delizoic acid</td> <td>DIFL</td> <td>1.51</td> <td>0.01</td> <td>-1.39</td>	Delizoic acid	DIFL	1.51	0.01	-1.39
D-Futconic acid DHPL 1.51 0.00 -1.70 Amino levulinic acid* DHPL 1.57 0.01 -1.61 Creatinine* DHFL 1.65 0.00 -1.72 Glutarate DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.63 0.00 -2.02 3-Indole actic acid* DHFL 1.76 0.00 -2.02 3-Indole actic acid* DHFL 1.68 0.00 -1.68 1-Cyclohexencarboxylic acid DHFL 1.68 0.00 -1.81 Pseudouridine DHFL 1.77 0.00 -1.71 Tetradecanoic acid* DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.53 0.01 -1.57 Pyrazinoic acid* DHFL 1.53 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -1.73 Qiycine* NDHHB	Dulyrate	DIFL	1.54	0.01	-1.65
Animo levulinic add DHFL 1.54 0.00 -1.62 Glutarate DHFL 1.57 0.01 -1.61 Creatinine* DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -2.02 3-Indole acetic acid* DHFL 1.63 0.00 -2.02 3-Indole butanoic acid* DHFL 1.68 0.00 -1.68 1-Schole acetic acid* DHFL 1.68 0.00 -2.09 (R)-Mandelic acid* DHFL 1.68 0.00 -1.68 3-Indole butanoic acid* DHFL 1.68 0.00 -1.61 S-Indole bexenecarboxylic acid DHFL 1.68 0.00 -1.61 1-Schoexenecarboxylic acid DHFL 1.68 0.00 -1.71 Glutaconic acid* DHFL 1.77 0.00 -1.72 Glutaconic acid* DHFL 1.53 0.01 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -1.83 2,22-trifluoroethanone DHFL 1.63 0.00 -1.83	D-Gluconic acid	DIFL	1.51	0.00	-1.70
Onticate DHFL 1.57 0.01 -1.61 Creatinine* DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.65 0.00 -2.02 3-Indole acetic acid* DHFL 1.72 0.00 -2.09 (R)-Mandelic acid* DHFL 1.76 0.00 -1.73 3-Indole acetic acid* DHFL 1.76 0.00 -1.73 3-Indole butanoic acid* DHFL 1.68 0.00 -1.68 1-Cyclobexenecarboxylic acid DHFL 1.86 0.00 -1.81 Pseudouridine DHFL 1.77 0.00 -1.71 Glutaconic acid* DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -1.83 2,22-trifluoroethanone 0.01 -1.57 0.00 +1.82 I-Glycine*	Chitanata	DIFL	1.54	0.00	-1.82
Creatmine DHFL 1.65 0.00 -1.69 Gulonic acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.63 0.00 -2.09 3-Indole acetic acid* DHFL 1.72 0.00 -2.09 (R)-Mandelic acid* DHFL 1.76 0.00 -1.75 3-Indole acetic acid* DHFL 1.68 0.00 -1.68 I-Cyclohexenecarboxylic acid DHFL 1.68 0.00 -1.81 Pseudouridine DHFL 1.77 0.00 -1.71 Glutaconic acid* DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pteridine DHFL 1.53 0.00 -2.19 1-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB </td <td>Giularale</td> <td>DIFL</td> <td>1.57</td> <td>0.01</td> <td>-1.61</td>	Giularale	DIFL	1.57	0.01	-1.61
Gutanic acid DHFL 1.65 0.00 -1.72 Glucaric acid DHFL 1.63 0.00 -2.02 3-Indole acetic acid* DHFL 1.72 0.00 -2.02 3-Indole acetic acid* DHFL 1.76 0.00 -2.02 3-Indole butanoic acid* DHFL 1.76 0.00 -1.75 3-Indole butanoic acid* DHFL 1.68 0.00 -1.68 1-Cyclohexenecarboxylic acid DHFL 1.86 0.00 -1.81 Pseudouridine DHFL 1.77 0.00 -1.71 Glutaconic acid* DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -1.73 Pyrazinoic acid* DHFL 1.63 0.00 -1.73 Pyrazinoic acid* DHFL 1.63 0.00 -1.73 Qiycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB </td <td>Creatinine</td> <td>DHFL</td> <td>1.65</td> <td>0.00</td> <td>-1.69</td>	Creatinine	DHFL	1.65	0.00	-1.69
Chicard acid DHFL 1.65 0.00 -2.02 3-Indole aceita caid* DHFL 1.72 0.00 -2.09 (R)-Mandelic acid* DHFL 1.76 0.00 -1.75 3-Indole butanoic acid* DHFL 1.68 0.00 -1.68 1-Cyclohexenecarboxylic acid DHFL 1.86 0.00 -1.81 Pseudouridine DHFL 1.77 0.00 -2.10 Glutaconic acid* DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -1.73 Pyrazinoic acid* DHFL 1.63 0.00 -1.73 Qicne* DHFL 1.63 0.00 -1.83 2.22-triflororethanone DHFL 1.63 0.00 +1.82 Glycine* NDHHB	Guionic acid	DIFL	1.05	0.00	-1.72
3-Indole actic acid DHFL 1.72 0.00 -2.09 (R)-Mandelic acid* DHFL 1.76 0.00 -1.75 3-Indole butanoic acid* DHFL 1.68 0.00 -1.68 1-Cyclohexenecarboxylic acid DHFL 1.86 0.00 -1.81 Pseudouridine DHFL 1.77 0.00 -2.10 Glutaconic acid* DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.77 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pteridine DHFL 1.53 0.00 -2.19 1-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 1.89 0.02 +1.56 Glycine* NDHHB 1.89 0.02 +1.56 Z,3-Butanedione NDHHB<		DHFL	1.63	0.00	-2.02
(R)-Mandelic acid DHFL 1.76 0.00 -1.75 3-Indole butanoic acid* DHFL 1.68 0.00 -1.68 1-Cyclohexenecarboxylic acid DHFL 1.86 0.00 -1.81 Pseudouridine DHFL 1.79 0.00 -2.10 Glutaconic acid* DHFL 1.77 0.00 -1.74 Ethylene DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pyrazinoic acid* DHFL 1.53 0.00 -2.19 1-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.15 0.01 +1.62 D-Xylose* NDHHB 1.87 0.00 +1.85 Ribiol NDHHB 1.89 0.02 +1.56 73-Butanedione NDHHB 1.94 0.05 +1.42 74-Tionic acid NDHHB 1.94 0.05 +1.42	3-Indole acetic acid	DHFL	1.72	0.00	-2.09
3-Indole butanoic acid DHFL 1.68 0.00 -1.68 I-Cyclohexenecarboxylic acid DHFL 1.86 0.00 -1.81 Pseudouridine DHFL 1.79 0.00 -2.10 Glutaconic acid* DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pyrazinoic acid* DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -2.19 I-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.62 D-Sylose* NDHHB 1.89 0.01 -1.57 Sibitol NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB </td <td>(R)-Mandelic acid</td> <td>DHFL</td> <td>1./6</td> <td>0.00</td> <td>-1./5</td>	(R)-Mandelic acid	DHFL	1./6	0.00	-1./5
I-Cyclonexenecarboxylic acidDHFL1.86 0.00 -1.81 PseudouridineDHFL1.79 0.00 -2.10 Glutaconic acid*DHFL1.77 0.00 -1.71 Tetradecanoic acidDHFL1.68 0.00 -1.74 EthyleneDHFL1.51 0.01 -1.57 PteridineDHFL1.57 0.00 -1.73 Pyrazinoic acid*DHFL1.53 0.00 -1.73 Pyrazinoic acid*DHFL1.63 0.00 -1.83 2,2,2-trifluoroethanoneDHFL1.63 0.00 -1.83 Glycine*NDHHB2.11 0.01 $+1.62$ D-Sylose*NDHHB2.15 0.01 -1.57 D-Gluconic acidNDHHB1.87 0.00 $+1.85$ RibitolNDHHB2.19 0.01 -1.57 Z,3-ButanedioneNDHHB1.89 0.02 $+1.56$ Tartronic acidNDHHB2.21 0.00 -1.66 Vanillylmandelic acidNDHHB1.94 0.05 $+1.42$	3-Indole butanoic acid	DHFL	1.68	0.00	-1.68
Pseudouridine DHFL 1.79 0.00 -2.10 Glutaconic acid* DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pteridine DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -2.19 I-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.94 0.05 +1.42 Yamino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	I-Cyclonexenecarboxylic acid	DHFL	1.86	0.00	-1.81
Glutaconic acid DHFL 1.77 0.00 -1.71 Tetradecanoic acid DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pteridine DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.57 0.00 -2.19 I-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -2.19 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Pseudouridine	DHFL	1.79	0.00	-2.10
letradecanoic acid DHFL 1.68 0.00 -1.74 Ethylene DHFL 1.51 0.01 -1.57 Pteridine DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.57 0.00 -2.19 1-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -2.19 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Glutaconic acid	DHFL	1.77	0.00	-1.71
Ethylene DHFL 1.51 0.01 -1.57 Pteridine DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -2.19 1-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	letradecanoic acid	DHFL	1.68	0.00	-1.74
Pteridine DHFL 1.57 0.00 -1.73 Pyrazinoic acid* DHFL 1.53 0.00 -2.19 I-(I-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Ethylene	DHFL	1.51	0.01	-1.57
Pyrazinoic acid DHFL 1.53 0.00 -2.19 l-(1-Benzyl-1H-indol-3-yl)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone DHFL 1.63 0.00 -1.83 Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 2.21 0.00 -1.66 Vanillylmandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Pteridine	DHFL	1.57	0.00	-1.73
I-(I-BenzyI-IH-indol-3-yI)- DHFL 1.63 0.00 -1.83 2,2,2-trifluoroethanone NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 2.21 0.00 -1.66 VanillyImandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Pyrazinoic acid	DHFL	1.53	0.00	-2.19
Glycine* NDHHB 2.11 0.01 +1.62 D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 2.21 0.00 -1.66 VanillyImandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	l-(l-Benzyl-IH-indol-3-yl)- 2.2.2-trifluoroethanone	DHFL	1.63	0.00	-1.83
D-Xylose* NDHHB 2.15 0.01 -1.57 D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 2.21 0.00 -1.66 VanillyImandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Glvcine*	NDHHB	2.11	0.01	+1.62
D-Gluconic acid NDHHB 1.87 0.00 +1.85 Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.66 Tartronic acid NDHHB 2.21 0.00 -1.66 Vanillylmandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	D-Xvlose*	NDHHB	2.15	0.01	-1.57
Ribitol NDHHB 2.19 0.01 -1.57 2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 2.21 0.00 -1.66 VanillyImandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -148	D-Gluconic acid	NDHHB	1.87	0.00	+1.85
2,3-Butanedione NDHHB 1.89 0.02 +1.56 Tartronic acid NDHHB 2.21 0.00 -1.66 VanillyImandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2-4-triazole NDHHB 2.31 0.03 -1.48	Ribitol	NDHHB	2.19	0.01	-1.57
Tartronic acidNDHHB2.210.00-1.66Vanillylmandelic acidNDHHB1.940.05+1.423-Amino-1.2-4-triazoleNDHHB2.310.03-1.48	2.3-Butanedione	NDHHB	1.89	0.02	+1.56
VanillyImandelic acid NDHHB 1.94 0.05 +1.42 3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Tartronic acid	NDHHB	2.21	0.00	-1.66
3-Amino-1.2.4-triazole NDHHB 2.31 0.03 -1.48	Vanillylmandelic acid	NDHHB	1.94	0.05	+1 42
	3-Amino-1,2,4-triazole	NDHHB	2.31	0.03	-1.48

^aVIP: variable importance in the project.

^b *P*(M-W) value was obtained from Mann-Whitney test (syndromes compared to healthy control). ^cFN is fold change of mean ranks calculated by the Mann-Whitney test (syndromes compared to healthy control). "+" means upregulated and "–" means downregulated. * These metabolites were identified by NIST library and standards; others were only identified by NIST library.

TABLE 3: List of the macro-micro biomarkers of DH in CHB and NFL.

Biomarkers	Category
(R)-mandelic acid	Metabolites
1-Cyclohexenecarboxylic acid	Metabolites
3-Indole acetic acid	Metabolites
3-Indole butanoic acid	Metabolites
Acetic acid	Metabolites
Amino levulinic acid	Metabolites
Butyrate	Metabolites
Creatinine	Metabolites
Glutaconic acid	Metabolites
Pteridine	Metabolites
Pyrazinoic acid	Metabolites
Succinic acid	Metabolites
Aspartate aminotransferase	Indicators
Thick fur	Symptoms
Slimy and curdy fur	Symptoms
Tongue color	Symptoms
Fur color	Symptoms
String-like pulse	Symptoms

TABLE 4: Summary of the modeling quality of OPLS analysis.

Name	No ^a	$R^2 X_{cum}^{b}$	$R^2 Y_{cum}^{c}$	$Q^2 Y_{cum}^{d}$
1A	$1P + 1O^{e}$	0.24	0.97	0.91
1B	1P + 1O	0.16	0.96	0.78
1C	1P + 1O	0.17	0.98	0.93
2A	1P + 2O	0.50	0.89	0.70
2B	1P + 3O	0.56	0.90	0.48
2C	1P + 3O	0.49	0.91	0.57

^aNo represents the number of components.

 $^{b,c}R^2X_{cum}$ and R^2Y_{cum} represent the cumulative sum of squares (SSs) of all the X's and Y's explained by all extracted components.

 ${}^{d}Q^{2}Y_{cum}$ is an estimate of how well the model predicts the *Y*'s.

^e1P + IO: one predictive component and one orthogonal component for establishing the OPLS model.

Tongue diagnosis is of great importance for syndrome differentiation in TCM, determining the treating principle, prescribing a formula, and predicting the prognosis [21]. Except string-like pulse, other differential symptoms are the characterization of tongue, which is one of the direct objective bases for TCM clinical diagnosis and treatment. In our opinion, pulse diagnosis is as important as tongue diagnosis, so ultimately a more comprehensive analysis for the combination of them is needed.

Although only metabonomics was utilized in this study, we suggest that it would be valuable to expand beyond Metabonomics to system biology owing to the similarity between the various omics. Including a full System Biology approach to the determination of informative biomarkers will provide a more comprehensive and accurate syndrome differentiation. We thus suggest that genes, proteins, metabolites, and clinical information should all be integrated in future analyses.

5. Conclusion

This study is the first time that biomarkers of DH were obtained by a macro-micro approach with the integration of omic and clinical information to provide an effective and objective and repeatable approach for Chinese personalized medicine. Moreover, the preliminary verification indicated the feasibility and robustness of the approach for dampnessheat syndrome differentiation. Thus, these DH biomarkers could be used to provide a foundation on which we can to develop a possible population-screening tool for selecting target individuals and for creating an evaluation index for personalized treatment based on syndrome differentiation.

Authors' Contribution

Jianye Dai, Shujun Sun, and Jinghua Peng contributed equally to this work and should be considered cofirst authors.

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