Elsevier Editorial System(tm) for Journal of Ethnopharmacology
Manuscript Draft

Manuscript Number: JEP-D-10-00094R1

Title: Application of bioactivity database of Chinese herbal medicine on the therapeutic prediction, drug development, and safety evaluation

Article Type: Full Length Article

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*Cover Letter

Dear Dr. Guo,

We have revised the manuscript (JEP-D-10-00094) according to reviewers' comments. Our point-by-point reply to the reviewers' comments is described as follows.

Please handle our manuscript at your convenience. Thank you very much.

Sincerely yours,

Tin-Yun Ho

Journal of Ethnopharmacology
AUTHOR CHECKLIST

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Revised manuscripts

- **Have you addressed each remark from the referees?** ☐

Revision notes (JEP-D-10-00094)

Reviewer #1

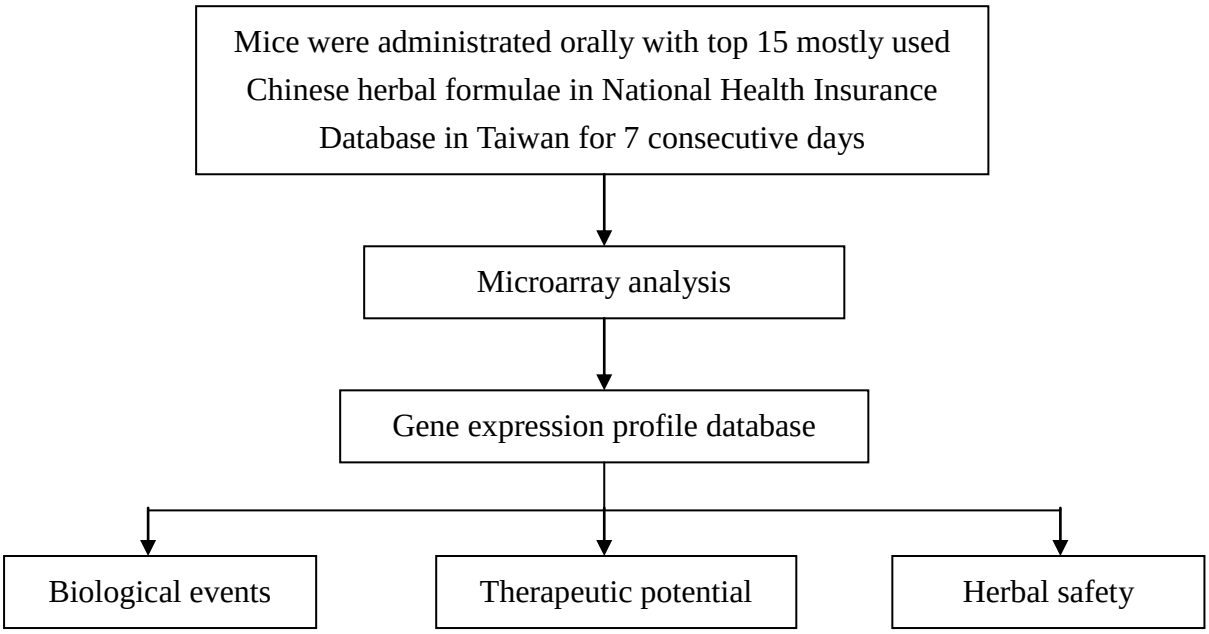
1. We have requested the phytochemical profile (including HPLC and TLC data) of each formula from a GMP pharmaceutical company, Sun Ten Pharmaceutical Co, in Taiwan. These profiles have been deposited in Molecular Biology Laboratory, School of Chinese Medicine, China Medical University. We have supplemented this statement in the "Materials and Methods" section (p. 6, line 13).
2. In this study, we would like to analyze the biological events induced by herbal formulae, predict the therapeutic potentials of formulae, and evaluate the safety of formulae. Therefore, the dose (150 mg/kg) for animal study is equivalent to the dose (9 g/adult) for human usage. We have supplemented this statement in the "Materials and Methods" section (p. 6, line -7).
3. The organs from three mice were combined into a single sample and the number of microarray replicates was three. We have supplemented this statement in the "Materials and Methods" section (p. 7, line 4).
4. We have revised the writing according to reviewer's suggestion.
   - "large-scaled" → "large-scale" (p. 4, lines -7 and -10)
   - shown "on" Table → shown "in" Table (p. 6, line -10; p. 11, line -6)

Reviewer #2

1. Table 1 shows the constituents of each herbal formula and the ration of each constituent in the formula. The herbal constituents are represented by scientific names (genus and species) and the non-herbal constituents are expressed by English names.
2. We have supplemented the ethnopharmacological usage of each formula in Table 1.
3. We have supplemented the detail information about the preparation of each formula in Table 1.
4. We have revised the figure legends to make them more informative (p. 26-27). We also have supplemented the interpretation of each figure in "Materials and Methods" and "Results" sections.
5. In this study, we would like to analyze the biological events induced by herbal formulae, predict the therapeutic potentials of formulae, and evaluate the safety of formulae. Therefore, the dose (150 mg/kg) for animal study is equivalent to the dose (9 g/adult) for human usage. Additionally, organs from three mice were combined into a single sample and the number of microarray replicates was three. We have supplemented this statement in the "Materials and Methods" section (p. 6, line -7; p. 7, line 4).
6. The aim of this report is to apply transcriptomic tools as a novel platform of translational medicine for Chinese herbal medicine. We illustrate the usefulness of this platform by analyzing the biological events induced by formulae, predicting the therapeutic potential of formulae, and evaluating the safety of formulae. Additionally, the linkage of other literature studies and our microarray analysis also show the reliability of bioactive database. Therefore, this platform will be used not only for the understanding of therapeutic mechanisms involving Chinese herbal medicine and gene interactions, but also for the new theories in drug discovery.
7. We have revised the writing according to reviewer's suggestion.
   - Title: Application of bioactivity database of Chinese herbal medicine on the therapeutic prediction, drug development, and safety evaluation (p. 1)
   - have "be" used → have "been" used (p. 4, line 15)
   - "Mouse experiments…" → "Mouse protocols…." (p. 6, line 10)

Graphical Abstract

We applied transcriptomic tools as a novel platform of translational medicine for Chinese herbal medicine. The usefulness of this platform was illustrated by analyzing the biological events induced by formulae, predicting the therapeutic potential of formulae, and evaluating the safety of formulae.



Abstract

Aim of the study: Chinese herbal medicine has been used for the treatments of various diseases for years. However, it is often difficult to analyze their biological activities and molecule mechanisms because of their complex nature. In this study, we applied DNA microarray to analyze the biological events induced by herbal formulae, predict the therapeutic potentials of formulae, and evaluate the safety of formulae.

Materials and methods: Mice were administrated orally with 15 formulae for 7 consecutive days, and the gene expression profiles in liver or kidney were further analyzed by transcriptomic tools.

Results: Our data showed that most formulae altered the metabolic pathways, such as glutathione metabolism and oxidative phosphorylation, and regulatory pathways, such as antigen processing and presentation and insulin-like growth factor signaling pathway. By comparing the gene expression signatures of formulae with those of disease states or drugs, we found that mice responsive to formula treatments might be related to disease states, especially metabolic and cardiovascular diseases, and drugs, which exhibit anti-cancer, anti-inflammatory, and anti-oxidative effects. Moreover, most formulae altered the expression levels of cytochrome p450, glutathione S-transferase, and UDP glycosyltransferase genes, suggesting that caution should be paid to possible drug interaction of these formulae. Furthermore, the similarities of gene expression profiles between formulae and toxic chemicals were low in kidney, suggesting that these formulae might not induce nephrotoxicities in mice.

Conclusions: This report applied transcriptomic tools as a novel platform of translational medicine for Chinese herbal medicine. This platform will not only for understanding the therapeutic mechanisms involving herbal formulae and gene interactions, but also for the new theories in drug discovery.

Keywords: Chinese herbs, formulae, DNA microarray, transcriptomic

Application of bioactivity database of Chinese herbal medicine on the therapeutic prediction, drug development, and safety evaluation

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Abbreviations: SHXXT, San-Huang-Xie-Xin-Tang; PBS, phosphate-buffered saline; MeSH, Medical Subject Headings; GSTs, glutathione S-transferases; UGT, UDP glycosyltransferase; EDGE, Environment, Drugs and Gene Expression; IGF,

insulin-like growth factor; CCCTS, Chuan-Chiong-Chaa-Tyau-Saan; MHC-I, major histocompatibility complex class I.

Abstract

Aim of the study: Chinese herbal medicine has been used for the treatments of various diseases for years. However, it is often difficult to analyze their biological activities and molecule mechanisms because of their complex nature. In this study, we applied DNA microarray to analyze the biological events induced by herbal formulae, predict the therapeutic potentials of formulae, and evaluate the safety of formulae.

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Conclusions: This report applied transcriptomic tools as a novel platform of translational medicine for Chinese herbal medicine. This platform will not only for understanding the therapeutic mechanisms involving herbal formulae and gene interactions, but also for the new theories in drug discovery.

Keywords: Chinese herbs, formulae, DNA microarray, transcriptomic

1. Introduction

Chinese herbal medicine has been used for the treatments of various diseases for years. Extracts prepared from medicinal plants and other natural sources contain a variety of molecules with potent biological activities. Unfortunately, it is often difficult to analyze the biological activities of these extracts because of their complex nature and the possible interaction of their components. Therefore, the genome-wide expression monitoring system with high-density microarrays may provide a simple way to test biochemical effects of herbs and thereby gain insights into their potential beneficial effects and negative side effects.

DNA microarray is a popular research and screening tool for differentially expressed genes (Schena et al., 1995). Although certain limitations of the current technology exist and have become more apparent during the past couple of years, their ability to monitor the expression of thousands of genes simultaneously is unsurpassed (Draghici et al., 2006). Microarray-based gene expression patterns have been used as fingerprints of cellular physiology and cancer researches (Altenhein et al., 2006; Bild et al., 2006; Pittman et al., 2004). The large-scale gene expression analysis of toxin-treated cells and animals has yielded a highly accurate capacity to recognize the toxic potentials of novel drug candidates (Ganter et al., 2005). Moreover, the large-scale gene expression profile has been applied to identify the disease target for drug development (Whitfield et al., 2006). Furthermore, the therapeutic efficacy can be predicted on the basis of gene expression signatures *in vitro* (Gunther et al., 2003).

Chinese herbal medicine has been applied for the treatments of diseases or served as corroborants to improve general physical weakness and fatigue for thousands of years. The clinical trials of herbal formulae have demonstrated their efficacies in

patients with illness. However, the molecular mechanisms and molecular effects of most herbal formulae are still unclear. Microarray data have been used to characterize the mechanisms of herbal formulae or herbal compounds. For examples, PC-SPES is used among patients with prostate carcinoma as an alternative to conventional forms of therapy. Biochemical assays and clinical observations suggest that the cytotoxic effects of PC-SPES are mediated at least in part through estrogenic activity (DiPaola et al., 1998). Microarray analysis indicates that alternations in specific genes involved in modulating the cell cycle, cell structure, and androgen response may also be responsible for PC-SPES-mediated cytotoxicity (Bonham et al., 2002). San-Huang-Xie-Xin-Tang (SHXXT) has been used to treat gastritis, gastric bleeding, and peptic ulcers. Microarray data show that SHXXT exhibits an anti-proliferation effect via p53 and DNA damage signaling pathways. Moreover, Rhizoma Coptis shares a similar gene expression profile with SHXXT, suggesting that Rhizoma Coptis is the principle herb that exerts the major effect in SHXXT (Cheng et al., 2008). EGb 761, a well-defined extract from *Ginkgo biloba*, has been widely used in patients with cerebral disorders. Transcriptomic analysis shows that EGb 761 alters unique pathways and regulates the expressions of some specific neuronal receptor genes exclusively in frontal cortex (Su et al., 2009). Vanillin is one of the most widely used flavor compounds in food and personal products. Microarray data show that vanillin exhibits the anticancer potential by the regulation of cell cycle and apoptosis. Moreover, its regulation may involve the suppression of a central molecule, activator protein 1 (Cheng et al., 2007). Quinoclamine is a chemically synthesized naphthoquinone compound. Comprehensive evaluation of quinoclamine by transcriptomic analysis shows that quinoclamine is a novel nuclear factor-κB inhibitor with anti-cancer potential (Cheng, et al., 2009).

In this study, we applied DNA microarray to analyze the transcriptomic patterns of top 15 mostly used Chinese herbal formulae in National Health Insurance Database in Taiwan. Our data showed that transcriptomic analysis of formulae can be used as a novel platform of translational medicine to analyze the biological events, predict the therapeutic potentials, and evaluate the safety of herbal formulae.

2. Materials and methods

2.1. Animal experiments

Mouse protocols performed in all experiments have been approved by the China Medical University Animal Ethics Committee. Fifteen herbal formulae were purchased from GMP pharmaceutical company (Sun Ten Pharmaceutical Co., Taipei, Taiwan). The phytochemical profiles of herbal formulae have been deposited in Molecular Biology Laboratory, School of Chinese Medicine, China Medical University. The composition and ethnopharmacological usage of each formula is shown in Table 1. For formula groups, three mice were administered orally with one formula (150 mg/kg), which was resuspended in phosphate-buffered saline (PBS, 137 mM NaCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 2.7 mM KCl, pH 7.2), for 7 consecutive days. The dose for mouse study is equivalent to the dose (9 g/adult) for human usage. For mock group, three mice were administrated orally with the same volume of PBS for 7 consecutive days. Mice were then sacrificed for RNA extraction.

2.2. Total RNA isolation

Total RNA was extracted from liver or kidney using a RNeasy Mini kit (Qiagen, Valencia, CA, USA). Total RNA was quantified using the Beckman DU800

spectrophotometer (Beckman Coulter, Fullerton, CA, USA). Samples with A260/A280 ratios greater than 1.8 were further evaluated using Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA sample with a RNA integrity number greater than 7.0 was accepted and the RNA samples from three mice were combined into a single sample for microarray analysis.

2.3. Microarray and pathway analysis

Microarray analysis was performed as described previously (Hsiang et al., 2009). Briefly, fluorescent amplified RNA targets were hybridized to the Mouse Whole Genome OneArray™ (Phalanx Biotech Group, Hsinchu, Taiwan), and the fluorescent signals on the array were scanned by an Axon 4000 scanner (Molecular Devices, Sunnyvale, CA, USA). Number of replicates was three. The fluorescent intensity of each spot was analyzed by genepix 4.1 software (Molecular Devices, Sunnyvale, CA, USA). The signal intensity of each spot was corrected by subtracting background signals in surroundings. We filtered out spots that signal-to-noise ratio was less than 1 or control probes. Spots that passed these criteria were normalized by the limma package of the R program (Smyth, 2005). Normalized data were analyzed using the "geneSetTest" function implemented in the "limma" package of R program to detect groups of up-regulated genes in biological pathways. This function computes a *p*-value to test the hypothesis that the selected genes tend to be up-regulated. Then, we defined the score of each pathway in formula treatments as follows: score = -log (*p*) if *p*-value $\leqq$ 0.5 or score = log (2(1-*p*)) if *p*-value > 0.5. Three-hundred and fifty-two pathways, including Kyoto Encyclopedia of Genes and Genomes pathways (http://www.genome.jp/kegg/pathway.html) and PathArt™ pathways (http://www.jubilantbiosys.com/pathart.html), were extracted from ArrayTrack

(http://www.fda.gov/nctr/science/centers/toxicoinformatics/ArrayTrack/) and used in this analysis. Finally, the scores of pathways were displayed using TIGR Multiexperiment Viewer (http://www.tm4.org/index.html) (Eisen et al., 1998).

2.4. Connection analysis of formulae-altered genes and diseases-altered genes

To connect the gene expression profiles of herbal formulae with those of diseases, we built the diseases-genes gene sets from the genetic association database (Becker et al., 2004) according to Medical Subject Headings (MeSH) terms (http://www.nlm.nih.gov/mesh/meshhome.html). There are 735 MeSH disease terms used in this analysis. We performed the "geneSetTest" to detect groups of differentially-expressed genes in MeSH disease terms. This function computes a *p*-value to test the hypothesis that the selected genes tend to be differentially expressed. Then, we calculated the score of each MeSH disease term in formula treatments as the negative logarithm of its *p*-value computed by "geneSetTest" function and ranked the MeSH disease terms in descending of MeSH disease term scores in each formula treatment. We calculated the average rank for each MeSH disease term in 15 formula treatments and the weighted MeSH disease term scores in each formula treatment were calculated by multiplying the original MeSH disease term scores with a ratio of the rank of MeSH disease terms over the average rank of those MeSH disease terms.

2.5. Connectivity Map analysis of formulae-altered genes and small molecules-altered genes

To connect the expression signatures of formulae-regulated genes with those of drugs-regulated genes, we analyzed gene expression profiles by the Connectivity Map

(Lamb et al., 2006; Lamb, 2007). We first converted the symbols of top 500 up-regulated and down-regulated genes in each formula group into Affymetrix ID according to U133A probe set. Then, we uploaded and analyzed these gene lists on the Connectivity Map (http://www.broad.mit.edu/cmap/). Using Connectivity Map, an imported query was compared with predefined signatures of therapeutic compounds and ranked according to a connectivity score, representing relative similarity to the imported gene list.

2.6. Analysis of expression levels of genes associated drug metabolism and toxicity

The expression levels of phase I drug metabolism genes, including alcohol dehydrogenases, aldehyde dehydrogenases and cytochrome P450 families genes, and phase II drug metabolism genes, including glutathione S-transferases (GSTs), sulfotransferase families and UDP glycosyltransferase (UGT) families genes, in formula-treated kidney were analyzed. Furthermore, we compared the alterations in gene expression resulting from formula treatments and chemical exposures. The transcriptional profiles of 22 chemicals and 223 treatment conditions built in Environment, Drugs and Gene Expression (EDGE) website (http://edge.oncology.wisc.edu/edge.php) were used in this analysis (Hayes et al., 2005b). The comparison were performed by hierarchical clustering analysis and visualized by TIGR Multiexperiment Viewer.

3. Results

3.1. Pathway analysis of formulae-regulated gene expression profiles in livers

We elucidated the gene expression profiles of livers responsive to 15 formula

treatments by transcriptomic analysis. The "geneSetTest" function was used to test which biological pathways were transcriptionally regulated by formulae in livers. Scores of pathways calculated using aforementioned method were then visualized by TIGR Multiexperiment Viewer. As shown in Fig. 1, >90% biological pathways were regulated by formulae. The scores of metabolism-associated pathways were positive in most formula treatments, indicating that most formulae could upregulate the metabolism processes. Additionally, these formulae shared several pathways in common in livers. For examples, low-density lipoprotein signaling pathway involved in atherosclerosis, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation involved in biosynthesis of secondary metabolites, and serum response factor-mediated pathway involved in cell adhesion were negatively regulated by all formulae. Regulatory pathways, including antigen processing and presentation, complement and coagulation cascades, limonene and pinene degradation, metabolism of xenobiotics by cytochrome p450 and type I diabetes mellitus, were positively regulated by all formulae. Insulin-like growth factor (IGF) signaling pathway, Gas6 signaling pathway, and prothymosin signaling pathway, which are involved in cell cycle, and retinoic acid signaling pathway and Wnt signaling pathway, which are involved in neurogenesis, were upregulated by all formulae. Moreover, metabolic pathways, including glutathione metabolism, oxidative phosphorylation and tyrosine metabolism, were also upregulated by all formulae. These findings suggested that these formulae might alter similar biological pathways in livers.

3.2. Gene expression connection between formula treatments and disease states

We next tested groups of regulated genes in MeSH disease terms by the "geneSetTest", and the weighted MeSH disease term scores of each formula treatment

were calculated to interpret whether the mice treated with these formulae were related to disease states. Among 735 tested MeSH disease terms, top 30 disease terms were selected, and their weighted MeSH disease term scores were visualized by TIGR Multiexperiment Viewer. As shown in Fig. 2, half of diseases belonged to metabolic and cardiovascular diseases, such as diabetes, myocardial infarction, hyperlipoproteinemia, thrombosis, hypolipoproteinemia, diabetic angiopathies, hypertriglyceridemia, hyperlipidemia, coronary restenosis, arteriosclerosis, and carotid artery diseases. Additionally, gene expression signatures of neuroskeletal diseases, such as Parkinson disease, apnea, dyskinesias, eclampsia, and seizures, were related to formula treatments. These findings suggested that mice responsive to formula treatments might be related to disease states, especially metabolic and cardiovascular diseases.

3.3. Gene expression connection between formula treatments and drugs

To further interpret whether the formula treatments in livers were similar to those induced by drugs, we connected the gene expression signatures by Connectivity Map. The top 500 up- and down-regulated genes were selected and analyzed to obtain the connectivity score. Top five drugs that exhibited highly connectivity scores in each formula were selected and further ranked in the order of the gene expression similarities with 15 formulae. As shown in Table 2, six compounds, including fulvestrant, 17-allylamino-geldanamycin, sirolimus, staurosporine, LY-294002 and resveratrol, were ranked top five drugs in at least five formulae. Fulvestrant, 17-allylamino-geldanamycin, and staurosporine have been known to exhibit the anti-cancer effects (Chia and Gradishar, 2008; Sharp and Workman, 2006; Stepczynska et al., 2001). Sirolimus is an immunosuppressant drug and resveratrol

exhibits a number of beneficial health effects, such as anti-aging, anti-inflammatory, and antioxdiative effects (Baur and Sinclair, 2006; Morelon et al., 2001). These findings suggested that mice responsive to formula treatments might be similar to drugs, which exhibit anti-cancer, anti-inflammatory, and antioxidative effects.

3.4. Formulae affected the expression levels of several genes involved in drug metabolism

To evaluate whether formula treatments affected drug metabolism in liver, we analyzed the expression levels of genes encoding phase I and II drug metabolism enzymes. Most of genes encoding cytochrome p450 family 4, GSTs, and UGTs, were significantly altered by formulae (Fig. 3). ALDH6A1, CYP24A1, CYP2B13, CYP2C38, SFT1B1, and UGT2A1 genes were down-regulated by most formulae, while UGT1A1 gene was up-regulated by most formulae. Among 15 formulae, Chuan-Chiong-Chaa-Tyau-Saan (CCCTS) upregulated the expression levels of almost all genes involved in drug metabolism. These findings suggested that most formulae altered the expression levels of CYP4, GSTs, and UGT genes. Moreover, CCCTS differed from other formulae in upregulating most genes involved in drug metabolism.

3.5. Analysis of nephrotoxicities of formulae

To analyze the nephrotoxicities of 15 formulae in kidneys, we compared the gene expression profiles of 223 chemical treatments with those of 15 formula treatments. One thousand five hundred and eighteen genes were selected from EDGE websites, and the comparison was performed by hierarchical clustering analysis. As shown in Fig. 4, the distances among formula treatments, except CCCTS, were close to each other and very far from most chemical treatments. Furthermore, the gene expression

patterns between chemical and formula treatments were different. Therefore, these findings suggested that these formulae might not induce nephrotoxicities in mice.

4. Discussion

In this study, we analyzed the gene expression profiles of 15 Chinese herbal formulae in mice by transcriptomic tools. The gene expression signatures were further applied to analyze the biological events induced by formulae, to predict the therapeutic potentials of formulae, and to evaluate the safety of formulae. By pathway analysis, we found that more than 90% of biological pathways were regulated by formulae. In addition to the metabolic pathways, formulae upregulated several regulatory pathways, such as antigen processing and presentation and IGF signaling pathways. Major histocompatibility complex class I (MHC-I) genes, such as H2-Q1, H2-Q2, H2-Q5, H2-Q6, H2-Q7, H2-Q8, H2-K1 and H2-T23 genes, in the antigen processing and presentation pathway were upregulated by all formulae in the livers. MHC-I antigen presentation pathway is active in most cell types to present peptides which are synthesized in the cells on the cell surface. MHC-I antigens play critical roles in the interaction between tumors and immune system by presenting tumor-associated peptides to cytotoxic T cells and regulating the cytotoxic activity of natural killer cells. Additionally, MHC-I antigen presents viral peptides to cytotoxic T cells, evoking the protective immunity to viral infection (Purcell and Elliott, 2008). It has been shown that Xiao-Qing-Long-Tang inhibits influenza virus and ganciclovir-resistant human cytomegalovirus through stimulation of mucosal immune system and induction of interferon-β, respectively (Murayama et al., 2006; Nagai and Yamada, 1998). Moreover, Ge-Gen-Tang suppresses the replication of influenza

viruses by increasing body temperature, enhancing the phagocytic activity of macrophages, and increasing interleukin-12 level (Kurokawa et al., 2002; Muraoka et al., 2004). All formulae upregulated the MHC-I antigen presentation pathway, suggesting that these formulae might exhibit anti-viral and anti-cancer effects through MHC-I pathway.

By comparing the similarities of gene expression signatures between formulae and disease states, we found that mice responsive to formula treatments might be related to metabolic, cardiovascular, neuroskeletal, and hepatic diseases, such as diabetes, myocardial infarction, arteriosclerosis, Parkinson disease, liver cirrhosis, and chronic hepatitis C virus infection. Indeed, Liow-Wey-Diyh-Huang-Wan has been used for the treatment of diabetes and anti-aging for a long time (ChPC, 2000). Xiao-Chia-Hu-Tang and Yin-Chen-Hao-Tang have been used to treat liver diseases, such as hepatitis and liver cirrhosis. Moreover, administration of Jia-Wey-Shiau-Yau-Saan is effective against the tremor associated with Parkinsonism (Ishikawa et al., 2000). Previous studies indicate that IGF-1 is associated with diabetes-associated, cardiovascular, and hepatic diseases. It has been shown that levels of IGF-1 in the blood are related to the risks of arteriosclerosis, type II diabetes, and myocardial infarction (Sievers et al., 2008). Additionally, increasing the level of IGF has been shown to suppress liver cirrhosis and to exhibit hepatoprotective effects (Tutau et al., 2008). Our findings showed that all formulae positively regulated the IGF signaling pathways and upregulated the expression of IGF-1 gene. These data suggested that these formulae might be used for the metabolic, cardiovascular, neuroskeletal, and hepatic diseases through the regulation of IGF signaling pathway.

We further linked the formulae-altered genes associated with drugs or compounds by Connectivity Map. Connectivity Map is a method that compares lists of

differential expressed genes to a library of experiments assessing the effects of small molecules and genetic events on gene expression (Lamb et al., 2006; Lamb, 2007). Connectivity Map finds connections among molecules sharing similar mechanisms of action. By connecting the gene expression signatures of formulae with those of drugs, we found that gene expression profiles induced by formulae were similar to those induced by anti-cancer, anti-inflammatory, anti-oxidative, and anti-diabetic drugs. For examples, fulvestrant is used for the treatment of hormone receptor-positive metastatic breast cancer in postmenopausal women (Chia and Gradishar, 2008). 17-Allylamino-geldanamycin that binds heat shock protein 90 has shown a promising anti-tumor activity in preclinical studies (Sharp and Workman, 2006). Staurosporine is a potent protein kinase C inhibitor and has the potential for anti-cancer (Stepczynska et al., 2001). Sirolimus is an immunosuppressant drug that blocks the signal transduction pathway required for cytokine-stimulated T cells replication and has been used to prevent rejection in organ transplantation and certain autoimmune disorders (Morelon et al., 2001). Resveratrol exhibits a number of beneficial effects, such as anti-cancer, anti-aging, neuroprotective, antioxidant, and anti-angiogenic effects (Baur and Sinclair, 2006). Troglitazone is an insulin sensitizer and a peroxisome proliferator-activated receptor γ agonist and has been used for diabetes (Ghanim et al., 2001). Based on the similarity of gene expression profile, we proposed that 15 formulae might display the anti-cancer, anti-inflammatory, antioxidative, and anti-diabetic effects in mice.

The expression levels of genes encoding phase I and phase II drug metabolism enzymes in formulae-treated mice were analyzed. Most of genes encoding cytochrome p450, GSTs, and UGTs were significantly altered by formulae. Cytochromes p450 are external monooxygenases that catalyze the incorporation of a

single atom of molecular oxygen into a substrate with the concomitant reduction of the other atom to water. Cytochrome p450 is involved in the biotransformation of drugs, the bioconversion of xenobiotics, the metabolism of chemical carcinogens, and the degradation of herbicides and insecticides (Bernhardt, 2006). Many chemical compounds which can induce or inhibit the cytochrome p450 enzyme function can also alter the metabolism of themselves or other compounds (Elbarbry et al., 2008). GSTs are considered to contribute to the phase II biotransformation of xenobiotics. GSTs conjugate xenobiotics with reduced glutathione to facilitate the solubility of xenobiotics in the aqueous cellular and extracellular environments and therefore enhance the excretion of xenobiotics. This activity is useful for the detoxification of endogenous compounds, such as peroxidised lipids (Hayes et al., 2005a; Margis et al., 2008). UGT superfamily is comprised of 2 families (UGT1 and UGT2) and 3 subfamilies (UGT1A, UGT2A, and UGT2B). A glucuronidation reaction catalyzed by UGT enzymes makes the drug molecule more hydrophilic and thus the drug molecule tends to be excreted easily (Ouzzine et al., 2003). Glucuronidation usually abolishes the pharmacological activity in most cases. The upregulation of UGTs by formulae might increase UGT-mediated glucuronidation and improve the drug excretion. Among 15 formulae, CCCTS differed from others in upregulating almost all genes involved in drug metabolism. CCCTS has been used for the treatment of common cold in traditional Chinese medicine. CCCTS consists of eight herbs, including *Glycyrrhiza uralensis*, *Ligusticum chuanxiong*, *Notopterygium incisium*, *Angelica dahurica*, *Mentha haplocalyx*, *Asarum sieboldii*, *Schizonepeta multifida* and *Saposhnikovia divaricata*, and five out of eight herbs have been shown to alter drug metabolism. For examples, falcarindiol from *Notopterygium incisium* induces phase II drug-metabolizing enzymes (Ohnuma et al., 2009). *Glycyrrhiza uralensis*,

Ligusticum chuanxiong, *Notopterygium incisium*, *Angelica dahurica*, and *Saposhnikovia divaricata* modulate cytochrome p450 activity and participate in the interaction with conventional drugs (Ioannides et al., 2002; Tang et al., 2006). Therefore, our findings suggested that caution should be paid to possible drug interactions of these formulae, especially CCCTS.

The nephrotoxicities of formulae were further evaluated by comparing the gene expression profiles between formulae and other chemicals established in EDGE. The pattern of transcriptional activity is a highly sensitive indicator of chemical exposure and can be used as diagnostic fingerprint to predict toxicity and/or carcinogenicity (Thomas et al., 2001). EDGE is a prototype resource for sharing toxicogenomics information and used to develop algorithms for efficient chemical classification and hazard prediction (Hayes et al., 2005b). By comparing the gene expression signatures, we found that the distances of most formulae were close to each other and far from chemicals treatments, such 2,3,7,8-tetrachlorodibenzo-p-dioxin, phenylhydrazine, lipopolysaccharide, and phenobarbital . These data suggested that the nephrotoxicities of these formulae might be ignored. However, among 15 formulae, the distance of CCCTS was far from other formulae. The aristolochic acid I, which is a known nephrotoxin, is found in *Asarum sieboldii*, might explain why CCCTS displayed the different transcriptomic pattern with others (Jong et al., 2003).

5. Conclusion

In conclusion, this report applied transcriptomic tools as a novel platform of translational medicine for Chinese herbal medicine. The usefulness of this platform was illustrated by analyzing the biological events induced by formulae, predicting the

therapeutic potential of formulae, and evaluating the safety of formulae. This platform will be used not only for the understanding of therapeutic mechanisms involving Chinese herbal medicine and gene interactions, but also for the new theories in drug discovery.

Acknowledgements

This work was supported by grants from National Research Program for Genomic Medicine, National Science Council, and Committee on Chinese Medicine and Pharmacy at Department of Health (CCMP 96-RD-201 and CCMP 97-RD-201), Taiwan.

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Figure captions

Fig. 1. Pathway analysis of gene expression profiles in livers responsive to formula treatments. Normalized data were analyzed using the "geneSetTest" to compute a *p*-value and to detect groups of up-regulated genes in biological pathways. The score of each pathway in formula treatments was defined as follows: score = -log (*p*) if *p*-value ≦ 0.5 or score = log (2(1-*p*)) if *p*-value > 0.5. The scores of pathways were then displayed by TIGR Multiexperiment Viewer. Scores are color-coded according to the legend on the top.

Fig. 2. Gene expression connection between formulae and disease states. Normalized data were analyzed using the "geneSetTest" to compute a *p*-value and to detect groups of differentially-expressed genes in MeSH disease terms. The score of each MeSH disease term in formula treatments was calculated as the negative logarithm of its *p*-value computed by "geneSetTest" function. The weighted MeSH disease term scores in each formula treatment were calculated by multiplying the original MeSH disease term scores with a ratio of the rank of MeSH disease terms over the average rank of those MeSH disease terms. The weighted MeSH disease term scores were displayed by TIGR Multiexperiment Viewer. Scores are color-coded according to the legend on the top.

Fig. 3. Expression levels of genes involved in drug metabolism. The expression levels of alcohol dehydrogenase genes, aldehyde dehydrogenase genes, cytochrome P450 family genes, GST genes, sulfotransferase genes, and UGT family genes in formula-treated kidney were selected. Normalized $\log_2$ expression values of these genes were displayed by TIGR Multiexperiment Viewer. Scores are color-coded

according to the legend on the top.

Fig. 4. Hierarchical clustering analysis of gene expression profiles altered by chemicals and formulae. The transcriptional profiles of 22 chemicals and 223 treatment conditions built in EDGE website were used in this analysis. Normalized $\log_2$ expression values are color-coded according to the legend on the top. DEHP, Di-(2-ethylhexyl)phthalate; LPS, lipopolysaccharide; IL1B, interleukin-1β; PHB, phenobarbital; PHNZ, phenylhydrazine; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Table 1 Herbal formulae, their constituents, and ethnopharmacological usage.

Herbal formulae	Constituents[a]	Ethnopharmacological usage
Gui-Zhi-Tang (GZT)	*Cinnamomum cassia*[b] (6), *Paeonia lactiflora*[c] (6), *Zingiber officinale*[d] (6), *Zizphus jujube*[e] (5), *Glycyrrhiza uralensis*[c] (4)	Respiratory diseases
Ma-Xing-Shi-Gan-Tang (MXSGT)	*Ephedra sinica*[f] (4), gypsum ($CaSO_4 \cdot 2H_2O$) (8), *Prunus armeniaca*[g] (3), *Glycyrrhiza uralensis*[c] (2)	Respiratory diseases and asthma
Xiao-Qing-Long-Tang (XQLT)	*Ephedra sinica*[f] (4), *Cinnamomon cassia*[b] (4), *Glycyrrhiza uralensis*[c] (4), *Paeonia lactiflora*[c] (4), *Schisandra chinensis*[e] (1.5), *Asarum sieboldii*[h] (1.5), *Pinellia ternate*[d] (4), *Zingiber officinale*[d] (4)	Respiratory diseases and asthma
Xiao-Chai-Hu-Tang (XCHT)	*Bupleurum chinense*[c] (8), *Scutellaria baicalensis*[c] (3), *Pinellia ternate*[d] (5), *Zingiber officinale*[d] (3), *Panax ginseng*[c] (3), *Ziziphus jujube*[e] (2), *Glycyrrhiza uralensis*[c] (3)	Liver diseases
Wu-Ling-Saan (WLS)	*Alisma orientalis*[i] (7.5), *Poria cocos*[j] (4.5), *Polyporus umbellatus*[j] (4.5), *Cinnamomum cassia*[b] (3), *Atractylodes macrocephala*[d] (4.5)	Cardiovascular diseases
Zhu-Ling-Tang (ZLT)	*Polyporus umbellatus*[j] (1), *Poria cocos*[j] (1), *Alisma orientalis*[i] (1), talc ($Mg_3Si_4O_{10}(OH)_2$) (1), donkey-hide gelatin (1)	Cardiovascular diseases
Ge-Gen-Tang (GGT)	*Pueraria lobata*[c] (6), *Ephedra sinica*[f] (4.5), *Ramulus cinnamomi*[b] (3), *Paeonia lactiflora*[c] (3), *Zingiber officinale*[d] (4.5), *Zizphus jujube*[e] (4), *Glycyrrhiza uralensis*[c] (3)	Fever
Yin-Chen-Hao-Tang (YCHT)	*Artemisia scoparia*[f,l] (3), *Gardenia jasminoides*[e] (1), *Rheum officinale*[d] (1)	Liver diseases
Jia-Wey-Shiau-Yau-Saan (JWSYS)	*Angelica sinensis*[c] (4), *Atractylodes macrocephala*[d] (4), *Gardenia jasminoides*[e] (2.5), *Paeonia suffruricosa*[c] (2.5), *Poria cocos*[j] (4), *Mentha haplocalyx*[l] (2), *Bupleurum chinense*[c] (4), *Paeonia lactiflora*[c] (4), *Zingiber officinale*[d] (4), *Glycyrrhiza uralensis*[c] (2)	Liver diseases

Shu-Jing-Hwo-Shiee-Tang (SJHST)	*Angelica sinensis*[c] (2), *Glycyrrhiza uralensis*[c] (1), *Ligusticum chuanxiong*[d] (1), *Prunus persica*[g] (2), *Achyranthes bidentata*[c] (2), *Citrus reticulata*[k] (2), *Rehmannia glutinosa*[d] (2), *Atractylodes japonica*[d] (2), *Clematis chinensis*[d] (2), *Notopterygium incisium*[c,d] (1), *Angelica dahurica*[c] (1), *Poria cocos*[j] (1), *Saposhnikovia divaricata*[c] (1), *Zingiber officinale*[d] (3), *Gentiana scabra*[c,d] (1), *Stephania tetrandra*[c] (1), *Paeonia lactiflora*[c] (2.5)	Pain in muscle, joint, and lumbar
Shin-Yi-Ching-Fey-Tang (SYCFT)	*Ophiopogon japonicus*[c] (3), *Glycyrrhiza uralensis*[c] (1.5), *Eriobotya japonica*[l] (3), *Scutellaria baicalensis*[c] (3), *Bupleurum chinense*[c] (3), *Anemarrhena asphodeloides*[d] (3), *Lilium brownie*[f,l] (3), gypsum ($CaSO_4 \cdot 2H_2O$) (3), *Magnolia lilifora*[m] (2), *Cimicifuga foetida*[d] (1)	Respiratory diseases
Chuan-Chiong-Chaa-Tyau-Saan (CCCTS)	*Glycyrrhiza uralensis*[c] (2), *Ligusticum chuanxiong*[d] (4), *Notopterygium incisium*[c,d] (2), *Angelica dahurica*[c] (2), *Mentha haplocalyx*[l] (8), *Asarum sieboldii*[h] (1), *Schizonepeta multifida*[h] (4), *Saposhnikovia divaricata*[c] (1.5)	Pain
Yn-Chyau-Saan (YCS)	*Glycine max*[g] (2.5), *Platycodon grandiflorum*[c] (3), *Lophatherum gracile*[l] (2), *Forsythia suspense*[e] (5), *Lancer japonica*[m] (5), *Schizonepeta tenuifolia*[h] (2), *Arctium lappa*[e] (3), *Glycyrrhiza uralensis*[c] (2.5), *Mentha haplocalyx*[l] (3)	Respiratory diseases
Dwu-Hwo-Jih-Sheng-Tang (DHJST)	*Angelica sinensis*[c] (1), *Eucommia ulmoides*[n] (1), *Glycyrrhiza uralensis*[c] (1), *Ligusticum chuanxiong*[d] (1), *Panax ginseng*[c] (1), *Paeonia suffruticosa*[c] (1), *Achyranthes bidentata*[c] (1), *Rehmannia glutinosa*[d] (1), *Angelica pubescens*[c] (1.5), *Gentiana macrophylla* [c] (1), *Poria cocos*[j] (1), *Asarum sieboldii*[h] (1), *Saposhnikovia divaricata*[c] (1), *Cinnamomum cassia*[n] (1), *Taxillus chinensis*[f,l] (1)	Respiratory diseases and pain
Liow-Wey-Diyh-Huang-Wan (LWDHW)	*Dioscorea opposite*[i] (4), *Cornus officinalis*[e] (4), *Alisma plantago*[i] (3), *Poria cocos*[j] (3), *Rehmannia glutinosa*[d] (8), *Paeonia suffruticosa*[c] (3)	Diabetes and anti-aging

[a] The ratio of each constituent in the formula was enclosed in the parenthesis.

[b] twig, [c] root, [d] rootstock, [e] fruit, [f] stem, [g] seed, [h] whole plant, [i] tuber, [j] fungi ,[k] pericarp, [l] leaf, [m] flower, [n] bark

Table 2 Gene expression similarities between formulae and drugs.

	CCCTS	XCHT	XQLT	WLS	LWDHW	JWSYS	SYCFT	GZT	YCHT	MXSGT	SJHST	GGT	YCS	ZLT	DHJST
Fulvestrant	7	4	2	13	8	4	3	-	1	3	4	6	4	2	2
17-Allylamino-geldanamycin	1	11	1	3	1	1	5	18	7	11	1	5	7	-	15
Sirolimus	20	18	4	2	14	15	8	16	3	1	6	3	1	14	-
Staurosporine	-	19	-	1	2	2	-	-	-	-	-	2	2	17	1
LY-294002	9	12	7	7	3	16	11	29	12	2	2	4	6	13	3
Resveratrol	3	2	5	5	11	28	1	-	20	10	15	-	8	19	-
Trichostatin A	-	-	-	-	7	18	19	3	-	-	21	28	16	1	17
Sulindac sulfide	-	-	3	10	15	9	4	-	10	-	3	-	15	-	10
Exisulind	23	13	6	6	6	13	12	-	23	8	18	1	3	-	11
Geldanamycin	2	3	11	12	18	-	-	25	-	-	13	9	-	-	-
Iloprost	12	20	12	16	9	5	2	-	24	16	23	-	-	-	-
Monastrol	22	8	16	11	19	29	14	-	22	23	8	20	20	-	4
Troglitazone	-	-	-	27	-	-	-	4	4	-	-	23	26	-	13
5253409	-	-	-	-	-	-	-	5	-	-	-	18	-	-	-
Alpha-estradiol	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Fisetin	-	-	27	17	-	17	21	27	-	28	-	15	-	18	5
Haloperidol	-	1	-	23	-	-	-	-	-	-	-	-	-	30	-
N-Phenylanthranilic acid	-	-	-	30	27	-	-	12	5	27	-	-	29	-	-
Prazosin	-	-	-	-	-	-	-	1	-	-	-	25	27	-	-
Rfecoxib	-	-	-	-	-	-	-	-	-	17	-	-	-	3	27
Tolbutamide	5	-	-	19	10	-	-	-	-	-	-	-	-	-	-
Valproic acid	8	5	22	24	-	-	16	-	11	-	-	-	-	-	-

[a] The value was the ranking of drug in each formula.

Figure 1

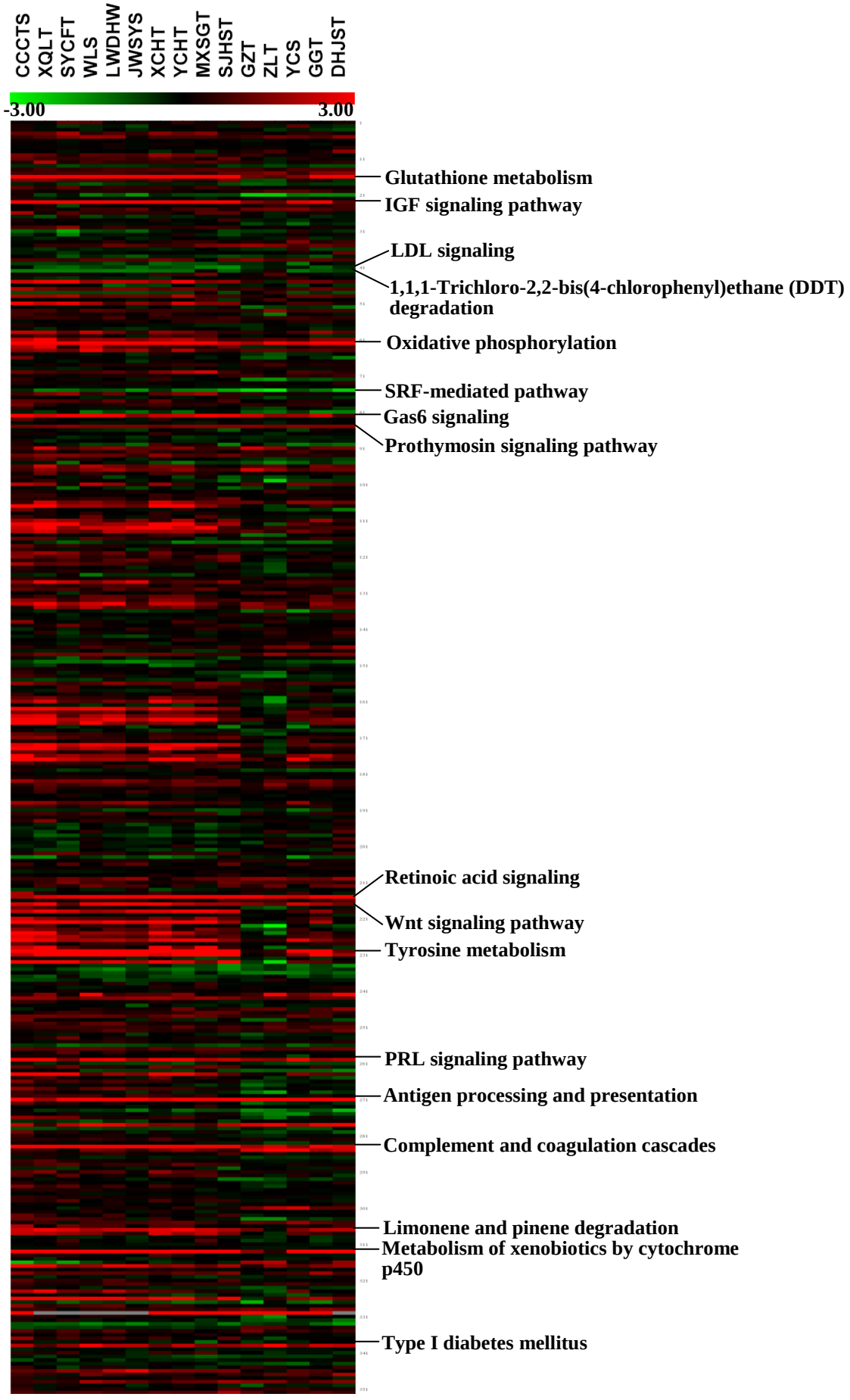
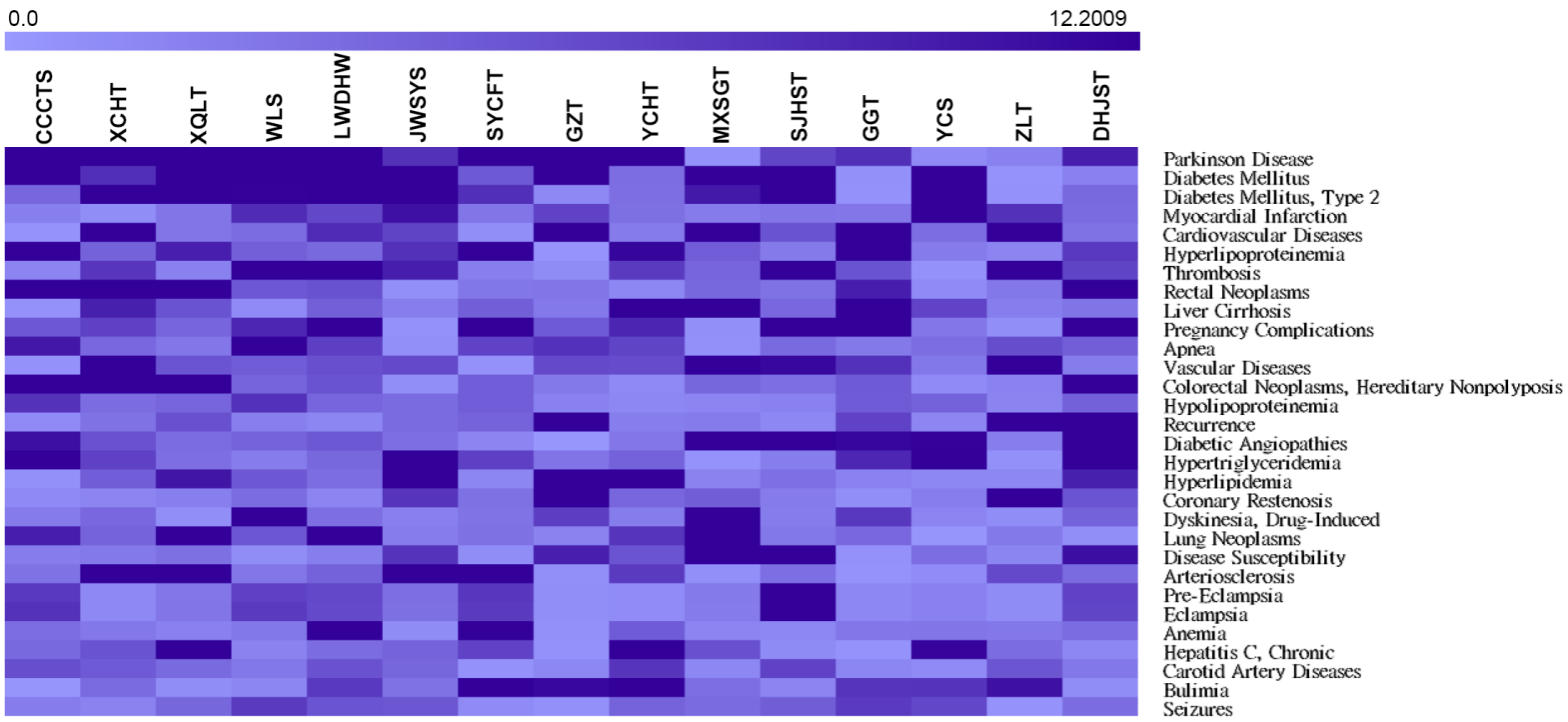

CCCTS
XQLT
SYCFT
WLS
LWDHW
JWSYS
XCHT
YCHT
MXSGT
SJHST
GZT
ZLT
YCS
GGT
DHJST
-3.00
3.00
Glutathione metabolism
IGF signaling pathway
LDL signaling
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation
Oxidative phosphorylation
SRF-mediated pathway
Gas6 signaling
Prothymosin signaling pathway
Retinoic acid signaling
Wnt signaling pathway
Tyrosine metabolism
PRL signaling pathway
Antigen processing and presentation
Complement and coagulation cascades
Limonene and pinene degradation
Metabolism of xenobiotics by cytochrome p450
Type I diabetes mellitus

Figure 2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12.2009
CCCTS
XCHT
XQLT
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GZT
YCHT
MXSGT
SJHST
GGT
YCS
ZLT
DHJST
Parkinson Disease
Diabetes Mellitus
Diabetes Mellitus, Type 2
Myocardial Infarction
Cardiovascular Diseases
Hyperlipoproteinemia
Thrombosis
Rectal Neoplasms
Liver Cirrhosis
Pregnancy Complications
Apnea
Vascular Diseases
Colorectal Neoplasms, Hereditary Nonpolyposis
Hypolipoproteinemia
Recurrence
Diabetic Angiopathies
Hypertriglyceridemia
Hyperlipidemia
Coronary Restenosis
Dyskinesia, Drug-Induced
Lung Neoplasms
Disease Susceptibility
Arteriosclerosis
Pre-Eclampsia
Eclampsia
Anemia
Hepatitis C, Chronic
Carotid Artery Diseases
Bulimia
Seizures

Figure 3

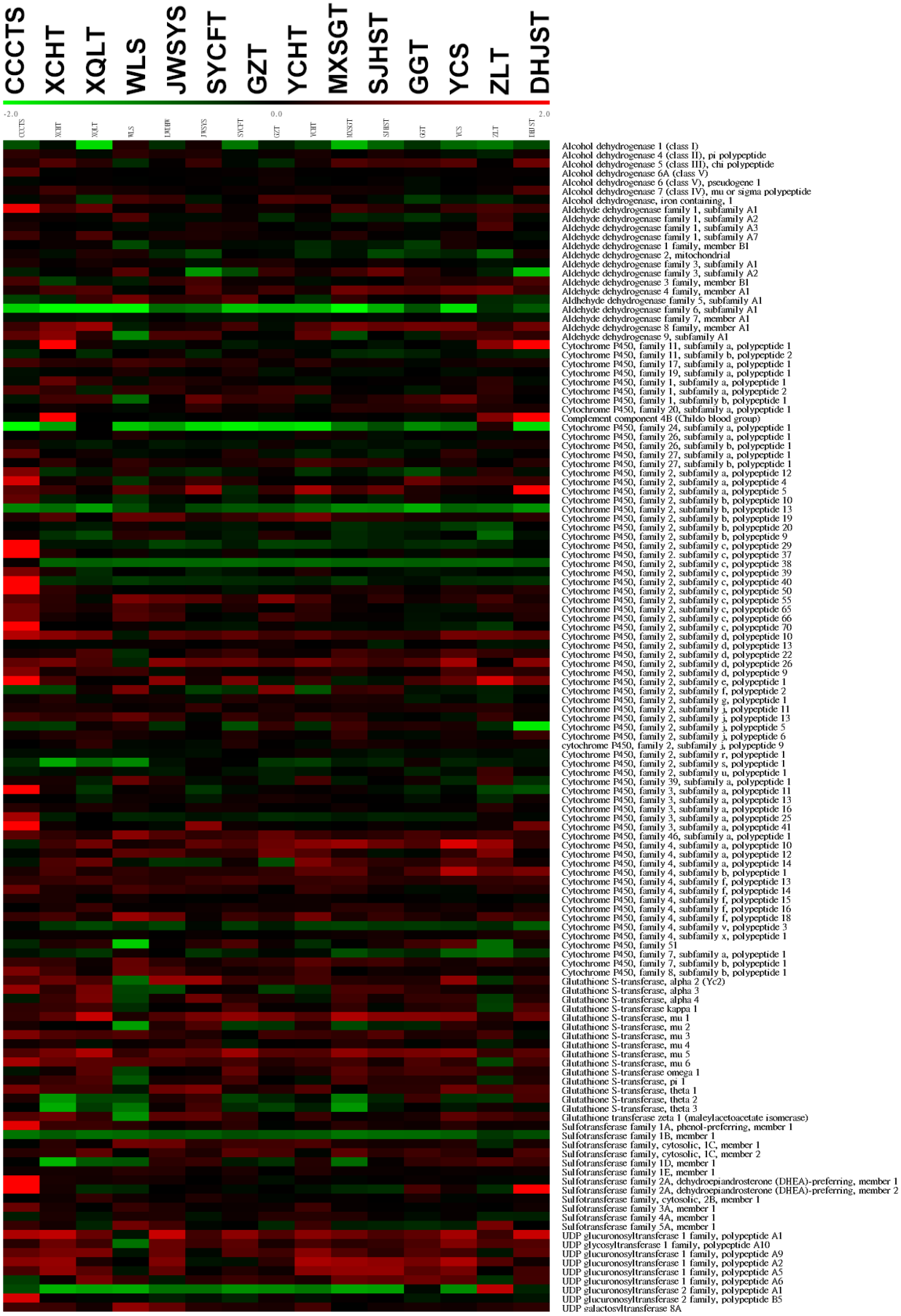
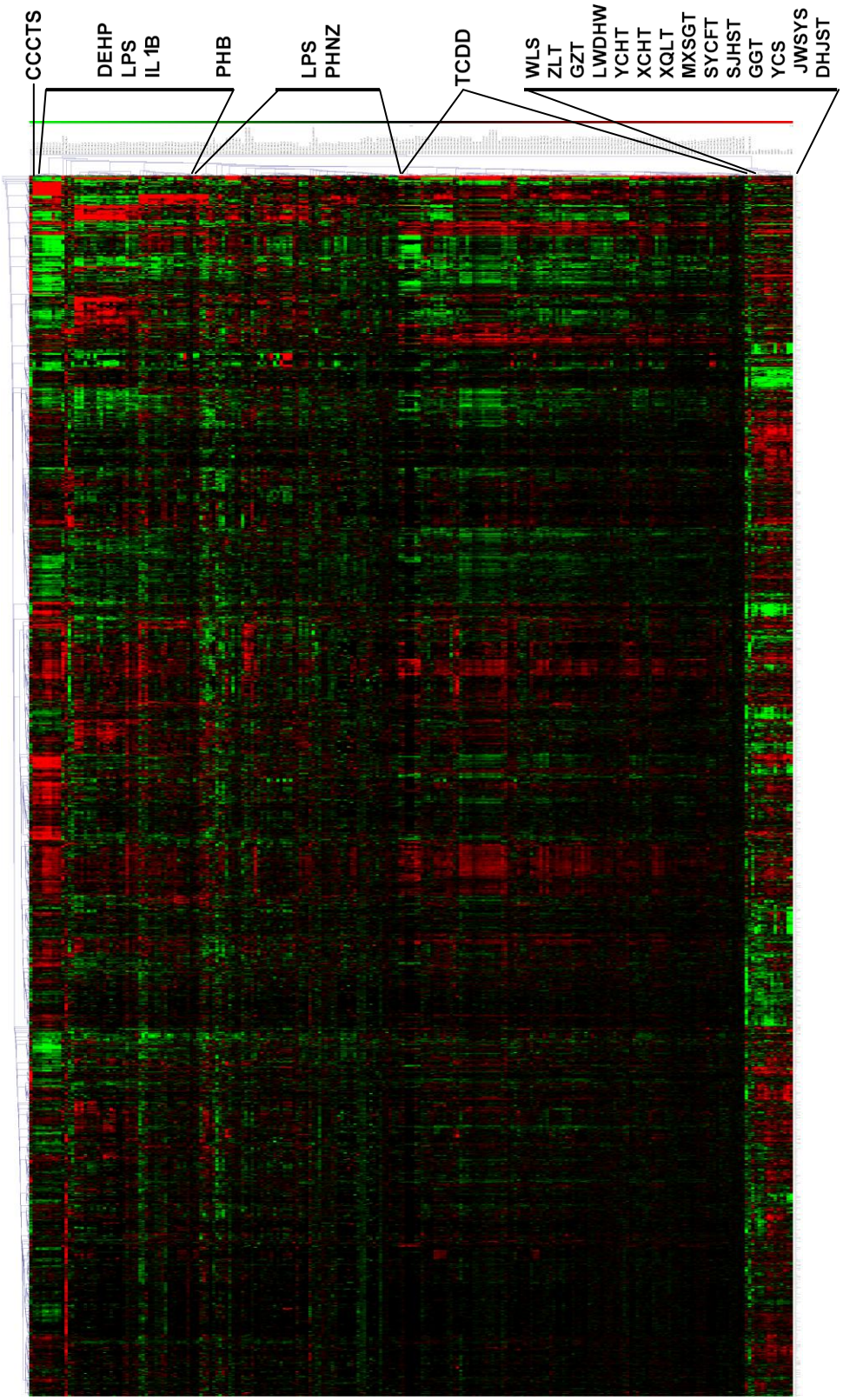

CCCTS
XCHT
XQLT
WLS
JWSYS
SYCFT
GZT
YCHT
MXSGT
SJHST
GGT
YCS
ZLT
DHJST
-2.0
0.0
2.0
Alcohol dehydrogenase 1 (class I)
Alcohol dehydrogenase 4 (class II), pi polypeptide
Alcohol dehydrogenase 5 (class III), chi polypeptide
Alcohol dehydrogenase 6A (class V)
Alcohol dehydrogenase 6 (class V), pseudogene 1
Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide
Alcohol dehydrogenase, iron containing, 1
Aldehyde dehydrogenase family 1, subfamily A1
Aldehyde dehydrogenase family 1, subfamily A2
Aldehyde dehydrogenase family 1, subfamily A3
Aldehyde dehydrogenase family 1, subfamily A7
Aldehyde dehydrogenase 1 family, member B1
Aldehyde dehydrogenase 2, mitochondrial
Aldehyde dehydrogenase family 3, subfamily A1
Aldehyde dehydrogenase family 3, subfamily A2
Aldehyde dehydrogenase 3 family, member B1
Aldehyde dehydrogenase 4 family, member A1
Aldhehyde dehydrogenase family 5, subfamily A1
Aldehyde dehydrogenase family 6, subfamily A1
Aldehyde dehydrogenase family 7, member A1
Aldehyde dehydrogenase 8 family, member A1
Aldehyde dehydrogenase 9, subfamily A1
Cytochrome P450, family 11, subfamily a, polypeptide 1
Cytochrome P450, family 11, subfamily b, polypeptide 2
Cytochrome P450, family 17, subfamily a, polypeptide 1
Cytochrome P450, family 19, subfamily a, polypeptide 1
Cytochrome P450, family 1, subfamily a, polypeptide 1
Cytochrome P450, family 1, subfamily a, polypeptide 2
Cytochrome P450, family 1, subfamily b, polypeptide 1
Cytochrome P450, family 20, subfamily a, polypeptide 1
Complement component 4B (Childo blood group)
Cytochrome P450, family 24, subfamily a, polypeptide 1
Cytochrome P450, family 26, subfamily a, polypeptide 1
Cytochrome P450, family 26, subfamily b, polypeptide 1
Cytochrome P450, family 27, subfamily a, polypeptide 1
Cytochrome P450, family 27, subfamily b, polypeptide 1
Cytochrome P450, family 2, subfamily a, polypeptide 12
Cytochrome P450, family 2, subfamily a, polypeptide 4
Cytochrome P450, family 2, subfamily a, polypeptide 5
Cytochrome P450, family 2, subfamily b, polypeptide 10
Cytochrome P450, family 2, subfamily b, polypeptide 13
Cytochrome P450, family 2, subfamily b, polypeptide 19
Cytochrome P450, family 2, subfamily b, polypeptide 20
Cytochrome P450, family 2, subfamily b, polypeptide 9
Cytochrome P450, family 2, subfamily c, polypeptide 29
Cytochrome P450, family 2. subfamily c, polypeptide 37
Cytochrome P450, family 2, subfamily c, polypeptide 38
Cytochrome P450, family 2, subfamily c, polypeptide 39
Cytochrome P450, family 2, subfamily c, polypeptide 40
Cytochrome P450, family 2, subfamily c, polypeptide 50
Cytochrome P450, family 2, subfamily c, polypeptide 55
Cytochrome P450, family 2, subfamily c, polypeptide 65
Cytochrome P450, family 2, subfamily c, polypeptide 66
Cytochrome P450, family 2, subfamily c, polypeptide 70
Cytochrome P450, family 2, subfamily d, polypeptide 10
Cytochrome P450, family 2, subfamily d, polypeptide 13
Cytochrome P450, family 2, subfamily d, polypeptide 22
Cytochrome P450, family 2, subfamily d, polypeptide 26
Cytochrome P450, family 2, subfamily d, polypeptide 9
Cytochrome P450, family 2, subfamily e, polypeptide 1
Cytochrome P450, family 2, subfamily f, polypeptide 2
Cytochrome P450, family 2, subfamily g, polypeptide 1
Cytochrome P450, family 2, subfamily j, polypeptide 11
Cytochrome P450, family 2, subfamily j, polypeptide 13
Cytochrome P450, family 2, subfamily j, polypeptide 5
Cytochrome P450, family 2, subfamily j, polypeptide 6
cytochrome P450, family 2, subfamily j, polypeptide 9
Cytochrome P450, family 2, subfamily r, polypeptide 1
Cytochrome P450, family 2, subfamily s, polypeptide 1
Cytochrome P450, family 2, subfamily u, polypeptide 1
Cytochrome P450, family 39, subfamily a, polypeptide 1
Cytochrome P450, family 3, subfamily a, polypeptide 11
Cytochrome P450, family 3, subfamily a, polypeptide 13
Cytochrome P450, family 3, subfamily a, polypeptide 16
Cytochrome P450, family 3, subfamily a, polypeptide 25
Cytochrome P450, family 3, subfamily a, polypeptide 41
Cytochrome P450, family 46, subfamily a, polypeptide 1
Cytochrome P450, family 4, subfamily a, polypeptide 10
Cytochrome P450, family 4, subfamily a, polypeptide 12
Cytochrome P450, family 4, subfamily a, polypeptide 14
Cytochrome P450, family 4, subfamily b, polypeptide 1
Cytochrome P450, family 4, subfamily f, polypeptide 13
Cytochrome P450, family 4, subfamily f, polypeptide 14
Cytochrome P450, family 4, subfamily f, polypeptide 15
Cytochrome P450, family 4, subfamily f, polypeptide 16
Cytochrome P450, family 4, subfamily f, polypeptide 18
Cytochrome P450, family 4, subfamily v, polypeptide 3
Cytochrome P450, family 4, subfamily x, polypeptide 1
Cytochrome P450, family 51
Cytochrome P450, family 7, subfamily a, polypeptide 1
Cytochrome P450, family 7, subfamily b, polypeptide 1
Cytochrome P450, family 8, subfamily b, polypeptide 1
Glutathione S-transferase, alpha 2 (Yc2)
Glutathione S-transferase, alpha 3
Glutathione S-transferase, alpha 4
Glutathione S-transferase kappa 1
Glutathione S-transferase, mu 1
Glutathione S-transferase, mu 2
Glutathione S-transferase, mu 3
Glutathione S-transferase, mu 4
Glutathione S-transferase, mu 5
Glutathione S-transferase, mu 6
Glutathione S-transferase omega 1
Glutathione S-transferase, pi 1
Glutathione S-transferase, theta 1
Glutathione S-transferase, theta 2
Glutathione S-transferase, theta 3
Glutathione transferase zeta 1 (maleylacetoacetate isomerase)
Sulfotransferase family 1A, phenol-preferring, member 1
Sulfotransferase family 1B, member 1
Sulfotransferase family, cytosolic, 1C, member 1
Sulfotransferase family, cytosolic, 1C, member 2
Sulfotransferase family 1D, member 1
Sulfotransferase family 1E, member 1
Sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 1
Sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 2
Sulfotransferase family, cytosolic, 2B, member 1
Sulfotransferase family 3A, member 1
Sulfotransferase family 4A, member 1
Sulfotransferase family 5A, member 1
UDP glucuronosyltransferase 1 family, polypeptide A1
UDP glycosyltransferase 1 family, polypeptide A10
UDP glucuronosyltransferase 1 family, polypeptide A9
UDP glucuronosyltransferase 1 family, polypeptide A2
UDP glucuronosyltransferase 1 family, polypeptide A5
UDP glucuronosyltransferase 1 family, polypeptide A6
UDP glucuronosyltransferase 2 family, polypeptide A1
UDP glucuronosyltransferase 2 family, polypeptide B5
UDP galactosyltransferase 8A

Figure 4


CCCTS
DEHP
LPS
IL1B
PHB
LPS
PHNZ
TCDD
WLS
ZLT
GZT
LWDHW
YCHT
XCHT
XQLT
MXSGT
SYCFT
SJHST
GGT
YCS
JWSYS
DHJST