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Use of transcriptomics in understanding mechanisms of drug-induced toxicity

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Abstract

Adverse drug reactions (ADRs) are an important clinical issue and a serious public health risk. Understanding the underlying mechanisms is critical for clinical diagnosis and management of different ADRs. Toxicogenomics can reveal impacts on biological pathways and processes that had not previously been considered to be involved in a drug response. Mechanistic hypotheses can be generated that can then be experimentally tested using the full arsenal of pharmacology, toxicology, molecular biology and genetics. Recent transcriptomic studies on drug-induced toxicity, which have provided valuable mechanistic insights into various ADRs, have been reviewed with a focus on nephrotoxicity and hepatotoxicity. Related issues have been discussed, including extrapolation of mechanistic findings from experimental model systems to humans using blood as a surrogate tissue for organ damage and comparative systems biology approaches.

Keywords

adverse drug reaction; hepatotoxicity; idiosyncratic toxicity; mechanism; nephrotoxicity; toxicogenomics

An adverse drug reaction (ADR) is defined as 'an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product' [1]. While most ADRs in human are mild and may disappear after decreasing the dose or withdrawal of the drug, some ADRs can produce serious and even fatal outcomes. According to a meta-analysis of hospitalized patients during the period of 1966–1996, serious ADR accounts for 6.7% of overall hospitalization and, in 1994, it was estimated that there were more than a million deaths related to ADRs in the USA, making ADRs one of the top six causes of death [2]. From 1998 to 2005, the serious adverse drug events reported to the US FDA increased 2.6-fold from 34,966 to 89,842 cases per year and the fatal adverse drug events increased 2.7-fold from 5519 to 15,107 cases per year [3]. This suggests drug-related adverse effects are not only an important issue in the clinical practice but also a public health risk [4,5]. Elucidating and understanding the underlying mechanisms for these ADRs is critical for the clinical diagnosis and management of different ADRs.

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With the rapid growth in the field of molecular biology and genomics, an increased understanding regarding the complexity of ADRs has been gained in both animal models and humans. With the recent development of high-content, “omics” technologies in the last decade, such as transcriptomics (genome-wide gene-expression profiling predominately utilizing microarray technology), proteomics (cell- and tissue-wide protein-expression profiling predominately utilizing high-resolution 2D gel electrophoresis and mass spectrometry technology), and metabolomics/metabonomics (profiling of metabolites predominately utilizing mass spectrometry and nuclear magnetic resonance spectroscopy technology), a systems biology approach promises to be a very important tool in furthering the understanding of adverse drug effects. Toxicogenomics as a concept was developed by considering the structure and dynamics of the entire genome in the study of chemical toxicity [6,7]. Within this concept, the overall adverse effects are evaluated in the context of a biological network of interactions. A broader concept of toxicogenomics, that is, systems toxicology, would involve the application of a wide array of high-content “omics” technologies, including genetics, transcriptomics, proteomics and metabolomics, in combination with conventional toxicology [8]. By phenotypic anchoring of genome-wide gene-expression signatures, protein profiling and metabolic profiling, toxicogenomic studies have generated an incredible volume of information regarding drug exposure response on the molecular level. Notably, more recent ADR studies are employing multiple “omics” technologies and integrating the results in order to provide more power to elucidate the mechanisms of an ADR in model systems, and thus, facilitate extrapolation of the findings to humans. In this review, we focus mainly on the application of transcriptomics in the mechanistic study of ADRs, through which genes and pathways have been discovered that provide novel and valuable insights into the mechanisms of the adverse effects caused by different drugs. Additional “omics” technologies are discussed in the context of integration with transcriptomic approaches towards these goals.

Beginnings of toxicogenomics in ADR studies

DNA microarrays are one of the technologies that have revolutionized the field of biomedical research in the last decade. They allow examination of the expression of thousands of genes simultaneously and provide a large amount of information regarding the structure and the dynamic change of genome-wide gene expression. Soon after its introduction in the mid-1990s, microarray technology was embraced by toxicologists to address the relationships between chemical exposure and the various adverse effects that were elicited [9]. One of the pioneer toxicogenomic studies examined the transcription profile of *Saccharomyces cerevisiae* upon exposure to the alkylating agent methyl methanesulfonate using Affymetrix (CA, USA) GeneChip® oligonucleotide arrays representing the whole yeast genome [10]. It was found that the transcription of more than 300 genes were induced by the exposure, far more than the number of genes known to be induced by a DNA-damaging agent at the time, and the expression of 76 genes were decreased. In addition, the changes in the gene-expression profile also provided evidence for the initiation of a process to eliminate and replace alkylated proteins in the cell. This work demonstrated that a global gene-expression profile is useful in the discovery of novel genes and pathways involved in chemical toxicity. In order to evaluate whether gene-expression profiles could distinguish between chemicals with different mechanisms of action, Burczynski *et al.* conducted a human *in vitro* study to examine the gene expression of HepG2 cells after exposure to mechanistically unrelated drugs (cytotoxic anti-inflammatory drugs and DNA-damaging agents) using a low-density DNA microarray containing a set of 250 human genes [11]. It was demonstrated that a reproducible gene-expression pattern containing a small set of genes was obtained from cells treated with the DNA-damaging agent cisplatin, and the NSAIDs diflunisal and flufenamic acid. This gene set was able to distinguish compounds from these two classes based on a cluster type analysis. A gene set generated by a computer optimization algorithm, which best discriminated between DNA-damaging agents and the NSAIDs, included genes that were involved in DNA repair, xenobiotic metabolism,

transcriptional activation, structural maintenance, cell-cycle control, signal transduction and apoptosis. These early transcription-profiling studies and others [12,13] demonstrated that drug treatment can trigger expressional changes in many genes, which can be potentially linked to the mode of action of the chemical agent. Toxicogenomics became an emerging subdiscipline in the field of toxicology.

With the rapidly accumulated sequence information from the genome projects and the fast growth of microarray technology, the biological information that could be retrieved from microarray experiments increased exponentially. When transcriptional data was linked to phenotype, toxicogenomics became very useful in predicting drug-induced toxicity and understanding the underlying mechanisms. A large number of microarray studies were carried out in order to develop transcription 'fingerprints' to classify or predict chemical agents with different toxic mechanisms, with the assumption that drug toxicity is accompanied by transcriptional changes in gene expression that are causally linked to or downstream of the toxicity [14–19]. Waring and colleagues analyzed liver transcription profiles of rats treated with 15 hepatotoxic agents that cause a variety of hepatocellular injuries, attempting to discover whether gene-expression signature profiles can predict whether or not a compound will lead to a toxic response, and whether different mechanisms of toxicity can be determined from gene expression [16]. In this study, rats were treated with one of 15 known hepatotoxicants daily for 3 days. On the fourth day, blood was drawn for clinical chemistry analysis and liver samples were collected for both gene-expression analysis and histopathology evaluation. After clustering the transcription profiles and then comparing with rat liver histopathology, they found a strong correlation between the histopathology, clinical chemistry and gene-expression profiles induced by the agents. Similar observations were also obtained in rat hepatocytes by the same group, suggesting that gene-expression profile is indeed a sensitive measure of toxic response [17]. In another study, Hamadeh *et al.* investigated the transcription profiles of rat liver tissues derived from animals treated with two different classes of compounds, three peroxisome proliferators (clofibrate, Wyeth 14643 and gemfibrozil) and an enzyme inducer (phenobarbital), on a high-density rat cDNA array [18,19]. In this study, rats were treated with one of the four compounds once or daily for 2 weeks. Gene-expression profiling, blood chemistry analysis and liver histopathology evaluation were performed on all experimental animals. Drug-induced hepatocellular hypertrophy was observed in all animals treated for 2 weeks, with distinguishable characteristics between the peroxisome proliferators and the enzyme inducer, but no drug-related histopathology changes were found in the animals examined 24 h after a single dose. However, pattern recognition of the 24-h gene-expression data using a variety of statistical methods, including clustering, principle component analysis and cross-validation, revealed that compounds from the same class generated similar gene-expression signature while a compound from a different class produced a very distinct gene-expression profile. They reasoned that these differences between expression signatures are related to different adverse effects caused by different drug treatments. For example, exposure to peroxisome proliferators was associated with gene-expression changes in pathways including stimulation of triglyceride hydrolysis, fatty acid uptake and conversion to acyl CoA derivatives, and stimulation of the β -oxidation pathway, which was consistent with previous findings of the toxic mechanism of action of these compounds [20,21]. Exposure to phenobarbital, on the other hand, was associated with gene-expression changes in the previously described metabolic, pharmacologic and toxicologic effects of pheno barbital, such as up regulation of cytochrome P450 genes, epoxide hydrolase, diaphorase and glutathione *S*-transferases. In addition, gene-expression profiling also revealed several novel effects in phenobarbital-treated rats, such as the upregulation of carboxylesterase precursor and the downregulation of carnitine palmitoyl transferase 1 (*CPT1*), which may explain the serum carnitine reduction observed in phenobarbital-treated patients [22]. Further investigation integrating these genes in the context of chemical toxicity may provide new mechanistic insights into the liver injury elicited by these drugs. While some of these studies, along with

many others, tried to bridge animal toxicity with the potential mechanisms by which chemical agents or drugs induce adverse effects in humans, many of the analyses were limited to description of alterations in known toxicity-associated target genes and identification of gene-expression signatures. This was partially due to the lack of annotation of genomes at the time. However, these pioneering studies successfully demonstrated that specific gene-expression profiles/signatures can be linked to specific toxicity end points. Further investigation of these expression signatures may greatly expand the understanding of the underlying mechanisms of the adverse effects elicited. These studies also laid the foundation for further application of toxicogenomics in ADR research, especially with regards to experimental methodology and the need for toxicogenomics databases.

Moving forward with toxicogenomic studies in ADR research

In the past few years, toxicogenomics approaches have become more powerful and more widely available with advances in genome annotation and bioinformatics, as well as the decreased costs of microarray technology itself. Toxicogenomic studies have provided more mechanistic insights into the adverse effects caused by various chemical agents and drugs, especially when gene-expression profiles from different exposure windows and exposure levels were combined. A large number of animal and human studies have been conducted, aiming to illustrate different aspects of ADRs using toxicogenomic approaches. These studies not only provide a global view of the molecular response of the drug reaction, but help point out possible biological pathways involved in the process, as well as directions for further detailed study, thus making considerable contribution towards our understanding of the underlying mechanisms. Although drugs have been reported to cause a wide array of adverse effects in a variety of organs in humans, drugs that have the potential to cause liver or kidney damage appear to be among the most frequently studied. Therefore, in the following section, we will focus on some of the toxicogenomic studies targeting nephrotoxicity and hepatotoxicity, especially those that clearly advanced our understanding of the mechanisms of toxicity.

Nephrotoxicity

Nephrotoxicity is one of the common adverse effects seen in many therapeutic drugs. The vast majority of pharmacological compounds and their metabolites are excreted via the urine, some of which may either exert direct toxicity by actively interacting with the complex structure of the kidney or have indirect effects by disturbing the electrolyte balance or renal blood flow [23]. In a clinical study conducted by Taber and colleagues, a group of 182 drugs, prioritized by the six adult intensive care units included in their study, were evaluated for nephro toxicity [24]. Of these top 182 drugs, 41 (22.5%) have nephrotoxic potential, including causing allergic interstitial nephritis, acute tubular necrosis and hemodynamically mediated effects, especially in older patients and patients with pre-existing renal diseases. Similarly, 38 (25.2%) of the top 151 medications prioritized by the pediatric intensive care units investigated in the study could cause kidney damage.

A number of toxicogenomic studies have been carried out to understand the mechanisms of nephrotoxicity caused by different drugs, some of which are listed in Table 1. Some of the nephrotoxic drugs, such as cisplatin, which is a platinum-based chemotherapy drug used to treat various types of cancers, and gentamicin, which is an aminoglycoside antibiotic used to treat many types of infections, have been used as model nephrotoxicants for mechanistic research and discovery of injury markers of nephro toxicity in a number of studies [25–32]. Both cisplatin and gentamicin are tubule toxicants, causing cell death in the proximal tubules. In a study conducted by the Nephrotoxicity Working Group of the Genomics Technical Committee of the International Life Sciences Institute Health and Environmental Science Institute (HESI; Washington DC, USA), temporal transcriptional changes in a rat kidney that were associated with administration of three different nephrotoxicants, cisplatin, gentamicin

and puromycin, were examined [31]. The analysis of gene-expression profiles not only revealed sample separation based on dose, time and degree of renal injuries but also reflected gene-expression alterations that correlated with biological processes relevant to nephron segment-specific toxicity. For instance, a group of genes was found to be strongly downregulated in samples that exhibited proximal tubular necrosis. Many of these genes, which are known to be involved in sugar metabolism (such as glucose-6-phosphatase), xenobiotic metabolism (such as glutathione *S*-transferase, α -hydroxyacid oxidase and peroxisomes), or peptide/amino acid metabolism (such as aminopeptidases), are functionally localized to the proximal tubules. This suggested that the downregulation of these genes may be related to the proximal tubule-specific toxicity. Further study of these genes may provide a detailed mechanistic insight on how tubular nephrotoxics cause kidney injuries. It was also found that after high-dose cisplatin and gentamicin treatment a grouping of genes appeared to be upregulated in a dose- and time-dependent fashion. These genes include kallikrein, hemeoxygenase-1, clusterin, osteopontin and KIM-1, which have been implicated in the mechanisms of renal toxicity, and some of these genes, such as KIM-1 and osteopontin, have become among the most promising biomarkers of nephrotoxicity today. In a recent study conducted by Xu and colleagues, an integrated systems biology approach was used to compare urine metabolomic profiles and kidney transcriptomic profiles in male Sprague Dawley rats treated with cisplatin and gentamicin, in order to identify nephrotoxicant-associated biochemical processes [29]. The analysis revealed that cisplatin- or gentamicin-induced renal Fanconi-like syndromes manifested by glucosuria, hyperaminoaciduria, lactic aciduria, and ketonuria were strongly correlated with the downregulation of luminal transporters that handle the respective elevated urine metabolites. For instance, cisplatin- and gentamicin-induced increase of urine glucose strongly correlated with the mRNA decrease of sodium-dependent glucose transporters SLC5A1 and SLC5A2. The downregulation of these genes may be a downstream effect of the reduction in hepatocyte nuclear factor 1 α , which is known to control the transcription of both genes. The elevation of urinary amino acids after cisplatin and gentamicin treatment significantly correlated with the transcriptional downregulation of SLC6A18, which, according to some studies in the published literature, functions as a sodium-dependent reabsorption transporter of neutral amino acids [33]. They concluded that cisplatin and gentamicin-induced renal dysfunction may be better explained by reduction of these transporters in the proximal tubules rather than perturbation of metabolic pathways inside the kidney cells. This study also demonstrated the advantage of the integrated use of multiple 'omics technologies in exploring new mechanisms of drug-induced toxicity. Furthermore, since urine or serum samples can be easily obtained from human patients, comparison of the metabolic profiles of these samples between species could facilitate the extrapolation of a mechanistic hypothesis from animal models to humans. The experimental approaches used in these studies, such as phenotypic anchoring of gene-expression profiles or expression signatures, and integration of different systems biology approaches, may also be applied to the mechanistic study of other nephrotoxics having different modes of action.

Acetaminophen-induced hepatotoxicity

The liver is the major site for metabolism of most drugs, and drug-induced liver injury is the leading cause of death from acute liver failure in the USA. Numerous toxicogenomic studies have been carried out to investigate liver injuries caused by a variety of medications, herbal supplements and dietary supplements. Table 2 summarizes a few of the most frequently studied drugs. According to a prospective study among a variety of drugs and supplements, acetaminophen (APAP) is the most common cause of drug-induced liver injury [34]. In addition, APAP is also among the most frequently suspected drugs in death and serious but nonfatal outcomes related to ADRs during the period of 1998–2005, according to the Adverse Event Reporting System operated by the FDA [3]. In the following section, we will use APAP as an example to illustrate how gene-expression profiling has been used to elucidate mechanisms underlying the adverse effects elicited by this drug. Some of the issues we will

address may also apply to similar mechanistic study of other drugs. Additional toxicogenomic studies of other drugs are listed in Table 2 and have been reviewed by others [35].

The mechanism of APAP-induced liver injury has been widely studied both in animal models and a variety of hepatic cell cultures. It was known that the toxic dose of APAP can cause oxidative stress by depleting GSH and result in mitochondrial damage and cellular death. The precise mechanism of how APAP leads to toxicity remains unclear or disputed on the molecular level, although it is known that the reactive metabolite *N*-acetyl-*p*-benzoquinone imine plays a key role [36]. In an *in vivo* animal study conducted by the National Center of Toxicogenomics of the National Institute of Environmental Health Sciences (NIEHS; NC, USA) at the US NIH, APAP-induced liver injuries and gene-expression changes were investigated in male Fisher 344 rats at multiple time points (6, 24 or 48 h post-drug treatment) following treatment with a single subtoxic dose (50 or 150 mg/kg) or toxic dose (1500 mg/kg) of APAP [37]. Although subtoxic doses of APAP did not cause any morphological or functional changes compared with controls at any time points studied, both subtoxic and toxic doses of APAP resulted in downregulation of genes involved in energy-consuming biochemical pathways including gluconeogenesis (glucose-6-phosphatase), fatty acid synthesis (fatty acid synthase; sterol-C4-methyl oxidase-like), cholesterol synthesis (3-hydroxy-3-methylglutaryl-coenzyme A synthase 1) and porphyrin synthesis (aminolevulinic acid synthase 1), and upregulation of genes involved in energy-producing biochemical pathways, such as glycolysis/ gluconeogenesis (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 1) and mitochondrial ω -hydroxylation (rat Cyp4a locus, encoding cytochrome p450 [IVA3] mRNA), as well as upregulation of stress-response-related genes, such as metallothionein and phospholipase C γ 1, which have been implicated in the cellular defense system against oxidative stress. All of these changes in gene expression were APAP dose dependent with increased magnitude and number of genes changed in the same pathway and accompanied by mitochondrial damage and ATP depletion, providing mechanistic cues of APAP-induced mitochondrial injury and oxidative stress on the molecular level. This study also suggested the importance of using subtoxic dose treatment in mechanistic toxicogenomic studies. The oxidative stress induced by both the subtoxic dose and toxic dose of APAP administration was further assessed by measuring a wide array of oxidative stress end points, such as liver tissue glutathione depletion, presence of nitrotyrosine protein adducts and 8-hydroxy-deoxyguanosine accumulation in the same liver tissues used for gene-expression analysis [38]. It was demonstrated that a subtoxic dose of APAP produced significant accumulation of nitrotyrosine protein adducts, while both subtoxic and toxic doses caused a significant increase in 8-hydroxydeoxyguanosine in liver tissues, validating the previous findings through toxicogenomic studies. APAP-induced mitochondrial damage and oxidative stress in rats and mice has also been reflected in gene- and protein-expression patterns discovered in other genomic [39–42], as well as proteomic, studies [41,43]. While the relationship between APAP toxicity and the genes and pathways that were revealed from these studies are only beginning to be understood, these results provide new hypotheses for further detailed biochemical study.

As with many other types of animal model studies, one of the greatest challenges in APAP toxicogenomic studies is an interspecies extrapolation of the results. Comparative genomic approaches are powerful tools to extrapolate mechanistic hypothesis from one species to another. When attempting to compare toxicogenomic data derived from different species, comparisons at the biochemical pathways and biological processes level usually provides more transferable information than comparisons made at the individual gene level. For instance, a recent study compared gene-expression profiles derived from APAP-treated rat liver and human hepatocytes as well as rat *in vivo* exposure [44]. Comparison at the modulated biochemical pathway and biological process level revealed more overlap than at the individual gene level. Similar changes were found in repression of energy-consuming biochemical pathways, mitochondrial function, and oxidoreductase activity between rat and human *in*

vitro, rat *in vivo* and *in vitro*. However, in some cases, focusing on critical, conserved individual genes that play important roles in the toxicological response may also be an effective approach to extrapolate the results gained from one species to another. CD44 is a ubiquitous multifunctional cell surface protein involved in cell–cell and cell–matrix interactions. It has been recently reported that *Cd44* polymorphisms are associated with susceptibility to APAP hepatotoxicity in inbred mouse strains [45,46]. It was speculated that variations in *Cd44* may significantly affect liver necrosis through effects on leukocyte signaling via cytokine modulation. When the human ortholog *CD44* was evaluated for APAP susceptibility relevance in two independent cohorts, a significant association was found between an individual's genotype of *CD44* polymorphism and APAP-induced elevation in serum alanine aminotransferase [46].

Although many adverse drug events can be reproduced in animal models, the reproducibility of these ADRs may only be partial owing to species differences, suggesting that extrapolation of toxic mechanisms from animal models to humans should be very cautious. Sometimes, species differences, which may arise from differences in pharmacological kinetics or genome responses, can lead to different toxicity outcomes in different species. For example, peroxisome proliferators including ciprofibrate, Wyeth 14643 and clofibrate, are liver carcinogens in rodents and the biological effects are mediated by PPAR- α . However, there is a lack of evidence in primates, including humans, for an increased risk of liver cancer associated with these compounds [47]. In a comparison of hepatic transcriptional profiling of a ciprofibrate-treated monkey with a PPAR- α agonist-treated rodent, transcriptomic data suggested that the magnitude of induction in α -oxidation pathways was substantially greater in the rodent than the primate, which could be related to the greater liver oxidative damage observed in rodents [48]. By contrast, PPAR- α humanized mice appear to be resistant to peroxisome proliferator-induced cell proliferation and cancer [49]. Further study revealed that the mechanism of hepatocellular proliferation in mice but not in humans involves PPAR- α -mediated downregulation of the microRNA *let-7c* gene, which controls levels of proliferative c-myc by destabilizing its mRNA.

Using blood as a surrogate tissue to study APAP-related hepatotoxicity

Peripheral blood gene-expression profiling can provide a great deal of information reflecting the physiological status of the body. It is particularly attractive as a tissue source in clinical pharmacogenomic studies because of the ease of collection from patients. The use of transcriptional profiling of peripheral blood cells in differential diagnosis and monitor of disease status has been described in several review articles [50–53]. Peripheral blood has also been used as a surrogate tissue in the mechanistic study of ADRs, including APAP-induced liver injury [54–56]. Bushel *et al.* tested the hypothesis that gene-expression patterns derived from peripheral blood cells could predict acute APAP exposure levels well before the liver damage could be detected by traditional measurements [57]. In this study, genomic classifiers of APAP toxicity that were derived from blood gene-expression analysis of APAP-treated rats were able to predict exposure to subtoxic/nontoxic versus toxic doses in rats with very high accuracy (88.9–95.8%). These findings were extended to humans using the human orthologs of the rat classifiers, and those genes clearly distinguished APAP-intoxicated human patients from normal individuals, further supporting the original hypothesis. Pathway analysis of these blood predictor genes revealed a major alteration in inflammatory pathways involving IL-1 and NF- κ B. This inflammatory response could be a response of blood cells to APAP-induced release of cytokines and chemokines from the liver, or a direct response of blood cells to APAP exposure, or both. Interestingly, this blood gene-expression pattern also demonstrated a specificity of APAP-toxic exposure in comparison with other known hepatotoxicants, suggesting the usefulness of using blood in understanding APAP-related toxicity. The success of using rat-derived classifiers to separate APAP-intoxicated human patients from normal

individuals also indicated that blood may serve as a mediator in extrapolating findings from animal models to human patients and then utilizing these findings in support of clinical diagnosis and treatment of APAP overdose patients. Recently, Fannin *et al.* studied both the blood transcriptomic profiles and the blood metabolomic profiles of human volunteers that received a single dose of 4 g of APAP [58]. It was found that APAP dosing produced a gene-expression signature in peripheral blood cells 48 h post-treatment, in the absence of liver injury as evaluated by traditional liver chemistries. This gene-expression signature was characterized by downregulation of oxidative phosphorylation genes and the magnitude of the change in expression was positively correlated with the production of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine, which is known to play a key role in APAP-induced liver toxicity, and the increase in serum lactate, as revealed by serum metabolomic analysis. Although there is a lot to be understood regarding the organ-to-organ communication (e.g., liver-to-blood communication), these studies demonstrated the possibility of using blood as a surrogate tissue to study the mechanisms of adverse drug effects, and probably more importantly the potential of using blood transcription profiles for the early detection of adverse drug effects. To better explore and utilize the information that peripheral blood gene-expression profiling may carry in the event of ADRs will require the integrated use of transcriptomics and additional high-content technologies, including metabolomics and bioinformatics.

Idiosyncratic hepatotoxicity

While many ADRs are time or dose related and can be managed or prevented, some are idiosyncratic and unpredictable [1]. Idiosyncratic ADRs occur at a very rare rate among the general population (e.g., idiosyncratic drug-induced liver injury from any single medication is less than one per 10,000–100,000 patients who take the drug), and it is hard to investigate or predict this type of toxicity during drug development [59]. Idiosyncratic drug reaction is usually dose independent and the mechanisms are poorly understood. The occurrence is believed to be related to a number of biological and environmental factors, including immunoallergic reaction to a drug or drug metabolites, and abnormalities in drug metabolism. Hepatotoxicity is one of the most common types of idiosyncratic ADRs in humans. Trovafloxacin is a fluoroquinolone antibiotic that has been withdrawn from the market in Europe because of idiosyncratic hepatotoxicity, and it is restricted in use in the USA. Studies of trovafloxacin-related toxicity in human hepatocytes and animal models using toxicogenomic approaches revealed a potential mechanism leading to idiosyncratic hepatotoxicity. Liguori *et al.* compared the gene expression of human hepatocytes treated with trovafloxacin and those treated with drugs of the same pharmacologic class but devoid of idiosyncratic toxicity. It was found that trovafloxacin uniquely regulates a larger number of genes that are important in major biological processes, such as inflammatory response, RNA processing, regulation of transcription and mitochondrial function, which could be the potential underlying mechanisms of trovafloxacin-induced idiosyncratic toxicity [60]. Trovafloxacin-induced hepatotoxicity was not observed in rat toxicity studies when animals were exposed to trovafloxacin alone [60]. However, the combination of trovafloxacin, but not levofloxacin, a fluoroquinolone without human idiosyncratic liability, with nonhepatotoxic doses of bacterial lipopolysaccharide (LPS) resulted in hepatotoxicity in rats and mice [61,62]. Liver gene-expression profiling identified distinct gene-expression patterns induced by trovafloxacin/LPS co-exposure, including changes in expression of genes involved in interferon signaling, which led to the mechanistic finding that IFN- γ and IL-8 play critical roles in trovafloxacin/LPS induced liver injury. In this manner, one can identify risk factors and critical genetic components that determine individual susceptibility through mechanistic study in genetically homozygous cell culture and animal models. More detailed information regarding studies on trovafloxacin-induced idiosyncratic hepatotoxicity can be found in the review by Blomme *et al.* [35].

Conclusion & future perspective

Toxicogenomics is a powerful new tool to study the adverse effects of drugs and can provide valuable insight into the mechanism of ADRs. However, analysis of toxicogenomic data can be challenging. Gene expression is a sensitive measure of the overall status of a cell or tissue as reflected by the genome. A number of experimental factors, in addition to drug treatment, may contribute to the overall gene-expression changes in the microarray data. A flow chart of a toxicogenomic study is displayed in Figure 1. Although not all of the factors in the flow chart will be involved in a single toxicogenomic study, many of them may greatly improve the efficiency of the study in some cases. For instance, one of the difficulties in the interpretation of toxicogenomic data generated from drug exposure is the differentiation of the intended drug pharmacology from off-target adverse effects. Although phenotypic anchoring of transcriptional data is a key in linking cause to effect in order to generate biologically meaningful hypotheses of toxic mechanism, gene regulation caused by therapeutic effects may also change in the same fashion as that caused by off-target toxic effects. In human patients it is even more complicated considering the differences in the health status of the subjects and possible drug interactions. However, in animal models, it has been demonstrated that the use of a reference drug from the same pharmacological class that is devoid of toxicity is very helpful in terms of differentiating gene-expression changes caused by pharmacological effects from those caused by toxic effects [60,61]. In addition, timing is a critical factor in the experimental design. It is important to capture the transcriptional changes in early exposure preceding the occurrence of toxicity, but after any nonspecific general acute stress responses, because these changes carry important mechanistic information regarding the mode of action of the drug, while transcriptional changes at the occurrence of toxicity can be more related to an adaptive response to the toxicity or to secondary effects of the toxicity.

Currently, there are numerous techniques developed for microarray data analysis, the description of which is beyond the scope of this review. However, statistical modeling and pattern recognition (such as principle component analysis, clustering and support vector machines) are among the most basic approaches. The application of these analyses provides a high-level view of the overall transcriptomic data by reducing the dimensionality and complexity of the data, and may also generate biologically relevant expression signatures. In addition, although pathway analysis, assisted by a variety of bioinformatics software tools and databases, has been widely adopted in gene-expression data analysis, literature searching is nevertheless important in connecting different bodies of knowledge to discover new genes and pathways involved in a particular adverse response and gain a better interpretation of the toxicogenomic data. Last but not least, transcriptomic data has its own limitations and it can not reflect post-transcriptional changes. Therefore, hypotheses generated based on toxicogenomic studies need to be validated by additional scientific approaches and the extrapolation of a mechanistic hypothesis to human can be assisted by comparative genomic approaches, as well as comparative systems biology approach.

One of the most important issues in the management of ADRs is to develop noninvasive biomarkers that can be used in clinic for the early detection of drug-induced adverse effects. Blood, and specifically the blood transcriptome, is an excellent resource for such noninvasive early-detection makers. The use of human peripheral blood transcriptional profiling to assess the status of diseases, including cancer and cardiovascular diseases, and environmental exposure risks has been described by several reviews [50–53]. It has also been reported recently that a gene-expression signature in the peripheral blood mononuclear cells, which consists of 29 genes, can distinguish individuals with early-stage non-small-cell lung cancer from individuals with nonmalignant lung disease with approximately 80% accuracy [63]. Although there have not been many successful clinical applications of peripheral blood transcriptome analysis in the detection of drug-induced toxicity, the study of APAP toxicity in both rats and

humans has demonstrated that the peripheral blood transcriptome certainly holds the potential to be used as a biomarker for the early detection of ADRs [57,58].

In the last decade, there have been collaborative efforts among academia, government and industry in generating toxicogenomic data on a variety of industry chemicals and pharmaceutical compounds. The purpose was to better understand the mode of action of these compounds, generate detection biomarkers, and also improve the data analysis strategy. Several databases have been generated as an outcome of these efforts and provide a wonderful resource for queries about genome response to a variety of compounds, including some pharmaceuticals. For example, the Chemical Effects in Biological Systems database at NIEHS hosts rodent toxicogenomic data, which was generated by in-house studies at the NIEHS, the National Toxicology Program and a number of consortia, including the HESI toxicogenomics projects and the Toxicogenomics Research Consortium, as well as research laboratories from various universities and companies [64]. Hepatotoxicity and nephrotoxicity data have also been generated by several additional consortia, including the FDA and GB Medicine Liver Toxicity Biomarker Study, the C-Path: Predictive Safety Testing Consortium, and projects carried out in Europe and Japan. Information regarding these consortia and associated databases have been reviewed in two papers [65,66]. In addition to these specific databases, Gene Expression Omnibus is another important public database for repository of array- and sequence-based data, including toxicogenomic data. The usefulness of these databases is still underappreciated, owing to limitations, such as variation in array platforms, and lack of integrated high-content data and analysis tools, although some of the databases accept other experimental data in addition to transcriptomic data. For example, Chemical Effects in Biological Systems contains traditional toxicology data and a small number of proteomic studies. Currently, many novel genes and pathways that were discovered in transcriptomic studies, which may play important roles in the mechanism of specific toxicities, need further study in order to draw solid conclusions. Full appreciation of the toxicogenomic data needs collaborative efforts from the field of toxicology. Hopefully, in the foreseeable future, integrated 'omics data analyses will be available from these datasets within these databases, which will significantly improve data interpretation and serve as a better resource for the toxicology community.

Toxicogenomics must be placed in the context of traditional scientific approaches to investigate the mechanisms of adverse effects from drugs. No technology is able to stand alone and great progress can be made as information is integrated from disparate data sources. One of the clear strengths of toxicogenomics is to reveal novel roles for biological pathways and processes that had not previously been considered to be involved in a drug response. Thus, mechanistic hypotheses can be generated that can then be experimentally tested using the full arsenal of pharmacology, toxicology, molecular biology and genetics. Extrapolation of mechanistic information from model systems into human cell systems and ultimately to human populations can then be fulfilled. As more information is obtained regarding the functional significance of genetic polymorphisms through genome-wide association studies, this information will need to be interpreted with toxicogenomic information regarding critical pathways and processes in ADRs. Then one may begin to be able to achieve the elusive goal of predictive toxicology associated with drugs in development and perhaps even be able to anticipate an 'idiosyncratic' adverse response in a specific subpopulation. The challenges are great but the prospects have never been brighter.

Executive summary

Beginnings of toxicogenomics in adverse drug reaction studies

- Drug-induced gene-expression changes can be causally linked to or downstream of the toxicity that is elicited.

- Toxicogenomic approaches are very useful, not only in identifying potential predictors for drug-induced toxicity, but also in understanding the underlying mechanisms.

Moving forward with toxicogenomic studies in adverse drug reaction research

- Toxicogenomic studies of nephrotoxic drugs revealed genes and pathways that are involved in the toxic mechanism and provided novel hypotheses for further studies.
- Toxicogenomic studies of acetaminophen-induced hepatotoxicity revealed genes and pathways that are involved in the toxic mechanism and provided novel hypotheses for further studies.
- Although using blood as a surrogate tissue to study the mechanisms of acetaminophen-related hepatotoxicity is challenging, it may provide valuable insight in the future as more is understood regarding organ-to-organ communication.
- Toxicogenomic studies can help identify environmental risk factors and critical genetic components in idiosyncratic drug reactions.

Conclusion & future perspective

- Analysis of toxicogenomic data can be challenging and a well-designed experiment is the base of an informative toxicogenomic study.
- The peripheral blood transcriptome may be used for early detection of drug-induced adverse effects.
- Toxicogenomic databases with integrated analysis tools and collaborative efforts from the field of toxicology are needed for better use of available toxicogenomic data.
- The strength of a toxicogenomics approach in investigating the mechanisms of adverse effects will be further enhanced when the toxicogenomic study is integrated with traditional scientific approaches and systems biology.

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Bibliography

Papers of special note have been highlighted as:

- of interest
- ■ of considerable interest

1. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. *Lancet* 2000;356(9237):1255–1259. [PubMed: 11072960]
2. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients – a meta-analysis of prospective studies. *JAMA* 1998;279(15):1200–1205. [PubMed: 9555760]
3. Moore TJ, Cohen MR, Furberg CD. Serious adverse drug events reported to the Food and Drug Administration, 1998–2005. *Arch. Intern. Med* 2007;167(16):1752–1759. [PubMed: 17846394]

4. Gandhi TK, Weingart SN, Borus J, et al. Adverse drug events in ambulatory care. *N. Engl. J. Med* 2003;348(16):1556–1564. [PubMed: 12700376]
5. Pirmohamed M, James S, Meakin S, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18,820 patients. *Br. Med. J* 2004;329(7456):15–19. [PubMed: 15231615]
6. Harrill AH, Rusyn I. Systems biology and functional genomics approaches for the identification of cellular responses to drug toxicity. *Expert Opin. Drug Metab. Toxicol* 2008;4(11):1379–1389. [PubMed: 18950280]
7. Heijne WHM, Kienhuis AS, van Ommen B, Stierum RH, Groten JP. Systems toxicology: applications of toxicogenomics, transcriptomics, proteomics and metabolomics in toxicology. *Expert Rev. Proteomics* 2005;2(5):767–780. [PubMed: 16209655]
8. Paules R. Phenotypic anchoring: linking cause and effect. *Environ. Health Perspect* 2003;111(6):A338–A339. [PubMed: 12760838]
9. Nuwaysir EF, Bittner M, Trent J, Barrett JC, Afshari CA. Microarrays and toxicology: the advent of toxicogenomics. *Mol. Carcinog* 1999;24(3):153–159. [PubMed: 10204799]
10. Jelinsky SA, Samson LD. Global response of *Saccharomyces cerevisiae* to an alkylating agent. *Proc. Natl. Acad. Sci. USA* 1999;96(4):1486–1491. [PubMed: 9990050]
11. Burczynski ME, McMillian M, Ciervo J, et al. Toxicogenomics-based discrimination of toxic mechanism in HEPG2 human hepatoma cells. *Toxicol. Sci* 2000;58(2):399–415. [PubMed: 11099651]
12. Bammert GF, Fostel JM. Genome-wide expression patterns in *Saccharomyces cerevisiae*: comparison of drug treatments and genetic alterations affecting biosynthesis of ergosterol. *Antimicrob. Agents Chemother* 2000;44(5):1255–1265. [PubMed: 10770760]
13. Wang YX, Rea T, Bian JH, Gray S, Sun Y. Identification of the genes responsive to etoposide-induced apoptosis: application of DNA chip technology. *FEBS Lett* 1999;445(2–3):269–273. [PubMed: 10094470]
14. Bulera SJ, Eddy SM, Ferguson E, et al. RNA expression in the early characterization of hepatotoxicants in wister rats by high-density DNA microarrays. *Hepatology* 2001;33(5):1239–1258. [PubMed: 11343254]
15. Thomas RS, Rank DR, Penn SG, et al. Identification of toxicologically predictive gene sets using cDNA microarrays. *Mol. Pharmacol* 2001;60(6):1189–1194. [PubMed: 11723225]
16. Waring JF, Jolly RA, Ciurlionis R, et al. Clustering of hepatotoxins based on mechanism of toxicity using gene expression profiles. *Toxicol. Appl. Pharmacol* 2001;175(1):28–42. [PubMed: 11509024]
17. Waring JF, Ciurlionis R, Jolly RA, Heindel M, Ulrich RG. Microarray analysis of hepatotoxins *in vitro* reveals a correlation between gene expression profiles and mechanisms of toxicity. *Toxicol. Lett* 2001;120(1–3):359–368. [PubMed: 11323195]
18. Hamadeh HK, Bushel PR, Jayadev S, et al. Gene expression analysis reveals chemical-specific profiles. *Toxicol. Sci* 2002;67(2):219–231. [PubMed: 12011481] ■ Demonstrated that specific gene-expression profiles and/or signatures can be linked to specific toxicity end points.
19. Hamadeh HK, Bushel PR, Jayadev S, et al. Prediction of compound signature using high density gene expression profiling. *Toxicol. Sci* 2002;67(2):232–240. [PubMed: 12011482]
20. Amacher DE, Beck R, Schomaker SJ, Kenny CV. Hepatic microsomal enzyme induction, β -oxidation, and cell proliferation following administration of clofibrate, gemfibrozil, or bezafibrate in the CD rat. *Toxicol. Appl. Pharmacol* 1997;142(1):143–150. [PubMed: 9007043]
21. Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res* 1996;37(5):907–925. [PubMed: 8725145]
22. Hug G, McGraw CA, Bates SR, Landrigan EA. Reduction of serum carnitine concentrations during anticonvulsant therapy with phenobarbital, valproic acid, phenytoin, and carbamazepine in children. *J. Pediatr* 1991;119(5):799–802. [PubMed: 1941389]
23. Pannu N, Nadim MK. An overview of drug-induced acute kidney injury. *Crit. Care Med* 2008;36(4):S216–S223. [PubMed: 18382197]
24. Taber SS, Mueller BA. Drug-associated renal dysfunction. *Crit. Care Clin* 2006;22(2):357–374. [PubMed: 16678005]

25. Huang Q, Dunn RT, Jayadev S, et al. Assessment of cisplatin-induced nephrotoxicity by microarray technology. *Toxicol. Sci* 2001;63(2):196–207. [PubMed: 11568363]
26. Fielden MR, Eynon BP, Natsoulis G, Jarnagin K, Banas D, Kolaja KL. A gene expression signature that predicts the future onset of drug-induced renal tubular toxicity. *Toxicol. Pathol* 2005;33(6):675–683. [PubMed: 16239200]
27. Thukral SK, Nordone PJ, Hu R, et al. Prediction of nephrotoxicant action and identification of candidate toxicity-related biomarkers. *Toxicol. Pathol* 2005;33(3):343–355. [PubMed: 15805072]
28. Suzuki H, Inoue T, Matsushita T, et al. *In vitro* gene expression analysis of nephrotoxic drugs in rat primary renal cortical tubular cells. *J. Appl. Toxicol* 2008;28(2):237–248. [PubMed: 18172885]
29. Xu EY, Perlina A, Vu H, et al. Integrated pathway analysis of rat urine metabolic profiles and kidney transcriptomic profiles to elucidate the systems toxicology of model nephrotoxicants. *Chem. Res. Toxicol* 2008;21(8):1548–1561. [PubMed: 18656965] ■■ Integrated quantitative analysis of both urine metabolomic and kidney transcriptomic profiles.
30. Thompson KL, Afshari CA, Amin RA, et al. Identification of platform-independent gene expression markers of cisplatin nephrotoxicity. *Environ. Health Perspect* 2004;112(4):488–494. [PubMed: 15033599]
31. Amin RA, Vickers AE, Sistare F, et al. Identification of putative gene-based markers of renal toxicity. *Environ. Health Perspect* 2004;112(4):465–479. [PubMed: 15033597] ■ Comprehensive nephrotoxicity study that revealed gene-expression alterations associated with nephron segment-specific toxicity.
32. Kramer JA, Pettit SD, Amin RP, et al. Overview of the application of transcription profiling using selected nephrotoxicants for toxicology assessment. *Environ. Health Perspect* 2004;112(4):460–464. [PubMed: 15033596]
33. Singer D, Camargo SMR, Huggel K, et al. Orphan transporter SLC6A18 is renal neutral amino acid transporter B(0)AT3. *J. Biol. Chem* 2009;284(30):19953–19960. [PubMed: 19478081]
34. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann. Intern. Med* 2002;137(12):947–954. [PubMed: 12484709]
35. Blomme EAG, Yang Y, Waring JF. Use of toxicogenomics to understand mechanisms of drug-induced hepatotoxicity during drug discovery and development. *Toxicol. Lett* 2009;186(1):22–31. [PubMed: 18996174]
36. Jaeschke H, Bajt ML. Intracellular signaling mechanisms of acetaminophen-induced liver cell death. *Toxicol. Sci* 2006;89(1):31–41. [PubMed: 16177235]
37. Heinloth AN, Irwin RD, Boorman GA, et al. Gene expression profiling of rat livers reveals indicators of potential adverse effects. *Toxicol. Sci* 2004;80(1):193–202. [PubMed: 15084756] ■ Comprehensive rat study using both toxic and subtoxic doses, providing mechanistic cues of acetaminophen (APAP)-induced mitochondrial injury and oxidative stress.
38. Powell CL, Kosyk O, Ross PK, et al. Phenotypic anchoring of acetaminophen-induced oxidative stress with gene expression profiles in rat liver. *Toxicol. Sci* 2006;93(1):213–222. [PubMed: 16751229]
39. Zidek N, Hellmann J, Kramer PJ, Hewitt PG. Acute hepatotoxicity: a predictive model based on focused Illumina microarrays. *Toxicol. Sci* 2007;99(1):289–302. [PubMed: 17522070]
40. Reilly TP, Bourdi M, Brady JN, et al. Expression profiling of acetaminophen liver toxicity in mice using microarray technology. *Biochem. Biophys. Res. Commun* 2001;282(1):321–328. [PubMed: 11264010]
41. Ruepp, SU.; Tonge, RP.; Shaw, J.; Wallis, N.; Pognan, F. Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. 2002. p. 135-150.
42. Beyer RP, Fry RC, Lasarev MR, et al. Multicenter study of acetaminophen hepatotoxicity reveals the importance of biological endpoints in genomic analyses. *Toxicol. Sci* 2007;99(1):326–337. [PubMed: 17562736]
43. Welch KD, Wen B, Goodlett DR, et al. Proteomic identification of potential susceptibility factors in drug-induced liver disease. *Chem. Res. Toxicol* 2005;18(6):924–933. [PubMed: 15962927]

44. Kienhuis AS, van de Poll MCG, Wortelboer H, et al. Parallelogram approach using rat–human *in vitro* and rat *in vivo* toxicogenomics predicts acetaminophen-induced hepatotoxicity in humans. *Toxicol. Sci* 2009;107(2):544–552. [PubMed: 19008212]
45. Harrill AH, Ross PK, Gatti DM, Threadgill DW, Rusyn I. Population-based discovery of toxicogenomics biomarkers for hepatotoxicity using a laboratory strain diversity panel. *Toxicol. Sci* 2009;110(1):235–243. [PubMed: 19420014]
46. Harrill AH, Watkins PB, Su S, et al. Mouse population-guided resequencing reveals that variants in CD44 contribute to acetaminophen-induced liver injury in humans. *Genome Res* 2009;19(9):1507–1515. [PubMed: 19416960]
47. Klaunig JE, Babich MA, Baetcke KP, et al. PPAR α agonist-induced rodent tumors: modes of action and human relevance. *Crit. Rev. Toxicol* 2003;33(6):655–780. [PubMed: 14727734]
48. Cariello NF, Romach EH, Colton HM, et al. Gene expression profiling of the PPAR- α agonist ciprofibrate in the cynomolgus monkey liver. *Toxicol. Sci* 2005;88(1):250–264. [PubMed: 16081524]
49. Gonzalez FJ, Shah YA. PPAR α : mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology* 2008;246(1):2–8. [PubMed: 18006136]
50. Burczynski ME, Dörner AJ. Transcriptional profiling of peripheral blood cells in clinical pharmacogenomic studies. *Pharmacogenomics* 2006;7(2):187–202. [PubMed: 16515398]
51. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J. Lab. Clin. Med* 2006;147(3):126–132. [PubMed: 16503242]
52. Mohr S, Liew CC. The peripheral-blood transcriptome: new insights into disease and risk assessment. *Trends Mol. Med* 2007;13(10):422–432. [PubMed: 17919976]
53. Staratschek-Jox A, Classen S, Gaarz A, Debey-Pascher S, Schultze JL. Blood-based transcriptomics: leukemias and beyond. *Expert Rev. Mol. Diagn* 2009;9(3):271–280. [PubMed: 19379085]
54. Lobenhofer EK, Auman JT, Blackshear PE, et al. Gene expression response in target organ and whole blood varies as a function of target organ injury phenotype. *Genome Biol* 2008;9(6):R100. [PubMed: 18570634]
55. Chao A, Wang TH, Lee YS, et al. Analysis of functional groups of differentially expressed genes in the peripheral blood of patients with cervical cancer undergoing concurrent chemoradiation treatment. *Radiat. Res* 2008;169(1):76–86. [PubMed: 18159954]
56. Sonis S, Haddad R, Posner M, et al. Gene expression changes in peripheral blood cells provide insight into the biological mechanisms associated with regimen-related toxicities in patients being treated for head and neck cancers. *Oral Oncol* 2007;43(3):289–300. [PubMed: 16920386]
57. Bushel PR, Heinloth AN, Li J, et al. Blood gene expression signatures predict exposure levels. *Proc. Natl. Acad. Sci. USA* 2007;104(46):18211–18216. [PubMed: 17984051] ■■ First study using classifiers derived from rat APAP exposure to distinguish APAP-intoxicated human patients from normal individuals.
58. Fannin RD, Russo M, O'Connell TM, et al. Acetaminophen dosing of humans results in blood transcriptome and metabolome changes consistent with impaired oxidative phosphorylation. *Hepatology* 2010;51(1):227–236. [PubMed: 19918972] ■■ Blood transcriptome study of APAP toxicity in humans utilizing an integrated systems biology approach.
59. Chalasani N, Fontana RJ, Bonkovsky HL, et al. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology* 2008;135(6):1924–1934. [PubMed: 18955056]
60. Liguori MJ, Anderson MG, Bukofzer S, et al. Microarray analysis in human hepatocytes suggests a mechanism for hepatotoxicity induced by trovafloxacin. *Hepatology* 2005;41(1):177–186. [PubMed: 15619227]
61. Waring JF, Liguori MJ, Luyendyk JP, et al. Microarray analysis of lipopolysaccharide potentiation of trovafloxacin-induced liver injury in rats suggests a role for proinflammatory chemokines and neutrophils. *J. Pharmacol. Exp. Ther* 2006;316(3):1080–1087. [PubMed: 16299187]
62. Shaw PJ, Ditewig AC, Waring JF, et al. Coexposure of mice to trovafloxacin and lipopolysaccharide, a model of idiosyncratic hepatotoxicity, results in a unique gene expression profile and interferon γ -dependent liver injury. *Toxicol. Sci* 2009;107(1):270–280. [PubMed: 18930950]

63. Showe MK, Vachani A, Kossenkov AV, et al. Gene expression profiles in peripheral blood mononuclear cells can distinguish patients with non-small cell lung cancer from patients with nonmalignant lung disease. *Cancer Res* 2009;69(24):9202–9210. [PubMed: 19951989]
64. Waters M, Stasiewicz S, Merrick BA, et al. CEBS – chemical effects in biological systems: a public data repository integrating study design and toxicity data with microarray and proteomics data. *Nucleic Acids Res* 2008;36:D892–D900. [PubMed: 17962311]
65. Gallagher WM, Tweats D, Koenig J. Omic profiling for drug safety assessment: current trends and public-private partnerships. *Drug Discov. Today* 2009;14(7–8):337–342. [PubMed: 19340928]
66. Mattes, WB. Public Consortium Efforts in Toxicogenomics. In: Mendrick, DL.; William, B., editors. *Essential Concepts in Toxicogenomics*. Humana Press; NJ, USA: 2008. p. 221–238.
67. Blomme EAG, Ciurlionis R, Marsh KC, Waring JF, Yang Y. Evaluation of the effects of serial phlebotomy on the transcriptome of major tissues and on the response to toxicants in rats. *Toxicol. Lett* 2008;176(2):138–148. [PubMed: 18164877]
68. Jiang Y, Gerhold DL, Holder DJ, et al. Diagnosis of drug-induced renal tubular toxicity using global gene expression profiles. *J. Transl. Med* 2007;5:47. [PubMed: 17908307]
69. Leussink BT, Baelde HJ, Broekhuizen-van den Berg TM, et al. Renal epithelial gene expression profile and bismuth-induced resistance against cisplatin nephrotoxicity. *Hum. Exp. Toxicol* 2003;22(10):535–540. [PubMed: 14655719]
70. Oh JH, Park HJ, Lim JS, et al. Gene expression profiling of early renal toxicity induced by gentamicin in mice. *Mol. Cell. Toxicol* 2006;2(3):185–192.
71. Jennings P, Aydin S, Bennett J, et al. Inter-laboratory comparison of human renal proximal tubule (HK-2) transcriptome alterations due to cyclosporine A exposure and medium exhaustion. *Toxicol. Vitro* 2009;23(3):486–499.
72. Pallet N, Rabant M, Xu-Dubois YC, et al. Response of human renal tubular cells to cyclosporine and sirolimus: a toxicogenomic study. *Toxicol. Appl. Pharmacol* 2008;229(2):184–196. [PubMed: 18331748]
73. Rokushima M, Fujisawa K, Furukawa N, et al. Transcriptomic analysis of nephrotoxicity induced by cephaloridine, a representative cephalosporin antibiotic. *Chem. Res. Toxicol* 2008;21(6):1186–1196. [PubMed: 18500788]
74. Huang QH, Jin XD, Gaillard ET, et al. Gene expression profiling reveals multiple toxicity endpoints induced by hepatotoxicants. *Mutat. Res* 2004;549(1–2):147–168. [PubMed: 15120968]
75. Minami K, Saito T, Narahara M, et al. Relationship between hepatic gene expression profiles and hepatotoxicity in five typical hepatotoxicant-administered rats. *Toxicol. Sci* 2005;87(1):296–305. [PubMed: 15976192]
76. Heinloth AN, Boorman GA, Foley JF, Flagler ND, Paules RS. Gene expression analysis offers unique advantages to histopathology in liver biopsy evaluations. *Toxicol. Pathol* 2007;35(2):276–283. [PubMed: 17366322]
77. Uehara T, Hirode M, Ono A, et al. A toxicogenomics approach for early assessment of potential non-genotoxic hepatocarcinogenicity of chemicals in rats. *Toxicology* 2008;250(1):15–26. [PubMed: 18619722]
78. Kier LD, Neft R, Tang L, et al. Applications of microarrays with toxicologically relevant genes (*TOX* genes) for the evaluation of chemical toxicants in sprague dawley rats *in vivo* and human hepatocytes *in vitro*. *Mutat. Res* 2004;549(1–2):101–113. [PubMed: 15120965]
79. de Longueville F, Atienzar FA, Marcq L, et al. Use of a low-density microarray for studying gene expression patterns induced by hepatotoxicants on primary cultures of rat hepatocytes. *Toxicol. Sci* 2003;75(2):378–392. [PubMed: 12883083]
80. Noel S, Sharma S, Rath SK. Simultaneous application of *t*-test and fold change criteria to identify acetaminophen and carbon tetrachloride affected genes in mice liver. *Environ. Toxicol. Pharmacol* 2008;26(2):150–161.
81. Suzuki H, Inoue T, Matsushita T, et al. *In vitro* gene expression analysis of hepatotoxic drugs in rat primary hepatocytes. *J. Appl. Toxicol* 2008;28(2):227–236. [PubMed: 18246545]
82. Reilly TP, Brady JN, Marchick MR, et al. A protective role for cyclooxygenase-2 in drug-induced liver injury in mice 2001;14(12):1620–1628.

83. Liu J, Li CX, Waalkes MP, et al. The nitric oxide donor, v-pyrro/no, protects against acetaminophen-induced hepatotoxicity in mice. *Hepatology* 2003;37(2):324–333. [PubMed: 12540782]
84. Shankar K, Vaidya VS, Corton JC, et al. Activation of PPAR- α in streptozotocin-induced diabetes is essential for resistance against acetaminophen toxicity. *FASEB J* 2003;17(10):1748–1750. [PubMed: 12958197]
85. Priyadarsiny P, Khattar SK, Malik R, et al. Differential gene expression analysis of a known hepatotoxin, *N*-acetyl-*p*-amino-phenol (APAP) as compared with its non-toxic analog, *N*-acetyl-*m*-amino-phenol (AMAP) in mouse liver. *J. Toxicol. Sci* 2008;33(2):163–173. [PubMed: 18544908]
86. Jeong YK, Kang JS, Kim JW, et al. Differences in gene expression profiles reflecting differences in drug sensitivity to acetaminophen in normal and transformed hepatic cell lines *in vitro*. *Mol. Cell. Toxicol* 2009;5(1):32–43.
87. Harris AJ, Dial SL, Casciano DA. Comparison of basal gene expression profiles and effects of hepatocarcinogens on gene expression in cultured primary human hepatocytes and HEPG2 cells. *Mutat. Res* 2004;549(1–2):79–99. [PubMed: 15120964]
88. Hamadeh HK, Knight BL, Haugen AC, et al. Methapyrilene toxicity: anchorage of pathologic observations to gene expression alterations. *Toxicol. Pathol* 2002;30(4):470–482. [PubMed: 12187938]
89. Waring JF, Ulrich RG, Flint N, et al. Interlaboratory evaluation of rat hepatic gene expression changes induced by methapyrilene. *Environ. Health Perspect* 2004;112(4):439–448. [PubMed: 15033593]
90. Ulrich RG, Rockett JC, Gibson GG, Pettit SD. Overview of an interlaboratory collaboration on evaluating the effects of model hepatotoxicants on hepatic gene expression. *Environ. Health Perspect* 2004;112(4):423–427. [PubMed: 15033591]
91. Craig A, Sidaway J, Holmes E, et al. Systems toxicology: integrated genomic, proteomic and metabolomic analysis of methapyrilene induced hepatotoxicity in the rat. *J. Proteome Res* 2006;5(7):1586–1601. [PubMed: 16823966]
92. Auman JT, Chou J, Gerrish K, et al. Identification of genes implicated in methapyrilene-induced hepatotoxicity by comparing differential gene expression in target and nontarget tissue. *Environ. Health Perspect* 2007;115(4):572–578. [PubMed: 17450226]
93. Beekman JM, Boess F, Hildebrand H, Kalkuhl A, Suter L. Gene expression analysis of the hepatotoxicant methapyrilene in primary rat hepatocytes: an interlaboratory study. *Environ. Health Perspect* 2006;114(1):92–99. [PubMed: 16393664]
94. Shaw PJ, Hopfensperger MJ, Ganey PE, Roth RA. Lipopolysaccharide and trovafloxacin coexposure in mice causes idiosyncrasy-like liver injury dependent on tumor necrosis factor- α . *Toxicol. Sci* 2007;100(1):259–266. [PubMed: 17709330] ■ Toxicogenomic study targeting idiosyncratic hepatotoxicity with mechanistic findings.
95. Liguori MJ, Blomme EAG, Waring JF. Trovafloxacin-induced gene expression changes in liver-derived *in vitro* systems: Comparison of primary human hepatocytes to HEPG2 cells. *Drug Metab. Dispos* 2008;36(2):223–233. [PubMed: 17967932]
96. Jung JW, Kim SJ, Kim JS, et al. Gene expression analysis of methotrexate-induced hepatotoxicity between *in vitro* and *in vivo*. *Mol. Cell. Toxicol* 2005;1(4):256–261.
97. Lee GJ, Kim YS, Jung JW, et al. Gene expression analysis of anticancer drug induced hepatotoxicity using cDNA microarray. *Mol. Cell. Toxicol* 2006;2(2):141–149.
98. Ganter B, Tugendreich S, Pearson CI, et al. Development of a large-scale chemogenomics database to improve drug candidate selection and to understand mechanisms of chemical toxicity and action. *J. Biotechnol* 2005;119(3):219–244. [PubMed: 16005536]
99. Lee MH, Hong I, Kim M, et al. Gene expression profiles of murine fatty liver induced by the administration of methotrexate. *Toxicology* 2008;249(1):75–84. [PubMed: 18502557]

Figure 1. Complete toxicogenomic study

Factors that need to be considered in each stage of the study are listed in the right column.

Table 1

Kidney gene-expression profiling of drugs with nephrotoxicity.

Drug	Model used	Ref.
Cisplatin	Rat <i>in vivo</i>	[25,26,29–31,67,68]
	Rat <i>in vitro</i>	[28,69]
	Human <i>in vitro</i>	[11]
Gentamicin	Rat <i>in vivo</i>	[26,29,31,68]
	Rat <i>in vitro</i>	[28]
	Mouse <i>in vivo</i>	[70]
Puromycin	Rat <i>in vivo</i>	[27,31,68]
Ciclosporin	Rat <i>in vivo</i>	[26,68]
	Rat <i>in vitro</i>	[28]
	Human <i>in vitro</i>	[71,72]
Cephaloridine	Rat <i>in vivo</i>	[73]
	Rat <i>in vitro</i>	[28]

Table 2

Liver gene-expression profiling of drugs with hepatotoxicity.

Drug	Model used	Ref.
Acetaminophen	Rat <i>in vivo</i>	[37,39,44,57,74–78]
	Rat <i>in vitro</i>	[44,79–81]
	Mouse <i>in vivo</i>	[40–42,45,82–85]
	Mouse <i>in vitro</i>	[86]
	Human <i>in vivo</i>	[57,58]
	Human <i>in vitro</i>	[44,78,87]
Methapyrilene	Rat <i>in vivo</i>	[16,74,77,88–92]
	Rat <i>in vitro</i>	[17,93]
Trovafloracin	Rat <i>in vivo</i>	[61]
	Mouse <i>in vivo</i>	[62,94]
	Human <i>in vitro</i>	[60,95]
Methotrexate	Rat <i>in vivo</i>	[16,74,96,97]
	Rat <i>in vitro</i>	[17,96,98]
	Mouse <i>in vivo</i>	[99]