Idiosyncratic drug-induced liver injury (DILI) is a rare disease that develops independently of drug dose, route, or duration of administration. Furthermore, idiosyncratic DILI is not a single disease entity but rather a spectrum of rare diseases with varying clinical, histological, and laboratory features. The pathogenesis of DILI is not fully understood. Standardization of the DILI nomenclature and methods to assess causality, along with the information provided by the LiverTox Web site, will harmonize and accelerate research on DILI. Studies of new serum biomarkers such as glutamate dehydrogenase, high mobility group box protein 1, and microRNA-122 could provide information for use in diagnosis and prognosis and provide important insights into the mechanisms of the pathogenesis of DILI. Single nucleotide polymorphisms in the HLA region have been associated with idiosyncratic hepatotoxicity attributed to fluvoxacinil, ximelagatran, lapatinib, and amoxicillin-clavulanate. However, genome-wide association studies of pooled cases have not associated any genetic factors with idiosyncratic DILI. Whole genome and whole exome sequencing analyses are under way to study cases of DILI attributed to a single medication. Serum proteomic, transcriptome, and metabolome as well as intestinal microbiome analyses will increase our understanding of the mechanisms of this disorder. Further improvements to in vitro and in vivo test systems should advance our understanding of the causes, risk factors, and mechanisms of idiosyncratic DILI.

Keywords: Acetaminophen; Liver Failure; Side Effect; Epidemiology.

There is a growing interest in developing “personalized medicine” wherein a specific drug or treatment is offered to a given patient based on its predicted efficacy derived from host genomic data and/or tissue-specific gene expression.1 Examples of personalized medicine include interleukin (IL)-28B genotyping before interferon therapy for hepatitis C virus infection and use of CD117 expression in gastrointestinal stromal tumors to guide decisions regarding chemotherapy.2-4 Genomic and transcriptomic approaches may also improve patient safety by avoiding use of potentially hazardous drugs in susceptible patients. For example, avoidance of abacavir therapy in HLA-B*5701-positive patients with HIV infection has reduced the incidence of a potentially severe hypersensitivity reaction from 15% to nearly 0%.5,6 Although severe adverse drug reactions (ADRs) such as drug-induced acute liver failure (ALF) are very uncommon, the inability to reliably identify high-risk patients has prevented many promising drugs from gaining regulatory approval and led to the removal of other drugs from the marketplace.7 Currently, the role of host genetic, immunologic, and metabolic factors as well as drug and environmental influences on the pathogenesis of idiosyncratic drug-induced liver injury (DILI) is poorly understood. This is in part attributable to the lack of reliable in vitro and in vivo test systems to study DILI and the difficulty in reliably diagnosing and tracking patients with DILI.8,9 The aim of this review is to summarize recent advances in the epidemiology and diagnosis of idiosyncratic DILI, the development of sensitive and specific biomarkers for DILI, and insights gleaned from pharmacogenetic studies. As our understanding of the role of the immune system in idiosyncratic DILI evolves, studies of other host factors such as the gut microbiome will hopefully further improve our understanding of the causes and mechanisms of idiosyncratic DILI.

Advances in the Epidemiology of Idiosyncratic DILI

Intrinsic and “idiosyncratic” DILI are commonly believed to arise by different pathophysiological mechanisms. Intrinsic hepatotoxins such as acetaminophen (APAP) are typically dose dependent and have reproducible animal models that help inform our understanding of the pathways leading to hepatocyte injury.10 In contrast, most instances of DILI seen in clinical practice are termed “idiosyncratic” (ie, a mixture of characteristics unique to that patient) because they are not clearly related to the dose, route, or duration of drug administration (Figure 1). The aim of this review is to provide an update on advances in research on idiosyncratic DILI.

Abbreviations used in this paper: ADR, adverse drug reaction; ALF, acute liver failure; APAP, acetaminophen; ALT, alanine aminotransferase; DILI, drug-induced liver injury; DILIN, Drug-Induced Liver Injury Network; GWAS, genome-wide association studies; HIV, human immunodeficiency virus; HMGB-1, high mobility group box protein 1; IL, interleukin; K-18, keratin 18; SDH, sorbitol dehydrogenase.
ICD-9 diagnostic coding has proven to be labor intensive and idiosyncratic DILI will remain a challenge to reliably diagnose and study (Table 1). Nevertheless, idiosyncratic DILI is a leading cause of ALF in the United States and is likely underdiagnosed because of the need to exclude other more common causes of liver injury and show improvement after drug discontinuation or “dechallenge.” Furthermore, idiosyncratic DILI attributed to a specific drug may present with variable laboratory, clinical, and histopathological features, making it even more difficult to reliably diagnose and study (Table 1). Until an objective and reliable confirmatory test is developed, idiosyncratic DILI will remain a “clinical diagnosis of exclusion” that requires a high index of suspicion.

Studies of the epidemiology of idiosyncratic DILI have largely been retrospective case series with highly variable estimates of the incidence and natural history. The recent adaptation of electronic medical records into routine medical practice has created a unique opportunity to track and study various rare ADRs. Identification of idiosyncratic cases of DILI from administrative databases using International Classification of Diseases, Ninth Revision (ICD-9) diagnostic coding has proven to be labor intensive with low sensitivity and specificity. However, recent studies that use natural language processing algorithms that can search for key words in a text field such as “hepatotoxicity” or “toxic hepatitis” have shown improved sensitivity and specificity for DILI. In addition, the linking of clinical, laboratory, and pathology databases with text-searching algorithms may allow for more real-time identification of cases of idiosyncratic DILI.

**Registries of Idiosyncratic DILI**

In 2004, the Drug-Induced Liver Injury Network (DILIN) was established by the National Institutes of Health to improve our understanding of the causes, mechanisms, and outcomes of idiosyncratic DILI in adults and children. Similar multicenter networks have been established in Spain, Iceland, the United Kingdom, Europe, Japan, China, and Korea. These networks are leading efforts to develop standardized nomenclature, grading systems, and causality assessment methods in research on DILI. Harmonization of the approach to DILI phenotyping and causality assessment will hopefully provide an increased number of cases of DILI for pooling in genetic association studies (Supplementary Table 1). In addition, the National Institutes of Health in conjunction with the National Library of Medicine has developed a comprehensive, multilayered, and interactive database of the published literature of human drug hepatotoxicity. The LiverTox Web site (http://www.liver.tox.nih.gov) has concise overview sections on DILI phenotypes, severity grading, and likelihood scales. In addition, chapters that summarize the clinical and laboratory features of DILI associated with more than 650 individual drugs are available, along with illustrative cases and annotated references with available hyperlinks to the full PubMed reference. The LiverTox Web site has already increased awareness of DILI and will likely prove to be a valuable resource for basic, translational, and clinical research into the pathogenesis of DILI for years to come.

Several reports of the etiologies and outcomes of idiosyncratic DILI from around the world have recently been published (Table 2). The laboratory profile of DILI at presentation is defined by the ratio of serum alanine aminotransferase (ALT) to alkaline phosphatase levels (ie, R value = [ALT/upper limit of normal]/[alkaline phosphatase/upper limit of normal]) and can be categorized as hepatocellular (R > 5), mixed (R = 2–5), or cholestatic (R < 2). Similarities across the DILI cohorts include the proportion of female patients (49%–65%), the median age of the subjects (48–55 years), and the proportion of patients with acute hepatocellular injury at presentation (42%–58%). Although the DILIN and Spanish networks tend to enroll sicker patients who are more likely to be hospitalized, the proportion of patients who have died or required liver transplantation is remarkably similar. Antibiotics are the most commonly identified drug class leading to idiosyncratic DILI, but the specific implicated agents differ substantially. In addition, DILIN has reported a significant proportion of cases attributed to herbal and dietary supplements, increasing from 7% in 2004–2005 to 20% in...
The most frequently implicated herbal and dietary supplements in DILIN are bodybuilding supplements and weight loss products that contain green tea extract.36,37 DILIN is prospectively following all study subjects for a minimum of 6 months after enrollment.22,33 A competing cause of liver injury has been identified in up to 15% of patients in DILIN, including previously unsuspected acute idiosyncratic DILI.

<table>
<thead>
<tr>
<th>Table 1. Clinicopathological Presentations of Idiosyncratic DILI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenotype</strong></td>
</tr>
<tr>
<td>Acute fatty liver with lactic acidosis</td>
</tr>
<tr>
<td>Acute hepatic necrosis</td>
</tr>
<tr>
<td>Autoimmune-like hepatitis</td>
</tr>
<tr>
<td>Bland cholestasis</td>
</tr>
<tr>
<td>Cholestatic hepatitis</td>
</tr>
<tr>
<td>Fibrosis/cirrhosis</td>
</tr>
<tr>
<td>Immunoallergic hepatitis</td>
</tr>
<tr>
<td>Nodular regeneration</td>
</tr>
<tr>
<td>Nonalcoholic fatty liver</td>
</tr>
<tr>
<td>Sinusoidal obstruction syndrome</td>
</tr>
<tr>
<td>Vanishing bile duct syndrome</td>
</tr>
</tbody>
</table>

Table 2. Presenting Clinical Features and Outcomes in Prospective Studies of DILI

<table>
<thead>
<tr>
<th>Feature</th>
<th>DILIN US (N = 300)</th>
<th>Spain (N = 446)</th>
<th>Japan (N = 1676)</th>
<th>Iceland (N = 96)</th>
<th>France (N = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causality method</td>
<td>Expert opinion</td>
<td>RUCAM</td>
<td>RUCAM</td>
<td>RUCAM</td>
<td>CIOMS</td>
</tr>
<tr>
<td>Duration of follow-up (mo)</td>
<td>6–24</td>
<td>3</td>
<td>NA</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>48</td>
<td>53</td>
<td>55</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>49</td>
<td>57</td>
<td>56</td>
<td>65</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>79</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Black</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of liver injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>57</td>
<td>58</td>
<td>59</td>
<td>42</td>
<td>53</td>
</tr>
<tr>
<td>Mixed/cholestatic</td>
<td>20/23</td>
<td>22/20</td>
<td>20/21</td>
<td>26/32</td>
<td>26/21</td>
</tr>
<tr>
<td>Jaundice</td>
<td>69</td>
<td>71</td>
<td>NA</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>50</td>
<td>25</td>
<td>NA</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>60</td>
<td>53</td>
<td>NA</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Died or underwent transplantation</td>
<td>10</td>
<td>7</td>
<td>3.7</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Chronic</td>
<td>14</td>
<td>10</td>
<td>8.4</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td>Suspected drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>45</td>
<td>32</td>
<td>14</td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td>Psychotropic drugs</td>
<td>15</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Herbal and dietary supplements</td>
<td>9</td>
<td>0</td>
<td>17.1</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Hypolipidemic agents</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

NOTE. All values are percentages unless otherwise noted.
RUCAM, Roussel Uclaf Causality Assessment Method; CIOMS, Council for International Organizations of Medical Sciences; NA, not available.
Epidemiology of Idiosyncratic DILI: Mechanistic Insights

Although drugs undergo extensive safety testing in vitro test systems and various animal species before clinical development, this testing frequently fails to identify potentially hepatotoxic drugs. In addition, the low overall incidence of idiosyncratic DILI with most available drugs of only 1 in 10,000 to 1 in 100,000 patient-years prevents most hepatotoxic drugs from being identified in clinical trials. Recent studies have suggested that drugs that are administered at a daily dose >50 to 100 mg/day with greater lipophilicity are more prone to cause DILI compared with agents given at a lower daily dose with less lipophilicity. Possible explanations for these simple but intriguing observations include the fact that drugs given in high daily doses may lead to higher intrahepatic levels of the parent drug or a metabolite involved in the pathogenesis of DILI. In addition, lipophilic drugs require greater metabolism to be eliminated from the body, which may increase their likelihood of causing liver damage. It is also possible that extensively metabolized drugs may induce covalently bound hapten, which can elicit an adverse adaptive immune response in a genetically susceptible patient. However, the daily dose of a medication, its lipophilicity, and the extent of hepatic metabolism are inadequate features to reliably predict the risk of DILI from individual drugs. In addition, studies of chemoinformatics have failed to identify chemical moieties in drugs that are more prone to lead to idiosyncratic hepatotoxicity.

In Western patients with DILI, antibacterial antibiotics and psychoactive drugs are the most frequently implicated therapeutic drug classes (Table 2). Although antibacterial antibiotics are used by millions of Americans each day, they are usually only taken for a few days or weeks. This suggests that an active or recent infection may increase the susceptibility for an ADR via aberrant innate or adaptive immune pathways or alterations in the gut microbiome in susceptible patients. In support of the “danger hypothesis” (Figure 2), administration of tumor necrosis factor to hepatocyte cultures as well as in animal models of DILI can potentiate the hepatotoxicity of multiple drugs. In addition, the greater frequency of DILI in immunosuppressed liver transplant recipients (1 in 100 patient-years) compared with the general population (1 in 10,000 patient-years) suggests that specific patient populations may be at increased risk for idiosyncratic DILI. However, a plausible explanation as to why the majority of patients with an acute or chronic illness who receive multiple medications, including antibiotics, do not develop DILI remains unclear.

A recent population-based study from Iceland provides the best estimates of the incidence of idiosyncratic DILI in a
In this study, all suspected episodes of DILI regardless of severity among the 250,000 inhabitants of Iceland over a 2-year period were reviewed. The crude overall annual incidence of idiosyncratic DILI was 19.1 cases per 100,000 population, which is remarkably similar to the rate of 13.9 cases per 100,000 reported from France.64 These rates are likely higher than prior estimates because of the prospective nature of these studies, the inclusion of subclinical cases, and the ability to systematically canvass an entire population. Although no differences in sex were noted, there was a strong association of the risk of DILI with patient age, which varied from 9 per 100,000 (age 15–29 years) to 41 per 100,000 (age >70 years). Although amoxicillin-clavulanate was the most frequently implicated drug, the estimate of the risk of DILI was 1 per 2350 users; this was substantially lower than that observed with azathioprine (1 per 133 users) and infliximab (1 per 148 users). These data lend support to the notion that some drugs used in clinical practice are intrinsically more hepatotoxic than others. Although DILIN is not a population-based study, amoxicillin-clavulanate is the most commonly implicated agent (>120 cases in DILIN) but is prescribed to more than 70 million Americans each year. In contrast, isoniazid (>50 cases in DILIN and <200,000 prescriptions per year) and nitrofurantoin (>50 cases of DILIN and <500,000 prescriptions each year) appear to be overrepresented in the DILIN and ALF Study Group databases.64 Similarly, the infrequent reporting of statins, beta-blockers, and calcium channel blockers in DILI registries despite their widespread use suggests that these drug classes are probably not as intrinsically hepatotoxic as previously suggested.64,65 The LiverTox Web site has developed a categorization of the likelihood of DILI from a specific agent based on the frequency of bona fide cases in the literature that varies from category A (“Well known with > 50 cases described,” such as amoxicillin-clavulanate and phenytoin) to category E (“Despite extensive use, no evidence of liver injury,” such as felodipine or propranolol).

**Inferences of the Pathogenesis of DILI From Liver Histology**

DILI is a well-known imitator of most forms of acute and chronic liver injury (Table 1). Furthermore, some drugs, such as valproate, may cause differing patterns of liver injury in individual patients that can vary from hepatic steatosis to massive necrosis.66 A careful review of liver histopathology in 249 patients in DILIN indicated that 5 liver injury patterns accounted for 83% of the cases.67 In addition, poorer outcomes were associated with higher degrees of necrosis, microvesicular steatosis, and a ductular reaction, whereas subjects with intrahepatic eosinophilic and/or granulomas tended to fare better. The prognostic use of these histological features is consistent with prior reports and suggests that peripheral or intrahepatic eosinophilia is protective.58,59

Other studies have suggested potential histopathological differences in patients with idiopathic and drug-induced autoimmune hepatitis.60 Similarly, studies of lymphocyte subsets in liver tissue samples have shown that subjects with DILI are less likely to have natural killer cells, CD4+ T-helper cells, and B cells compared with patients with acute viral hepatitis (Foureau et al, manuscript in submission). The predominance of CD8+ cytotoxic T cells in the livers of patients with idiosyncratic DILI is consistent with the hypothesis that the intrahepatic generation of neoantigens from the drug or its metabolite may lead to the recruitment and activation of T lymphocytes that can initiate or perpetuate the liver injury.61 In contrast, neutrophil and macrophage infiltration into the liver is believed to be a late event in patients with APAP overdose and treated with other drugs that directly injure pericentral hepatocytes.62 Similarly, patients with bland cholestasis due to use of anabolic steroids may develop liver injury through direct toxicity to bile salt or other drug transporters.63

Drugs that disrupt mitochondrial function via depletion of mitochondrial DNA or triggering of outer mitochondrial membrane permeabilization can lead to variable patterns of liver tissue injury. Acute impairment of mitochondrial function is a distinctive and frequently dramatic clinical syndrome characterized by small droplets of fat (microvesicular) that accumulate in the hepatocyte cytosol from impaired beta oxidation of fatty acids as reported with tetracycline and valproic acid.65,66 In contrast, drugs that lead to partial but chronic depletion of mitochondrial function can lead to accumulation of large fat droplets (macrovesicular) that are often eccentrically located in the cell. Drugs associated with the latter subacute or chronic phenotype include tamoxifen and various dideoxynucleoside analogues used to treat patients with human immunodeficiency virus (HIV) infection.65,66 Other drugs, such as oxaliplatin, can damage liver endothelial cells and lead to nodular regenerative hyperplasia and portal hypertension in some patients.67,68

**In Vitro Test Systems to Study the Mechanisms of DILI**

Extensive in vitro and animal toxicology testing is required in drug development, but these approaches have consistently failed to predict the development of various ADRs, including DILI. In 2007, the US National Academy of Sciences suggested greater use of human biological test systems to improve detection of and provide mechanistic insight into human ADRs.69 In addition, the need for pathway analysis of toxicological mechanisms was emphasized using metabolomic, transcriptomic, and genomic approaches. Currently, potential drug-drug interactions can be reliably predicted from in vitro inhibition of known phase 1 and 2 polymorphisms in cultured human hepatocytes. Recently, several chimeric mice strains expressing highly differentiated human hepatocytes that can be used in cell culture experiments as well as human hepatoma cell lines with highly differentiated cellular function have been developed.70,71 In addition, the discovery that human and mouse fibroblasts and somatic cells can be preprogrammed
into inducible pluripotent stem cells has generated a great deal of interest in the use of this novel technology to study drug hepatotoxicity, liver regeneration, and various genetic diseases.\textsuperscript{72–74} Derivation of hepatocytes from inducible pluripotent stem cells of patients who develop DILI could allow for a long-term and renewable source of cells to study the mechanisms of idiosyncratic DILI in affected patients compared with treated controls.\textsuperscript{75}

Advances in the development of in vitro culture methods of T cells implicated in various hypersensitivity drug reactions have also recently been reported, including techniques to expand the number of available T cells derived from the peripheral blood of patients with allergic drug reactions.\textsuperscript{76} Because antigens are processed and presented on HLA molecules of antigen-presenting cells to the T-cell receptor on T cells, additional studies of T-cell physiology can now be conducted (Figure 3). Patients with known hypersensitivity to piperacillin, which by itself is unlikely to stimulate an immune response, have shown alterations in the binding site of albumin that can lead to highly immunogenic drug metabolite–albumin conjugates.\textsuperscript{77,78} In addition, modifications of the lymphocyte proliferation assay have been developed with readouts of T-cell cytokine expression and microarrays rather than pure cellular proliferation.\textsuperscript{79} Whether these modified lymphocyte transformation assays will improve drug-specific diagnoses or provide mechanistic insights into the pathogenesis of DILI remains to be determined.

Biomarkers for the Pathogenesis of DILI and Outcomes

Biomarkers are analytes from blood, urine, or other biological samples that may provide insight into the severity, cause, or outcome of an episode of DILI. In addition, biomarkers may improve the speed or accuracy of diagnosing DILI.\textsuperscript{80} Ideally, direct examination of liver tissue would provide the greatest insight into the pathophysiological steps involved in idiosyncratic DILI but is impractical for obvious reasons. The serum biomarkers most commonly used to detect and manage most forms of acute and chronic liver injury are serum ALT, alkaline phosphatase, and total bilirubin levels. Serum ALT is more liver specific than serum aspartate aminotransferase but is not etiology specific, and levels can also be elevated in subjects with extensive hepatic glycogen, hepatocyte autophagy, and hepatic steatosis.\textsuperscript{81,82} Furthermore, monitoring for elevated serum ALT levels in subjects receiving a potentially hepatotoxic drug such as isoniazid has consistently failed to identify subjects at risk for developing DILI compared with the larger group of

Figure 3. Proposed role of aberrant immunity in the pathogenesis of DILI. Drugs are small molecules capable of binding to serum proteins under normal physiological circumstances for transport, metabolism, and elimination. In most instances, a drug-protein conjugate will not elicit a host immune response. However, a minority of individuals with specific class II HLA alleles that are ubiquitously expressed may be uniquely predisposed to have the native drug-protein or drug-metabolite–protein conjugate activate an antigen presenting cell such as a dendritic cell or macrophage. The processing and handling of drug-protein conjugates in these subjects can then inadvertently activate T-cell receptors, which may proliferate and mediate end-organ damage. MHC, major histocompatibility complex.
patients who “adapt” with normalization of ALT levels during continued treatment.83,84 Similarly, serum alkaline phosphatase levels are not liver specific and may be spuriously elevated in other disease states.85 Total bilirubin levels are a sensitive marker for most forms of liver disease, increasing only when there has been extensive liver damage or via direct inhibition of biliary transporters. Fractionation of total bilirubin levels can help exclude benign elevations due to intravascular hemolysis and genetic polymorphisms in uridine glucuronyl transferase activity (Gilbert syndrome), which are present in 1% to 5% of the general population.

**New Serum Biomarkers**

Ongoing efforts to identify new biomarkers for DILI include the large-scale initiative of the Safer and Faster Evidence-based Translation (SAFE-T) Consortium in Europe.85 Proposed biomarker candidates include serum markers of liver injury (sorbitol dehydrogenase [SDH], glutathione S-transferase α) and mitochondrial dysfunction from disrupted hepatocytes (glutamate dehydrogenase) (Table 3). In addition, the discovery of circulating microRNAs (miRNAs) in the serum has shown the novel tissue specificity of miR-122 for liver injury. Assessment of full-length keratin-18 (K-18) and high mobility group box protein 1 (HMGB-1) in the serum has been shown to be a sensitive biomarker for necrotic cell death, while caspase cleaved K-18 fragments are noted in subjects with ongoing apoptosis. However, neither of these markers are liver disease specific. M-30 is a serum protein that selectively recognizes caspase cleaved neo-epitopes of K-18 released from hepatocytes undergoing apoptotic death, while serum M-65 reflects total hepatocyte death (apoptosis and necrosis).86,87 An index of serum M-30 levels in combination with other laboratory parameters was recently shown to be superior to the King’s College criteria and Model for End-Stage Liver Disease (MELD) score in predicting spontaneous survival in ALF.88,89

Heparins are a widely used class of biological agents that are frequently associated with mild and nonprogressive elevations of serum ALT levels but rarely, if ever, lead to clinically significant liver injury.90 A recent study of 48 healthy men given a heparin formulation for 5 days showed asymptomatic elevations in serum aspartate aminotransferase and ALT levels in 90% of the treated patients.91 In addition, significant elevations in the levels of serum SDH, glutamate dehydrogenase, miR-122, and HMGB-1 in both its native and acetylated form were noted.92 However, serum K-18 fragment levels indicative of apoptosis remained unchanged. These data suggest that heparins cause a self-limited and mild hepatocyte necrosis with secondary activation of the innate immune system. HMGB-1, a damage-associated molecular pattern protein, can bind to Toll-like receptor 4 and initiate an innate immune response at its site of release in the liver.93 The detection of acetylated HMGB-1 is suggestive of activated innate immune cells, which may be linked to tissue repair. However, the reason for a lack of progressive liver injury despite continued dosing with heparins remains unclear.94

**Table 3. Proposed Biomarkers for DILI**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Source and significance</th>
<th>Test performance to date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver injury markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDH, Glutathione S-transferase α</td>
<td>Hepatocyte-specific injury; Centrilobular liver damage and kidney damage</td>
<td>Earlier marker of acute liver injury; Early marker of acute liver (serum) and kidney (urine) injury</td>
</tr>
<tr>
<td>Bile acids</td>
<td>Synthesized in the liver; disruption of hepatic excretion</td>
<td>More sensitive than bilirubin for excretory abnormalities</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>Mitochondrial disruption</td>
<td>Increased in some patients with chronic liver disease</td>
</tr>
<tr>
<td>Serum cytokine profiles</td>
<td>Intra and extrahepatic origin</td>
<td>Associated with prognosis</td>
</tr>
<tr>
<td>miRNAs, miR-122</td>
<td>Liver-specific release from damaged hepatocytes</td>
<td>Released into plasma with acute and chronic injury; validation ongoing</td>
</tr>
<tr>
<td>Mechanistic biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMGB-1</td>
<td>Necrosis marker</td>
<td>Not liver specific</td>
</tr>
<tr>
<td>Acetylated HMGB-1</td>
<td>Innate immune activation marker</td>
<td>Acetylation requires mass spectroscopy for detection</td>
</tr>
<tr>
<td>Cytokeratin-18 fragments</td>
<td>Marker of caspase cleaved proteins in apoptotic cell death</td>
<td>Not liver specific</td>
</tr>
<tr>
<td>M-30</td>
<td>Apoptosis marker</td>
<td>Ability to distinguish therapeutic dosing from drug overdose being tested</td>
</tr>
<tr>
<td>M-65</td>
<td>Total apoptosis and necrosis marker</td>
<td></td>
</tr>
<tr>
<td>Serum Cys-APAP adducts</td>
<td>Marker of APAP overdose</td>
<td></td>
</tr>
<tr>
<td>Metabolomics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine or serum metabolome</td>
<td>Amount and type of endogenous substances</td>
<td>Exploratory with substantial drug, dietary, environmental, and microbiome influences; bioinformatics for data reduction ongoing</td>
</tr>
</tbody>
</table>
treated patients experienced an increase in serum ALT levels. There was also an increase in serum SDH, glutamate dehydrogenase, and HMGB-1 levels. A panel of these early biomarkers for DILI was recently tested in patients presenting to the hospital after an APAP overdose with initially normal serum aminotransferase levels. A combination of miR-122, HMGB-1, and K-18 levels identified the development of liver injury with a higher degree of accuracy than the initial serum ALT level, international normalized ratio, and plasma APAP level. In addition, acetylated HMGB-1 levels reflective of immune activation were noted later in the course of APAP overdose and associated with a poorer outcome in an independent cohort of 78 patients. Several studies have shown that detection of APAP-protein adducts in serum can confirm a diagnosis of APAP hepatotoxicity. The premise of this test is based on the formation of covalent adducts between intrahepatic protein and the major reactive metabolite of APAP formed in the liver, N-acetyl-p-benzoquinone imine. Because serum APAP-protein adducts have a longer half-life in serum compared with the parent drug or its metabolites, this test may be particularly informative in patients presenting late after an unintentional APAP overdose. In addition, 15% of patients with indeterminate ALF also had detectable APAP-protein adducts. However, low levels of APAP-protein adducts have also been detected in the blood of patients taking therapeutic doses of APAP over 5 days. The development of tests to detect circulating drug-protein adducts in other patients with idiosyncratic DILI would be desirable. However, idiosyncratic DILI due to most drugs occurs at lower daily doses, and toxic metabolites implicated in the pathogenesis of DILI are believed to represent a smaller fraction of the total testable drug in the serum compared with APAP, making it technically difficult to identify and quantify drug-protein adducts.

Serum Proteomics
Proteomic studies that simultaneously identify and quantify thousands of proteins use high-pressure liquid chromatography and tandem mass spectroscopy. With advanced bioinformatics software, the protein(s) and/or pathways involved in the pathogenesis of DILI can then be studied. The serum proteomic profiles of 74 patients in DILIN who had a baseline sample collected within 2 weeks of onset of DILI and 40 healthy controls were recently analyzed using a label-free, mass spectrometry quantitative approach. Several proteins were expressed at a higher level in subjects with hepatocellular versus cholestatic DILI, including fructose-bisphosphate aldolase B. This hepatic enzyme correlated with serum ALT and aspartate aminotransferase levels at baseline and returned to normal during follow-up. Of note, autoantibodies to this protein have previously been reported in patients with troglitazone hepatotoxicity. In addition, elevations in levels of apolipoprotein E, an abundant lipoprotein of triglyceride-rich chylomicrons, had the greatest ability to distinguish patients with DILI from controls. A longitudinal analysis of 21 patients with baseline and 6-month samples showed that expression of 53 priority 1 proteins either increased or decreased over time, including components of inflammatory, immune system activation, and several hepatotoxicity-specific pathways. A proteomics platform in combination with metabolomics was recently used to distinguish patients who developed ximelagatran hepatotoxicity from unaffected controls. In that study, biomarkers that predicted patients at risk for elevation of ALT levels included apolipoproteins A-II, A-IV, and E. These provocative data suggest that further proteomic studies are indicated particularly in samples obtained before or shortly after the onset of DILI.

Serum Cytokines and Chemokines
Serum cytokine and chemokine levels may also prove to be useful diagnostic or prognostic biomarkers for DILI. DILIN recently completed an analysis of 27 immune analytes in 78 subjects who were enrolled within 2 weeks of onset of DILI and 6 months after enrollment. Disparate patterns of immune responses were evident, and low values of IL-9, IL-17, platelet-derived growth factor BB, and RANTES combined with serum albumin were predictive of early death. Lower levels of the cytokines associated with innate immunity (ie, low IL-9 and IL-17 levels) were associated with a poorer prognosis, suggesting a role for these biomarkers and immune pathways in the pathogenesis of DILI. These data are consistent with recent studies showing a role of the Th17 adaptive immunity pathway in the pathogenesis of idiosyncratic DILI. However, a study from the ALF Study Group failed to show a difference in circulating IL-17 levels of patients with ALF due to idiosyncratic DILI compared with those with APAP overdose.

Transcriptomics and Metabolomics
Transcriptomics represents the detection and quantification of transcribed genes or messenger RNA in the serum and other fluids. In contrast, metabolomics represents the study of endogenous small molecules and metabolites in the serum and urine that reflect normal physiological and diseased states. Transcriptomic studies require the collection of RNA from specialized tubes and then detection of relative gene expression levels using oligo array chips and quantitation of messenger RNA expression using polymerase chain reaction. In metabolomic studies, samples are analyzed using nuclear magnetic resonance spectroscopy or mass spectral techniques to simultaneously detect and quantify thousands of endogenous metabolites. A recent study of 8 hepatotoxins administered to rats showed that transcriptomes present in peripheral blood correlated with direct measures of these genes in the liver. In fact, these studies suggested that peripheral blood transcriptomic data might be more sensitive to liver injury than traditional liver injury tests such as serum ALT and that unique signatures for individual drugs, including APAP hepatotoxicity, could be determined. Furthermore, human whole blood transcriptome data from patients with overt APAP overdose could differentiate patients with...
toxicity from those without. However, marked down-regulation of genes involved in oxidative phosphorylation and mitochondrial function as well as metabolic changes in the blood and urine were observed in patients receiving a therapeutic dose of APAP compared with those with overt APAP hepatotoxicity.

Advantages of metabolomic and transcriptomic studies in biomarker discovery include the large amount of data that can be generated and quantified from small sample aliquots. Furthermore, pathways analysis can be conducted using bioinformatics platforms. Finally, the relationship between changes in gene expression from the transcriptomic platforms can be integrated with the physiological changes detected from metabolomics. However, the transcriptome in whole blood may chiefly reflect the transcriptional activity of lymphocytes, and it appears that these approaches may not be able to distinguish the pharmacological effect of a drug like APAP from a toxic dose. In addition, serum and urine metabolomes in patients can be influenced by dietary and environmental factors, including the gut microbiome. Therefore, further studies of the drivers of interindividual differences in the metabolome and transcriptome are needed as part of a systems biology approach to DILI susceptibility.

miRNAs

miRNAs are small regulatory, noncoding RNAs that are 18 to 25 nucleotides in length and can be detected in micro-vesicles in the serum. Although a number of miRNAs are widely expressed, certain miRNAs appear to be tissue specific. Liver-specific miRNAs include miR-122, miR-21, and miR-192, and these molecules are believed to repress a set of cellular proteins and cell phenotypes. Liver-derived miRNAs may be a highly sensitive and specific biomarker of APAP hepatotoxicity that parallel ALT levels but increase earlier in the course of liver injury. In addition, they appear to have prognostic significance for patients with APAP overdose in need of transplant versus those more likely to survive. However, serum miR-122 levels have not been directly correlated to hepatic expression in humans. Nonetheless, their short half-life, liver tissue specificity, and quantifiability make them attractive biomarkers for severe acute liver injury.

Pharmacogenetic Studies of Idiosyncratic DILI

Because of its low incidence in the general population, a genetic variation in host receptors, immune response, and metabolic pathways have been implicated in the pathogenesis of idiosyncratic DILI. Previous genetic association studies have largely focused on candidate genes involved in the uptake, metabolism, transport, or detoxification of a drug that can be used to predict drug pharmacokinetic and pharmacodynamic parameters. For example, reduced activity in the NAT2 gene and increased activity in CYP2E-mediated oxidative metabolism have been implicated in isoniazid hepatotoxicity. However, these hypothesis-driven, biologically plausible approaches have yielded only weak associations that are frequently not replicated in independent cohorts.

An alternative approach to identifying genetic associations is to scan the entire human genome in affected cases and population controls without a specific a priori hypothesis. In most genome-wide association studies (GWAS), the frequency of single nucleotide polymorphisms is at least 1% to 5% in the general population. This “discovery” platform allows for the unbiased detection of up to 1 million single nucleotide polymorphisms that may associate with the disease trait of interest. The first successful GWAS in DILI identified a very strong association between flucloxacillin-induced liver injury and the HLA-B*5701 allele on chromosome 6. Other GWAS have identified additional HLA alleles associated with lumiracoxib, ximelagatran, and lapatinib hepatotoxicity (Supplementary Table 2).

Genetic Studies of Flucloxacillin Hepatotoxicity

Flucloxacillin is a parenterally administered β-lactam antibiotic that is widely used in Europe and other countries to treat staphylococcal and streptococcal infections. Flucloxacillin is associated with a rare but potentially severe cholestatic hepatitis that is more common in female patients, in elderly patients, and after prolonged courses of treatment. Daly et al performed a GWAS in 51 patients with bona fide flucloxacillin cholestasis compared with 64 ethnically matched treated controls as well as population controls. In the initial and validation cohorts, possession of the HLA-B*5701 allele was associated with an 80-fold increased risk of developing DILI ($P = 9 \times 10^{-19}$). This HLA allele has also been associated with abacavir hypersensitivity in patients with HIV infection. Although this is one of the strongest genetic associations ever reported from a GWAS, the high frequency of HLA-B*5701 in the general population (6%–8%) coupled with the low incidence of DILI in treated patients (1 in 10,000) leads to a low positive predictive value (0.12%) for identifying patients at risk for flucloxacillin cholestasis but a high negative predictive value (99.99%). Therefore, testing for HLA-B*5701 may help diagnose flucloxacillin cholestasis in exposed patients who develop jaundice, but use of the drug should not be withheld in HLA-B*5701–positive patients.

Flucloxacillin is also an agonist of the human pregnane X receptor, and further studies have shown that patients with flucloxacillin cholestasis are more likely to have polymorphisms in the pregnane X receptor promoter region. Other studies have shown an important role for the adaptive immune system, with T-cell clones of afflicted patients and previously unexposed HLA-B*5701–positive patients showing increased reactivity to flucloxacillin-albumin conjugates in a dose-dependent manner. These studies also showed that the reactive T-cell clones from afflicted patients had cell surface markers that are associated with hepatic localization. These data confirm that flucloxacillin-protein binding is critical for the formation of functional T-cell
antigens. This is in contrast to recent mechanistic studies of abacavir and carbamazepine hypersensitivity reactions wherein the native drug itself has been shown to bind in the antigen-binding cleft of an HLA molecule and directly stimulate an immune response. Additional studies to further understand how flucloxacinil mediates liver damage and the role of hapten formation versus direct stimulation of T cells are ongoing (Figure 3).

Genetic Studies in Pooled Patients With Idiosyncratic DILI

DILIN and other groups have been collecting DNA from patients with idiosyncratic DILI for pooled pharmacogenetic studies. Hypothesizing that susceptibility to DILI may be shared across multiple drugs, a GWAS was recently undertaken in 783 white patients who experienced DILI from more than 200 implicated agents. Unfortunately, no genome-wide significant associations were noted, and further stratification of cases according to clinical phenotypes such as injury pattern, latency, severity, drug class, and patient age did not reveal any significant associations. The lack of GWAS findings in the pooled DILIN cases supports the notion that genetic determinants of risk of idiosyncratic DILI may be largely drug specific or due to rarer genetic variants not assessed on the GWAS chip. Going forward, newer techniques, including exome arrays that can assess for functional genetic variants present in 1 in 1000 to 1 in 5000 patients at more than 250,000 loci, are being undertaken. In addition, improvements in the speed, accuracy, and costs of whole exome and whole genome sequencing now allow for a more in-depth search of causal variants from smaller samples of well-phenotyped, high-causality cases attributed to a single drug. The role of DNA methylation, copy number variants, and epigenetics in most forms of acute liver injury is largely unknown but also worthy of further study.

Mechanistic Inferences Into the Pathogenesis of DILI

The strong and consistent association of susceptibility to DILI with various single nucleotide polymorphisms in the HLA region suggests that the host immune response plays a key role in the pathogenesis of DILI. HLAs are highly polymorphic proteins that are designed to initiate immunity by presenting pathogen-derived peptides to T cells. Polymorphisms in HLA genes mostly map to the antigen-binding cleft, which allows diversification of the repertoire of self-derived and pathogen-derived peptide antigens to be presented to T cells. A growing number of other immunologically based ADRs, including dermatologic reactions and idiosyncratic DILI, are also associating with various HLA alleles. In most of these instances, the implicated drug does not directly bind to the antigen-binding cleft of the HLA–molecule. Rather, a series of drug-protein modification steps or conversion of the drug to an intermediate or reactive metabolite is required to form an immunogenic hapten. However, it is likely that other host genetic or intracellular pathways may also be required for an ADR to develop. Furthermore, because many susceptible patients with a given HLA haplotype do not develop DILI or other ADRs on drug exposure, the role of other intracellular “bioactivation” and “detoxification” pathways that may allow adaptation to occur need to be evaluated (Figure 2). In addition, because HLA polymorphisms are ethnically restricted, the absence of a genetic association of susceptibility to DILI in one patient population will not preclude a positive association in another group, as recently noted in differing HLA susceptibility alleles in Han Chinese and European subjects to carbamazepine hypersensitivity reactions.

The potential for the gut microbiome to affect susceptibility and outcome to DILI is another intriguing hypothesis worthy of further study. Early studies have shown interesting alterations in the gut microbiome of patients with obesity compared with nonobese controls and in animal models of fatty liver. Recent studies of complex biliary tract disorders also show that variance in microbiome content may exceed that explained by genomic variation. There have been no studies of the gut microbiome in patients with idiosyncratic DILI, but there are interesting animal data showing a significant impact of the gut microbiome in mediating melamine-related kidney injury.

Future Directions in Research on DILI

Over the next 5 to 10 years, additional studies of host genetic polymorphisms and susceptibility to idiosyncratic DILI attributed to individual agents will be completed using next-generation sequencing. To conduct such studies, DNA samples collected from bona fide cases of DILI in which competing viral, immunologic, and metabolic causes of liver disease have been definitively excluded are needed, as well as validation samples from independent cohorts. It is hoped that mining of electronic medical records with natural language processing algorithms will improve the speed and accuracy of the acquisition of cases of DILI and inform pharmacoepidemiological studies regarding the causes of DILI in a given population. In addition, continued efforts from multicenter research networks such as DILIN will help provide biological samples for mechanistic studies. Improved causality assessment tools, case definitions, and further development of a web-based portal of human hepatotoxicity such as LiverTox will also be essential. Finally, the integration of data from divergent research platforms (ie, proteomics, transcriptomics, metabolomics, genomics) using a systems biology approach as well as data derived from improved in vitro and in vivo test systems may provide an unprecedented opportunity to study human drug metabolism and idiosyncratic DILI.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at www.gastrojournal.org and at http://dx.doi.org/10.1053/j.gastro.2013.12.032.
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Conflicts of interest
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### Supplementary Table 1. Causality Assessment Methods Used in Studies of DILI

<table>
<thead>
<tr>
<th>Likelihood of DILI</th>
<th>World Health Organization (4 levels)</th>
<th>Naranjo (4 levels)</th>
<th>Roussel Uclaf Causality Assessment Method (5 levels)</th>
<th>Maria and Victorino (5 levels)</th>
<th>DILIN (5 levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;75%</td>
<td>Level 1 (certain)</td>
<td>Definite</td>
<td>Highly probable</td>
<td>Definite</td>
<td>Definite (1) and highly likely (2)</td>
</tr>
<tr>
<td>50%–75%</td>
<td>Level 2 (probable)</td>
<td>Probable</td>
<td>Probable</td>
<td>Probable</td>
<td>Probable</td>
</tr>
<tr>
<td>25%–50%</td>
<td>Level 3 (possible)</td>
<td>Possible</td>
<td>Possible</td>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td>&lt;25%</td>
<td>Level 4 (unlikely)</td>
<td>Doubtful</td>
<td>Not likely and excluded</td>
<td>Not likely and excluded</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

Adapted from the LiverTox Web site (http://www.livertox.nih.gov).

### Supplementary Table 2. GWAS of Susceptibility for DILI

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of cases of DILI</th>
<th>Control</th>
<th>Gene</th>
<th>Minor allele frequency (%)</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flucloxacillin (β-lactam antibiotic)</td>
<td>51</td>
<td>282 population controls</td>
<td>HLA-B*5701</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>Ximelagatran (thrombin inhibitor)</td>
<td>74</td>
<td>130 treated controls</td>
<td>DRB1*07</td>
<td>8.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Lumiracoxib (cyclooxygenase-2 inhibitor)</td>
<td>41</td>
<td>176 treated controls</td>
<td>DQA1*02</td>
<td>15</td>
<td>5.0</td>
</tr>
<tr>
<td>Laptinib (kinase inhibitor)</td>
<td>37</td>
<td>286 treated controls</td>
<td>DRB1*1501</td>
<td>21</td>
<td>9.0</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate (antibiotic)</td>
<td>201</td>
<td>532 population controls</td>
<td>DRB1*02</td>
<td>16</td>
<td>2.8</td>
</tr>
</tbody>
</table>