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Update on Advances in Research on Idiosyncratic Drug-Induced Liver Injury

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Drug-induced liver injury (DILI) is a major concern for public health, as well as for drug development in the pharmaceutical industry, since it can cause liver failure and lead to drug withdrawal from the market and black box warnings. Thus, it is important to identify biomarkers for early prediction to increase our understanding of mechanisms underlying DILI that will ultimately aid in the exploration of novel therapeutic strategies to prevent or manage DILI. DILI can be subdivided into 'intrinsic' and 'idiosyncratic' categories, although the validity of this classification remains controversial. Idiosyncratic DILI occurs in a minority of susceptible individuals with a prolonged latency, while intrinsic DILI results from drug- induced direct hepatotoxicity over the course of a few days. The rare occurrence of idiosyncratic DILI requires multicenter collaborative investigations and phenotype standardization. Recent progress in research on idiosyncratic DILI is based on key developments in 3 areas: (1) newly developed high-throughput genotyping across the whole genome allowing for the identification of genetic susceptibility markers, (2) new mechanistic concepts on the pathogenesis of DILI revealing a key role of drug-responsive T lymphocytes in the immunological response, and (3) broad multidisciplinary approaches using different platform "-omics" technologies that have identified novel biomarkers for the prediction of DILI. An association of a specific human leukocyte antigen (HLA) allele with DILI has been reported for several drugs. HLA-restricted T-cell immune responses have also been investigated using lymphocytes and T-cell clones isolated from patients. A microRNA, miR-122, has been discovered as a promising biomarker for the early prediction of DILI. In this review, we summarize recent advances in research on idiosyncratic DILI with an understanding of the key role of adaptive immune systems.

Keywords: Drug-induced liver injury; human leukocyte antigen; T-cell; immune response; hapten; biomarker

INTRODUCTION

Idiosyncratic drug-induced liver injury (DILI) is an adverse drug reaction in the liver that is differentiated from intrinsic DILI by not showing simple dose-dependency. Idiosyncratic DILI has become a major clinical challenge because of its high morbidity, mortality, unpredictable nature, frequent hospitalization, and need for liver transplantation.[1] Given the low incidence of idiosyncratic DILI, it cannot be detected in preclinical testing or clinical trials.

Prospective population-based epidemiological studies reported an annual crude incidence of 13.9 cases per 100,000 inhabitants in France in 1997-2000,[2] 12 cases per 100,000 inhabitants in Korea in 2005-2007,[3] and 19.1 cases per 100,000 inhabitants in Iceland in 2010-2011.[4] Antibiotics, nonsteroidal anti-inflammatory drugs, and isoniazid are the most common medications associated with idiosyncratic DILI. In Asian countries, herbal medications are the principal cause of DILI.[3] A retrospective population-based study in the UK estimated the incidence of DILI as 2.4 per 100,000 inhabitants,[5] showing 6 times lower incidence in the prospective study of DILI.

Based on the ratio of abnormalities in liver function (R ratio, a ratio of the alanine aminotransferease [ALT] to alkaline phosphatase [ALP] relative to their respective upper limits of normal [ULN]), DILI can be categorized as hepatocellular (R>5), cholestatic (R<2), or mixed type (2<R<5) of liver injury.[6] It can be applied to classify DILI into endotypes for genetic association

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studies.

Risk factors for DILI are complex; however, host genetic, immunological, and metabolic factors play an important role. DILI is more likely to occur in the elderly,[7] females,[4] and patients with underlying chronic liver disease.[8] Several characteristics of a drug, such as medication dose, drug lipophilicity, and extent of hepatic metabolism, also play a role. Environmental factors, such as alcohol intake, pre-existing disease, and viral infections, are important patient characteristics. Genetic factors are associated with genes that influence drug metabolism and/or immunological reactions to the drug. The frequency, severity, and clinical manifestations vary according to the drug, the underlying disease, and ethnicity of the patient.

Although mechanisms for idiosyncratic DILI remain unclear, the delayed onset of the reaction and genetic associations between the expression of particular HLA molecules and susceptibility to drug-induced liver injury[9-12] are indicative of an immunogenetic basis.

The present review focuses on current pharmacogenomics, recent advances in mechanistic studies, and newly identified biomarkers for idiosyncratic DILI.

Genetic predisposition to idiosyncratic DILI

Genetic association studies on DILI remain challenging because of the relative rarity of this condition, phenotypic diversity in DILI cohorts, ethnic diversity, and complex culprit drugs. The limitations are magnified when performing genome-wide association studies on DILI because it requires sufficient study subjects to reach a genome-wide level of significance, as well as a replication study cohort based on different populations to exclude frequently occurring false-positive associations. To overcome these limitations, international multicenter research networks have been established in accordance with case definition and phenotype standardization as well as with regard to grading the severity of DILI.[13] Multicenter research networks for studying idiosyncratic DILI have been established in the USA (DILIN),[14] UK (DILIGEN),[10] Europe (EUDRAGENE),[15] Spain (Spain DILI registry),[16] Canada (CPNDS),[17] and the international Severe Adverse Event consortium (SAE).[18]

An association between a specific human leukocyte antigen (HLA) allele with idiosyncratic DILI has been reported for several drugs (*e.g.*, flucloxacillin [*B*57:01*],[10] ximelagatran [*DRB1*07:01* and *HLA-DQA1*02*],[9] co-amoxiclave *[DRB1*15:01*],[12,19,20] lumiracoxib [*DRB1*15:01*],[11] lapatinib [*HLA-DRB1*0701-DQA1*0202/DQB1*0203*],[21] antituberculosis drugs [*HLA-DQB1*0502*],[22] and isoniazid [*HLA-DRB1*03*], rifampin [*HLA-DQA1*0102*], and ethambutol [*HLA-DQB1*0201*][23]).

Recently, *in silico* analysis of HLA alleles associated with DILI revealed a link between different HLA alleles.[24] DILI caused by several chemically unrelated drugs, such as ticlopidine, lumiracoxib, and co-amoxiclav, resides on similar haplotypes, namely, *DQB1*0604-DQA1*0102* and *DQB1*0602-DQA1*0102*. Given the same peptide binding capability between *DQB1*0604* and *DQB1*0602*, common causal alleles within the major histocompatibility complex (MHC) class II may be genetic predisposing factors for DILI. However, further genotyping is required using next-generation sequencing techniques.

New insights from GWAS can provide good clinical utility by avoiding the use of potentially harmful drugs in susceptible patients having an HLA risk allele.[25,26] For example, avoidance of carbamazepine therapy in *HLA-B*1502*-positive patients has reduced the incidence of a potentially severe cutaneous hypersensitivity, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in Taiwan.[25] *HLA-B*5701* testing significantly reduces the incidence of abacavir-induced hypersensitivity.[26] The food and drug administration (FDA) recommended pretreatment HLA screening for abacavir and carbamazepine therapies and this has been helpful for the management of adverse events. Furthermore, a validation study of *HLA-DRB1*0701* using a prospective, randomized, placebo-controlled clinical trial of lapatinib monotherapy in early-stage breast cancer demonstrated good clinical utility for *HLA-DRB1*0701* typing for the management of patients experiencing hepatotoxicity during lapatinib treatment.[27] *HLA-DRB1*0701* allele carriers show increased ALT above background levels in the placebo treatment, which supports a previous finding of *HLA-DRB1*0701* as a predictive risk factor for lapatinib-induced liver injury.

However, recent studies on predictive genetic testing for DILI demonstrated a low positive predictive value of 0.12%, even in the strongest association of *HLA-B*5701* with flucloxacillin-induced DILI.[28] Therefore, a multidisciplinary approach that relates immune responses to clinical outcomes is required to improve predictability by combining biomarkers with HLA typing tests. The association between particular HLA alleles and susceptibility to DILI drives studies on how small drugs can induce drug-specific T cell responses, which are restricted by the host HLA allele.

Predisposition to immunological drug reactions

The association of a specific HLA allele with DILI gives rise to several major questions: (1) how are small drugs antigenic (*i.e.*, interact with MHC proteins to activate T lymphocytes); (2) how and why do drug-specific T cell responses arise in a small portion of patients; and (3) how is a specific immune response restricted by host HLA alleles?

T lymphocytes are thought to be involved in the pathogenesis of certain immune-mediated adverse drug reactions, indirectly causing tissue damage through the action of cytokines or directly by the secretion of cytolytic molecules (*e.g.*, perforin, granulysin, and FAS ligand). To stimulate a T-cell response, a drug must act as an antigen and ligate specific T-cell receptors that ultimately results in the activation of specific T cells.

Hapten theory—originating from the studies of Landsteiner

and Jacobs in the 1930s relating skin sensitization potential to protein reactivity [29]—states that a drug must bind irreversibly to self-protein to break immune tolerance. T cells are subsequently stimulated by peptides liberated from the modified protein following antigen processing. Drugs have also been shown to be associated directly with peptides embedded in the MHC to stimulate a T-cell response.[30-37] Despite this, very few studies have attempted to define drug-protein antigens in patients and hence relate the chemistry of antigen formation to the immune response.

Drug antigenicity

Traditionally, drugs were thought to be too small to interact directly with MHC molecules. For this reason, hapten formation is believed to be an important step in the generation of drug-specific immune responses. With reference to T cell-mediated reactions, the protein conjugate is broken down or "processed" by antigen-presenting cells, liberating antigenic peptides that are associated with MHC molecules prior to presentation to T cells. For β-lactam antibiotics, the β-lactam ring is targeted directly by nucleophilic lysine residues on proteins, leading to ring opening and binding of the penicilloyl group.[38-40] Most of the other drug classes associated with a high incidence of immunological reactions are chemically inert; however, through normal processes of drug metabolism, protein-reactive intermediates may be generated.[41-43] Using advanced technologies, such as mass spectrometry, it is possible to define the chemistry of drug-protein conjugation in patients and the nature of the drug-derived epitopes, which can function as an antigen to stimulate T cells.[44-48]

There is another mechanism underlying drug-specific T-cell activation. This concept, referred to as the "pharmacological interaction of drugs with immune receptors (p-i concept)," states that drugs by themselves act as antigens interacting in a reversible fashion with immunological receptors.[49] By characterizing T-cell clones from patients with immunological drug reactions, the pharmacophore of several drugs, including sulfamethoxazole,[30,34,50] carbamazepine,[51,52] abacavir,[53] and penicillin,[54] have been shown to interact with MHC molecules directly and provide a sufficiently strong signal to stimulate T cells.

Recently, new *in vitro* assays using cells from healthy volunteers have been designed to study drug antigenicity.[55] Several key elements are required for the initiation of drug-specific T-cell responses, and each must be incorporated into an *in vitro* assay: (1) drug delivery in an appropriate antigenic form, (2) the provision of maturation signals for professional antigen-presenting cells, and (3) appropriate co-stimulatory/co-inhibitory receptor ligand interactions. Furthermore, one must consider the phenotype and functionality of antigen-specific T cells, appropriateness of the T-cell readout, and the genetic background of the volunteer. This recently developed assay relies on the isolation and culture of highly pure T-cell and antigen presenting-cell populations. Immature monocyte-derived dendritic cells and naive T cells are used as antigen presenting cells and responder cells, respectively. After a 10-day culture period, T cells are re-exposed to the drug antigen and dendritic cells, and antigen specificity is measured shortly thereafter. In addition to classical readouts for proliferation, cytokine secretion, and cytotoxicity, a change in phenotype from naive to memory can be quantified using flow cytometry.

Drug immunogenicity

Not all carriers of the risk allele develop pathogenic immunological drug reactions. To initiate an immune response, 2 pathways must be triggered, namely, the antigenic signal, sensed by specific T-cell receptors, and the maturation signal, sensed by dendritic cells, which subsequently provide co-stimulatory signals to T cells upon activation. The latter is thought to function as a "danger signal" regarding the "drug-inflammation interaction," suggesting that a modest inflammation can enhance some drug-induced immunogenicity and potentially predispose an individual to hepatotoxicity.[56] Our understanding of drug-dendritic cell interactions and the role of bystander cells in the provision of dendritic-cell maturation signaling is limited.[57-59] By establishing an HLA-typed cell bank[24] and the availability of a novel assay to detect the stimulation of naïve T cells with drugs, it is beginning to become possible to define whether an environment rich in dendritic-cell maturation signals contributes to the conversion of antigenic signals into an immune response.

For T cells to exert a cytotoxic response in a particular organ, they must be recruited from the circulation. The expression of specific chemokines and their subsequent interaction with chemokine receptors on the surface of lymphocytes is known to promote T-cell migration through the endothelium and into tissues. T cells infiltrating skin express high levels of cutaneous lymphocyte-associated antigen (CLA) and the chemokine receptors CCR4 and CCR10, and drug-specific T cells from patients with cutaneous drug hypersensitivity reactions express high levels of these receptors.[52,60-62] Although the expression of chemokine receptors on liver homing T cells is less defined, CXCR3, CCR5, CCR9, and CXCR6 have been found on T cells isolated from patients with various forms of liver disease.[63] Lymphocytes from a patient with trimethoprim-induced liver injury express high levels of CXCR3 and CCR9, but only low levels of skin-homing receptors.[64] Thus, it may be possible to monitor chemokine receptor expression profiles to define the role of drug-specific T cells in hepatic (CXCR3, CXCR6, CCR5, and CCR9) and cutaneous (CCR4 and CCR10) side-effects of immunological drug reactions.[63]

HLA allele-specific immune response

Several studies suggest that the hapten/pro-hapten immunogenic complex may be restricted to a specific HLA allotype.[65]

First, a haptenated peptide with a drug occupies an anchor pocket in a specific HLA molecule. Second, incorporation of the drug or metabolite into an anchor pocket of the associated HLA molecule can alter its peptide-binding groove and generate a shift in the peptide repertoire it can subsequently bind. Third, a modification of a peptide that specifically binds a particular HLA allotype by solvent-exposed side chains of the drug. Using affinity chromatography via immobilized monoclonal antibodies specific for the HLA allotypes, cell-surface HLA-peptide complexes can be isolated, and peptide ligand can be dissociated from HLA proteins. The peptide can be identified using tandem mass spectrometry and then used to detect the specific interaction with the HLA molecule based on peptide-binding assays.[66,67] However, to date the identity of naturally eluted T-cell stimulatory hapten-peptide complexes is not known for drugs associated with a high incidence of idiosyncratic DILI.

Immunologic basis for the pathogenesis of DILI

The presence of drug-responsive T lymphocytes in blood and liver biopsies of patients with DILI supports their involvement in disease pathogenesis. In 1997, Maria and Victorino demonstrated lymphocyte responses to drugs in over 50% of patients with DILI. The detection of drug-specific lymphocyte responses in certain patients with liver injury suggests that drug reactions are immune-mediated. Histological examination of inflamed liver tissue from a patient with a sulfasalazine-induced drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome revealed infiltration of granzyme B secreting T lymphocytes.[69] This finding supports a direct role of drug-responsive T lymphocytes in the pathogenesis of DILI.

The characterization of the phenotype and function of drug-responsive T lymphocytes has been reported in patients with trimethoprim-induced liver injury.[64] The proliferation of $CD4^+$ and $CD8^+$ T cells against trimethoprim is reported, and the proliferative response is associated with enhanced IFN-γ and IL-13 secretion.

Recent studies on flucloxacillin-induced liver injury[70,71] suggest 2 distinct immunological mechanisms based on hapten and p-i concepts. Monshi *et al.*[70] reported that flucloxacillin-responsive T cells stimulate IFN-γ secretion from peripheral blood mononuclear cells (PBMC) isolated from DILI patients as well as healthy volunteers expressing *HLA-B*5701*. Covalent binding of flucloxacilin to specific lysine residues on albumin is also observed using mass spectrometric analysis. The level of drug binding showed a significant correlation with the proliferative response of T-cell clones against flucloxacillin. The T-cell response is dependent on antigen processing, which suggests that T cells may be activated by peptides derived from the haptenated protein. Furthermore, naïve T cells isolated from healthy volunteers expressing *HLA-B*5701* are found to be activated by flucloxacillin. The drug-specific T-cell response is dependent on the presence of antigen-presenting cells expressing *HLA-B*5701*.

Wuillemin *et al.*[71] suggested that HLA haplotypes determine distinct immunologic mechanisms, hapten- or p-i, based T-cell reactivity toward flucloxacillin. They used T-cell assays with PBMC from healthy human donors to evaluate mechanisms of drug antigen presentation by characterizing 3 aspects: (1) stable or labile presentation of flucloxacillin by antigen presenting cells, (2) dependency on proteasomal processing, and (3) activation kinetics of T-cell clones on the stimulation of flucloxacillin in solution. In healthy volunteers expressing *HLA-B*5701*, flucloxacillin is presented in a labile manner, independent of antigen processing, and restricted by the host HLA allele, *HLA-B*5701*. Thus, p-i based T-cell reactivity is found in *HLA-B*5701* carriers. On the other hand, in healthy volunteers expressing other HLA-B alleles, T-cell reactivity against flucloxacillin is dependent on antigen processing, suggestive of a hapten mechanism. However, Yassen *et al.*[72] demonstrated preferential T-cell responses to flucloxacillin-hapten in patients with liver injury expressing *HLA-B*5701* via a hapten mechanism. This finding suggested promiscuous T-cell responses to flucloxacillin and flucloxacillin-hapten may co-exist and predominant T-cell response may be affected by individual immunological factors. Furthermore, the infiltration of cytotoxic $CD8^+$ T lymphocytes is found in the liver biopsies of a patient with flucloxacillin-induced liver injury,[73] where cytotoxic T-cells could kill hepatocytes in a perforin/granzyme B-dependent manner. Moreover, bystander killing caused by FasL could lead to exacerbation of liver injury caused by flucloxacillin.[73]

An alternative hypothesis has been suggested as a mechanism behind interactions between drug and HLA molecules in abacavir-induced hypersensitivity.[74-76] Abacavir can bind inside the peptide-binding groove of *HLA-B*5701*, changing the shape, finally leading to the alteration in the repertoire of peptide that can bind *HLA-B*5701*. However, there has been no experimental evidence supporting this hypothesis as a mechanism for DILI. In particular, Norcross *et al.*[76] reported that antigen presenting cell exposure to flucloxacillin does not alter the repertoire of *HLA-B*5701* binding peptides displayed on the cell surface.

In our most recent study, we compared/contrasted β-lactam and β-lactam hapten-specific T-cell responses, but importantly focused on cells from patients with DILI and healthy donors. Soluble drugs activate T cells from healthy donors in an HLA-allele-unrestricted fashion that does not occur in patients. In contrast, T cells from patients with DILI are activated via a hapten mechanism. The drug hapten-specific T-cell response is HLA-allele-restricted.[72]

Strong evidence of an immune-mediated mechanism of DILI has recently been reported in isoniazid (INH)-induced liver injury.[77] Anti-INH antibody and anti-CYP autoantibodies are detected in patients with liver failure, but not in patients with mild injury or no. Impaired immune tolerance in severe cases of

INH-induced liver injury has also been suggested, while mild cases can resolve with immune tolerance.

It is likely that complex mechanisms can occur together in DILI patients, although the predominant mechanism under various conditions remains unclear. Several aspects of these mechanisms require further studies. First, protein modification with the drug is likely to occur, even in tolerant patients.[44] The formation of the drug-protein complex may be an important initiator to induce allergic reactions, but it does not ensure T-cell activation. Additional signals to convert an antigenic signal into an aberrant T-cell response may exist in patients with DILI. Second, the high generation rate of flucloxacillin-responsive T cells from *HLA-B*5701*$^+$ healthy donors *in vitro* does not match with the low incidence of DILI in a general population. Only 1 to 1,000 individuals carrying the *HLA-B*5701* allele will develop liver injury when treated with flucloxacillin. Third, the activation of flucloxacillin-responsive T cells requires a high concentration of drug, which exceeds the therapeutic dose in clinics. This may favor the expansion of drug-responsive T cells unrestricted by the HLA allele. Fourth, other manifestations, such as DRESS, may coexist with DILI, which could mask an alternative activation mechanism.

In a recent meeting of drug hypersensitivity researchers in Bern, the drug-responsive T cells are also reported to be detected in patients with co-amoxiclave (amoxicillin-clavulanate)- and antituberculosis drug-induced liver injuries. Amoxicillin- or clavulanic acid-responsive T-cell clones isolated from patients with DILI show drug-specific T-cell proliferation in a dose- and antigen processing-dependent manner, suggesting a hapten mechanism for antigen presentation.[78] The proliferative response of amoxicillin-specific CD4$^+$ T-cell clones is restricted by MHC class II, especially the DR molecule.[78] This finding is consistent with the previous identification of *HLA-DRB1*1501* as a risk genotype for co-amoxiclave-induced liver injury.[12,19,20,79] In patients with antituberculosis drug-induced liver injury, enhanced proliferation and IFN-γ secretion are found in PBMC against isoniazid, but not rifampicin, pyrazinamide, or ethambutol. Antituberculosis drugs may be the next target for immunological studies on DILI.

Animal model of idiosyncratic DILI

In vitro studies with human PBMC provide knowledge of the nature of the immune response and the signals that are specific for DILI; however, little information can be acquired regarding the pathways that are activated when the immune response develops and/or the ability of T cells to damage an intact liver. Thus, an animal model that mimics the human condition (delayed onset, similar pathogenesis) would greatly assist the study of mechanisms of idiosyncratic DILI. To date, however, attempts to develop such models have been largely unsuccessful. This is because of difficulties in mimicking human drug exposure and the absence of relevant human HLA alleles. Moreover, the co-stimulatory/co-inhibitory signals that occur during a pathogenic immune response have not yet been defined and are therefore almost impossible to model.

In 2003, Shenton *et al.*[80] described a rat model of nevirapine-induced tissue injury. Rats developed a mild-moderate skin rash following drug exposure. The reaction has a delayed onset (2-3 weeks), is dose-dependent, is strain-specific, and is only detected in a portion of animals. Furthermore, rechallenge with nevirapine results in the development of clinical symptoms much more rapidly. Each of these phenomena is indicative of an immune-mediated reaction against the drug, and indeed depletion of CD4$^+$ T-cells was found to decrease the incidence of skin rash. Importantly, rats do not develop DILI, which is observed in several nevirapine-exposed human patients.

To investigate whether the absence of DILI involves an acquired tolerance against the drug-derived antigen, Metushi *et al.*[81,82] administered the hepatotoxin amodiaquine and anti- cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibodies to programmed cell death-1 (PD-1) knockout mice. Both CTLA4 and PD-1 are known to be negative regulators of antigen-specific T-cell responses. Interestingly, mice exposed to amodiaquine develop liver injury with a delayed onset. In subsequent experiments, it will be interesting to see whether similar observations are detected with other DILI drugs, including those that cause reactions in human patients expressing specific HLA alleles.

Predictive biomarkers of DILI

Clinicians and drug manufacturers recognize 2 different types of DILI. Dose-dependent intrinsic DILI can be detected during the early stages of drug development, while idiosyncratic DILI not showing simple dose-dependency cannot be predicted. Over 1,000 approved drugs have been associated with DILI.

The most common serum biomarkers for the detection of liver injury are serum total bilirubin and the enzyme activity of ALT, ALP, and aspartate aminotransferase (AST). Increased enzyme activities and total bilirubin may represent liver injury and declining liver function.[83] The enzyme activity of ALT in serum has been considered the gold standard to predict hepatocellular damage;[84] however, the elevated activity is actually asymptomatic and not specific for liver injury. ALT activity can also increase from other extrahepatic injuries, such as skeletal muscle injury due to inflammation.[85] Serum alkaline phosphatase is not liver- specific and can be increased in other disease states.[84] Total bilirubin is an insensitive marker of liver disease.

A considerable effort to identify novel biomarkers of DILI has been made by the international research consortia of clinicians and scientists, such as the Safer and Faster Evidence-based Translation (SAFE-T) and the Mechanism Based Integrated Systems for the Prediction of Drug Induced Liver Injury (MIP-DILI). The main purpose is to identify a more specific, reliable, non-invasive biomarker for early prediction, treatment, or pre-

vention of DILI. In advanced technologies on "-omics" platform fields, a few promising biomarkers have been identified to date.[86] Circulating glutamate dehydrogenase representing mitochondrial leakage may be used as a DILI biomarker since it is released during hepatocellular necrosis.[83] High-mobility group box 1 has been identified as an early serum indicator of hepatocellular necrosis in acute liver injury.[87] Liver-enriched microRNA, miR-122, may be a promising biomarker for the prediction of DILI because it is highly restricted to the liver, detected during the early stages of hepatocellular damage, and well-correlated with histopathologic features.[88] It is a more sensitive biomarker compared to currently used clinical chemistry parameters for acute and chronic drug-induced liver injury.[89,90]

Future perspectives

The mechanism involved in the development of DILI is complicated, and the hypotheses that exist today are likely to be only a portion of the mechanisms involved in DILI. A better understanding of underlying diseases could lead to improved preclinical tests to detect whether a new drug candidate has the potential to cause DILI as well as the form of DILI. Furthermore, the development of diagnostic tests based on the drug-specific immune response may lead to the prediction of DILI in the early stages of a reaction and identification of a culprit drug in a patient taking multiple drugs.

HLA genotypes serve as biomarkers of DILI as well as other genotypes, such as those of drug-metabolizing enzymes and transporters, and the use of more genetic markers could be effective in preventing idiosyncratic DILI. The next challenge will be to identify rare variants that confer the susceptibility of DILI using next generation sequencing and to understand how the pattern of genetic variants predicts disease susceptibility.

Recent studies have attempted to develop an *in vitro* model system based on differentiated hepatocyte-like cells (HLCs) from human-induced pluripotent stem cells (hiPSC) to study and screen for idiosyncratic DILI.[91] In addition, hiPSC-derived HLCs from individuals suffering from idiosyncratic DILI could be used for mechanistic studies on the pathophysiology of idiosyncratic DILI. However, further efforts to refine the differentiation and characterization of HLCs *in vitro* will be applicable for safety pharmacology and toxicology assessment.

New developments in the fields of bioinformatics, genomics, and proteomics will expedite the identification of predictive biomarkers of DILI. These studies can be used to improve early diagnosis and generate novel therapies for DILI.

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