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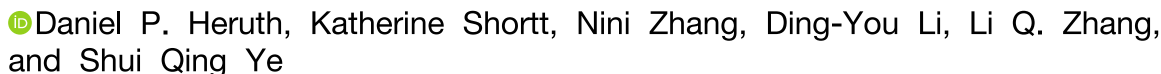
Minireviews

Genetic Association of Single Nucleotide Polymorphisms with Acetaminophen-Induced Hepatotoxicity



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ABSTRACT

Acetaminophen is commonly used to reduce pain and fever. Unfortunately, overdose of acetaminophen is a leading cause of acute liver injury and failure in many developed countries. The majority of acetaminophen is safely metabolized in the liver and excreted in the urine; however, a small percentage is converted to the highly reactive *N*-acetyl-*p*-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is inactivated by glutathione *S*-transferases, but at toxic levels, excess NAPQI forms reactive protein adducts that lead to hepatotoxicity. Individual variability in the response to both therapeutic and toxic levels of acetaminophen suggests a genetic component is involved in acetaminophen metabolism. In this review, we evaluate the genetic association studies that have identified 147 single nucleotide polymorphisms linked to acetaminophen-induced hepatotoxicity. The identification of novel genetic markers for acetaminophen-induced hepatotoxicity provides a rich resource for further evaluation and may lead to improved prognosis, prevention, and treatment.

Introduction

Acetaminophen (paracetamol) is a commonly used analgesic and antipyretic; however, high nontherapeutic doses can cause severe liver injury. Although acetaminophen overdose is a major cause of hepatotoxicity in many developed countries, drug-induced liver injury (DILI) is not unique to acetaminophen, as more than 50% of acute liver failure (ALF) cases have been attributed to the hepatic biotransformation of a wide array of small-molecule drugs (Lee, 2003). Acetaminophen should be considered a dose-dependent hepatotoxin since most cases of toxicity are secondary to excessive dosing of acetaminophen (Nourjah et al., 2006). Thus, the DILI induced by acetaminophen is classified as intrinsic (dose dependent) instead of idiosyncratic (dose independent) (Lee, 2013; Mosedale and Watkins, 2017).

Approximately 90% of a therapeutic dose of acetaminophen is metabolized completely by multiple enzymes in the liver and, along with 5% of nonmetabolized acetaminophen, eventually excreted in urine and bile (Raheja et al., 1983; Vermeulen et al., 1992). The majority of acetaminophen is detoxified via formation of either glucuronide (~50%) or sulfate (~40%) conjugates by uridine 5′-diphospho-glucuronosyltransferases (e.g., UGT1A1, UGT1A6, UGT1A9, UGT2B7, UGT2B15) and sulfotransferases (e.g., SULT1A1, SULT1A3, SULT1A4, SULT1E1, SULT2A1), respectively. However, a small percentage of ingested acetaminophen (~5%) is metabolized by oxidation via the microsomal cytochrome P450 pathway into *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Corcoran et al., 1980, 1985). The cytochrome P450 (P450) enzymes, including CYP1A2, CYP2A6, CYP2D6, CYP2E1, and CYP3A4, convert acetaminophen into the highly reactive NAPQI metabolite (Raucy et al., 1989; Thummel et al., 1993; Lee et al., 1996; Chen et al., 1998; Dong et al., 2000). At toxic acetaminophen levels, CYP3A4 presented with the highest relative capacity for acetaminophen bioactivation to NAPQI by oxidation, followed by CYP2E1, CYP2D6, and CYP1A2 (Laine et al., 2009). At therapeutic acetaminophen levels, CYP3A4 again had the

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ABBREVIATIONS: ALF, acute liver failure; ALI, acute liver injury; ALT, alanine aminotransferase; DILI, drug-induced liver injury; GPX, glutathione peroxidase; GST, glutathione *S*-transferase; GWAS, genome-wide association study; MAF, minor allele frequency; NAPQI, *N*-acetyl-*p*-benzoquinone imine; OR, odds ratio; P450, cytochrome P450; rs, rs accession number; SNP, single nucleotide polymorphism; SULT, sulfotransferase; UGT, uridine 5′-diphospho-glucuronosyltransferase.

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highest rate of conversion to NAPQI, whereas the other P450 enzymes possessed a significantly lower capacity for bioactivation (Laine et al., 2009). NAPQI, a strong oxidizer that is toxic to liver tissue, is reduced (inactivated) by conjugation with glutathione by glutathione *S*-transferases (GSTs), a family of enzymes (e.g., GSTT1, GSTP1) responsible for the detoxification of many drugs (Dahlin et al., 1984). The toxicity of NAPQI is associated with its ability to bind to cysteine residues in proteins to form NAPQI-protein adducts (Jollow et al., 1973; Davern et al., 2006). At therapeutic doses, the small amount of NAPQI-protein adducts produced are removed effectively by autophagy (McGill et al., 2013; Ni et al., 2016). However, in the case of acute and chronic overdoses of acetaminophen, the SULT and UGT enzymes become saturated, shifting the metabolism of acetaminophen through the P450 enzymes to produce increased levels of NAPQI. Subsequently, NAPQI depletes hepatic glutathione and accumulates in hepatocytes where excess NAPQI binds to cysteine residues (Leeming et al., 2015) on cellular (Cohen et al., 1997) and mitochondrial proteins (Tirmenstein and Nelson, 1989), leading to acute liver injury (ALI) or the more severe ALF.

The current model of acetaminophen-induced hepatic necrosis links the NAPQI-protein adducts with amplified cascades of reactive oxygen and nitrogen species, resulting in the swift loss of hepatic cells and liver function (Ramachandran and Jaeschke, 2017; Wang et al., 2017). This model has been reviewed thoroughly (Russmann et al., 2009, 2010; Hinson et al., 2010; Fontana, 2014; Krasniak et al., 2014), but in brief, the reactive oxygen species/reactive nitrogen species induce increased mitochondrial permeability, resulting in impaired mitochondrial function (McGill et al., 2012; Jiang et al., 2015) and leading to the initiation of massive necrotic cell death. Subsequently, necrotic hepatocytes release damage-associated molecular patterns resulting in an immune response mediated by various cytokines and innate immune cells (Bourdi et al., 2007; Wang et al., 2015; Fannin et al., 2016).

Although acetaminophen is a dose-dependent hepatotoxin, elevated alanine aminotransferase (ALT) serum levels were measured in some healthy adults following a 7–14-day administration of the maximum daily dose of 4 g per day (Watkins et al., 2006; Harrill et al., 2009b). Additional case studies, although rare, have reported the development of ALI even at therapeutic doses (Kurtovic and Riordan, 2003; Satirapoj et al., 2007). These findings confirm that some healthy individuals experience mild to severe liver injury in response to therapeutic doses of acetaminophen, suggesting that genetic components are involved in acetaminophen metabolism. Thus, several groups have proposed that NAPQI toxicity can be enhanced by alterations in the metabolism of acetaminophen due to genetic polymorphisms in the corresponding enzymes (Adjei et al., 2008; Zhao and Pickering, 2011; Krasniak et al., 2014). Single nucleotide polymorphisms (SNPs) in metabolic enzymes have been predicted to explain both the ethnic and interindividual differences in acetaminophen degradation and hepatotoxicity (Critchley et al., 1986, 2005). This concept is not unique to acetaminophen-induced hepatotoxicity, as genetic variations in cellular stress, drug metabolism, and immune response genes are associated with DILI susceptibility (Daly and Day, 2012; Chen et al., 2015). Numerous SNPs have been identified that alter the activity of drug-metabolizing enzymes, including SULT, UGT, and P450 (Chambers et al., 2011; Zhao and Pickering, 2011; Krasniak et al., 2014), yet very few SNPs have been experimentally associated directly with acetaminophen-induced hepatotoxicity (Ueshima et al., 1996; Court et al., 2013, 2014). Genome-wide association studies (GWAS) provide a powerful tool to scan for SNPs that associate with a disease phenotype, such as hepatotoxicity. GWAS in idiosyncratic DILI have identified pharmacogenetic polymorphisms (Chambers et al., 2011; Urban et al., 2012; Petros et al., 2017) associated with liver injury following treatment with statins (Nicoletti et al., 2017), flucloxacillin (Daly et al., 2009), amoxicillin-clavulanate (Lucena et al., 2011), flupirtine (Nicoletti et al., 2016), and antituberculosis drugs (Petros et al., 2016). Unfortunately, large-scale GWAS for acetaminophen-induced hepatotoxicity have not been performed to date.

To overcome the limitations of GWAS in human cohorts, several groups have performed innovative experiments using mouse models and/or human tissue culture assays to identify and characterize SNPs associated with acetaminophen-induced hepatotoxicity. Therefore, this review explores the candidate gene and genome-wide approaches that have identified 147 SNPs associated with either protection against or susceptibility to acetaminophen-induced hepatotoxicity. The inclusion criteria used in this review were to include all human SNPs that: 1) have been experimentally identified for a significant association with acetaminophen-induced hepatotoxicity using candidate gene and genome-wide approaches, and 2) have been annotated with an rs accession number (rs).

The clinical utilities of pharmacogenetics and pharmacogenomics are becoming increasingly important for optimizing individual patient care. Although the application of genetic information has not yet been applied formally to acetaminophen dosing, the studies presented here provide the foundation for critical translational research in DILI. The identification of SNPs associated with a significant risk for acetaminophen-induced hepatotoxicity will provide potential targets for improved prognosis, prevention, and treatment.

Candidate Gene Approaches to Identify SNPs Associated with Acetaminophen-Induced Hepatotoxicity

Ueshima et al. (1996) described a *CYP2E1* promoter SNP (rs2031920, C>T) that was associated with altered acetaminophen metabolism. rs2031920 is common in East Asian populations with a minor allele frequency (MAF) of 0.20 but is rare in other ethnic groups. Homozygous carriers of the rs2031920 variant T allele presented with a 2-fold increase in the elimination rate of acetaminophen compared with CC and CT individuals (Ueshima et al., 1996), which correlated with increased promoter activity due to the homozygous minor genotype (Hayashi et al., 1991) and higher hepatic levels of CYP2E1 (Tsutsumi et al., 1994). We can predict that elevated levels of CYP2E1 would result in increased production of NAPQI and could lead to a greater risk of acetaminophen-induced hepatotoxicity in homozygous TT individuals. However, GWAS in acetaminophen-induced hepatotoxicity cohorts have not yet identified rs2031920 as a susceptibility locus.

Court et al. (2013) identified three 3′untranslated region SNPs (rs8330, C>G; rs10929303, C>T; rs1042640, C>G) in the *UGT1A* gene that were associated with increased

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glucuronidation activity following acetaminophen exposure. The *UGT1A* rs8330 MAF (G) was significantly lower in the unintentional acetaminophen hepatotoxicity group (0.16) compared with the other ALF subgroups (0.22), with an odds ratio (OR) of 0.53 (0.30–0.94; $P = 0.027$) (Court et al., 2014) (Table 1). This finding was consistent with a protective effect of the variant rs8330 G allele through enhancement of acetaminophen glucuronidation and detoxification, as demonstrated by a series of in vitro mechanistic studies by Court et al. (2013). rs8330 increased glucuronidation activity due to altered splicing of the primary UGT1A transcript, resulting in the preferential retention of exon 5A versus exon 5B. Translation of UGT1A mRNA containing exon 5B produces a truncated UGT1A protein, termed isoform two variant, which lacks enzymatic activity and further represses enzymatic activity through heterodimerization with the wild-type isoform (Court et al., 2013). Similar to rs2031920, the rs8330 MAF varies among ethnic populations.

Court et al. (2014) evaluated the association with acetaminophen-induced hepatotoxicity in a panel of polymorphisms from genes encoding known acetaminophen-metabolizing enzymes, including *UGT1A*, *UGT1A1*, *UGT1A6*, *UGT1A9*, *UGT2B15*, *SULTA1*, *CYP2E1*, and *CYP3A5*. They also analyzed a polymorphism in *CD44* that was associated with elevated serum ALT levels in healthy volunteers who consumed the maximum recommended dose of acetaminophen for up to 2 weeks (Watkins et al., 2006; Harrill et al., 2009b). Three genes, *CYP3A5*, *UGT1A*, and *CD44*, contained SNPs with relatively weak associations with acetaminophen-induced liver injury in an acute liver failure study group cohort of 260 Caucasian individuals, which consisted of 78 patients with intentional acetaminophen overdose, 79 patients with unintentional acetaminophen overdose, and 103 patients with ALF due to nonacetaminophen-associated causes.

The CYP3A5 splice donor variant (rs776746, G>A) is associated with acetaminophen-induced hepatotoxicity (Table 1). The minor A allele (also known as *CYP3A5*1*) encodes a functional cytochrome P450 family 3 subfamily A member 5 protein, whereas a nonfunctional protein is produced from *CYP3A5* genes containing the major G allele (rs776746; *CYP3A5*3*) (Kuehl et al., 2001). The *CYP3A5*1* A allele was observed more frequently in intentional acetaminophen overdose cases compared with all other acute liver failure patients (Court et al., 2014). The heterozygous GA genotype was an "at risk" genotype with OR = 2.3 (1.1–4.9; $P = 0.034$) (Court et al., 2014). The homozygous AA genotype was not observed in this cohort. Subsequently, the *CYP3A5* diplotypes have been correlated with phenotypes for the metabolism of drugs, such as tacrolimus: **1/*1*, extensive metabolizer; **1/*3*, intermediate metabolizer; **3/*3*, poor metabolizer (Tanaka et al., 2014; Birdwell et al., 2015). The MAF for the rs776746 A allele (**1*) in this cohort of 260 Caucasians was 0.06, which is the same as the European population. Although the rs776746 MAF varies among ethnic groups, it did not correlate with the incidence of acetaminophen-induced hepatotoxicity across stratified ethnic groups (Critchley et al., 1986; Patel et al., 1992; Russo et al., 2004; Marzilawati et al., 2012).

The *CD44* rs1467558 (C>T) TT minor allele genotype was over-represented in the unintentional hepatotoxicity group, with OR = 4.0 (1.0–17.2; $P = 0.045$) (Court et al., 2014), suggesting that rs1467558 TT is an "at risk" genotype (Table 1). This observation was supported by previous studies that revealed rs1467558 is associated with elevated serum ALT levels (Watkins et al., 2006; Harrill et al., 2009b). In silico mechanistic structural analysis predicted that rs1467558 can alter many of the complex, alternative *CD44* transcripts, including a potentially damaging amino acid change from threonine to isoleucine (Harrill et al., 2009b). Interestingly, *CD44* is not an acetaminophen-metabolizing enzyme, but rather a cell surface receptor involved in cell-cell interactions, cell adhesion, and cell migration in inflamed tissue (Kimura et al., 2010).

The rs1902023 (G>T) missense polymorphism in *UGT2B15* (termed *UGT2B15*2*) was associated with lower acetaminophen glucuronide-to-acetaminophen concentration ratios in urine (Navarro et al., 2011) and blood (Mehboob et al., 2017). Court et al. (2017) demonstrated that *UGT2B15*2* was associated with increased plasma concentrations of NAPQI-protein adducts, and that the plasma concentrations of the protein adducts negatively correlated with acetaminophen glucuronidation. Thus, carriers of rs1902023 may be slower metabolizers of acetaminophen glucuronidation, resulting in increased availability of acetaminophen for oxidative metabolism to NAPQI and subsequent liver damage.

Although a major limitation of these studies is the population size, the results are compelling. The association of these polymorphisms with acetaminophen-induced hepatotoxicity, along with their ethnic variations, should be investigated further. To overcome the challenges of the candidate gene approach in human populations with ALF resulting from acetaminophen toxicity, additional studies have used alternative approaches, such as GWAS, to identify SNPs that may serve as biomarkers for acetaminophen susceptibility.

Genome-Wide Approaches to Identify SNPs Associated with Acetaminophen-Induced Hepatotoxicity

To test the hypothesis that genetic polymorphisms downstream of NAPQI formation contribute to hepatotoxicity, Moyer et al. (2011) used a human variation panel of 176 lymphoblastoid cell lines established from healthy donors. The growth inhibitory effect of NAPQI (IC_{50}) was determined for

TABLE 1

Top human SNPs associated with acetaminophen-induced hepatotoxicity [Court et al. (2014), n = 78 intentional, 79 unintentional, 103 control]

SNP	Gene	Name	Alleles[a]	MAF	Category	Odds Ratio[b]	P Value
rs8330	*UGT1A*	UDP glucuronosyltransferase family 1 member A complex locus	C/G	0.26	3′UTR[c]	0.53 (0.3–0.94)	0.027
rs776746	*CYP3A5*	Cytochrome P450 family 3 subfamily A member 5	G/A	0.38	Intron	2.3 (1.1–4.9)	0.034
rs1467558	*CD44*	CD44 molecule	C/T	0.06	Missense	4.0 (1.0–17.2)	0.045

[a]Major allele/minor allele.
[b]OR (95% confidence interval).
[c]Untranslated region.

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each cell line following 24 hours of treatment with seven doses (0–100 μM) of NAPQI. Large variations in NAPQI IC_{50}, ranging from 1 to 25 μM (6.5 ± 4.5 μM; mean ± S.D.), were detected between the 176 cell lines, suggesting a genetic component in NAPQI metabolism. To identify SNPs associated with NAPQI-induced hepatotoxicity, GWAS was performed using Illumina (San Diego, CA) Infinium HumanHap 550K and 510S bead chips and Affymetrix (Santa Clara, CA) 6.0 GeneChips.

Initially, Moyer et al. (2011) examined the association of 716 SNPs, located in 31 glutathione pathway genes, with NAPQI IC_{50}. Only 45 SNPs had significant *P* values (<0.05), 24 of which were located in the multidrug resistance ATP-binding cassette, sub-family C (CFTR/MRP), member 3 (*ABCC3*), and member 4 (*ABCC4*) genes. Expression of *Abcc3* and *Abcc4* in mice upon acetaminophen-induced hepatotoxicity has been shown to be dependent upon the transcription factor, Nrf2 (Aleksunes et al., 2008). Nrf2 has been shown to play a protective role in acetaminophen-induced hepatotoxicity as $Nrf2^{-/-}$ knockout mice were more susceptible to acetaminophen-induced liver damage compared with their wild-type *Nrf2+/+* controls (Enomoto et al., 2001). The remaining significant SNPs were located in or near glutamate cysteine ligase (*GCLC*), glutathione peroxidase (*GPX2*, *GPX3*, *GPX4*, and *GPX7*), glutathione synthetase (*GSS*), and glutathione transferase (*GSTA2*, *GSTA3*, and *GSTP1*). Two SNPs, E4p254 (*GSTM1*, $P = 0.13$) and I6m18 (*GSTP1*, $P = 0.04$), are not annotated in dbSNP database and, therefore, are not discussed further in this review.

Moyer et al. (2011) extended their study to a genome-wide SNP analysis in which 1,008,202 SNPs were screened for association with NAPQI IC_{50}. Ninety-six SNPs ($P < 1 \times 10^{-4}$) were associated with NAPQI IC_{50}. Interestingly, 15 of the top-20 significant SNPs mapped to intergenic regions. Ten of these 15 intergenic SNPs were clustered in a region of chromosome 3, between the *C3orf38* and *EPHA3* genes. Functional analysis of rs2880961, which lies 317 kb downstream of *C3orf38*, demonstrated binding of transcription factors, including NF-κB, HSF1, and HSF2 (Moyer et al., 2011). However, significant differences in NF-κB, HSF1, and HSF2TF binding were not detected by chromatin immunoprecipitation assays between wild-type and variant SNPs (Moyer et al., 2011). However, this does not preclude differential binding of other transcription factors. Thus, further analyses of these potential regulatory islands and their roles in NAPQI hepatotoxicity are warranted. The top-10 intragenic SNPs are located in the introns of genes (Table 2). These genes are involved in gene regulation (*LMX1A*), signal transduction (*ETKN2*, *KCNJ3*, *MCTP1*), immune response (*IL23R*, *UBASH3A*), extracellular matrix (*SPAG16*, *LAMA4*), and the detoxification of aldehydes generated by lipid peroxidation (*ALDH1A3*). The remaining gene, *RFPL4B*, which is poorly characterized, encodes a zinc-finger protein. To identify potential *cis* effects of SNPs on gene expression, Moyer et al. (2011) measured mRNA expression using Affymetrix U133 Plus 2.0 GeneChips. Interestingly, 19 probe sets, representing 17 genes, were associated significantly with NAPQI IC_{50}, with $P < 0.0001$. However, none of these 17 genes overlapped with genes containing SNPs, suggesting that the SNPs may have a *trans* effect on the expression of these genes (Moyer et al., 2011).

Two studies by Harrill et al. (2009a,b) identified potential susceptibility targets using a panel of 36 inbred mouse strains to model genetic diversity. Fasting mice were treated with 300 mg/kg acetaminophen by intragastric dosing. Food was reintroduced after 3 hours of acetaminophen dosing. After 24 hours, the mice were euthanized for analysis. The extent of liver injury was quantified by serum ALT levels. Haplotype-associated mapping and targeted sequencing revealed that polymorphisms in *Ly86*, *Cd44*, *Cd59a*, and *Capn8* correlated with increased ALT levels. To determine if the orthologous human genes were also associated with acetaminophen-induced liver injury, genomic DNA from two independent cohorts, University of North Carolina (Harrill et al., 2009b) and Purdue Pharma (Watkins et al., 2006), was sequenced. Although Harrill et al. (2009b) did not detect SNP associations within *LY86* and *CD59*, rs3749166 in *CAPN10* (the human ortholog of mouse *Capn8*) ($P = 0.045$) and rs1467558 in *CD44* ($P = 0.002$) were associated with elevated ALT levels in both cohorts. To validate these findings further, liver damage was measured in C57BL/6J Cd44 knockout mice administered acetaminophen. Cd44 knockout mice presented with greater liver injury (61% ± 7%, mean liver necrosis ± S.E.) compared with wild-type controls (40% ± 4%) following a 24-hour dose of acetaminophen (300 mg/kg). These results indicate a role for CD44 in modulation of susceptibility to acetaminophen hepatotoxicity, as supported by Court et al. (2014). Further investigations of *CD44* and *CAPN10*, as well as *LY86* and *CD59*, are needed to determine if they are indeed potential markers of enhanced risk of acetaminophen-induced hepatotoxicity. Harrill et al. (2009a) also performed mRNA microarray analyses on an Agilent (Santa Clara, CA) Mouse Toxicology Array (#4121A) to identify gene-expression biomarkers for

TABLE 2
Intronic SNPs associated with NAPQI-induced hepatotoxicity [Moyer et al. (2011), $n = 176$]

SNP	Gene	Name	Alleles[a]	MAF	Category	*P* Value[b]
rs1532815	*LMX1A*	LIM homeobox transcription factor 1 alpha	A/T	0.33	Intron	6.04E−7
rs3795578	*ETNK2*	Ethanolamine kinase 2	C/T	0.40	Intron	7.18E−6
rs3825924	*ALDH1A3*	Aldehyde dehydrogenase 1 family member A3	C/T	0.21	Intron	1.73E−5
rs1343151	*IL23R*	Interleukin 23 receptor	G/A	0.34	Intron	1.91E−5
rs17640676	*KCNJ3*	Potassium voltage-gated channel subfamily J member 3	G/T	0.23	Intron	2.09E−5
rs4869233	*MCTP1*	Multiple C2 and transmembrane domain containing 1	T/C	0.32	Intron	2.28E−5
rs16851554	*SPAG16*	Sperm associated antigen 16	T/G	0.15	Intron	2.46E−5
rs3208829	*LAMA4*	Laminin subunit alpha 4	C/G	0.16	Intron	2.54E−5
rs11153350	*RFPL4B*	Ret finger protein like 4B	G/A	0.15	Intron	2.54E−5
rs3746923	*UBASH3A*	Ubiquitin associated and SH3 domain containing A	C/T	0.43	Intron	2.56E−5

[a]Major allele/minor allele.
[b]The top-10 intronic SNPs based upon *P* value are presented.

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acetaminophen hepatotoxicity in their panel of 36 inbred mouse strains. Gene-expression profiling identified 26 genes which were associated significantly with liver damage. Similar to the Moyer et al. (2011) study, these genes did not overlap with the hepatotoxicity SNPs identified in their mouse panel. This observation further supports the hypothesis that, in addition to affecting protein-coding regions, SNPs may disrupt noncoding regulatory regions. An alternative explanation is that the 26 genes function either upstream or downstream of the SNP-modified genes.

Discussion

As a leading cause of ALF, DILI both increases the cost of medical care and limits access to drugs which would normally be beneficial (Lee, 2003). In this review, we explored the SNPs associated with the intrinsic DILI associated with acetaminophen by highlighting the difficulties of genetic studies in cohorts with limited case and control populations while presenting a potentially useful perspective to elucidate additional insight into genetic variations that can be applied to all DILI GWAS studies.

Acetaminophen overdose is a major cause of hepatotoxicity in many developed countries and has been linked to the formation of reactive NAPQI-protein adducts resulting in increased oxidative damage, an enhanced immune response, and mitochondrial dysfunction, leading to apoptosis and/or necrosis. Currently, the primary therapy for acetaminophen overdose is the administration of *N*-acetylcysteine, a glutathione precursor; however, the effective therapeutic window is limited once liver injury has occurred. Liver transplantation is the only effective therapy for patients who do not recover from primary therapy and management. Therefore, it is necessary to identify genetic markers that identify individuals who are at risk for acetaminophen-induced hepatotoxicity. Unfortunately, as demonstrated in this review, there remains very little human data investigating acetaminophen-induced hepatotoxicity. The majority of data have been generated using either in vitro or animal models. However, the studies reviewed in this article provide a strong starting point for the validation of these findings and the further investigation of potentially promising acetaminophen-susceptible biomarkers. Ultimately, these 147 SNPs will have to be examined experimentally to determine if they are intricately involved in acetaminophen metabolism or simply false positives due to experimental limitations. The identification of SNPs associated with acetaminophen-induced hepatotoxicity will provide novel insights into the mechanisms of acetaminophen metabolism and the potential for therapeutic interventions. Additional GWAS studies, including whole-genome sequencing and SNP-array assays, on larger cohorts of acetaminophen-induced ALI or ALF and the inclusion of large control populations are critical for the identification of additional biomarkers. Furthermore, the complex, and perhaps redundant, biochemical metabolism of acetaminophen in the liver suggests that it might be necessary to perform haplotype and diplotype multiloci analyses to identify a combination of SNP alleles associated with acetaminophen-induced hepatotoxicity rather than a single polymorphic allele.

In addition, the coupling of GWAS studies with transcriptome, metabolome, and expression quantitative trait loci analyses will facilitate mechanistic studies to elucidate the immunologic, mitochondrial, apoptotic, and necrotic pathways involved in acetaminophen-induced hepatotoxicity. These mechanistic and systematic studies will allow the identification of additional, and hopefully more effective, therapeutic targets not only to counter acetaminophen-induced hepatotoxicity but also to understand DILI on a broader scale.

Authorship Contributions

Participated in research design: Heruth, Li, L. Zhang, Ye.

Performed data analysis: Heruth, Shortt, N. Zhang.

Wrote or contributed to the writing of the manuscript: Heruth, Shortt, Ye.

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