

Genetics of Immune-Mediated Adverse Drug Reactions: a Comprehensive and Clinical Review

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Abstract Adverse drug reactions (ADRs) are common and are a major problem in drug therapy. Patients experience unnecessary morbidity and mortality whilst many effective drugs are withdrawn because of ADRs in a minority of patients. Recent studies have demonstrated significant associations between human leukocyte antigens (HLA) and predisposition to ADRs such as drug-induced skin injury (DISI) and drug-induced liver injury (DILI). *HLA-B*58:01* has been significantly associated with allopurinol-induced hypersensitivity. Associations between HLA and carbamazepine hypersensitivity reactions demonstrate both ethnicity and phenotype specificity; with *HLA-B*15:02* associated with Stevens-Johnson syndrome and toxic epidermal necrolysis in South East Asian patients only whilst *HLA-A*31:01* is associated with all phenotypes of hypersensitivity in multiple ethnicities. Studies of ximelagatran, an oral direct thrombin inhibitor withdrawn because of hepatotoxicity, found associations between *HLA-DRB1*07:01* and *HLA-DQA1*02:01* and ximelagatran DILI. Interestingly, *HLA-B*57:01* is associated with both abacavir DISI and flucloxacillin DILI but the reasons for the different phenotype of ADR remains unknown. Pharmacogenetic screening for *HLA-B*57:01* prior to abacavir therapy has significantly reduced the incidence of abacavir hypersensitivity syndrome in clinical practice. No other HLA associations have been translated into clinical practice because of multiple reasons including failure to replicate, inadequate sample sizes, and our lack of understanding of pathophysiology of ADRs. Here, we review genetic associations that have been reported with ADRs and discuss the

challenges that scientists, clinicians, pharmaceutical industry and regulatory agencies face when attempting to translate these associations into clinically valid and cost-effective tests to reduce the burden of ADRs in future.

Keywords Adverse drug reactions · Drug-induced skin injury · Drug-induced liver injury · Human leukocyte antigen · Maculopapular exanthema · Hypersensitivity syndrome · Stevens-Johnson syndrome · Hypersensitivity · Abacavir · Allopurinol · Carbamazepine · Flucloxacillin · Ximelagatran

Introduction

Adverse drug reactions (ADRs) are defined as ‘an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product; adverse effects usually predict hazard from future administration and warrant prevention, or specific treatment, or alteration of the dosage regimen, or withdrawal of the product [1]’. ADRs are common and account for 6.5 % of all hospital admissions, and occur in 15 % of in-patients increasing healthcare and societal costs [2–4]. ADRs are a major problem in drug therapy and lead to increased morbidity and mortality, unnecessary hospital admissions and drug withdrawals. In the US, they are responsible for >100,000 deaths, making them between the fourth and sixth commonest cause of death with similar estimates in the UK [2, 4]. A report by the FDA revealed that 28 drugs had been withdrawn from the US market between 1978 and 2005 because of safety issues including high profile drugs such as COX-II inhibitors [5]. This has huge financial implications for the pharmaceutical industry.

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Genetics and Adverse Drug Reactions

ADRs are classified into two basic types: types A and B [6]. Type A (or augmented) ADRs are predictable, and based on an exaggerated response to the known primary and/or secondary pharmacological action of the drug. This is illustrated by hypotension in a patient receiving anti-hypertensive medications or bleeding episodes with warfarin therapy. In contrast, type B (or bizarre) ADRs are unpredictable, totally aberrant effects that are not to be expected from the known pharmacological action of the drug when given in the usual therapeutic doses to a patient whose body handles the drug in the normal way. However, as our understanding of ADRs increases, some of these type B reactions will become predictable and tests can be developed to identify susceptible patients [7]. More recently, genetic risk factors have been implicated in the development of both type A and type B ADRs. The most significant associations that have been reported with ADRs are associated with the human leukocyte antigen (HLA) [8–11]. This is the focus of this review.

HLA alleles can be divided into class I and II and are involved in antigen presentation to T cells and subsequent activation of the immune response. They are encoded by the major histocompatibility complex (MHC) on chromosome 6, which is a highly polymorphic region with considerable linkage disequilibrium. It is proposed that the drug/metabolite is presented on HLA molecules with HLA restriction predisposing to an inappropriate immune response and subsequent ADR [12].

Studies into the genetics of ADRs are important because ADRs are a significant public health problem as outlined above, but also to identify pathophysiological mechanisms of these reactions so that safer drugs can be designed in future and biomarkers for diagnosis/prevention and treatments for patients who suffer with ADRs can be developed. Genetic association studies for ADRs are challenging because of the heterogeneity of clinical presentation and the wide spectrum of drugs that can cause ADRs. Investigators need to ensure that adequate numbers of cases and appropriate controls are recruited as some genetic effects may have only small clinical effects and therefore require large sample sizes. Genetic associations that are discovered will also require replication cohorts to confirm their significance. Therefore, in order to obtain enough cases, international multi-centre collaboration will be necessary—this is highlighted by the formation of International Consortium on Drug Hypersensitivity (ITCH), which is supported by the International Serious Adverse Events Consortium [13]. A second challenge is that different ADRs are likely to have different pathophysiological mechanisms even if they present in the same organ, e.g. maculopapular exanthema and Stevens-Johnson syndrome are different phenotypes of drug-induced skin injury [14]. Incorporating a mixture of phenotypes into genetic association

studies may therefore reduce the likelihood of identifying significant associations. The development of phenotype standardisation criteria for ADR will help to improve this in the future [14, 15]. Thirdly, initial pharmacogenetic studies utilised candidate gene approaches that focused on drug metabolising genes but were largely unsuccessful [16]. Genome-wide association studies and next-generation sequencing (NGS) technologies are becoming more widely available and less expensive, allowing unbiased hypothesis-generating approaches to genome investigation that may allow investigators to identify novel susceptibility factors. However, investigators still need to dedicate time and resources to unravel the mechanisms and biological plausibility of any significant hits as well as replicate the results in other cohorts [7]. The aim of this paper is to review our current understanding of the influence of genetic variation in ADRs and discuss the potential for predictive tests in clinical practice.

Drug-Induced Skin Injury

Drug-induced skin injury (DISI) is a term developed by the phenotype standardisation project and includes definitions and diagnostic criteria for severe cutaneous ADRs including Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), acute generalised exanthematous pustulosis (AGEP) and the hypersensitivity syndrome (HSS) (Table 1) [14]. The incidence of DISI is estimated to be approximately 5 to 15 % of all ADRs [17]. The most severe phenotypes, SJS/TEN, are associated with significant mortality (up to 30 %) and long-term morbidity [18]. There have been numerous studies investigating genetic factors predisposing to DISI, but the most significant results have been related to human leukocyte antigen (HLA) alleles (Table 2).

Abacavir Hypersensitivity and *HLA-B*57:01*

Abacavir is a nucleoside reverse transcriptase inhibitor frequently and effectively used in the treatment of HIV. However, approximately 5 % of patients experience an abacavir hypersensitivity syndrome (AHS), usually within 6 weeks of starting therapy, which necessitates immediate cessation of the medication. Re-exposure of these patients to abacavir resulted in rapid re-appearance of symptoms and the potential to induce more severe clinical symptoms [50]. The clinical features of AHS suggest an immune-based aetiology with the discovery of CD8⁺ T cell response in skin biopsies of abacavir patch test positive patients [51]. Genetic susceptibility to AHS was hypothesised because of reports of familial predisposition, reduced risk in black ethnicities and the observation that only a small group of patients developed hypersensitivity within 6 weeks whilst those that did not remained at low risk despite long-term therapy [52, 53]. Subsequently, an

Table 1 Phenotype definitions for drug-induced skin injury

Phenotype	Diagnostic criteria
Stevens-Johnson Syndrome (SJS)/Toxic epidermal necrolysis (TEN)	• Skin detachment 1–10 % (SJS), 10–30 % (overlap syndrome), >30 % (TEN) • Severe, often haemorrhagic erosions of mucus membranes • Other manifestations indicating systemic involvement (e.g. fever, liver chemistry elevations, intestinal and pulmonary manifestations, or the presence of lymphopenia)
Acute generalised exanthematous pustulosis (AGEP)	• Acute widespread oedematous erythema followed by a sterile pustular eruption. Often the pustules are first localised in the neck, groin and axillae and later become widely disseminated • Fever (temperature >38 °C) • Neutrophilia with or without mild eosinophilia
Hypersensitivity syndrome (HSS)	• Hospitalisation • Acute exanthema accompanied by fever • Lymphadenopathy • Internal organ involvement • Eosinophilia >10 % or >700 μl, or atypical lymphocytes or lymphopaenia <4,000, or lymphcytosis or thrombocytopaenia

association between *HLA-B*57:01* and AHS was first reported in an Australian population and replicated in patients from North America and the UK [8, 19, 20]. Later research focused on translating this association into a clinically applicable test for identification of susceptible individuals. Simple tests to detect *HLA-B*57:01* were developed, and studies in Australia, UK and France demonstrated that prospective screening for *HLA-B*57:01* in patients significantly reduced the incidence of AHS from 8 % to less than 1 % [54–58]. Pre-treatment testing was proven not only to be clinically effective but also to be cost-effective in several countries [20, 59, 60]. PREDICT-1 was a prospective randomised controlled clinical trial that confirmed the clinical utility of pre-treatment of *HLA-B*57:01* testing [21]. Following these results, the FDA and European Medicines Agency amended the drug label for abacavir mandating testing for *HLA-B*57:01* prior to prescription.

Although testing for *HLA-B*57:01* has significantly reduced the incidence of AHS, estimates suggest that approximately 50 % of patients that test positive for the allele will not develop AHS [21]. This suggests that other factors, as yet unidentified, play a role in AHS as well as genetic predisposition. Typing for *HLA-B*57:01* is now the most commonly used pharmacogenetic test in clinical practice and represents the paradigm for the translation of pharmacogenetic tests into clinical practice [7].

Carbamazepine Hypersensitivity and *HLA-B*15:02/HLA-A*31:01*

Carbamazepine (CBZ) is an important drug that is used in the treatment of epilepsy, trigeminal neuralgia and bipolar disorder [61–63]. Although generally well-tolerated, up to 10 % of patients can experience a cutaneous ADR [61]. These

reactions can range from mild maculopapular exanthema (MPE), where drug withdrawal is usually the only treatment necessary, to severe HSS or life-threatening SJS/TEN [18]. The clinical features of CBZ hypersensitivity are similar to AHS with an immune-based aetiology and suspected genetic predisposition [64]. Researchers from Taiwan were the first to report a very strong association ($OR > 1,000$) between *HLA-B*15:02* and CBZ-induced SJS amongst Han Chinese patients [9]. This association was subsequently replicated in other Han Chinese patients and Asian populations including Thai, Malay and Indians [23–25, 27–30]. However, this association was not replicated amongst Caucasian and Japanese patients [35, 65–67]. The discrepancy may be explained by differing background allele frequencies between populations: the prevalence of *HLA-B*15:02* is highest amongst Asian populations (0.057–0.145 in Han Chinese, 0.085–0.275 in Thais and 0.12–0.157 in Malays) compared with European (0.01–0.02), Japanese (0.002) and Korean (0.004) populations [68]. The association between *HLA-B*15:02* and CBZ-induced SJS/TEN is both ethnicity and phenotype specific. A systematic review conducted by ourselves demonstrated that carriage of the *HLA-B*15:02* allele was associated with approximately 100-fold risk of developing SJS/TEN with CBZ [69]. The clinical utility of pre-prescription *HLA-B*15:02* screening was recently demonstrated by a prospective cohort study in Taiwan [70]. Four thousand and eight hundred seventy-seven patients were genotyped and 7.7 % were positive for *HLA-B*15:02*. These patients were prescribed alternative therapy to CBZ and no patients developed SJS in the study when it was expected that there would be ten cases based on historical incidence. A study in Thailand also suggested that genotyping for *HLA-B*15:02* would be cost-effective if a test specific for *HLA-B*15:02* was utilised [71]. On the basis of such data, the FDA and European Medicines

Table 2 Drug-induced skin injury and HLA allele associations to commonly prescribed medications

Drug	Class of drug	HLA allele	Ethnicity	Phenotypes	References
Abacavir	Antiretroviral	<i>B*57:01</i>	Caucasians	HSS	[8, 19–21]
			African-Americans		[22]
Carbamazepine	Anticonvulsant	<i>B*15:02</i>	Han Chinese	SJS/TEN	[9, 23–26]
			Thai		[27, 28]
			Malaysian		[29]
			Indian		[30]
		<i>A*31:01</i>	Japanese	MPE	[31]
			Caucasian	HSS	[10]
			Korean	SJS/TEN	[32]
			Han Chinese		[26]
Allopurinol	Xanthine oxidase inhibitor	<i>B*58:01</i>	Han Chinese	HSS	[33]
			Thai	SJS/TEN	[34]
			Japanese		[35]
			Malay		[36]
Phenytoin	Anticonvulsant	<i>B*15:02</i>	Han Chinese	SJS/TEN	[23]
			Thai		[28]
Lamotrigine	Anticonvulsant	<i>B*58:01</i> , <i>A*68:01</i> , <i>DRB1*13:01</i>	Caucasians	HSS SJS/TEN	[37]
Nevirapine	Antiretroviral	<i>DRB1*01</i>	Caucasian	MPE HSS SJS/TEN	[38] [39]
		<i>Cw8,B14</i>	Caucasian		[40]
		<i>Cw8</i>	Japanese		[41]
		<i>B*35:05</i>	Thai		[42]
			Indian		[43]
		<i>Cw*04</i>	Thai		[44]
			Han Chinese		[45]
Sulphamethoxazole	Antibiotic	<i>B*38</i>	Caucasian	SJS/TEN	[46]
		<i>A*29, B*12, DR7</i>	Caucasian	SJS/TEN	[47]
Aminopenicillins	Antibiotic	<i>A2, Drw52</i>	Caucasian	HSS	[48]
Co-trimoxazole	Antibiotic	<i>A*30, B*13, Cw6</i>	Turkish	Fixed drug eruption	[49]

Agency have included warnings in the drug label advising the need for genotyping patients from certain areas of Asia before starting therapy with CBZ [72].

More recently, genome-wide association (GWAS) studies in Caucasian and Japanese populations have demonstrated an association between *HLA-A*31:01* and all phenotypes of CBZ hypersensitivity unlike *HLA-B*15:02*, which was phenotype specific for SJS/TEN [10, 31]. This association has also been reported in Han Chinese and Korean populations [26, 32]. Carriage of *HLA-A*31:01* was associated with a ninefold increased risk of CBZ cutaneous ADR, and the number needed to test to avoid one case was between 47 and 67 [69]. The pathogenesis of these CBZ hypersensitivity reactions need further research as it remains unclear whether *HLA-B*15:02* and *HLA-A*31:01* represent the true causal

alleles or if they are in linkage disequilibrium with another causal variant.

Allopurinol Hypersensitivity and *HLA-B*58:01*

Allopurinol is a xanthine oxidase inhibitor that is used in the treatment of gout and hyperuricaemia. In 2005, a case control study in a Han Chinese population from Taiwan reported an extremely strong association between *HLA-B*58:01* and allopurinol-induced SJS/TEN or HSS [33]. In this study, all 51 patients who developed allopurinol-induced SJS/TEN or HSS were positive for *HLA-B*58:01* compared with 20 out of 135 (15 %) allopurinol-tolerant patients and 19 out of 93 (20 %) population controls (OR=580, 95 % CI 34–9,781). This association was also detected in Thai, Japanese,

Caucasian and South Korean patients suggesting that *HLA-B*58:01* could be a potential biomarker that is applicable in multiple ethnic populations worldwide [34, 35, 46, 73]. However, further research is required as clinical utility of pre-prescription genotyping for *HLA-B*58:01* has not been confirmed; it is not clear if *HLA-B*58:01* also predisposes to milder cutaneous ADR such as MPE and we are not certain if *HLA-B*58:01* is the causal allele.

Drug-Induced Liver Injury

Drug-induced liver injury (DILI) is a rare, but potentially life-threatening, adverse hepatic reaction to medication that is unexpected on the basis of the pharmacological action of the drug administered. It is therefore distinct from DILI secondary to drug overdose which is illustrated by acetaminophen overdose, where the risk and severity of liver injury generally increase with dose taken [15]. Drugs that cause DILI are structurally diverse and belong to different therapeutic classes. In the majority of cases, drugs that cause DILI are safe over a wide range of dosages for the majority of treated patients, but a small subset of patients experience severe ADRs such as DILI. DILI accounts for up to 15 % of acute liver failure cases in the US and Europe and an incidence estimated at 1–10 per 100,000 in those exposed [74–76]. Because of this unpredictability and low incidence of events, a drug's hepatotoxic potential may only be realised after it has obtained market approval and large numbers of patients have been exposed. Because of this, DILI is the most common single adverse effect causing major drug problems including withdrawals and refusals to approve by the FDA [77].

DILI is difficult to diagnose correctly because of the variability in presentation, heterogeneity of responsible medications and our lack of understanding of the pathophysiology of liver injury. Recently published guidelines outline the case definition and provide a diagnostic algorithm for DILI which should help reduce some uncertainty [15]. The mechanism of DILI is a complicated multi-step process that involves direct injury to the hepatocytes and activation of inflammatory pathways. It has been hypothesised that initial events in DILI are drug specific, that the drug and/or its metabolites directly cause cell stress, trigger the immune response and/or directly impair mitochondrial function. Subsequent 'downstream' steps are less specific (common to all drugs) and represent a balance between protective and toxic pathways. In susceptible individuals, the toxic pathways overwhelm the protective pathways triggering 'mitochondrial permeability transition' which initiates apoptotic or necrotic cell death [78]. Genetic variation in drug metabolism pathways and variability in immune response have been hypothesised to influence susceptibility to DILI.

In a similar fashion to DISI, several HLA associations have been reported with DILI (Table 3). This is a little surprising because the clinical features of DILI with some drugs (e.g. lumiracoxib and ximelagatran) would not suggest an immune component [84]. Despite the strong associations reported by some studies, none of these tests have been translated into clinical practice.

Amoxicillin-Clavulanate

Amoxicillin-clavulanate (AC) is among the most commonly prescribed anti-microbial worldwide. It is an important cause of DILI and responsible for approximately 10–13 % of all DILI-related hospitalisations [89, 90]. Most patients with AC-induced DILI make a full recovery but reports of liver failure leading to death and transplantation have been reported [91, 92]. Hautekeete et al. were the first to demonstrate a HLA association with AC DILI. They reported in a European population that the frequency of the haplotype *HLA-DRB1*15:01-DRB5*01:01-DQB1*06:02* was significantly higher in AC DILI compared with controls (57.1 vs. 11.1 %) [11]. The association between AC DILI and *HLA-DRB1*15:01* was replicated in two UK populations with odds ratios between 2.3 and 9.3 [79, 80]. However, a third study in Spanish patients failed to replicate this association [93]. More recently, a GWAS study involving 201 patients with AC DILI confirmed the association between *HLA-DRB1*15:01* and AC DILI [OR 4.2 (95 % CI 2.7–6.6)]. Two novel genetic risk factors were also identified: *HLA-A*02:01* [OR 2.2 (95 % CI 1.6–3.2)] in all patients and *HLA-B*18:01* [OR 4.0 (95 % CI 1.5–11)] in Spanish patients only [81]. This suggests AC DILI associated with MHC class I molecules may be ethnicity dependent in a similar manner to *HLA-B*15:02* and CBZ-induced SJS/TEN which was only applicable in certain Asian populations (see above).

Flucloxacillin

Flucloxacillin is an anti-microbial agent used widely in Europe for the treatment for staphylococcal infections. Its use has been associated with DILI and more specifically cholestatic liver disease. A GWAS study in 51 patients with flucloxacillin DILI reported a strong association with *HLA-B*57:01* [OR 80.6 (95 % CI 22.8–284.9)] [82]. This association was still significant in the replication cohort [OR 100.0 (95 % CI 20.6–485.8)] and suggested that individual's positive for *HLA-B*57:01* would have an approximate 100-fold greater risk of developing DILI with flucloxacillin. This finding is particularly interesting because it is the same HLA allele associated with abacavir hypersensitivity (see above) which does not manifest as hepatic injury. Whether this represents a similar causal mechanism or is merely co-incidental is unclear. The incidence of flucloxacillin DILI is low and has been estimated

Table 3 Drug-induced liver injury and HLA allele associations

Drug	Class of drug	HLA allele	Ethnicity	References
Amoxicillin-Clavulanate	Antibiotic	<i>DRB1*15:01</i>	Caucasians	[11, 79–81]
		<i>A*02:01</i>	Spanish	[81]
		<i>B*18:01</i>		[81]
Flucloxacillin	Antibiotic	<i>B*57:01</i>	Caucasian	[82]
Ximelagatran	Oral direct thrombin inhibitor	<i>DRB1*07:01</i>	Various	[83]
		<i>DQA1*02:01</i>		
Lumiracoxib	COX-II inhibitor	<i>DRB1*15:01</i>	Various	[84]
Nevirapine	Antiretroviral	<i>DRB1*01:01</i>	Caucasian	[38]
			Australian	[85]
			Various	[86]
Lapatinib	Tyrosine kinase inhibitor	<i>DQA1*02:01</i>	Various	[86]
Ticlopidine	Antiplatelet	<i>A*33:03</i>	Japanese	[87]
		<i>A*33:03 & CYP2B6 *1H/*1J</i>	Japanese	[88]

in the UK at 8.5 in every 100,000 new users [94]. The sensitivity and specificity of pre-treatment testing for *HLA-B*57:01* was high, 87 and 94 %, respectively, but because of the low incidence of flucloxacillin DILI, the positive predictive value for the test would only be 0.12 %. This means that more than 13,500 patients would need to be tested to avoid one case of flucloxacillin DILI. Furthermore, many patients that would not have developed DILI with flucloxacillin would also be denied this effective treatment [95]. Therefore, isolated routine genetic screening for *HLA-B*57:01* prior to flucloxacillin treatment would not be economically or clinically viable for prevention of DILI.

Ximelagatran

Ximelagatran was the first commercially available oral direct thrombin inhibitor. Studies showed that it was just as effective as conventional anticoagulation without the need for regular monitoring. Standard pre-clinical toxicological studies did not suggest that ximelagatran could cause DILI but it was withdrawn in 2007 because long-term analyses found that it was associated with hepatotoxicity in patients [96]. A GWAS, completed after the drug was withdrawn, identified an association in both discovery and replication cohorts between ximelagatran DILI and HLA alleles *DRB1*07:01* [OR 4.4 (95 % CI 2.2–8.9)] and *DQA1*02:01* [OR 4.4 (95 % CI 2.2–8.8)] [83]. A pharmacogenetic test for *HLA-DRB1*07:01* prior to ximelagatran therapy would have a sensitivity 47–50 %, specificity 83–97 %, positive predictive value 19–59 % and negative predictive value 95–96 %. The reported incidence of elevated serum transaminase levels with ximelagatran was 7.9 %. Therefore, between 20 and 24 patients would need to be screened to prevent one case of ximelagatran DILI. In comparison with other screening tests, such as mammography in breast cancers which screen

between 233 to 746 patients to prevent one death, the numbers for *HLA-DRB1*07:01* appear to be favourable [95]. These results are only based on one study, which cannot be replicated because the drug has been withdrawn, so need to be interpreted with caution. The association between ximelagatran DILI and HLA alleles is a little surprising because the clinical phenotype of liver injury does not suggest an immune component but GWAS suggests an immune pathogenesis highlighting our lack of understanding of the pathophysiological mechanisms of DILI [83].

Other Drugs

Lumiracoxib, a cyclooxygenase-2 selective inhibitor, was withdrawn or not approved in 2007 following cases of severe hepatotoxicity. A GWAS in 41 patients with lumiracoxib DILI and 176 lumiracoxib-tolerant patients demonstrated a significant association with the MHC class II haplotype: *HLA-DRB1*15:01-HLA-DQB1*06:02-HLA-DRB5*01:01-HLA-DQA1*01:02* [OR 5.0 (95 % CI 3.6–7.0)] [84]. This association was replicated in a second cohort of 98 cases and 405 controls. Interestingly, *HLA-DRB1*15:01* had previously been implicated in DILI associated with amoxicillin-clavulanate suggesting possible overlap of pathophysiology despite differences in drug structure and phenotype of DILI [11].

Candidate gene studies have also identified HLA allele associations with nevirapine, lapatinib and ticlopidine [38, 85–88]. Nevirapine is a widely prescribed non-nucleoside reverse transcriptase inhibitor for the treatment of HIV which is associated with DILI in some individuals. *HLA-DRB1*01:01* was significantly associated with nevirapine DILI in white patients with high CD4 T cell counts from an Australian population [OR 3.02 (95 % CI 1.66–5.49)]. This association was not significant in Asian or Black patients and

was attributed to low background frequency of the allele *HLA-DRB1*01:01* amongst those populations and small sample sizes [85].

An HLA class II association has been reported with the tyrosine kinase inhibitor lapatinib, which is used in the treatment of metastatic breast cancer. *HLA-DQA1*02:01* was more common amongst patients with elevated ALT on lapatinib therapy compared with controls [OR 2.1 (95 % CI 1.1–5.7)]. This association was confirmed in a replication cohort [OR 9.0 (95 % CI 3.2–27.4)] [86].

Ticlopidine is an anti-platelet agent associated with liver injury especially amongst Japanese patients when compared with Caucasian populations. An initial case study reported that *HLA-A*33:03* was associated with ticlopidine DILI in a Japanese population [OR 13.04 (95 % CI 4.40–38.59)] [87]. A second study reported that Japanese patients were most susceptible to ticlopidine DILI if they carried both *HLA-A*33:03* and *CYP2B6 *1H* or **1J* [OR 38.82 (95 % CI 8.08–196.0)] [88]. *CYP2B6* is one of the key enzymes responsible for formation of the toxic S-oxide metabolite of ticlopidine, which is hypothesised to be responsible for the liver injury [97]. This is the first report combining genetic polymorphisms in drug metabolising enzymes and molecules in antigen presentation to investigate susceptibility to ADRs.

Discussion

Over the past 20 years, significant progress has been made in our understanding of the influences of genetics on susceptibility to ADRs. However, to date, apart from testing for *HLA-*

*B*57:01* in abacavir therapy, no other pharmacogenetic test for ADR susceptibility has been translated into clinical practice. This is due to a multitude of factors. Although numerous associations have been discovered, replication has proven difficult because of the large numbers of patients and controls required. Many studies are conducted in a retrospective fashion where patient phenotypes are poorly recorded or incomplete and other crucial factors, such as drug dosage/timings and concomitant medications, are missing. Reports suggest that only 30–50 % of reported cases of DILI are correctly diagnosed or classified [98, 99]. In the future, phenotype standardisation and diagnostic algorithms will help and the development of international networks for sample and data collection such as United States Drug-Induced Liver Injury Network (DILIN; <https://diln.dcri.duke.edu/>), European collaboration to establish a case-control DNA collection for studying the genetic basis of adverse drug reactions (EUDRAGENE; www.eudragene.org/) and International Serious Adverse Events Consortium (SAEC; <http://www.saeconsortium.org/>) will ensure more accurate data recording [14, 15].

Accurate phenotype recording is crucial because different phenotypes are likely to have diverse pathophysiological mechanisms. Combining different phenotypes will reduce the power of a study to detect significant genetic associations. This has been highlighted by the phenotype specificity of *HLA-B*15:02* and CBZ-induced SJS/TEN but not CBZ HSS or MPE [9]. Ethnicity also plays a major role in the susceptibility and development of ADRs. CBZ-induced SJS/TEN, ticlopidine DILI and AC DILI are all ethnicity specific. Carriage of *HLA-B*15:02* was associated with CBZ-SJS/TEN

Table 4 Drugs that have undergone labelling changes in response to genetic risk factors in drug metabolism

Drug	Class of drug	Gene	Clinical effects	Refs
Azathioprine and 6-Mercaptopurine	Immunosuppressant	TPMT	Patients that are TPMT deficient are highly sensitive to the myelosuppressive effects of azathioprine and mercaptopurine which manifests clinically as bone marrow suppression. TPMT genotyping is advised prior to therapy and individuals with low activity genotypes/phenotypes should have dose reduction of azathioprine/6-mercaptopurine.	[102]
Warfarin	Anticoagulant	CYP2C9 and VKORC1	CYP2C9 is responsible for metabolism of Warfarin. CYP2C9*2 and *3 are associated with reduced activity and therefore increased risk of bleeding episodes with warfarin therapy. Warfarin acts on VKORC1 to inhibit formation of clotting factors. Different haplotypes of VKORC are associated with altered warfarin requirements.	[103]
Irinotecan	Chemotherapy	UGT1A1	UGT1A1 is responsible for inactivation of SN-38, the active metabolite of Irinotecan, by glucuronidation. Patients with UGT1A1 genotypes with reduced or intermediate activity are at increased risk of severe neutropaenia. Reduced dosages should be considered in these patients.	[104]
Primaquine	Antimalarial	G6PD	Patients with G6PD deficiency are at increased risk of haemolytic reactions and methaemoglobinaemia with primaquine therapy. Physicians are advised to monitor patients more closely but no recommendations regarding dosages.	[105]

but only in patients from Asian populations and not in European or Japanese populations [69]. Similarly, the association of *HLA-A*33:03* with ticlopidine DILI was significant only in Japanese and not Caucasian populations [88]. The association between *HLA-DRB1*15:01* and AC DILI was reported in several European populations but not Spanish patients [89]. Ethnic specificity is believed to be due to differing background frequencies of the risk HLA alleles. For example, the frequency of *HLA-A*33:03* amongst Japanese is much higher (0.1280) than English (0.003), Portuguese (0.011) or Italian (0.000) populations (<http://www.allelefrequencies.net/>).

Many significant associations in ADRs have been reported with HLA alleles which are encoded by the MHC. There is considerable variation and linkage disequilibrium in this region meaning that it is very difficult to prove a particular HLA allele, or an allele that is in linkage disequilibrium, as causal for the ADR [12]. Indeed, a functional role for HLA alleles in the pathogenesis of hypersensitivity reactions has only been proven in the case of abacavir [100]. A recent study looking at HLA haplotypes in 400 healthy volunteers reported that HLA alleles associated with DILI induced by structurally diverse drugs (amoxicillin-clavulanate, flucloxacillin, lumiracoxib and ximelagatran) may be connected through peptide binding of one of the HLA alleles that defines the causal haplotype [101]. This raises the possibility that common causal alleles within the MHC may predispose to DILI and fine mapping of this area with NGS technologies along with functional studies may be able to identify the causal pathways.

Because of the low incidence of some ADRs (e.g. CBZ-induced SJS/TEN is 23 per 10,000 in Asian populations), many proposed pharmacogenetic screening tests have high negative predictive values but low positive predictive values [21, 69, 95]. Therefore, a significant number of patients who test positive for the suspected allele will never develop hypersensitivity or very large numbers of patients need to be tested to avoid one case of ADR. This has major implications as patients may be denied optimal treatment, require more costly alternative therapies or the pharmacogenetic test itself is not cost-effective for clinical practice [95]. However, given the low incidence of some of these reactions, it will not be practically possible to undertake randomised controlled trials to prove the utility that genetic testing for HLA alleles prevents these reactions. This raises the question as to what kind of evidence is needed to provide evidence of clinical utility that is acceptable to prescribing clinicians.

For some drugs, combinations of genetic polymorphisms may predispose to the ADR. Thus, in Japanese patients with ticlopidine DILI who are *HLA-A*3303* positive, concurrent carriage of *CYP2B6* *1H or *1J was associated with significantly increased risk than carriage of the HLA allele alone [OR 38.82 (95 % CI 8.08–196.0)] [88]. The importance of genetic variation in drug metabolising enzymes has been highlighted by several

drugs which have undergone FDA labelling changes (Table 4). It is likely that ADRs are polygenic and each gene has a small overall effect. In the future, large numbers of patients will need to be recruited and screening for multiple genetic polymorphisms (metabolising enzymes, drug transporters, drug receptors and immune system variation) may improve accuracy of prediction of ADRs. More recently, a pharmacogenetic score combining three SNPs accurately predicted those patients with stable coronary artery disease who would most likely benefit and suffer harm from perindopril therapy highlighting how such an approach may be successful [106].

Any potential genetic test for the identification of patients at risk of adverse drug reactions must also demonstrate cost-effectiveness in clinical practice. ADRs that have the following characteristics would enhance the cost-effectiveness of any proposed test: severe clinical or economic consequences of ADR, well-established association between genotype and clinical phenotype, the availability of a rapid and inexpensive genetic test and high frequency of associated variant gene [107].

At present, NGS has not been utilised to investigate the role of genetics in ADRs. NGS allows the analysis of rare variants that cannot be detected by GWAS, which have been postulated to have larger effect sizes and more likely to be functionally relevant compared with common variants [108]. Epigenetics is the study of mitotically heritable changes in gene expression that are not attributable to nucleic acid sequence alterations. In humans, the main epigenetic phenomena are DNA methylation and histone modifications [109]. The role of epigenetics in ADR is reviewed in a recent article, and with the advent of NGS, we are likely to significantly increase our understanding of the role of epigenome in drug response [110].

Translation of a biomarker into clinical practice is a difficult process but can be broadly split into four translational phases: T1 (discovery), T2 (clinical validity and utility), T3 (implementation) and T4 (effect on public health) [111]. Much of the research into biomarkers for ADR remains in the first translational phase with screening for *HLA-B*57:01* in AHS (T4) and *CYP2C9* and *VKORC1* in warfarin dosing (T2) the only exceptions. In order to translate other associations into clinical practice, collaboration will be required between scientists, clinicians, governing agencies and pharmaceutical and technology companies.

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