



HLA and pharmacogenetics of drug hypersensitivity

Immunologically mediated drug reactions have been traditionally classified as unpredictable based on the fact that they cannot be predicted strictly on the pharmacological action of the drug. Such adverse drug reactions are associated with considerable morbidity and include severe cutaneous adverse reactions such as Stevens–Johnson syndrome/toxic epidermal necrolysis and the drug hypersensitivity syndromes (drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome). Over the last decade there have been many associations between these syndromes and Class I and II HLA alleles of the MHC, which have enriched and driven our knowledge of their immunopathogenesis. Significant translation has also occurred in the case of *HLA-B*5701* screening being used to exclude at risk patients from abacavir and prevent abacavir hypersensitivity. The ultimate translation of the knowledge of how drugs interact with HLA would be applicable to preclinical drug screening programs to improve the safety and cost-effectiveness of drug design and development.

KEYWORDS: altered peptide repertoire ■ DILI ■ DRESS ■ human leukocyte antigen hypersensitivity ■ major histocompatibility complex ■ pharmacogenetics ■ pharmacogenomics ■ Stevens–Johnson syndrome ■ toxic epidermal necrolysis ■ translation

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Drug hypersensitivity syndromes

Adverse drug reactions (ADRs), cause significant morbidity and mortality for patients and are an expense to the healthcare system. They are clinically and epidemiologically classified as either the prevalent ‘type A’ reactions, which are predictable based on the pharmacological action of the drug, or ‘type B’ reactions, which are often allergic reactions that are less common, not as dependent on dose, not predictable based on drug pharmacology and more recently related to host pharmacogenetic factors. Delayed hypersensitivity reactions include the hypersensitivity syndromes, drug-induced hypersensitivity syndrome (DIHS), which is also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS) or hypersensitivity syndrome (HSS); and Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), which are sometimes included under the collective classification of severe cutaneous adverse reactions (SCAR). Single-organ involvement can also occur with the most common being drug-induced liver disease (DILI).

■ DIHS/DRESS/HSS

DIHS is the most common of the ADRs, constituting 13% of all ADRs and can be characterized by a minimum criteria of fever, rash, hepatitis and white cell abnormalities [1–3]. The

reaction typically occurs within 3 months of drug treatment with a prolonged recovery phase after withdrawal of the drug. The skin is most commonly affected by the syndrome although this may be accompanied by failure of other organs including the liver, kidneys, lungs and/or heart. Reactivation of human herpes virus, mainly human herpes virus -6, and less frequently cytomegalovirus, has been described during the course of DIHS [2–4]. These viral reactivations have been reported in association with recurrence of symptoms more than 2 weeks after the drug was discontinued [2,4]. The most commonly implicated drugs associated with DIHS across all populations are the anti-retrovirals (abacavir [ABC], nevirapine [NVP] and fosamprenavir), allopurinol, the anticonvulsants (carbamazepine [CBZ], phenytoin, phenobarbital and lamotrigine), β -lactam antimicrobials, NSAIDs and sulfa antimicrobials (aromatic sulfonamides).

■ Single-organ drug hypersensitivity syndromes

Drug hypersensitivity syndromes (DHS) affecting single organs that are distinct from DIHS/DRESS are most commonly DILI. DILI is a single-organ disease, which can be associated with fulminant hepatic failure, and may be associated with genes regulating drug

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transport, metabolism, bile acid homeostasis or immunologic mechanisms [5]. DILI is most often associated with flucloxacillin or amoxicillin–clavulanate or the tetracycline antimicrobials [6,7]. Other examples of single-organ drug-induced diseases include pancreatitis and tubulointerstitial nephritis.

■ SJS/TEN

SJS/TEN share some clinical and laboratory features with DIHS/DRESS, also having a delayed onset, and an association with fever and internal organ involvement. However, in addition, these syndromes are characterized by blistering skin disease and mucosal involvement [8]. These diseases are classified according to the presence of skin separation and the percentage of body surface area that is involved, representing the same condition at different severities across the spectrum. SJS occurs when 10% of body surface area is involved, TEN when more than 30% of total body surface area is affected, leaving an SJS/TEN overlap between 10 and 30%. More recently, drug-induced skin injury has been proposed as an overarching term for all drug-induced skin diseases [8]. Although SJS/TEN are relatively uncommon, with a prevalence of two to six cases per million per year, both syndromes are associated with high morbidity and mortality (1–5% mortality for SJS and 30–50% for TEN). The drugs causing SJS/TEN generally overlap with those driving DIHS/DRESS, with the most common being allopurinol, aromatic amine anticonvulsants, antiretrovirals (particularly NVP), NSAIDs and sulfa antimicrobials.

Models for drug-induced hypersensitivity reactions

Given the severity of various ADRs, knowledge of the immune mechanisms driving their pathogenesis is highly desirable. In delayed-type hypersensitivity reactions (or type IV reactions) symptoms typically occur after at least 3 days of exposure to the antigen or drug. It has been long proposed that T cells are primed on initial exposure and a memory pool is restimulated on repeat exposure. This concept has been reinforced by the observation that these reactions resolve upon removal of the drug and occur more rapidly on drug reintroduction. The key proteins that mediate T-cell responses are the HLA molecules encoded within the MHC, located on chromosome 6. MHC Class I molecules (HLA-A, HLA-B and HLA-C) and MHC Class II molecules (HLA-DR and HLA-DQ) are responsible for presenting peptides to CD8⁺

and CD4⁺ T cells, respectively. Studies examining drug-induced hypersensitivity reaction (HSR) mechanisms *ex vivo* have demonstrated the activation of T cells by drugs driving DHS and their metabolites [9–12] and delineated the role of antigen-presenting cells (APCs) in hypersensitive patients [13,14]. Since the advent of more accurate and higher-resolution HLA typing and more precise clinical phenotyping of drug reactions, a number of strong associations between HLA allele carriage and the development of various ADRs and DHS have been reported (TABLE 1 & BOX 1). Taken together, the knowledge provided by these studies makes it reasonable to assume that in the future at least some of the classically unpredictable ‘type B’ ADRs may in fact become predictable and preventable due to their strong genetic associations.

Several nonmutually exclusive models have been proposed to explain how small molecular synthetic compounds are recognized by T cells in an MHC-dependent fashion. These include the hapten concept/prohapten model, the p-i model, and the altered repertoire model.

■ The hapten/prohapten model

Haptens/prohaptens are small chemically reactive molecules that undergo a stable, covalent binding to a larger protein or peptide, which modifies the side chain of the bound residue. Through binding to the higher-molecular-weight proteins the small molecules then become antigenic. Theoretically, this modification can affect any kind of autologous protein and known examples are derivatives of penicillin that bind covalently to the lysine residue of serum albumin [15] and nitrosulfamethoxazole-modified peptides produced during sulfamethoxazole treatment [16]. The haptenated product is then hypothesized to undergo antigen processing by APCs to produce haptenated peptides (novel MHC ligands) that are presented on MHC molecules and subsequently activate antigen-specific T cells [17]. Re-exposure of sensitized individuals will then result in proliferation of memory T cells and an inflammatory response within 24–72 h. In the case of a prohaptens, a chemically inert drug is first metabolized to gain reactivity before it is haptenated.

■ The p-i model: pharmacological interaction of drugs with immune receptors

Several aspects of DHS cannot be explained by the hapten–prohapten model. There are cases where a specific immune response to a drug can

Table 1. Pharmacogenetics of HLA-associated drug hypersensitivity and related drug-induced syndromes.

Syndrome and drug	Alleles	Populations	Ref.
SJS/TEN (SCAR)			
Allopurinol	<i>B*5801</i> (or <i>B*58</i> haplotype)	Han Chinese, Thai, European, Italian, Korean	[97,98,101,103,104,106,115,139]
Carbamazepine	<i>B*1502</i>	Han Chinese, Thai, Malaysian, Indian	[46–52,54,55,140,141]
	<i>B*1511</i>	Korean, Japanese	[61,71]
	<i>B*1518</i> , <i>B*5901</i> and <i>C*0704</i>	Japanese	[72]
	<i>A*3101</i>	Japanese, northern European, Korean	[58–61]
Oxcarbazepine	<i>B*1502</i> and <i>B*1518</i>	Han Chinese, Taiwanese	[64,65]
Lamotrigine	<i>B*1502</i> – positive	Han Chinese	[64]
	<i>B*1502</i> no association found	Han Chinese	[66,67]
	<i>B*38</i>	European	[99]
Phenytoin	<i>B*1502</i> , <i>B*1301</i> , <i>Cw*0801</i> and <i>DRB1*1602</i>	Han Chinese	[48,64,140]
Sulfamethoxazole	<i>B*38</i>	European	[104]
Methazolamide	<i>B*5901</i> , <i>Cw*0102</i> alleles and <i>B*5901</i> – <i>Cw*0102</i> – <i>A*2402</i> haplotype	Korean and Japanese	[142]
Sulphonamides	<i>A*29</i> , <i>B*12</i> and <i>DR7</i>	European	[143]
Oxicam	<i>B*73</i>	European	[104]
	<i>A*2</i> and <i>B*12</i>	European	[143]
HSS/DIHS/DRESS			
Abacavir	<i>B*5701</i>	European, African	[30,31,36]
Allopurinol	<i>B*5801</i> (or <i>B*58</i> haplotype)	Han Chinese, Korean, Japanese, Thai, European	[98,100,101,103,106,107,115,144]
Nevirapine (hepatitis and low CD4 ⁺)	<i>DRB1*0101</i> and <i>DRB1*0102</i>	Australian, European, South African	[84,86,145]
Nevirapine (DIHS/DRESS)	<i>Cw*8</i> or <i>CW*8</i> – <i>B*14</i> haplotype	Italian, Japanese	[87,88]
	<i>Cw*4</i> and <i>DRB1*15</i>	Han Chinese	[89]
	<i>B*3505</i>	Asian	[86]
	<i>B*3501</i> and <i>B*15/DRB1*15</i>	Australian	[93]
Carbamazepine	8.1 AH (<i>HLA A*0101</i> , <i>Cw*0701</i> , <i>B*0801</i> , <i>DRB1*0301</i> , <i>DQA1*0501</i> , <i>DQB1*0201</i>)	Caucasians	[146]
	<i>A*3101</i>	Northern European, Japanese, Korean	[58–61]
	<i>A*11</i> and <i>B*51</i> (weak)	Japanese	[58]
Delayed rash (nonsystemic)			
Efavirenz	<i>DRB1*01</i>	French	[85]
Nevirapine	<i>DRB1*01</i>	French	[85]
	<i>Cw*04</i>	African, Asian, European, Thai	[86,92]
	<i>B*3505</i> ; rs1576*G <i>CCHCR1</i> status (GWAS)	Thai	[90,91]
^aStrontium ranelate a compound derived from a natural earth metal similar to beryllium has also recently been described in association with MPE, DRESS and SJS/TEN although no HLA associations have been described as of yet [175,176]. DIHS: Drug-induced hypersensitivity syndrome; DILI: Drug-induced liver disease; DRESS: Drug reaction with eosinophilia and systemic symptoms; GWAS: Genome-wide association study; HSS: Hypersensitivity syndrome; MPE: Maculopapular eruption; SCAR: Severe cutaneous adverse reaction; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.			

Table 1. Pharmacogenetics of HLA-associated drug hypersensitivity and related drug-induced syndromes (cont.).

Syndrome and drug	Alleles	Populations	Ref.
Delayed rash (nonsystemic) (cont.)			
Aminopenicillins	A*2 and DR*52	Italian	[147]
Carbamazepine (or MPE)	A*3101	Han Chinese, northern European	[47,59]
Oxcarbazepine-induced MPE	B*1502	Han Chinese	[63]
DILI			
Amoxicillin–clavulanate; coamoxiclav DILI	DRB1*1501, DRB107 protective, HLA-A*0201, HLA-DQB1*0602 and rs3135388, a tag SNP of HLA-DRB1*1501–DQB1*0602	European	[148–150]
Lumiracoxib	HLA-DRB1*1501–HLA-DQB1*0602–HLA-DRB5*0101–HLA-DQA1*0102 haplotype	International, multicenter	[151]
Ximelagatran	DRB1(*)07 and DQA1(*)02	Swedish	[152]
Diclofenac	ABCB11, C-24T, UGT2B7*2, IL-4 C-590-A	European	[6,7,153]
Isoniazid	NAT2 slow acetylator, CYP2E1*5 and *1B	European	[6,7]
Flucloxacillin	B*5701, DRB1*0107–DQB1*0103	European	[7,154]
Lapatinib	DRB1*0701–DQA2*0201–DQB1*0202/0202	International, multicenter	[155]
Ximelagatran	DRB1*07 and DQA1*02	European	[152]
Fixed-drug eruption			
Feprazone	B*22	Italian	[156,157]
Sulfamethoxazole	A*30–B*13–Cw*6 haplotype	Turkish	[158]
Agranulocytosis			
Clozapine	B*38, DR*4 and DQw*3	Jewish	[159,160]
	(6672G>C) in HLA-DQB1	North American	[161]
	Cw7/B*18 or B*39 or B*44/DRB*5	Caucasian	[162]
Levamisole	B*27	South American	[163]
Drug-induced lupus erythematosus			
Hydralazine	DR*4	European	[164]
Procainamide, Isoniazid, Methyldopa and Quinidine	DR*4	Italian	[165]
Other drug reactions			
Aspirin (urticaria/angioedema)	DRB1*1302–DQB1*0609–DPB1*0201 haplotype	Korean	[166]
Aspirin (asthma)	DPB1*0301	Korean	[167]
Gold sodium thiomalate (mucocutaneous reaction)	DR*5	Spanish	[168]

*Strontium ranelate a compound derived from a natural earth metal similar to beryllium has also recently been described in association with MPE, DRESS and SJS/TEN although no HLA associations have been described as of yet [175,176].

DIHS: Drug-induced hypersensitivity syndrome; DILI: Drug-induced liver disease; DRESS: Drug reaction with eosinophilia and systemic symptoms; GWAS: Genome-wide association study; HSS: Hypersensitivity syndrome; MPE: Maculopapular eruption; SCAR: Severe cutaneous adverse reaction; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

Table 1. Pharmacogenetics of HLA associated drug hypersensitivity and related drug-induced syndromes (cont.).

Syndrome and drug	Alleles	Populations	Ref.
Other drug reactions (cont.)			
Gold sodium thiomalate (proteinuria, thrombocytopenia or leukopenia)	<i>B*8</i> and <i>DR*3</i>	European	[169]
NSAIDS (anaphylactoid and cutaneous reactions)	<i>DR*11</i>	Spanish	[170]
D-penicillamine (myasthenia gravis)	<i>DR*1</i>	Mixed Caucasian	[171]
D-penicillamine (proteinuria)	<i>B*8</i> and <i>DR*3</i>	European	[172]
Beryllium (granulomatous lung disease) [†]	<i>HLA-DPB1</i> gene and <i>DPR1</i> gene polymorphisms, <i>DRB1*13</i> and <i>DQB1*06</i>	North American	[173,174]

[†]Strontium ranelate a compound derived from a natural earth metal similar to beryllium has also recently been described in association with MPE, DRESS and SJS/TEN although no HLA associations have been described as of yet [175,176].
 DIHS: Drug-induced hypersensitivity syndrome; DILI: Drug-induced liver disease; DRESS: Drug reaction with eosinophilia and systemic symptoms; GWAS: Genome-wide association study; HSS: Hypersensitivity syndrome; MPE: Maculopapular eruption; SCAR: Severe cutaneous adverse reaction; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

be elicited at the first encounter without a sensitization phase. In addition, there are several examples of DHS caused by drugs that are not known to be metabolized to a reactive compound that can stimulate T-cell clones *in vitro* via the T-cell receptor (TCR) in an MHC-dependent fashion. Examples include drug-specific T-cell responses generated for lidocaine, lamotrigine and sulfamethoxazole in their nonreactive forms [16,18–20]. The p-i concept hypothesizes that drugs are able to activate T cells by direct binding to the TCR or via the formation of HLA–drug complexes that can activate T-cell immune responses without the requirement for a specific peptide ligand. The p-i-stimulated T cells are thought to arise from previously primed effector cells and memory T cells.

■ The altered repertoire model

There are many known associations between specific HLA molecules and drug hypersensitivity reactions. Key interacting residues in the HLA peptide-binding cleft for particular alleles allow formation of noncovalent bonds with the drugs in question [21,22]. Therefore, strong MHC allele drug specificity can be explained by a steric complementarity together with other strong noncovalent interactions between the drug molecule and the antigen presentation groove. The altered repertoire concept proposes that drugs occupy a specific site within the antigen-binding cleft, thereby altering the repertoire of self-peptide ligands that can be bound and presented to T

cells [13,23]. These concepts of noncovalent interaction of the drugs and MHC–TCR molecules may occur with the parent drug or its metabolites. More recent evidence from three independent groups provides strong evidence that this model may be key in the development of ABC hypersensitivity (FIGURE 1) [24–26]. Accumulating evidence also supports the altered peptide model as important in the pathogenesis of CBZ-induced SJS/TEN [21,24].

Specific examples of HLA-driven HSR

Over the last decade, there has been a significant increase in the volume of publications associating various HLA alleles with different DHS (TABLE 1). In general, different ethnic populations have different genetic associations. These differences may be a reflection of different allele frequencies as well as the presence of other important genes that contribute to the development of the various disease syndromes, such as genes involved in drug metabolism. It appears that each unique phenotypic DHS is associated with a set of pharmacogenetic markers and should be considered to have its own pathogenic mechanisms. To date, the best characterized HLA-associated DHS are to ABC, NVP, CBZ and allopurinol.

■ ABC

ABC is a guanosine analogue that works by competitively inhibiting the reverse transcription of HIV, and has been in use in most developed

Box 1. Highlights of the chronology of HLA-associated drug hypersensitivity syndromes.**1980s**

- *Ex vivo* studies
- Several two-digit associations; for example, delayed hypersensitivity to aminopenicillins, *A*2* and *DR*52* (1998) [143]; allopurinol skin reactions and *B58/AW33* in southern Chinese (1989) [92]

1990s

- Classification and epidemiology of serious cutaneous adverse drug reactions (e.g., SCAR)
- HHV-6 infection/reactivation noted in association with DIHS (1997) [173]
- Amoxicillin–clavulanate DILI and *HLA-DRB1*1501* association (1999) [144]

2002

- *HLA-B*5701* and abacavir hypersensitivity studies [27,28]
- Abacavir patch testing [37]

2004

- CBZ-induced SJS/TEN and *HLA-B*1502* in Han Chinese [42]

2005

- Allopurinol SJS/TEN and DIHS and *HLA-B*5801* [93]
- NVP rash/hepatitis and *HLA-DRB1*0101* [81]
- Aspirin-induced urticaria and haplotype *DRB1*1302–DQB1*0609–DPB1*0201* [162]

2006–2007

- US FDA recommends *HLA-B*1502* screening before CBZ use
- NVP HSR and *HLA-B*1402* and *Cw8* [84,141]
- *HLA-B*1502* and SJS/TEN with other aromatic AEDs [44]

2008

- Randomized double-blind screening study (PREDICT-1) shows that *HLA-B*5701* screening eliminates immunologically mediated ABC HSR [31]
- *HLA-B*5701* screening for ABC HSR routine clinical use

2009

- Flucloxacillin DILI and *HLA-B*5701* [150]
- NVP rash and *HLA-B*3505* [86]

2010

- *B75* serotype and CBZ-induced SJS/TEN [68,69]
- Methazolamide and *HLA-B*5901* in Koreans [137]

2011/2012

- *HLA-A*0201/DRB1*1501/DQB1*0602* and amoxicillin–clavulanate DILI [145]
- *HLA-A*3101* with CBZ-induced SJS/TEN, DIHS and MPE in northern Europeans and Japanese [56,58,69]
- Standardization of SCARs
- First GWAS in NVP-induced rash and allopurinol HSR confirm HLA associations [87,101,102]

ABC: Abacavir; AED: Antiepileptic drug; CBZ: Carbamazepine; DIHS: Drug-induced hypersensitivity syndrome; DILI: Drug-induced liver disease; GWAS: Genome-wide association study; HHV: Human herpes virus; HSR: Hypersensitivity reaction; MPE: Maculopapular eruption; NVP: Nevirapine; SCAR: Severe cutaneous adverse reaction; SJS: Stevens–Johnson syndrome; TEN: Toxic epidermal necrolysis.

counties since 1998. ABC can be associated with a DHS characterized by fever, malaise, gastrointestinal symptoms and internal organ involvement in approximately 5–8% of patients who begin therapy with the drug [27]. The syndrome can be accompanied by a mild-to-moderate rash in 70% of patients with ABC hypersensitivity and is associated with severe hypotension and possible death upon re-challenge, in contrast to the complete abrogation of symptoms 72 h after withdrawal of the drug [28].

The ABC example provides a model roadmap for how genetic investigations, together with supportive clinical and basic science and laboratory systems, can lead to successful translation

of pharmacogenetics to the clinic. The first suggestion of a potential genetic association with ABC HSR was in 2001, during the postmarketing phase of the drug in a study that showed a lower frequency of ABC HSR in both black and Asian populations when compared with Caucasians [29].

Following the initial observations of racial variations in ABC HSR incidence, two groups independently described a strong association between the HLA Class I allele, *HLA-B*5701*, and ABC HSR in 2002 [30,31]. Early studies showed a low sensitivity of *HLA-B*5701* for clinically suspected ABC hypersensitivity, particularly apparent in black patients that had a

low carriage rate for *HLA-B*5701* [31,32]. The ABC example illustrates how overassignment of the HSR syndrome and low allele frequency in certain population groups can wrongly lead to the assumption that a HLA association to a particular drug HSR is restricted to race. *HLA-B*5701* is most prevalent in Europeans (5–8%), when compared with African-Americans (2.4%) and even less in other ethnic groups [33]. Later work improved the clinical diagnosis of true immunologically mediated ABC hypersensitivity through the use of patch testing [34–36] and included an American study that examined subjects of European and African origin [36]. The PREDICT-1 was a randomized double-blind controlled study enrolling almost 2000 patients of predominately Caucasian ethnicity and

randomized patients to either receive real-time *HLA-B*5701* screening and exclusion of ABC for those positive for *HLA-B*5701* or ABC initiation and clinical monitoring with retrospective *HLA-B*5701* analysis. The study demonstrated the 100% negative-predictive value of *HLA-B*5701* for ABC HSR and supported a *HLA-B*5701* screening test to effectively eliminate immunologically confirmed ABC hypersensitivity [34]. Following PREDICT, SHAPE, a case–control study of black and white patients in the USA, demonstrated that 100% of both white and black patch test-positive patients with a clinical history consistent with ABC HSR carried *HLA-B*5701* [36]. These studies provide strong evidence for the clinical utility of *HLA-B*5701* to prevent ABC HSR, generalizable across race. Another

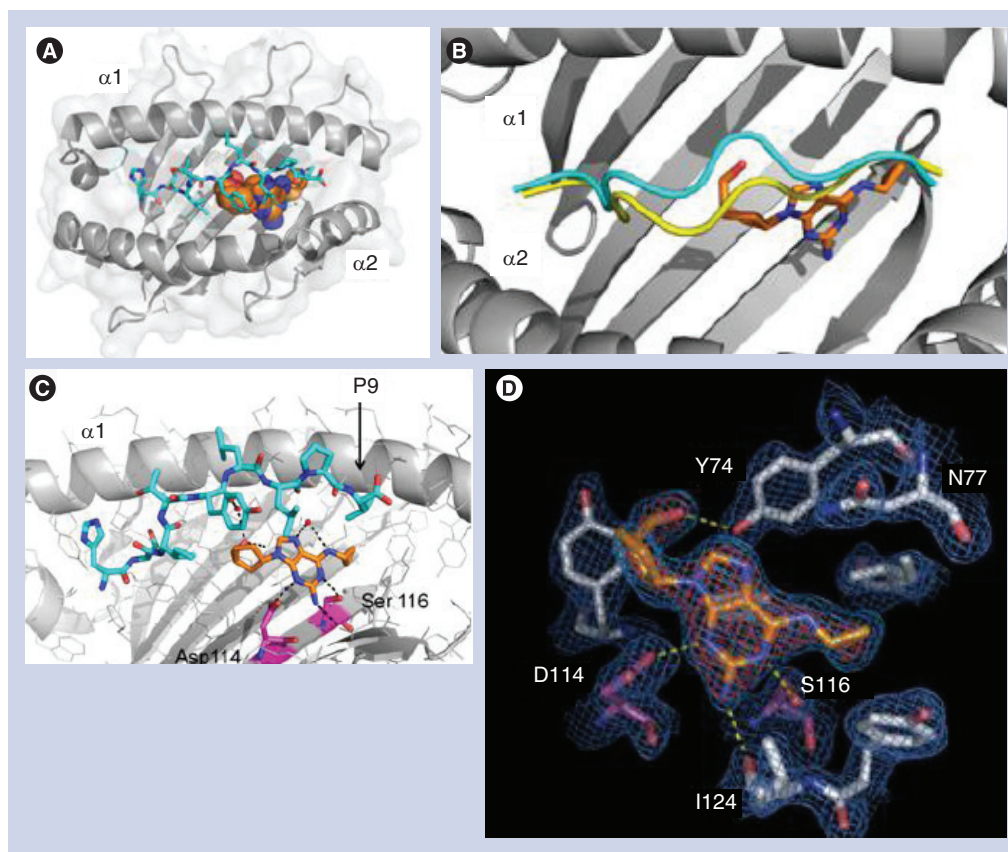


Figure 1. Crystal structure of the abacavir–MHC–peptide complex solved to a resolution limit of 2.0 Å. (A) Diagram of HLA-B*57:01 in gray. The peptide HSITYLLPV is shown in cyan carbons. Abacavir is shown as spheres, orange for carbon, blue for nitrogen and red for oxygen. **(B)** Drug binding influences the peptide backbone conformation by shifting the main chain. Peptide bound to abacavir and HLA-B*57:01 is shown in cyan. Peptide bound in the absence of abacavir is shown in yellow. **(C)** Abacavir forms H-bond interactions (black dashes) with both the peptide and HLAB*57:01. The residues that distinguish the abacavir-sensitive allele HLAB*57:01 from abacavir-insensitive HLA-B*57:03 are shown in magenta for carbon, blue for nitrogen and red for oxygen. **(D)** Experimental electron density corresponding to abacavir in a Fo-Fc difference map contoured at 5 σ (red mesh) following molecular replacement. Blue mesh depicts the final 2Fo-Fc electron density map of abacavir in the antigen-binding cleft of HLA-B*57:01 (contour level 1.5 σ). H-bond interactions between abacavir and HLA-B*57:01 are shown as yellow dashed lines. Reproduced with permission from [26].

important aspect of the PREDICT-1 study was that 45% of *HLA-B*5701* carriers were able to tolerate ABC. The study of ABC-exposed *HLA-B*5701*-positive subjects hold the key to understanding the additional factors required for the development of the syndrome in hypersensitive patients or, conversely, the protective factors in tolerant patients.

In parallel to the generation of a high level of clinical evidence, there was a considerable amount of work necessary to prepare for laboratory implementation. A specific quality assurance program for *HLA-B*5701* screening was validated and is currently actively administered by the Asia-Pacific Histocompatibility and Immunogenetics Association [37]. US FDA guidelines now recommend *HLA-B*5701* testing in advance of ABC prescription. Testing results in a reduction in the incidence of ABC HSR and is cost effective [34,38,39].

Strong scientific evidence strengthened the conclusions that the development of ABC hypersensitivity is *HLA-B*5701* restricted and mediated by CD8⁺ T lymphocytes. Infiltrating CD8⁺ T cells are present within the skin of ABC HSR patients with a rash [40] and TNF- α and IFN- γ are produced by ABC HSR patient peripheral blood mononuclear cells (PBMCs) *in vitro* [14,41]. In addition, CD8⁺ T cells from

ABC-naïve patients carrying the *HLA-B*5701* allele proliferate in response to ABC in long-term culture and are specifically activated by the drug. Until recently, this activation was thought to be dependent upon peptide processing via the conventional MHC Class-I presentation pathway [13], although the exact mechanism has remained unclear. Furthermore, cells from individuals with HLA allotypes closely related to ABC (*HLA-B*5801* and *HLA-B*5703*), which exhibit polymorphism in the antigen-binding F pocket, do not react with ABC, and substitution of the aspartate residue at position 114 or the serine at position 116 results in a >50% reduction in the number of IFN- γ -producing CD8⁺ T cells or complete abrogation of recognition by ABC-specific CD8⁺ T cells, respectively [13]. The serine at position 116 is particularly important in determining the structure of the F pocket and thus controls selection of the dominant anchor residue at the C terminus of bound peptides (FIGURE 2) [42,43].

Several new studies have presented evidence that ABC can undergo a metabolism-independent, direct and noncovalent interaction with the *HLA-B*5701* molecule, which forms an antigenic structure that is able to induce or restimulate a T-cell response. Adam and coauthors generated ABC-specific T-cell

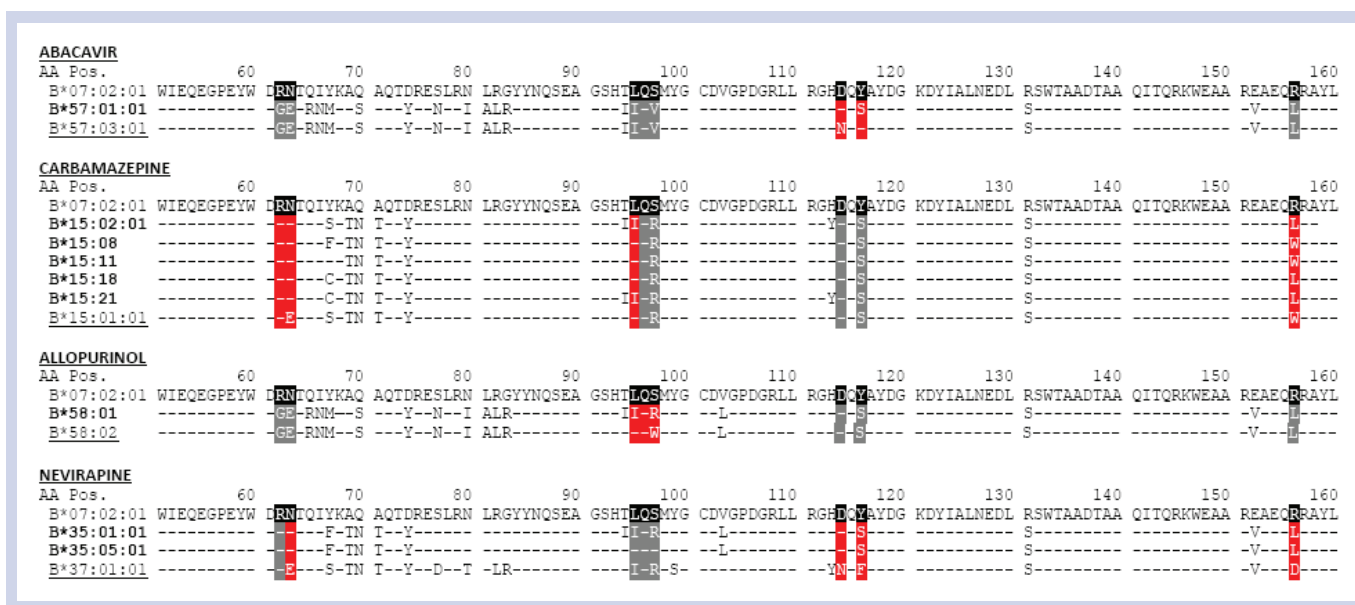


Figure 2. Alignments of *HLA-B* alleles associated with drug hypersensitivity indicating key residues for *HLA* drug hypersensitivity syndromes associated alleles aligned with the most similar nonassociated *HLA-B* alleles. The most similar nonassociated *HLA-B* alleles are underlined. *HLA-B*07:02:01* is used as the reference allele sequence. Key anchoring residues in the MHC binding pockets are highlighted. Red highlighting shows published regions of significance in *HLA* drug interaction or regions that are similar in the drug hypersensitivity syndromes associated alleles, yet differ to the most similar non-drug hypersensitivity syndromes associated alleles for a particular drug. Significant positions as published are 114 and 116 for abacavir, 62, 95 and 156 for carbamazepine. Allele comparisons suggest that residues 95–97 may be of importance in allopurinol and *HLA-B*58:01* binding and 63, 114, 116 or 156 may affect the interaction between nevirapine and *HLA-B*35:01* and *HLA-B*35:05*.

clones (ABC-TCC) from *HLA-B*5701* positive individuals and showed that proteasome inhibition in APCs did not affect the TCC reactivity to ABC [44]. Furthermore, ABC-reactive TCCs were shown to be heterogenous and their ABC-specific activation was dependent on the TCR avidity and drug concentration and was proportional to the level of *HLA-B*5701* molecules expressed on APCs, which is in keeping with rapid and noncovalent interactions between ABC and *HLA-B*5701* [44]. The effect of ABC concentration has been further investigated by others and shown to enhance binding of some self-peptides to *HLA-B*5701* in a dose-dependent manner [24,26]. Furthermore, investigation of peptides eluted from ABC-treated or -untreated *HLA-B*5701* B cells show variation in peptide sequence [24–26]. Novel drug-induced peptides lacked typical carboxyl (C) terminal aromatic hydrophobic amino acids characteristic of the *HLA-B*5701* peptide motif such as phenylalanine and tyrosine and instead contained predominantly hydrophobic aliphatic amino acids such as isoleucine, leucine or valine residues [24–26]. These differences can be explained by the binding of ABC within the floor of the *HLA-B*5701* F pocket peptide-binding groove, which results in altered specificity due to a change in the shape and chemistry of the antigen-binding cleft [24–26]. Two groups were able to resolve the crystal structure of the ABC–MHC–peptide complex based on an endogenous [24] and synthetic peptide [26], respectively (FIGURE 1). Taken together, these new studies support the ‘altered repertoire model’ that ABC can alter the repertoire of self-peptides presented to T cells and result in an immune response akin to an allo-reactive T-cell response. Indeed, these specific self-peptides presented only in the presence of ABC are recognized by T cells of hypersensitive patients [26].

■ CBZ

CBZ is an aromatic amine anticonvulsant associated with DIHS/DRESS that usually occurs 2–8 weeks following drug initiation [45]. These reactions present as fever, rash, internal organ development and lymphadenopathy. It has been traditionally thought that pathophysiology of this syndrome may be mediated through metabolism of the drug to its arene oxide metabolites, resulting in direct cellular toxicity [45]. In addition, CBZ also frequently causes cutaneous adverse drug reactions (cADRs), including a mild-to-moderate nonspecific exanthema without fever or internal organ involvement sometimes referred

to as maculopapular eruption, as well as the life-threatening diseases SJS and TEN. Although rare in Europeans, CBZ-induced SJS/TEN occurs with a prevalence of one to six in 10,000 individuals and occurs with an almost tenfold higher frequency in Han Chinese and other southeast Asian populations. Several HLA alleles have been associated with CBZ-related DHS in distinct ethnic groups, with the two most frequently observed being *HLA-B*1502* and, more recently, *HLA-B*3101*. The first report of a strong HLA association with CBZ-induced hypersensitivity was between the *HLA-B*1502* allele and SJS/TEN in Han Chinese populations where these reactions are most prevalent [46,47]. Since the initial studies, the association has been replicated several times in the Chinese population [48–51] and has also been observed in individuals of Thai, Malaysian and Indian ethnicities [52–55]. A study by Lonjou *et al.* examined the *HLA-B*1502* allele in 12 European patients with CBZ-induced SJS/TEN. Of these 12, four carried the *HLA-B*1502* and had Asian ancestry and the other eight did not carry the allele [56]. As this study would indicate, the *HLA-B*1502* allele has an extremely low frequency in European populations and its expression is essentially restricted to populations with ancestries derived from southeast Asia, India and Mexico [57,201].

The *HLA-A*3101* allele was first reported in a Han Chinese population associated with CBZ-induced maculopapular eruption/DIHS/DRESS/HSS together with SNPs from the motilin gene (rs2894342) located terminal to MHC Class II genes [47]. More recently, the *HLA-A*3101* allele has also been linked to CBZ-induced ADR (including SJS/TEN and DIHS) in Japanese and Caucasian populations [58,59]. A recent genome-wide association study (GWAS) in the Japanese examined CBZ ADRs including SJS, TEN and DIHS/DRESS/HSS. The most significant SNP identified was rs1633021 ($p = 1.18 \times 10^{-13}$), located within a genome block in linkage disequilibrium (LD) with the *HLA-A* locus. Subsequent examination of *HLA-A* found that *HLA-A*3101* was present in 60.7% of the patients with CBZ-induced cADRs, but in only 12.5% of the CBZ-tolerant controls ($p = 3.64 \times 10^{-15}$), implying that this allele has 60.7% sensitivity and 87.5% specificity when applied as a risk predictor for CBZ-induced cADRs in this population [60]. Similarly, in Koreans, the *HLA-A*3101* allele has been associated with CBZ-induced DIHS/DRESS/HSS and SCAR [61].

The *HLA-A*3101* allele, which is present in approximately 7% of Han Chinese, 7–12% of

Japanese and 5% of Koreans, also has a reasonably high prevalence in northern European populations of 2–5% [201] and may similarly be expected to be linked to CBZ HSRs in this population. Recently, this has been shown to be the case. Genome-wide analysis revealed that the SNP rs1061235, a marker for *HLA-A*3101* in people of European descent, was most strongly associated with the CBZ HSRs and this was confirmed with sequence-based HLA typing. The presence of *HLA-A*3101* had a sensitivity of 26% and a specificity of 96% as a predictor of CBZ-associated hypersensitivity [59].

An extension of the *HLA-B*1502* and *-A*3101* associations with CBZ-induced SJS/TEN have examined the association with other aromatic amine anticonvulsants including oxcarbazepine, phenytoin and lamotrigine. As expected, oxcarbazepine- and phenytoin-induced SJS has been associated with the *HLA-B*1502* allele in the Han Chinese [48,62–65]. Studies into lamotrigine associated DHS however, have produced conflicting results. Several studies could not show a link between lamotrigine-induced SJS and the carriage of *HLA-B*1502* in the Han Chinese [66,67]. Others, which have examined oxcarbazepine-, phenytoin- and lamotrigine-induced SJS/TEN together, have reported expression of *HLA-B*1502* in 33% of lamotrigine SJS cases, yet the reported differences from controls did not reach statistical significance [64]. Similarly, in Europeans, no single major HLA-related genetic risk factor has been identified for lamotrigine-induced SCARs [68], although suggestive evidence was obtained for *HLA-B*5801*, *HLA-A*6801*, *HLA-Cw*0718*, *HLA-DQB1*0609* and *HLA-DRB1*1301*, with significantly higher frequencies in the cases compared with the treated controls in one study [69]. Interestingly, the *HLA-B*5801* genotype has also been observed in a Chinese case of lamotrigine-induced HSR, but, given the prevalence of this allele in the Chinese population, further investigation is needed [70]. Taken together, these studies suggest that the aromatic anticonvulsants causing SJS/TEN in *HLA-B*1502* carriers may act on a similar pathogenetic mechanism, although other genetic/nongenetic factor(s) may also contribute to the development of the disease. *HLA-B*1502* carriers should avoid CBZ, oxcarbazepine and phenytoin, and caution should also be exercised for lamotrigine.

Although strong associations of *HLA-B*1502* with CBZ-induced SJS/TEN have been found in the Han Chinese and other Asian populations, these results have not been reproduced in the

Japanese. However, SJS patients carrying *HLA-B*1508*, *HLA-B*1511* or *HLA-B*1521*, which are members of the *HLA-B*75* serotype along with *HLA-B*1502*, were detected in studies in India and Thailand [53,54]. Further support for risks associated with the *HLA-B*75* group has been produced by a study in Korean patients, which found *B*1511* was much higher in those with CBZ–SJS than in the CBZ-tolerant control group ($p = 0.011$) [61]. A case of *HLA-B*1518* in association with oxcarbazepine-induced SJS has also been reported in a Taiwanese patient [65]. Similarly, *HLA-B*1511* has been reported as a risk factor for CBZ-induced SJS/TEN in the Japanese [71]. Another study that examined CBZ-induced cADRs in the Japanese have also reported a higher relative risk for those carrying the *HLA-B*1518* allele, as well as *HLA-B*5901* and *HLA-C*0704* [72]. In addition, the haplotype (*HLA-A*2402–B*5901–C*0102*) also had a higher relative risk for severe cADRs cases, suggesting *HLA-B*5901* may be a candidate marker for CBZ-induced SJS in the Japanese population [72].

Despite strong genetic predispositions, most *HLA-B*1502* or *HLA-A*3101* carriers are in fact able to tolerate CBZ. Ko *et al.* examined the TCR repertoire in CD8⁺ T cells derived from patients with CBZ-induced SJS/TEN in *HLA-B*1502*-positive patients to identify other factors that are involved in the T-cell-mediated immune reactions to CBZ-induced SJS/TEN [73]. After isolating and culturing CBZ-specific T cells from patients, drug stimulation was found to expand the cell population *in vitro* and activate granulysin release. A dominant T-cell clone, VB-11-ISGSY, was present in blister cells and PBMCs of several patients with *HLA-B*1502* associated CBZ-induced SJS/TEN at different disease stages and most highly expressed during the active phase. This clonotype was detected in 84% of patients with SJS/TEN, absent in all CBZ-tolerant patients (including two *HLA-B*1502* patients) and detected in 14% of healthy controls. CBZ-specific cytotoxicity could be primed *in vitro* in PBMCs from healthy individuals who carried *HLA-B*1502* and VB-11-ISGSY and then blocked by anti-TCR-Vb-11 antibodies. Furthermore, both a VB-11-ISGSY clone and specific VB-11-ISGSY transfectants displayed cytotoxicity against *HLA-B*1502* positive APCs in the presence of CBZ [73]. This study shows that the TCR usage provides important clues to the pathogenic mechanism of SJS/TEN caused by CBZ, and that the available TCR repertoire of an individual, together with the HLA

genotype will determine whether they will elicit an immune response to the drug. However, it is important to note that the identified drug-specific clonotypes were not present in all of the CBZ HSR patients and further studies are required to examine the role of whole drug-specific TCR clonotypes in the pathogenesis of hypersensitivity reactions.

Further insight into the interaction between CBZ and *HLA-B*1502* (and other members of the B75 serotype) has been provided by another recent study with findings supporting either the p-i or altered peptide model [21,74]. The study showed that CBZ cocultured with SJS/TEN patient PBMCs stimulated a specific population of cytotoxic T lymphocytes (CTLs) that exhibited cytotoxicity against B lymphoblastoid cell lines or keratinocyte transfectants expressing the *HLA-B*1502* allele and this could be blocked by anti-*HLA-B* antibodies. The study showed that endogenous peptide-loaded *HLA-B*1502* molecules presented CBZ to CTLs without the involvement of intracellular drug metabolism or antigen processing, yet endogenous peptide binding was required to stabilize the *HLA* Class I complex on the cell surface. Furthermore, CBZ binding was shown to be specific to members of the *HLA-B75* serotype and modifications of the ring structure of CBZ altered *HLA-B*1502* binding and CTL response. Finally, site-directed mutagenesis was used to show that the residues (Asn63, Ile95 and Leu156) in the peptide-binding groove of *HLA-B*1502* were involved in CBZ presentation and CTL activation. In particular, Asn63 shared by members of the B75 family was the key residue (FIGURE 2). Supporting this, computational modelling showed that CBZ compounds were preferentially bound in the B pocket and consistently observed in the binding groove near Arg62 [21]. This suggests that CBZ preferentially binds to specific residues within host *HLA* molecules without a requirement for drug metabolism. These findings have been supported by another study that has additionally shown that CBZ binding produces alterations in the repertoire of presented self-peptides in *HLA-B*1502* positive individuals [24]. The particular alleles associated with each drug appear to be dependent on noncovalent steric interactions between the *HLA* peptide-binding groove and the chemical structure of the drug in question. Further studies in other well-characterized *HLA*-associated drug HSR syndromes will illustrate whether this is a general mechanism in the pathogenesis of drug HSR.

■ NVP

NVP is a non-nucleoside reverse transcriptase inhibitor that noncompetitively inhibits reverse transcriptase with known efficacy in combination with other drugs for the treatment of HIV infection. Approximately 5% of patients initiating NVP may experience a HSR within the first 6 weeks that is characterized by a combination of rash, fever and/or hepatitis [75], and can be fatal in rare cases. Most NVP HSR occurs within 12–21 days after initiation of treatment and is more rapid and severe with NVP rechallenge [75,76]. In addition, lower pretreatment CD4⁺ T-cell counts are protective against the development of rash-associated hepatitis reactions, which are more severe and frequent in non-HIV-infected individuals receiving NVP treatment [77]. Other known associations with the development of NVP HSR include prolonged exposure to any antiretroviral therapy, hepatitis B virus or C coinfection and ALT or AST results at baseline [78–83]. As with CBZ, NVP HSR has been associated with different *HLA* alleles across different population groups, suggesting several *HLA*-mediated avenues for pathogenesis of the HSRs. However, the NVP case is somewhat more complex, as associations appear to be phenotype specific and involve both Class I and Class II *HLA* alleles.

A CD4⁺ T-cell dependent MHC Class II-restricted immune response directed against NVP or its metabolites was first reported as the association of hepatic symptoms with a combination of CD4⁺ T cells $\geq 25\%$ and *HLA-DRB1*0101* in the Western Australian population [84], and has since been observed in hepatic and cutaneous reactions in other Caucasian populations [85,86]. In addition, involvement of an MHC Class I response has also been implicated in NVP HSRs. The *HLA-Cw8-HLA-B*1402* haplotype has been shown to be associated with NVP HSR in Sardinians [87] and *HLA-Cw8* is also implicated in the Japanese [88]. Cutaneous specific adverse events due to NVP treatment are associated with *HLA-B*3505* and *HLA-Cw*04* in African-Americans and the Han Chinese [86,89], and these alleles are also associated with NVP-induced rash in Thai populations [86,90–92]. The varied Class I and Class II *HLA* associations with NVP rash and DIHS/DRESS across different populations suggest that varying genetic, immunological and metabolic pathways may lead to the development of these syndromes and, in particular, differences in drug metabolism that are ethnicity specific may trigger Class I-restricted CD8⁺-mediated immune

responses or Class II restricted CD4⁺-mediated immune responses in the presence of the relevant Class I and II MHC alleles, respectively.

To further characterize the subphenotypes of NVP HSR, our group recently updated and reanalyzed cases from the Western Australian population (TABLE 1) [84,93].

Two studies have recently implicated genes outside of the MHC as additional factors that are associated with the development of particular NVP HSR phenotypes. In a GWAS examining NVP-induced rash in Thai patients, two non-coding SNPs (rs1265112 and rs746647) within *CCHCR1* were identified that are in complete LD with nonsynonymous SNP rs1576, which has been associated with psoriasis [91]. The *CCHCR1* gene regulates keratinocyte proliferation and regulation of skin steroid metabolism [94,95]. In addition, the study by Yuan *et al.* found that in African-Americans, the risk for cutaneous adverse events was highest for individuals carrying both *HLA-Cw*04* and *CYP2B6* 516TT [86]. *CYP2B6* is the main isoenzyme involved in the major metabolic pathway 8-hydroxylation (and to a lesser extent in 7-hydroxylation) and is associated with plasma concentrations of NVP [96]. *CYP2B6* 516TT is a marker for the slow metabolizing haplotypes containing alleles *CYP2B6**6, *7, *9 and *13. These alleles result in a pronounced decrease in *CYP2B6* expression and activity. Therefore, in addition to the immune mechanisms required to elicit a response to NVP, genes within local tissues, such as *CCHCR1* in the skin, as well as other systemic factors, such as metabolism by *CYP2B6*, and their allele frequencies in various populations, will all impact on the development on NVP HSR in a phenotype-specific manner.

More recent work suggests that combinations of NVP-reactive Class I-restricted CD8⁺ T cells and Class II-restricted CD4⁺ T cells are important in explaining the pathophysiology of NVP HSR [93].

■ Allopurinol

Allopurinol is a xanthine oxidase inhibitor, used to prevent the common disorders gout and hyperuricemia, which may cause DIHS/DRESS and SJS/TEN. The first report of the HLA-associated haplotype *AW33-BW58* with SCARs was from a serological study in southern Chinese patients experiencing skin eruptions (TABLE 1 & BOX 1) [97]. This was followed by a case-control extensive study in the Han Chinese population that examined 823 SNPs in genes related to drug metabolism and immune response. Strong associations

were revealed within the MHC, and subsequent HLA typing determined that the *HLA-B*5801* allele was present in 100% of patients with allopurinol SCAR, but only 15% of tolerant patients [98]. As with the CBZ-associated HSRs, since these initial observations in the Han Chinese, the same alleles have been implicated in other ethnic groups. The first report in the Japanese was based on the detection of *HLA-B*58* in three case studies of a SJS, DIHS/DRESS and TEN patient, respectively [99], and this *HLA-B*5801* association was later confirmed in a case-control study of 58 SJS/TEN patients from across Japan [100]. The Thai population also has a high frequency of *HLA-B*5801*, and 100% of allopurinol-induced SJS/TEN patients were found to carry the allele, in contrast to only 12.96% of the control patients in one study [101]. Similarly, recently in Koreans a strong positive association of *HLA-B*5801* has been reported, together with a negative association of *HLA-A*0201* with the development of allopurinol-induced SCARs [102,103].

In Caucasians, the association of allopurinol induced SCARs and *HLA-B*5801* has been lower than in the Asian populations. This may reflect differences in the carriage rate of *HLA-B*5801*, which, in the Han Chinese population, is about 15% compared with less than 6% in Caucasians. The first study in Europeans found that 55% of patients with European ancestry and SJS/TEN carried *HLA-B*5801* and concluded that *HLA-B*5801* is neither sufficient nor necessary to explain the disease [104]. Similarly, in an Australian study, all patients who carried *HLA-B*5801* and had allopurinol-induced SJS/TEN were from a southeast Asian background [105]. This is in marked contrast to the high percentages reported in Han Chinese, Thai and Korean populations [99,101,103].

Two recent GWAS studies that have examined both European and Japanese cases of SJS/TEN have confirmed the association with *HLA-B*5801* and concluded that all identified SNPs that are associated with the disease are closely linked to HLA-B and HLA-C [106,107]. In the European cohort, 424 European cases of SJS/TEN included 57 allopurinol patients and, from a subset of HLA typed patients, 11 carried *HLA-B*58* and all had allopurinol-induced disease [106]. The most significant SNP identified was rs9469003, located 85 kb upstream of the *HLA-B* locus; however, five other SNPs located 250 kb telomeric from rs9469003 were also significant. Taking the six SNPs into account, the haplotype CACGAC was much

stronger in the subgroup of patients with an allopurinol-induced disease and the associated haplotype is in LD with the *HLA-B*5801* allele [106]. Interestingly, the region spanned by the haplotype SNPs encoded several genes, including *CCHCR1* and the *PSORSIC1* (SEEK1) gene known to be in LD with *HLA-C*. Both *CCHCR1* and *HLA-C* have been associated with psoriasis and a recent study has identified haplotypes from SNPs within these genes that are associated with the disease [108]. Similarly in the Japanese, in a GWAS that examined SJS/TEN, SNPs within or very near the genes *PSORSIC1* and *CCHCR1* (rs9267445 and rs9263726) were significantly associated with allopurinol-induced SJS/TEN and these were in almost complete LD with *HLA-B*5801* [107]. Additional significant SNPs were also identified in *BAT1* (rs2734583) and *HCP5* (rs3099844). *BAT1* has a role in the regulation of TNF- α and has been associated with rheumatoid arthritis [109,110], while *HCP5* is in strong LD with *HLA-B*5701* has been linked to HIV progression and ABC HSR [111–114]. It is intriguing that in GWAS of both NVP- and allopurinol-induced adverse skin reactions the same susceptibility genes have been identified in unrelated populations and that they are also associated with other inflammatory-based skin disorders [91,106,108].

A recent meta-analysis has looked at all studies from databases including MEDLINE, Pre-MEDLINE, Cochrane Library, EMBASE, International Pharmaceutical Abstracts (IPA), CINAHL, PsychInfo, the WHO International, Clinical Trial Registry and ClinicalTrial.gov from their inception to June 2011 to investigate the association between *HLA-B*5801* and allopurinol-induced SJS/TEN. Four studies used case-matched controls (allopurinol-tolerant patients) and five included controls from the general population. SJS/TEN cases were found to be significantly associated with *HLA-B*5801* allele in both groups of studies and subgroup analysis for Asian and non-Asian populations yielded similar findings [115]. This study suggests that the low associations seen in European populations may be due to the decreased frequency of the *HLA-B*5801* allele in combination with other as-yet-unknown factors that may predispose to allopurinol-induced SJS/TEN in these populations.

The evidence for ABC, NVP, CBZ and allopurinol ADRs strongly support MHC-dependent stimulation of both CD4⁺ and/or CD8⁺ T cells. It appears that the HLA alleles involved are specific to both the nature of the

drug and the phenotype of the DHS. Despite very strong associations between these HLA alleles and specific DHS, a high proportion of subjects carrying the susceptibility alleles are able to tolerate the drugs. The positive-predictive value is highest for ABC, where 55% of those carrying *HLA-B*5701* will develop ABC HSR. In addition, unlike the other syndromes, the 100% negative-predictive value of *HLA-B*5701* for ABC HSR has held up and there has yet to be another HLA allele associated with ABC HSR. From work to date, it is clear that other mechanisms must play a role in the development of these DHS and that these may be population specific and allele frequency dependent, or their role may be restricted to particular disease phenotypes, like the HLA alleles. For example, early evidence from the GWAS in NVP- and allopurinol-induced SCARs indicate that other genes controlling keratinocyte proliferation may contribute to the development of these cases [91,106,108], while the CBZ story focuses on TCR clonotype selection and availability [73].

HLA pharmacogenetic screening in clinical practice

Regardless of the underlying mechanism, a large number of hurdles need to be overcome to translate an HLA association into clinical practice [116,117]. The FDA has recently recommended genetic testing before starting ABC and CBZ therapies. Successful implementation of pharmacogenetic screening requires that a range of criteria be adequately addressed and these examples can be used to illustrate the requirements that must be met when considering a HLA-based screening program for a particular drug. Successful clinical translation requires evidence of the test's predictive value and generalizability, accurate clinical diagnostic criteria for the drug DHS, widespread availability and ease of adapting laboratory tests, and consideration for pharmaceutical factors such as alternative treatments to the drug in question that may provide improved cost-effectiveness, safety or efficacy [116,117].

Although many HLA DHS associations are now reported, as in the case for ABC or CBZ, the genetic association must be established in a large population with a diverse genetic background, and, crucially from a drug-safety standpoint, negative-predictive value of the test must be 100%. This data will provide an indication of the cases of hypersensitivity that would be prevented compared with the number of individuals inappropriately denied the drug treatment. In

some cases of HLA-associated DHS, however, the target population may be more selective. The FDA's recommendation for *HLA-B*1502* testing is restricted to all Asians beginning CBZ therapy given the extremely low prevalence of *HLA-B*1502* in Caucasian populations [57]. The situation is less clear for ABC, where generalizability of *HLA-B*5701* for ABC HSR occurs across populations. However, the ancestry of patients is often not easily discernible based on their phenotype, and global population admixture will mean that the carriage of *HLA-B*5701* is simply a marker of Caucasian admixture and carriage of *HLA-B*1502* a marker of Asian ancestry. Experience with ABC usage in countries with a low carriage rate of *HLA-B*5701* has been limited [36,118,119,201]. In addition, since ABC may be used in combination with other drugs that cause DHS, such as NVP, particularly in these countries, the ability to screen may have the benefit of making the decision to continue ABC in the face of a likely NVP HSR more feasible. Ultimately, the decision to screen needs to be made on the ground by clinicians weighing up the potential cost-benefit relationship as determined by prevalence of diagnosed DHS, resource constraints and availability of alternate safe and efficacious medications that do not require genetic screening.

Since the implementation of *HLA-B*5701* screening prior to ABC treatment and its endorsement in international HIV treatment guidelines [202], reports from several different countries have validated and supported the test. *HLA-B*5701* genotyping has demonstrated high sensitivity, specificity and positive-predictive values in Australia, Canada, Germany, France and Poland among others [34,116,120–126]. Therefore, cost-effectiveness has clearly been demonstrated in predominantly Caucasian populations, although *HLA-B*5701* screening may be less cost effective in diverse ethnic groups with lower rates of ABC HSR [38,39].

The avoidance of CBZ for *HLA-B*1502* carriers was first recommended by the FDA in December 2007. This was later updated to also warn against using phenytoin and fosphenytoin as alternatives for CBZ patients who test positive for *HLA-B*1502* [127]. In support of this recommendation, a retrospective study has demonstrated that the cost of *HLA-B*1502* screening is less than SJS treatment in Thailand [127]. Similarly, in a multicenter single-armed prospective study from Taiwan, 4877 candidate subjects who were CBZ naive were genotyped for *HLA-B*1502* and 7.7% who tested positive

were advised not to take CBZ. SJS/TEN did not develop in any of the *HLA-B*1502*-negative subjects receiving CBZ, while the historical incidence (0.23%) would have translated into approximately ten cases among the study subjects [128]. The implications for potential cross-reactivity with other aromatic amine anticonvulsants was not clear from this large study; however, without further knowledge of factors driving the positive-predictive value of CBZ-associated SJS/TEN in this population, the collective clinical and basic science of CBZ-induced SJS/TEN suggest it is prudent for all patients known to carry *HLA-B*1502* to avoid phenytoin and oxcarbazepine [129].

A key to the successful global implementation of HLA screening for ABC and CBZ is the availability and adaptability of cost-effective, user-friendly testing methodologies. When considering the laboratory designation of HLA typing, several alternative approaches can successfully be adopted. In the case of *HLA-B*5701* for example, simple PCR methods including simple sequence-specific (SSP)-PCR, sequence-based typing or real-time PCR melt curve analysis are all applicable [37,130–133]. Sequence-based typing or allele-specific PCR are the most definitive means of identifying the presence of *HLA-B*5701*; however, due to greater ease of use some clinical laboratories choose to perform SNP testing over allele-specific PCR. This method involves SNP typing of rs2395029, a SNP located in the nearby HLA complex P5 gene (*HCP5*) approximately 100 kb away from *HLA-B* and has been shown to significantly correlate with the presence of *HLA-B*5701* in Caucasians [111,134] and Hispanics [135]. However, it is currently known that *HLA-B*5701* is necessary for the development of ABC HSR and therefore caution should be used when using *HCP5* rs2395029 as a screening test as there is strong but incomplete LD between *HLA-B*5701* and this *HCP5* SNP leading to a lower positive-predictive value and also a less than 100% negative-predictive value when compared to *HLA-B*5701* specific tests [136]. A similar test has been developed for screening *HLA-B*5801* before initiating allopurinol, in which a PCR-RFLP assay has been successfully used for the genotyping of rs9263726 in the (*PSORS1C1*) gene, in LD with *HLA-B*5801* [137]. Although this is validated in the Japanese, the linkage analysis is not confirmed in other populations.

Such molecular methods may be preceded by simple monoclonal antibody screening (a B17 antibody would screen for both *HLA-B*57* and

*HLA-B*58*) for a more rapid ascertainment of low-risk patients who can begin immediate therapy and do not require further testing [132]. For *HLA-B*1502* screening, faster and cheaper approaches to conventional PCR and sequencing methods have been suggested to make the screening more suitable for an outpatient setting. A 1-h loop-mediated isothermal amplification procedure-based DNA amplification technique, and 4 h PCR test kit have been developed in response [127]. These methods are readily adaptable for determining other HLA types in most molecular biology laboratories. Such adaptability to allele designation testing is critical to provide the ability to rapidly achieve global distribution and commercialization of the allele-specific test and accompanying quality assurance programs.

Unlike ABC and CBZ, not all DHS with identified HLA associations will be directly amenable to pharmacogenetic testing. The NVP example demonstrates that the complexity of both hypersensitivity phenotypes and Class I and II associations across individuals of varied ancestry may make implementation of HLA screening challenging in most settings. Patch testing was a very useful research tool to improve the specificity of ABC HSR diagnosis by identifying those with true immunologically mediated ABC HSR and provide compelling clinical trial evidence that the negative-predictive value of *HLA-B*5701* testing for immunologically mediated patch test-positive ABC HSR was 100%. [31,34,138]. ABC patch testing is a useful research tool, however, it has a diagnostic sensitivity of 87% and should not be used as a standalone test to de-label patients with suspected ABC HSR. Phenotype standardization of various HSR syndromes with the goal to facilitating adequate and accurate patient recruitment in order to advance research in pharmacogenomic, immunological, mechanistic and epidemiological studies has recently been proposed by an international drug-induced skin injury working group and will prove to be important in the international collaboration of multiple groups to define pharmacogenomics associations of low prevalence, but life-threatening diseases such as SJS/TEN [8].

Conclusion & future perspective

Many more HLA-associated ADRs will be elucidated in the upcoming years and, as in the example of ABC, CBZ and other drugs, these will facilitate our understanding of biochemical and structural relationships between drugs and HLA and the functional consequences of these

interactions. Indeed, with a heightened understanding of HLA drug interactions, one could envision the next stage to be preclinical high-throughput 'pharmacogenomics' screening processes using a combination of *in silico* and biological approaches to inform drug design and screen out drugs at high risk for causing clinically significant immunologically mediated drug reactions based on their interaction with HLA and demonstrable functional consequences. The ABC and CBZ models strongly support a specific allele-restricted response, mediated by CD8⁺ T lymphocytes [13,21,73], whereby ABC and CBZ noncovalently bind to particular residues within the binding pockets of HLA molecules. Other examples of Class I-associated DHS, such as allopurinol, may potentially be further characterized by adopting similar approaches. The NVP example also illustrates the importance of both MHC Class I and II and the role of CD8⁺ and CD4⁺ T-cell interactions.

The history of ABC HSR false-positive clinical diagnosis and the role of ABC patch testing and the complicated NVP HSR phenotypes highlight the importance of precise clinical diagnosis and phenotypic characterization in the consideration of patient cohorts for genetic investigation of the DHS. The true heterogeneity seen in the clinic will be influenced by cofactors such as individual medical history, coadministered drugs, underlying diseases or autoimmune disorders, viral infections and regulatory T-cell population/function, all of which have the potential to influence association studies. Early GWAS with NVP and allopurinol-associated HSR with rash have uncovered little outside of the MHC [91,106,107]. Extended haplotype analysis in these cases may provide some insight into the true variation observed in the clinical setting. Given the dominant role of HLA in DHS it has proven best to characterize HLA associations using ancestral haplotype mapping before looking at associations outside of the MHC by GWAS or other techniques. Others have shown that, in the case of DILI, nonimmune mechanisms may play a role and hence genes of drug metabolism may also be significant [86].

Several international consortia have been established to undertake GWAS across various populations, considering specifically characterized drug DHS phenotypes. Examples include the International Serious Adverse Event Consortium (iSAEC; International Consortium on Hypersensitivity; Drug-induced Liver Injury) [203] and the RegiSCAR [204] projects, which are

working with academic collaborators to collect well defined cases of drug-induced skin and hypersensitivity reactions. These consortia are successfully establishing a scientific network of experts and establishing a multinational registry of biological samples from the various DHS with the ultimate goal to identify DNA variants useful in predicting the risk of drug-related serious adverse events. The work of these groups, together with continued dedicated laboratory investigations with well-characterized drug DHS models, will greatly enhance our understanding of the pathomechanisms driving DHS

and inform drug design and development in the future.

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Executive summary

Drug hypersensitivity syndromes & models for their immunopathogenesis

- Different phenotypes of drug hypersensitivity syndromes including eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome and Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) have unique characteristics, although they are often classified collectively as severe cutaneous adverse reactions.
- Knowledge of the immunopathogenetic basis of these syndromes has become more highly developed with different hypotheses that are not necessarily mutually exclusive to a particular drug:
 - The drug or a reactive metabolite of the drug may covalently bind to and modify an endogenous peptide (hapten model) leading to immune recognition of a neoantigen.
 - Certain drugs may directly activate T cells through noncovalent interaction between the drug and HLA and/or the T-cell receptor (p-i model).
 - Drug may noncovalently bind and occupy anchor sites within the HLA antigen-binding cleft and alter the repertoire of endogenous peptides that can be bound and presented to T cells (altered peptide repertoire model). There is currently evidence that this model is important in the pathogenesis of abacavir hypersensitivity and carbamazepine (CBZ)-induced SJS/TEN.

HLA-associated drug hypersensitivity

- Recent associations between HLA and drug hypersensitivity syndromes have underscored that these are often phenotype specific (e.g., *HLA-B*1502* associated with CBZ-induced SJS/TEN but not CBZ-induced DRESS).
- A common theme emerging is specific regions within HLA-B that have been associated with many of these syndromes.
- Defining an association between a specific HLA and drug hypersensitivity syndromes (DHS) can be used as the platform to gain further insights into the immunopathogenesis of these syndromes.
- International consortia and collaborations will facilitate the discovery of a number of new HLA and pharmacogenetic associations with less-common DHS.

Pharmacogenetic screening in clinical practice & future perspective

- Delineation of associations between HLA and DHS has large-scale implications for defining the immunopathogenetic basis for these diseases.
- The feasibility of translating HLA testing into routine care to prevent a specific DHS will depend on a number of characteristics of the test, the drug, the drug toxicity, the clinical and academic environment, supporting evidence and appropriate clinical and laboratory support. The translation of *HLA-B*5701* and abacavir hypersensitivity from discovery to a guideline-based test used routinely in HIV clinical practice in the developed world is a notable example.
- Increasing knowledge with regards to HLA associations, drug–HLA interactions (altered peptide model) and the immunopathogenetic basis of DHS will pave the way for the development of preclinical pharmacogenomic screening strategies that would both inform drug design and lead to safer and cost-effective drug design and development.

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