

# Drug hypersensitivity: Pharmacogenetics and clinical syndromes

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Severe cutaneous adverse reactions include syndromes such as drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS) and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN). An important advance has been the discovery of associations between HLA alleles and many of these syndromes, including abacavir-associated hypersensitivity reaction, allopurinol-associated DRESS/DIHS and SJS/TEN, and SJS/TEN associated with aromatic amine anticonvulsants. These HLA associations have created the promise for prevention through screening and have additionally shed further light on the immunopathogenesis of severe cutaneous adverse reactions. The rollout of HLA-B\*5701 into routine clinical practice as a genetic screening test to prevent abacavir hypersensitivity provides a translational roadmap for other drugs. Numerous hurdles exist in the widespread translation of several other drugs, such as carbamazepine, in which the positive predictive value of HLA-B\*1502 is low and the negative predictive value of HLA-B\*1502 for SJS/TEN might not be 100% in all ethnic

groups. International collaborative consortia have been formed with the goal of developing phenotypic standardization and undertaking HLA and genome-wide analyses in diverse populations with these syndromes. (*J Allergy Clin Immunol* 2011;127:S60-6.)

**Key words:** Drug hypersensitivity, drug reaction with eosinophilia and systemic symptoms, drug-induced hypersensitivity syndrome, Stevens-Johnson syndrome/toxic epidermal necrolysis, pharmacogenetics, severe cutaneous adverse reaction, abacavir, nevirapine, carbamazepine, allopurinol

Drug hypersensitivity remains an important clinical issue. It consists of a variety of phenotypes, mainly the cutaneous adverse reactions that range from milder skin reactions (eg, exanthem, urticaria, and angioedema) to severe cutaneous adverse reactions (SCARs). SCARs are life-threatening, including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS). An updated description of the clinical syndromes and pharmacogenetics of these entities as discussed during the recent 4th Drug Hypersensitivity Meeting 2010 in Rome, Italy, is provided below.

## PHARMACOGENETICS OF DRUG HYPERSENSITIVITY

Associations between HLA alleles and specific drug hypersensitivity syndromes, such as abacavir hypersensitivity, have been paradigm shifting in heralding the widespread use of a pharmacogenetic test in clinical practice to prevent the development of a specific life-threatening drug toxicity. More recently, HLA associations between DRESS/DIHS and SJS/TEN have been described (Table I).<sup>1-20</sup> Identifying the true phenotypic drug hypersensitivity entity with specificity has proved to be key to identifying the pharmacogenetic markers associated with these syndromes. In the case of abacavir, this was achieved by using the skin patch test, which identifies patients with true immunologically mediated abacavir hypersensitivity.<sup>21-23</sup> More recent work with nevirapine suggests that the specific phenotypic components of the drug hypersensitivity reaction are important for identifying specific HLA associations.<sup>20</sup> The association between the class I major histocompatibility allele HLA-B\*5701 and abacavir hypersensitivity has also furthered our understanding of the immunopathogenesis of this and other drug reactions and has provided a roadmap from discovery to widespread implementation of a pharmacogenetic association.<sup>24</sup> Most work currently has focused on the pharmacogenetics of drug hypersensitivity syndromes and SJS/TEN of drugs such as abacavir, nevirapine, anticonvulsants, and allopurinol. Further work and international collaborations will be needed to determine the pharmacogenetic basis of other

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*Abbreviations used*

CBZ:	Carbamazepine
CTL:	Cytotoxic T lymphocyte
DIHS:	Drug-induced hypersensitivity syndrome
DRESS:	Drug reaction with eosinophilia and systemic symptoms
SCAR:	Severe cutaneous adverse reaction
SJS:	Stevens-Johnson syndrome
TEN:	Toxic epidermal necrolysis

drugs and reactions, such as IgE-mediated reactions, and other syndromes, such as acute generalized exanthematous pustulosis.

## Abacavir

The pathway from discovery of a pharmacogenetic association to widespread clinical implementation is not without significant hurdles, as illustrated by the “abacavir example.” Abacavir, an antiretroviral drug approved by the US Food and Drug Administration for use since 1998, was known to be associated with a drug hypersensitivity syndrome in approximately 8% of those starting the drug. In 2002, 2 groups independently published a strong association between HLA-B\*5701 and abacavir hypersensitivity.<sup>12,13</sup> Early doubts as to the widespread applicability of HLA-B\*5701 as a potential routine screening test to prevent abacavir hypersensitivity were raised based on an apparent low sensitivity in black and Hispanic populations, in which there is a much lower carriage rate of HLA-B\*5701.<sup>25</sup> This apparent low sensitivity was actually the result of a high rate of clinical false-positive diagnosis in these populations with a low prevalence of HLA-B\*5701, and this is highlighted in abacavir double-blind, randomized clinical trials in which up to 7% of patients not receiving abacavir had a clinical diagnosis of abacavir hypersensitivity.<sup>24</sup>

To overcome this problem of false-positive clinical diagnosis, abacavir patch testing was used as a specific test to identify true immunologically mediated abacavir hypersensitivity.<sup>21-23</sup> Two clinical trials, the Prospective, Randomized Evaluation of DNA Screening in a Clinical Trial (PREDICT-1) and Study of Hypersensitivity to Abacavir and Pharmacogenetic Evaluation (SHAPE) studies, incorporated skin patch testing into their study design as a way of identifying the true phenotype of abacavir hypersensitivity.<sup>14,15</sup> The PREDICT-1 study was the first randomized, double-blind controlled study to prospectively test the clinical utility of a pharmacogenetic test to prevent a specific toxicity. This study, which enrolled 84% white subjects, was compelling in showing a 100% negative predictive value of HLA-B\*5701 as a screening test for the prevention of abacavir hypersensitivity.<sup>14</sup> The SHAPE study was a case-control study enrolling both black and white American patients that suggested a 100% negative predictive value of HLA-B\*5701 for abacavir hypersensitivity generalizable across black and white race.<sup>15</sup> Additional evidence from observational studies from different centers suggested HLA-B\*5701 screening to be cost-effective in real clinical practice not only by eliminating true immunologically mediated abacavir hypersensitivity but also by reducing false-positive clinical diagnosis.<sup>24</sup>

The abacavir story provides a translational roadmap from the discovery of a genetic association through to implementation of a pharmacogenetic test in routine clinical care (Fig 1). In addition,

important lessons were gleaned from abacavir clinical trials that can be applied to other drugs and pharmacogenetic markers. The PREDICT-1 study illustrated that using coprimary end points, where one was sensitive and not specific (clinical diagnosis) and the other was specific and not 100% sensitive (patch testing), was a powerful tool. The validation of a simple, inexpensive, allele-specific molecular test against the gold standard of high-resolution full allelic HLA typing in the PREDICT-1 study was also crucial to the widespread implementation of cost-effective and feasible methods for HLA-B\*5701 screening.

The abacavir story also clearly illustrated that any randomized controlled trial aiming to study the clinical utility of a pharmacogenetic marker to prevent a specific toxicity must look at the dominant ethnic group. Case-control studies, such as the SHAPE study, on the other hand are most ideally used to generalize the results from the dominant ethnic group to other groups with low prevalence of the allele in question.

Finally, observational and open screening studies are useful to define the role, practical issues surrounding implementation, and benefits of genetic testing in real clinical practice and can sometimes pick out different effects, such as the decrease in false-positive clinical diagnosis in addition to decreasing the rates of true hypersensitivity in the case of abacavir. Much of the success of the implementation of HLA-B\*5701 testing in clinical practice relates to the 100% negative predictive value of this pharmacogenetic marker, as well as the high (55%) positive predictive value.<sup>14</sup> Taking into account the high rates of false-positive diagnosis, this means that only 13 subjects would need to be screened to prevent 1 case of hypersensitivity.<sup>24</sup> Although many other HLA alleles associated with specific drug-induced diseases share a 100% or close to 100% negative predictive value, the positive predictive value and the prevalence of these diseases is much lower, creating challenges from the large number that would be needed to test to prevent 1 case (Fig 2).

## Nevirapine

Nevirapine is a nonnucleoside reverse transcriptase inhibitor used in the combination treatment of patients with HIV-1 infection and is associated with a drug hypersensitivity syndrome in approximately 5% of those starting the drug and SJS/TEN in 0.3% or less of those starting the drug.<sup>24</sup> Nevirapine differs from abacavir in that distinct class I and II associations have been described in association with nevirapine-associated rash and hypersensitivity across different populations. A population-based study from Western Australia associated the MHC class II allele HLA-DRB1\*0101 with rash-associated hepatitis in those with a CD4 percentage of 25% or greater.<sup>16</sup> This clinical work has been supported by *ex vivo* studies suggesting that nevirapine hypersensitivity is a CD4 cell-dependent process.<sup>24</sup>

Another case-control study in a Thai population associated nevirapine rash and hypersensitivity with HLA-B\*3505, which was present in 17.5% of patients with HIV with nevirapine-associated rash or hypersensitivity versus 1.1% of nevirapine-tolerant control subjects and less than 1% of the general Thai population.<sup>19</sup> This same group is attempting to validate findings through a prospective, blinded randomized screening study in which subjects randomized to the HLA-B\*3505 testing arm will be excluded from nevirapine treatment, if positive.<sup>26</sup>

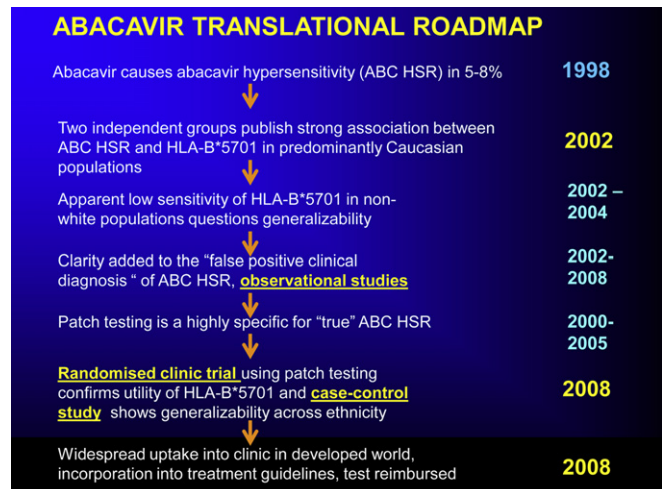
Additional studies have associated MHC class I alleles with nevirapine hypersensitivity, such as HLA-B\*1402 and HLA-Cw8,

**TABLE I.** Recent HLA associations with DIHS/DRESS and SJS/TEN

Drug toxicity syndrome/drug	Ethnicity	Allele	Reference
SJS/TEN			
Allopurinol	Han Chinese, Japanese, Thai	HLA-B*5801	1
			2
			3
			4
Carbamazepine	White subjects	HLA-B*1502	5-7
	Han Chinese, Thai		8
	Malaysians		
	Indian		
	Japanese	HLA-B*1511	9
			Ikezawa (Yokohama)
		HLA-B*5901	10
Oxcarbazepine	Han Chinese	HLA-B*1502	11
Phenytoin	Han Chinese, Thai	HLA-B*1502	6
			7
DIHS/DRESS			
Abacavir	Highest risk in Caucasians but generalizable across ethnicity	HLA-B*5701	12-15
Allopurinol	Han Chinese	HLA-B*5801	1
Nevirapine			
Rash associated hepatitis with CD4 <sup>+</sup> T cells 25% or greater	White subjects	HLA-DRB1*0101	16
DIHS/DRESS	Sardinian	HLA-Cw8-B14 haplotype	17
DIHS/DRESS	Japanese	HLA-Cw8	18
DIHS/DRESS	Thai	HLA-B*3505	19
with rash (no liver function tests done)			
DIHS/DRESS with rash	White subjects	HLA-B*3501	20

in a Sardinian population and HLA-Cw8 in a Japanese population.<sup>17,18</sup> Although familial occurrence of SJS/TEN associated with nevirapine has been described, suggesting a genetic basis, no HLA or genetic basis has currently been determined.<sup>24</sup>

An updated analysis of the Western Australia cohort by the investigative team of Phillips et al<sup>20</sup> (Perth, Australia) provides further insights into the potential pharmacogenetic basis of nevirapine hypersensitivity. In this study the original association between HLA-DRB1\*0101 and CD4 percentage of 25% or greater and nevirapine hypersensitivity with hepatitis held up; however, a new association was found between HLA-B\*3501 and nevirapine hypersensitivity with rash.<sup>20</sup> In addition, it appears that the phenotype of the drug hypersensitivity syndrome might be critical when attempting to delineate HLA associations. For instance, HLA-DRB1\*0101 was associated with rash only in the presence of hepatitis.<sup>20</sup> Specific HLA-B and HLA-DR pairings appeared to be important for the hepatitis phenotype and HLA-B for the rash phenotype. This could shed important light not only on the approach to studying the pharmacogenetics of drug hypersensitivity syndromes but also the cellular and immunopathogenetic basis of these drug-induced diseases.

**FIG 1.** Translational roadmap from discovery of HLA-B\*5701 association with abacavir hypersensitivity to widespread clinical implementation.

Various HLA-B types have been associated with SJS/TEN, drug hypersensitivity syndromes, or both, such as allopurinol and HLA-B\*5801; carbamazepine (CBZ) and HLA-B\*1502; HLA-B\*5701 and both abacavir hypersensitivity and flucloxacillin hepatotoxicity; and nevirapine hypersensitivity and HLA-B\*3505/01. It is intriguing that all of these HLA types share a similar chemistry to the F pocket of HLA-B with a serine at position 116, which might explain the propensity of haptenated peptides to bind.<sup>20</sup>

### Pharmacogenetics of other SCARs

**SCARs, clinical syndromes, and the RegiSCAR group.** Adverse drug reactions affecting the skin are frequent, and they present with a large variety of phenotypes. The term SCAR was proposed for very rare reactions that are associated with a significant morbidity and mortality, are nonpredictable (idiosyncratic and likely resulting from immunologic mechanisms), and are most often induced by drugs. A multinational collaborative research team was established in 1988 to study SCARs, bringing together dermatologists, epidemiologists, geneticists, immunologists, and pharmacologists.<sup>27,28</sup> It changed its name from the SCAR group to EuroSCAR and lately RegiSCAR when enlarging the scope of diseases of interest and aggregating new participating teams. At present, the RegiSCAR group is active in Austria, France, Germany, Italy, The Netherlands, South Africa, Taiwan, and the United Kingdom and should soon include several new European countries. It is operating as a registry collecting detailed clinical data and biological samples on 3 varieties of SCARs: (1) SJS/TEN, (2) DRESS/DIHS, and (3) acute generalized exanthematous pustulosis (AGEP).

The group defined consensus diagnostic criteria for each type of SCAR.<sup>29-31</sup> Potential cases of SCARs are detected in a large network of participating hospitals and investigated by means of direct interviews with standardized questionnaires to collect detailed information on the phenotype of the reaction (including clinical photographs and skin biopsy specimens in most cases), associated conditions, and exposure to medications. Potential cases are validated by an expert committee blinded for risk factors, including medications. Using this strict methodology, the group collected and curated detailed quality data on more than 1000 cases of SJS/TEN in Europe. A case-control analysis

DRUG	HLA Allele	HLA Carriage Rate	Prevalence of diagnosis	Negative Predictive Value	Positive Predictive Value	NNT to prevent One Case
Abacavir	B*5701	6-8% Caucasian <1% African/Asian 2.5% African American	8% (includes 3% true HSR and 2-7% false positive diagnosis)	100% for patch test confirmed	55%	13
Allopurinol	B*5801	9-11% Han Chinese 1-6% Caucasian	1/250-1/1000	100% in Han Chinese	3%	250
Carbamazepine	B*1502	10-15% Han Chinese <0.1% Caucasian	<1-6/1000	100% in Han Chinese	3%	1000
Flucloxacillin	B*5701	As for abacavir	8.5/100,000	99.99%	0.12%	13819

**FIG 2.** Number needed to test (NNT) to prevent 1 case of specific drug reaction. Numbers shown are for abacavir hypersensitivity, allopurinol-associated SJS/TEN/drug hypersensitivity, CBZ-associated SJS/TEN, and flucloxacillin-associated drug-induced liver disease. Adapted from Phillips and Mallal.<sup>24</sup>

indicated that a dozen “high-risk” medications accounted for more than one half of cases.<sup>28</sup>

During the course of these studies, it became evident that misdiagnoses were frequent and drug exposure was incompletely ascertained by reporting physicians, making it difficult to attribute causality to a particular drug. These findings point to the advantage of direct interviews with patients and relatives and structured and systematic questioning on drug use. The follow-up of a large cohort of patients with SJS/TEN has also shown that mortality and the prevalence of severe sequelae were higher than previously suspected.

These studies show that “undetermined” or “overlapping” cases of SCARs were rare if clear diagnostic criteria and a rigorous systematic approach to phenotyping was used. It also established that SJS and TEN should be considered as severity variants of a single disease distinct from erythema exsudativum multiforme majus, which has different demographic characteristics of patients, associated diseases, severity, causality, and prognosis.<sup>32</sup> The study team of Hashimoto (Ehime, Japan) presented new data to suggest that overlap can occur rarely between SJS/TEN and DRESS/DIHS. For the most part, however, SJS/TEN and DRESS are now thought to be distinct entities, although many of the same high-risk drugs have been implicated to precipitate the 2 syndromes.<sup>33</sup>

### Progress in pharmacogenetics of SCARs in Han Chinese

Although the incidences of SCARs are low, their complications and sequelae can result in death or disability in formerly healthy persons.<sup>28,34</sup> Several drugs have been withdrawn from the market because of their association with SCARs.<sup>35</sup> The culprit drugs associated with SCARs are distinct in different countries.<sup>28,34,36</sup> CBZ and allopurinol were 2 of the most common agents causing SJS and TEN in Taiwan, where Han Chinese form the largest ethnic group and make up approximately 98% of the population.<sup>1,36</sup> HLA-B\*1502 was initially found to be a genetic marker for CBZ-associated SJS/TEN in Han Chinese,<sup>5</sup> and this association has subsequently been found to be phenotype specific because the HLA-B\*1502 allele has shown no association with nonblistering cutaneous reactions, such as exanthem or DRESS. What was described as CBZ-associated maculopapular exanthem was

associated with HLA-A\*3101 in Han Chinese.<sup>37</sup> Recently, HLA-B\*1502 was found to be associated with an increased risk of SJS/TEN on exposure to aromatic antiepileptic drugs, including phenytoin, oxcarbazepine, and potentially lamotrigine, although the strength of these associations were weaker than that of CBZ.<sup>11</sup> Previous work in Han Chinese subjects also identified that the HLA-B\*5801 allele is a genetic marker for SJS/TEN/DRESS induced by allopurinol, a commonly prescribed medication for gout and hyperuricemia.<sup>1</sup> These data suggested that genetic susceptibility to drug hypersensitivity is both phenotype and drug specific.

### Progress of pharmacogenetics of SCARs in other countries

In 2006, researchers of the RegiSCAR group confirmed the same link between HLA-B\*1502 and CBZ-associated SJS/TEN in 4 European inhabitants who had Chinese ancestry. However, this association was not found in European patients with CBZ-associated SJS/TEN.<sup>4,38</sup> Man et al<sup>6</sup> enrolled Han Chinese living in Hong Kong and validated that HLA-B\*1502 was associated with SCARs induced by CBZ, phenytoin, and potentially lamotrigine. Two studies from Thailand replicated the strong association between the HLA-B\*1502 allele and patients with CBZ-associated SJS/TEN.<sup>3,7</sup> In addition, Chang et al<sup>8</sup> reported that up to 75% of patients with CBZ-associated SJS/TEN were the carriers of HLA-B\*1502 in Malaysia, a country composed of 4 races, with the majority Malay and Han Chinese showing carriage frequencies of HLA-B\*1502 of 15.7% and 5.7%, respectively. A recent study from India showed 6 (75%) of 8 patients with CBZ-associated SJS were positive for the HLA-B\*1502 allele, with an average HLA-B\*1502 carriage frequency of 2.5% in the general population.<sup>9</sup> By comparison, the same association was not found in the study populations from Japan, where the allele frequency of HLA-B\*1502 is very low (<0.1%).<sup>2,10</sup> The study team of Ikezawa (Yokohama, Japan) reported that CBZ-induced SJS/TEN in Japan was associated with HLA-B\*1511, a member of HLA-B75 type that also includes HLA-B\*1502, HLA-B\*1508, HLA-B\*1521, HLA-B\*1530, and HLA-B\*1531. These data suggested that not only HLA-B\*1502 but also the other HLA-B75 members are risk factors for CBZ-induced SJS/TEN in Asians. In addition, the association between HLA-B\*5801 and allopurinol-induced SJS/TEN/DRESS has been validated in



different populations, including Japanese, European, and Thai persons.<sup>2-4,38</sup> It is interesting to note that the strength of genetic associations that have been found relate to the prevalence of the susceptibility allele in the ethnic populations with consistent results in Southeast and South Asia, where the frequencies of the risk alleles are higher.<sup>39</sup>

The US Food and Drug Administration and regulatory agencies in some other countries have modified the drug label or product information.<sup>40</sup> For example, screening for HLA-B\*1502 before prescribing CBZ is recommended for subjects of Asian ancestry, particularly for those of Southeast Asian ancestry.

### RegiSCAR studies on pharmacogenetics and pharmacogenetics of SCARs in Europe

Since 2003, RegiSCAR has collected biological samples of patients with SCARs to allow future studies on the mechanisms and genetics of these reactions. The collection was assembled in compliance with existing regulations approved by relevant ethical committees, and all patients provided a signed informed consent form. Samples were recoded by a professional blood bank, and correlation between deidentified data on phenotype and samples was strictly protected and only accessible to the data center after formal agreement of the steering committee of RegiSCAR.

RegiSCAR initially took a candidate gene approach focusing on the usual suspects according to present knowledge or hypotheses of the physiopathology of SCAR. It is known that massive apoptosis of keratinocytes follows invasion by drug-specific cytotoxic T cells and natural killer cells and release of a variety of cytokines. Reactive metabolites might initiate the immune response, although it is also possible that the parent molecule directly interacts with the major histocompatibility complex-T-cell receptor to initiate the immune response (pharmacologic interaction of drugs with immune receptors [ie, the P-I concept]). The list of candidate genes included the genes related to the polymorphism of metabolizing enzymes, genes contributing to the regulation of immune response (including HLA region), and genes related to apoptosis (eg, death messengers and receptor and caspases).

The 2004 and 2005 publications by the Taiwanese group reported very strong associations between HLA and SCARs (HLA-B\*1502 and CBZ-related SJS/TEN and HLA-B\*5801 and allopurinol-related SCARs). This prompted RegiSCAR to look for HLA associations in European subgroups of SJS/TEN cases induced by high-risk medications. These studies<sup>38</sup> were disappointing in that an association could not be detected between CBZ-related cases in patients of European descent and HLA-B\*1502 or with another HLA allele. HLA-B\*5801 was associated with allopurinol-related SJS/TEN in European patients but only had a 55% sensitivity instead of the 100% sensitivity reported in Taiwan.<sup>4</sup> For other high-risk drugs, a few very rare alleles were found to be significantly associated, but the sensitivity of these tests were so low that it is very unlikely that any will be applied clinically.

The comparison of the European results with the findings from Taiwan has yielded interesting findings and conclusions with regard to CBZ-associated SJS and TEN, which occurs at a much lower prevalence (1/10,000) in European populations. In Europe, where the prevalence of HLA-B\*1502 is very low (<0.1%), although HLA-B\*1502 appears to be the strongest genetic risk factor for SJS/TEN, it lacks sensitivity. Other HLA alleles might

yet be implicated in the pathogenesis of CBZ-induced SJS/TEN in non-Asian populations.

The above considerations led the RegiSCAR group to collaborate with the Centre National du Genotypage (Evry, France) in a genome-wide association study with Illumina 317K chips. As presented by Mockenhaupt (Freiburg, Germany), more than 600 validated cases of SJS or TEN enrolled by RegiSCAR in 6 countries with a majority in France and Germany, adequate amounts of DNA were available in 563 cases (226 male and 337 female subjects). Several single nucleotide polymorphisms, all located in the HLA region on chromosome 6, were found to be significantly associated with *P* values of less than  $10^{-6}$ . The most significantly associated single nucleotide polymorphism was located close to the HLA-B locus. The association was much stronger in patients exposed to allopurinol than in those exposed to other drugs but remained significant after exclusion of all cases exposed to allopurinol. The involvement of genetic variants located in the HLA region in patients with SJS/TEN was therefore confirmed in European patients, especially in association with allopurinol, as previously demonstrated by HLA studies.<sup>4</sup> No other locus reached genome-wide association criteria in this study.

This sample of patients with SJS/TEN was the largest collected thus far. It is thus unlikely that in European populations any high-risk drug will have an association with a specific common allele strong enough to be useful as a predictive marker.

The probability that further studies on the genetics of SJS/TEN will find associations not detected in a European population by the RegiSCAR genome-wide association study is low unless performed in homogeneous ethnic groups with a high prevalence of reaction to a given medication.

### Functional studies and pathogenesis of SCARs in relation the pharmacogenetics

Hung (Taipei, Taiwan) proposed that the specific HLA-B allele is not only a genetic marker for SCARs but also plays functional roles in the pathogenesis of the diseases. By means of *in vitro* assay, Hung (Taipei, Taiwan) showed that HLA-B\*1502 is specific for the CBZ binding to activate cytotoxic T lymphocytes (CTLs) of patients with CBZ-related SJS/TEN. On stimulation, CTLs expressed a large amount of granulysin, which was identified as a key mediator responsible for the extensive epidermal necrolysis in patients with SJS/TEN. Chung et al<sup>41</sup> (Taipei, Taiwan) presented intriguing data to suggest that granulysin produced by the CTLs and natural killer cells causes widespread keratinocyte death, is a prognostic biomarker for SJS/TEN, and might be useful as a therapeutic target for developing new methods for the treatment of SJS/TEN. These studies of genetics, epidemiology, and immunologic mechanisms of SJS/TEN are improving our understanding of drug hypersensitivity and having a practical effect in the clinic.

### Other findings and future directions

The study team of Phillips and Mallal<sup>24</sup> (Perth, Australia) reviewed HLA-B\*5701 screening for abacavir hypersensitivity which has proved a success story that has created a translational roadmap for other drugs (Fig 1). Important elements driving this success have been cost-effective, simplified, and feasible methods for HLA-B\*5701 screening and an accompanying international quality assurance program. The generation of evidence that HLA-B\*5701 has 100% negative predictive value for abacavir hypersensitivity generalizable across race was key to the

routine clinical implementation of this test. The high positive predictive value and the low numbers of subjects needed to test to prevent 1 case of abacavir hypersensitivity have also contributed to the feasibility and cost-effectiveness of HLA-B\*5701 testing (Fig 2). The implementation of other predictive pharmacogenetic markers into clinical practice might be more challenging and population dependent. A large observational prospective study with HLA-B\*1502 screening in patients being initiated on CBZ was conducted in 25 hospitals in Taiwan and has now enrolled more than 4000 patients. This study incorporated an allele-specific PCR-based test for HLA-B\*1502 with a turnaround time of 3 days.<sup>42</sup> The study team of Chen et al<sup>42</sup> (Taipei, Taiwan) presented an updated analysis of this study, and no patients in this study had SJS/TEN compared with 8 patients in a historical control group (0.25%). Little information was gained from this study with regard to reactions associated with potentially cross-reactive aromatic amine anticonvulsants, such as phenytoin, where SJS/TEN also appears to be HLA-B\*1502 associated. Even in higher-prevalence groups such as Han Chinese, the positive predictive value of HLA-B\*1502 for CBZ-induced SJS/TEN appears to be low (Fig 2). Chen and Hung on behalf of their study team (Taipei, Taiwan) presented further evidence to suggest that specific V $\beta$ <sub>11</sub> T-cell receptor clonotypes might be necessary to evoke the T-cell responses leading to the phenotype of SJS/TEN in HLA-B\*1502-positive subjects. The US Food and Drug Administration recommendation to test for persons of Asian ancestry positive for HLA-B\*1502 before prescribing CBZ might be useful only for persons of Chinese and Southern Asian origin. Importantly, the CBZ example illustrates that unlike abacavir, the 100% negative predictive value of HLA-B\*1502 for SJS/TEN in Han Chinese and some other Asian groups appears not to be generalizable to people of European descent, as demonstrated by European cases of CBZ-associated SJS/TEN lacking HLA-B\*1502.<sup>38</sup> Since 2010, in Taiwan national health insurance has covered the expense of the genetic screening for HLA-B\*1502 in subjects initiating CBZ. In the case of allopurinol, in Europe, the EuroSCAR case-control study<sup>43</sup> showed that the majority of allopurinol-attributed cases of SJS/TEN were related to inappropriate use. Decreasing inappropriate use of allopurinol by reinforcing prescription rules might be an additional strategy to prevent SCARs related to allopurinol in European populations.

To date, most pharmacogenetic associations described have related to delayed reactions, such as drug hypersensitivity syndromes and SJS/TEN. Much less knowledge exists regarding the pharmacogenetics of IgE-mediated reactions, such as occur with  $\beta$ -lactam antimicrobials, which was reviewed in a plenary by Gueant-Rodriguez et al<sup>44</sup> (Nancy, France). For these reactions, there is the additional challenge of defining a durable phenotype because it is known that IgE reactivity can wane and disappear over the course of a lifetime. This is particularly known to occur with penicillin, for which approximately 10% of patients per year will lose skin test reactivity and the positive predictive value of testing is 50% to 60%. Specific HLA associations have not been described in association with IgE-mediated reactions, such as  $\beta$ -lactam allergy. However, genes related to atopy and IgE production, such as *IL4*, *IL4RA*, *IL13*, and *TNFA* have been examined, with specific polymorphisms of these genes associating with risk of  $\beta$ -lactam allergy as defined by clinical presentation, skin test positivity, or both in case-control studies.<sup>44-47</sup>

The future of defining the pharmacogenetic basis of the full spectrum of immunologically mediated drug reactions will rest on

international collaborative efforts to gather well-phenotyped cases for specific drugs and specific drug reactions and apply broad approaches, such as high-resolution HLA typing followed by whole-genome analysis, new technologies, or both as they become available. As reinforced by the studies teams of Mockenhaupt (Freiburg, Germany), Roujeau (Cr teil, France), and Shear (Toronto, Canada), registries such as RegiSCAR and the newly formed International Consortium on Drug Hypersensitivity are making important progress in this regard. Although an understanding of the pharmacogenetic basis for these reactions will not inevitably translate into clinically useful genetic markers for the majority of drugs, it will provide valuable insights into the immunopathogenesis of these reactions.

## REFERENCES

1. Hung SL, Chung WH, Liou LB, Chu CC, Lin M, Huang HP, et al. HLA-B\*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc Natl Acad Sci U S A* 2005;102:4134-9.
2. Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, et al. HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics* 2008;9:1617-22.
3. Tassaneeyakul W, Jantararoungtong T, Chen P, Lin PY, Tiamkao S, Khunarkornsiri U, et al. Strong association between HLA-B\*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population. *Pharmacogenet Genomics* 2009;19:704-9.
4. Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S, et al. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 2008;18:99-107.
5. Chung WH, Hung SI, Hong HS, Hsieh MS, Yang LC, Ho HC, et al. Medical genetics: a marker for Stevens-Johnson syndrome. *Nature* 2004;428:486.
6. Man CBL, Kwan P, Baum L, Yu E, Lau KM, Cheng ASH, et al. Association between HLA-B\*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 2007;48:1015-8.
7. Locharekul C, Loplumlert J, Limotai C, Korkij W, Desudchit T, Tongkobpetch S, et al. Carbamazepine and phenytoin induced Stevens-Johnson syndrome is associated with HLA-B\*1502 allele in Thai population. *Epilepsia* 2008;49:2087-91.
8. Chang C, Too C, Murad S, Hussein S. Association of HLA-B\*1502 with carbamazepine-induced toxic epidermal necrolysis and Stevens-Johnson syndrome in Malaysian populations. In: *Proceedings of the 7th Asian-Oceanian Epilepsy Congress*; 2008; Xiamen, China.
9. Mehta TY, Prajapati LM, Mittal B, Joshi CG, Sheth JJ, Patel DB, et al. Association of HLA-B\*1502 allele and carbamazepine-induced Stevens-Johnson syndrome among Indians. *Indian J Dermatol Venereol Leprol* 2009;75:579-82.
10. Ikeda H, Takahashi Y, Yamazaki E, Fujiwara T, Kaniwa N, Saito Y, et al. HLA Class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions. *Epilepsia* 2010;51:297-300.
11. Hung SI, Chung WH, Liu ZS, Chen CH, Hsieh MS, Hui RY, et al. Common risk allele in aromatic antiepileptic-drug induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese. *Pharmacogenomics* 2010;11:349-56.
12. Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, et al. Association between presence of HLA-B\*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002;359:727-32.
13. Hetherington S, Hughes AR, Mosteller M, Shortino D, Baker KL, Spreen W, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 2002;359:1121-2.
14. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, et al. HLA-B\*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008;358:568-79.
15. Saag MS, Balu R, Phillips E, Brachman P, Martorell C, Burman W, et al. High sensitivity of human leukocyte antigen-B\*5701 as a marker for immunologically confirmed abacavir hypersensitivity in white and black patients. *Clin Infect Dis* 2008;46:1111-8.
16. Martin AM, Nolan D, James I, Cameron P, Keller J, Moore C, et al. Predisposition to nevirapine hypersensitivity associated with HLA-DRB1\*0101 and abrogated by low CD4 T-cell counts. *AIDS* 2005;19:97-9.
17. Littera R, Carcassi C, Masala A, Piano P, Serra P, Ortu F, et al. HLA-dependent hypersensitivity to nevirapine in Sardinian HIV patients. *AIDS* 2006;20:1621-6.
18. Gatanaga H, Yazaki H, Tanuma J, Honda M, Genka I, Teruya K, et al. HLA-Cw8 primarily associated with hypersensitivity to nevirapine. *AIDS* 2007;21:264-5.
19. Chantarangsu S, Mushiroda T, Mahasirimongkol S, Kiertiburanakul S, Sungkanuparph S, Manosuthi W, et al. HLA-B\* 3505 allele is a strong predictor for

- nevirapine-induced skin adverse drug reactions in HIV-infected Thai patients. *Pharmacogenet Genomics* 2009;19:139-46.
20. Phillips E, Lucas M, Kean N, Lucas A, McKinnon E, Mallal S. HLA-B\*35 is associated with nevirapine hypersensitivity in the contemporary Western Australian HIV cohort study. *Eur Ann Allergy Clin Immunol* 2010;42:48.
  21. Phillips EJ, Sullivan JR, Knowles SR, Shear NH. Utility of patch testing in patients with hypersensitivity syndromes associated with abacavir. *AIDS* 2002;16:2223-5.
  22. Phillips EJ, Wong GA, Kaul R, Shahabi K, Nolan DA, Knowles SR, et al. Clinical and immunogenetic correlates of abacavir hypersensitivity. *AIDS* 2005;19:979-81.
  23. Shear NH, Milpied B, Bruynzeel DP, Phillips EJ. A review of drug patch testing and implications for HIV clinicians. *AIDS* 2008;22:999-1007.
  24. Phillips EJ, Mallal SA. Pharmacogenetics of drug hypersensitivity. *Pharmacogenomics* 2010;11:973-87.
  25. Hughes AR, Mosteller M, Bansal AT, Davies K, Haneline SA, Lai EH, et al. Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics* 2004;5:203-11.
  26. Genotype based personalized prescription of nevirapine (GENPART) NCT00986063. Available at: [www.clinicaltrials.gov](http://www.clinicaltrials.gov). Accessed June 11, 2010.
  27. Roujeau JC. Clinical heterogeneity of drug hypersensitivity. *Toxicology* 2005;209:123-9.
  28. Roujeau JC, Kelly JP, Naldi L, Rzany B, Stern R, Anderson T, et al. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N Eng J Med* 1993;333:1600-7.
  29. Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch Dermatol* 1993;129:92-6.
  30. Sidoroff A, Halevy S, Bavinck JNB, Vaillant L, Roujeau JC. Acute generalized exanthematous pustulosis (AGEP)—a clinical reaction pattern. *J Cutan Pathol* 2001;28:113-9.
  31. Kardaun SH, Sidoroff A, Valeyrie-Allanore L, Halevy S, Davidovici BB, Mockenhaupt M, et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br J Dermatol* 2007;156:609-11.
  32. Auquier-Dunant A, Mockenhaupt M, Naldi L, Correia O, Schroder W, Roujeau JC. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: Results of an international prospective study. *Arch Dermatol* 2002;138:1019-24.
  33. Kardaun SH, Sekula P, Mockenhaupt M, Chu CY, Creamer D, Sidoroff A, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): results from the RegiSCAR. *Eur Ann Allergy Clin Immunol* 2010;42:45.
  34. Mockenhaupt M. Epidemiology and causes of severe cutaneous adverse reactions to drugs. In: Pichler WJ, editor. *Drug hypersensitivity*. Basel: Karger; 2007. p. 18-31.
  35. Lasser KE, Allen PD, Woolhandler SJ, Himmelstein DU, Wolfe SM, Bor DH. Timing of new black box warnings and withdrawals for prescription medications. *JAMA* 2002;287:2215-20.
  36. Chung WH, Hung SI, Chen YT. Genetic predisposition of life-threatening antiepileptic-induced skin reactions. *Expert Opin Drug Saf* 2010;9:15-21.
  37. Hung SI, Chung WH, Jee SH, Chen WC, Chang YT, Lee WR, et al. Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet Genomics* 2006;16:297-306.
  38. Lonjou C, Thomas L, Borot N, Ledger N, deToma C, LeLouet H, et al. A marker for Stevens-Johnson syndrome: ethnicity matters. *Pharmacogenomics J* 2006;6:265-8.
  39. Dainichi T, Uchi H, Moroi Y, Furue M. Stevens-Johnson syndrome, drug-induced hypersensitivity syndrome and toxic epidermal necrolysis caused by allopurinol in patients with a common HLA allele: what causes the diversity? *Dermatology* 2007;215:86-8.
  40. Kuehn BM. FDA: epilepsy drugs may carry skin risks for Asians. *JAMA* 2008;300:2845.
  41. Chung WH, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, et al. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat Med* 2008;14:1343-50.
  42. Chen P, Shen C, Lin J, Ong C, Wu S, Tsai P, et al. A prospective study of HLA-B\*1502 genotyping in preventing carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis [abstract 305]. Presented at: 59th Annual Meeting of the American Society of Human Genetics; October 20-24, 2009; Honolulu, Hawaii.
  43. Halevy S, Ghislain PD, Mockenhaupt M, Fagot JP, Bouwes Bavinck JN, Sidoroff A, et al. Allopurinol is the most common cause of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe and Israel. *J Am Acad Dermatol* 2008;58:25-32.
  44. Gueant-Rodriguez RM, Gueant JL, Viola M, Tramoy D, Gaeta F, Romano A. Association of tumor necrosis factor-alpha-308G>A polymorphism with IgE-mediated allergy to beta-lactams in an Italian population. *Pharmacogenomics J* 2008;8:162-8.
  45. Guglielmi L, Fontaine C, Gougat C, Avinens O, Eliaou JF, Guglielmi P, Demoly P. IL-10 promoter and ILF4-Ralpha gene single nucleotide polymorphisms are associated with immediate beta-lactam allergy in atopic women. *Allergy* 2006;61:932-7.
  46. Gueant-Rodriguez RM, Romano A, Beri-Dexheimer M, Viola M, Gaeta F, Gueant JL. Gene-gene interactions of IL13 and IL4RA variants in immediate allergic reactions to beta lactam antibiotics. *Pharmacogenet Genomics* 2006;16:713-9.
  47. Qiao HL, Yang J, Zhang YW. Specific serum IgE levels and FcepsilonRI beta genetic polymorphism in patients with penicillins allergy. *Allergy* 2004;59:1326-32.