**Genetic and non-genetic factors that may predispose individuals to allergic drug reactions**

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**Abstract**

Purpose of review:

Defining predisposition to allergic drug reactions has largely focussed on HLA associations, but other genetic and non-genetic factors are also likely to be involved.

Recent findings:

Polymorphic genetic variants in cytokine genes, including IL-10, and co-signalling pathways, including CTLA4, have been associated with allergic drug reaction, but the effect size is lower than with HLA alleles and most associations have not been replicated. While TCR specificity seems to be important for CBZ-induced SJS/TEN in South East (SE) Asian patients, a distinct repertoire may not play a role in reactions to other drugs. New mass spectrometric techniques allowing for the identification of naturally eluted peptides from drug-exposed HLA alleles will allow for the antigenic source of T-cell activation to be defined and may shed light on the influence of disease. Indeed, preliminary data highlight the propensity of drug-responsive T-cells to cross-react with T-cells primed to viral antigens. Furthermore, the environment can epigenetically influence regulatory gene expression, suggesting that an individual’s family exposure history may alter immune thresholds and tip the balance toward activation.

Summary:

It is likely that predisposition to allergic drug reactions is multifaceted in most cases. This will require the study of large numbers of patients to detect genetic factors that have a lower effect size than HLA alleles. This should be accompanied by detailed clinical phenotyping of patients and the assessment of the immunological phenotype with respect to the presence and type of drug antigen-responsive T-cells.

Key words:

Predisposition, drug allergy, HLA supertypes, co-signalling, viral cross-reactivity

**Introduction**

Allergic drug reactions, also called drug hypersensitivity reactions (DHRs), occur to otherwise safe and efficacious drugs at therapeutic doses and are broadly classified into those with symptoms arising within 1 hr (immediate) of drug administration, or those thereafter (non-immediate or delayed). The 1h time-point was initially proposed by Terrados [1], but it should be noted that non-immediate reactions can occur within as little as a few hours [2]. Characterisation of these reactions based on time cut-offs is therefore empirical because of the possibility of some cross-over. A better classification is required

These adverse reactions have an immunological aetiology and are potentially life-threatening, with severe IgE-mediated immediate reactions triggering anaphylaxis, and delayed T-cell mediated reactions leading to Stevens Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), reactions characterised by skin detachment and mortality rates over 30% [3]. The reactions are sporadic, idiosyncratic and have a complex dose-response relationship. As such, allergic drug reactions are not detected by current pre-marketing screening and represent a significant burden to patients and pharmaceutical companies. Understanding the predisposing parameters that tip the immune balance from tolerance to allergy (figure 1) will inform the next generation of drug screening platforms to enhance drug safety.

**Genetic risk factors**

Associations between DHRs and HLA alleles have been the focus of the field, largely because of the large effect size, and continue to be described [4-8]. Single nucleotide polymorphisms (SNPs) in key immuno-regulatory [9], metabolic [9-13], and cytokine genes [9, 14-16] are also linked with disease predisposition. However, the majority have not been identified through genome-wide association studies, and thus their effect size is likely to be lower than that of HLA alleles.

**The role of metabolic variation**

Metabolic SNPs may simply determine the rate of formation or removal of the culprit antigen, but also the degree of ‘danger signalling’ where higher, more toxic concentrations infer increased tissue damage and signals capable of polarising the immune system [17]. A handful of clear metabolic associations have been described, most notably CYP2C9\*3, where metabolising capacity is reduced by up to 95%, leading to the occurrence of serious cutaneous adverse reactions with the anticonvulsant phenytoin [12, 13]. More recently, several studies have described an His645Asp substitution in diamino oxidase (+8956C/G) that reduces its capacity to metabolize circulating histamine, resulting in higher, prolonged histamine levels and increased risk of immediate NSAID-induced reactions [9-11]. Despite these data and the clinically observed increased likelihood of cutaneous reactions with higher doses of drugs [18], few other studies have reported associations with metabolic enzyme and transporter SNPs with DHRs [19]. This may relate to fractional clearance of the drug, i.e. if drug clearance is dependent on one pathway, variation in that pathway is more likely to result in predisposition to the adverse drug reaction. Where drugs can be detoxified through several pathways, reduced function of one pathway simply results in diversion to another pathway.

**The influence of cytokine signalling**

Cytokines function as signalling mediators during allergic reactions where distinct secretion patterns play a key role in the polarisation of T-cell differentiation and the subsequent effector response. The cytokine milieu, referred to as signal 3, is released from responding professional antigen presenting cells after antigen encounter. It is therefore critical to explore the vital role of dendritic cells during these reactions as the dynamics of an individual’s local and potentially imbalanced cytokine environment throughout a reaction remain to be defined. With regard to immediate reactions, most effort has been directed toward IL-4 and IL-13. Two key variants in the IL-4 receptor gene (*IL45RA*, I50V and Q551R) were shown to predispose patients to β-lactam allergy in separate Italian and Spanish cohorts [14, 16]. The Italian study further identified associations with IL13 –1055C>T and R130Q SNPs. Another IL-10 variant (*IL10* －592A) has also been associated with efavirenz allergy in HIV-infected patients [15]. As cytokines function in tandem with others, their individual impact may be limited unless they serve a critical function which leads to amplification of the immune response. It is however important to note that the association of DHRs with cytokine gene polymorphisms has usually been based on candidate gene studies, and not genome wide studies, and many of the associations have not been replicated. This may overall reflect their lower effect size and variability in phenotypes of patients considered in different studies where the patient heterogeneity impacts not only on the effect size but on replicability.

**Signal 1: specificity of HLA and TCR**

Drug-derived antigens are presented by HLA class I or II molecules on the surface of antigen-presenting cells (APCs) to passing CD8+ or CD4+ T-cells, respectively. The presentation of drug-antigen on HLA binding grooves to a corresponding T-cell receptor (TCR) constitutes signal 1 in T-cell activation and may occur via three routes: hapten mechanism, pharmacological interaction (PI), or altered self-peptide repertoire. Due to the heterogeneity of hypersensitivity reactions, it may be that these mechanisms are complementary and are all relevant in a single patient, for a single drug. Importantly, the HLA locus is the most polymorphic region of the human genome, and genetic association studies have identified a plethora of associations between drugs and allergic reactions, of which the most well-documented are abacavir (ABC) with HLA-B\*57:01 and carbamazepine (CBZ) with HLA-B\*15:02. Table 1 summarises all the HLA-associated DHRs to date.

**Table 1:** Summary of HLA-associated immune-mediated adverse reactions targeting the skin, liver and other organs (adapted from Pirmohamed et al., 2015)[20].

|  |  |  |
| --- | --- | --- |
| **HLA-associated drug-induced cutaneous reactions** | **HLA-associated drug-induced-liver injury** | **HLA-associated drug reactions targeting other organs** |
| * Carbamazepine (A\*31:01; B\*15:02)[21]
* Abacavir (B\*57:01)[22]
* Allopurinol (B\*58:01)[23]Carbamazepine & Phenytoin (B\*15:02)[24]
* Cold medicines (A\*02:06)[25]
* Cold Medicines (B\*44:03)[26]
* Dapsone (B\*13:01)[27]
* Lamotrigine (A\*68:01)[28]
* Nevirapine (C\*04:01 and B\*35:05)[29, 30]
* Phenytoin (B\*56:02)[31]
 | * Flucloxacillin (B\*57:01)[32]
* Co-amoxiclav, Lumiracoxib (DQB1\*06:02)[33, 34], Lapatinib (DQA1\*02:01)[35]
* Lumiracoxib, Co-amoxiclav (DRB1\*15:01)[34, 36]
* Lumiracoxib (DQA1\*01:02)[34]
* Minocycline (HLA-B∗35:02)[37]
* Ticlopidine (A\*33:03)[38]
* Ximelagatran, Lapatinib Asparaginase (DRB1\*07:01)[39-41]
* Ximelagatran (DQB1\*02:01)[40]
 | * Aspirin (DQB1\*06:09)[42]
* Clozapine (DQB1\*05:02)[43]
* Statins (DRB1\*11:01)[44]
 |

The remarkable specificity of ABC for HLA-B\*57:01 allowed for the adoption of prospective patient HLA-B\*57:01 screening in clinical practice to eliminate the risk of ABC-induced hypersensitivity [5, 8]. Similar to ABC, the association of CBZ with HLA-B\*15:02 has a negative predictive value of 100% [7]. However, while HLA-B\*15:02 is common in Asian populations where allele frequency can reach 15%, it is seldom expressed by European patients with CBZ-induced DHRs (<0.01%) [20, 45]. As such, a selective prospective screening approach has been implemented for CBZ in patients of Asian descent. In Caucasian patients, HLA-A\*31:01 has been shown to be a predisposing factor for a number of phenotypes of CBZ cutaneous reactions [21]. A recent guideline has recommended that if a patient is positive for HLA-A\*31:01, CBZ should be avoided unless the benefit is thought to exceed the risk, and in which case the patient should be more closely monitored [46].

Additional genetic factors are also important to predisposing to CBZ immune-mediated reactions, in particular SJS/TEN in SE Asians. For instance, Ko *et al* described that naïve T-cell priming to CBZ was possible in HLA-B\*15:02 donors who also expressed a specific T-cell receptor clonotype; TCR Vβ-11-ISGSY. Furthermore, this clonotype was absent in CBZ-tolerant patients, but present in 84% of affected patients [47]. However, inter-individual TCR specificity has not been described for other immune-mediated reactions as it appears that most individuals have T-cells within their repertoire capable of responding to drug antigens [48], but also because other drugs form multiple antigenic determinants and stimulate a diverse array of TCRs [49].

While drug-responsive T-cells restricted to HLA class II alleles remain elusive [50, 51], other class I associations confirmed by allele-restricted drug antigen-specific T-cell activation include HLA-B\*58:01 with allopurinol [6, 52], HLA-B\*13:01 with dapsone [4], and HLA-B\*57:01 with flucloxacillin [53]. Flucloxacillin-induced liver injury occurs in 8.5/100,000 patients, but is reduced to 1 in 1000 patients within the HLA-B\*57:01 population [32]. Still, such low frequency would necessitate the screening of >13,000 individuals to avoid just one flucloxacillin-induced cholestatic hepatitis. Therefore pre-prescription screening for flucloxacillin, and indeed other drugs where only a minority expressing the risk allele develop a reaction, may not be cost-effective [20], although knowledge of the genetic predisposition could be used in other clinically useful ways such stratifying, monitoring and diagnosis [54]. An alternative approach that has been proposed is to use HLA supertypes, groups of HLA alleles with overlapping binding specificity, although the clinical utility of such an approach needs to be proven [55].

**Signal 2: co-signalling and Tregs**

Signal 2 is the summation of co-stimulatory and co-inhibitory pathways that conversely communicate between APCs and T-cells to dictate the T-cell activation threshold. Using an *in vitro* model, it has been shown that blockade of two key co-inhibitory pathways, programmed death ligand-1 (PDL1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4), during the priming of naïve T-cells from healthy donors enhances drug-specific T-cell activation [49, 56]. These studies demonstrate the propensity for dysregulated co-signalling to confer susceptibility to allergic drug reactions. Importantly, these pathways have known SNPs that dysregulate T-cell responses and induce autoimmune disease [57-60]. Interestingly, few studies have focused on genetic variation in co-signalling molecules and allergic drug reactions. However, it has to be emphasised that there have been a large number of GWAS on allergic drug reactions, which have not identified SNPs in the genes for these co-signalling molecules as predisposing factors. This may reflect that their effect size individually is low, or that they interact with other predisposing genes to amplify the overall response and its severity. Clearly more than one signal 2 pathway exists in the interaction between a T-cell and an APC, and this may also account for their lower effect size.

An interesting area for further study is the role of signal 2 pathways in regulatory T-cells (Tregs), where they act as an important suppressive mechanism to induce tolerance. Indeed, addition of Tregs to TEN-models prevents injury, indicating defective Tregs in predisposition to allergic reactions [61, 62]. Interestingly, polymorphic variants of FOXP3, a critical transcription factor for Treg-mediated tolerance [63], has been shown to induce autoimmune disease [64, 65]. Further study in DHR is required but the challenge will be to ensure that the studies are adequately powered because of the likely low effect size.

**Emerging role of epigenetics**

The immune regulatory network is subject to genetic variation, but also to epigenetic influences from the environment. A complete antigenic exposure history is impossible to obtain, but distinct methylation patterns that modulate the expression of immuno-regulatory genes may be identifiable. Such exposures are likely to include viral infection, which utilise epigenetic modification to evade host immune response [66, 67], and air pollution, which through induced methylation of the FOXP3 locus can compromise Treg suppressive function [68]. Moreover, growing evidence from multi-generational monitoring studies have shown that while own environmental exposure history dictates your immune status, exposures imposed on your parents and grandparents may also be important [69, 70]. Thus, defining the exposome that shifts the immune response towards activation will be important in understanding predisposition to drug allergic reactions.

**Non-genetic risk factors**

Non-genetic risk factors including drug-related factors such as structural liabilities, protein reactivity, degree of exposure, route of administration, and cross sensitisation [71-75] may be important predisposing factors. Additionally, individual biology (age and gender) [76, 77] and specific disease characteristics, may also be relevant.

**Influence of disease**

In the pre-highly active antiretroviral therapy (HAART) era, patients infected with the human immunodeficiency virus (HIV) showed a significantly higher incidence of drug-induced cutaneous reactions compared with healthy subjects [78]. Trimethoprim-sulfamethoxazole, sulfadiazine, trimethoprim-dapsone and aminopenicillins had the highest rates of delayed cutaneous reactions in HIV patients, which often had a variety of clinical manifestations [79]. Progressive deterioration of immune function (very low CD4+ T cell count) and polypharmacy for multiple opportunistic infections were suggested to be predisposing factors for the higher rate of these reactions [80]. However, with the advent of HAART, where HIV is largely controlled, and has become a chronic disease and patients have maintained their immune function, the rate of these allergic reactions has diminished, despite continuing polypharmacy in these patients.

Cystic fibrosis (CF) is an autosomal recessive disorder characterised by abnormal transport of Cl- across the cell membrane resulting in the accumulation of mucus in the lungs. This provides a suitable environment for persistent bacterial infections, usually *Pseudomonas aeruginosa* [81]. As such, CF patients are often administered large doses of parenteral antibiotics, usually β-lactams, aminoglycosides or cephalosporins for the treatment of recurrent respiratory tract infections. Unfortunately, the incidence of β-lactam allergy is significantly higher in CF patients compared with non-CF patients exposed to similar medications. The nature and mechanisms of β-lactam allergy in CF patients is thought to be the same as in non-CF patients [82]. Hence, the significantly higher incidence of β-lactam allergy in CF patients may possibly be due to the increased frequency of exposure to high drug doses.

**The role of antiviral T-cell response**

DRESS (drug reaction with eosinophilia and systemic symptoms) is an idiosyncratic reaction with a mortality rate of 10% induced by a number of drugs including phenobarbital, carbamazepine, phenytoin, sulphonamides and allopurinol [83, 84], which targets the skin but also multiple other organs [85]. Drug-induced reactivation of human herpesviruses (HHV-6 and HHV-7) and Epstein-Barr virus (EBV) play a critical role in the pathogenesis and can complicate clinical diagnosis [86, 87]. Picard *et al* performed a retrospective study involving 40 patients with carbamazepine-, sulfamethoxazole-or allopurinol-induced DRESS using peripheral blood mononuclear cells (PBMC) from these patients. They exposed PBMC to ‘culprit’ drugs and then evaluated T-cell phenotype, CD4+ and CD8+ repertoire, cytokine activity and viral reactivation. The authors concluded that the clinical symptoms of DRESS are partly due to the activation of cytotoxic T-cells directed to kill herpes viruses such as EBV [87].

**The role of drug-protein adducts**

Both sulfamethoxazole (SMX) and ABC can trigger an immunological response via a non-covalent interaction with immune receptors [88, 89]. However, covalent modification of proteins by certain drugs and metabolites may represent a critical step in the pathogenesis of DHRs [90-93]. SMX undergoes CYP450-mediated biotransformation to form a non-reactive intermediate, SMX hydroxylamine which is then converted to nitroso-SMX (SMX-NO) through auto-oxidation. SMX-NO binds covalently to multiple cysteine residues on human and mouse proteins to generate antigenic determinants for immune activation [94-97]. Dapsone also displays a similar metabolism and covalent protein binding profile to SMX [98, 99]. Remarkably, not all drugs require metabolism to generate protein reactive molecules. For example, the broad-spectrum β-lactams flucloxacillin and piperacillin covalently bind to multiple lysine residues on human serum albumin (HSA) via a metabolism-independent pathway because of an intrinsically unstable β-lactam ring [100, 101].

Paradoxically, piperacillin-HSA adducts with similar modifications have been detected in the serum of both piperacillin-hypersensitive and tolerant patients [101, 102]. Using innovative mass spectrometry techniques, Meng *et al* demonstrated that the level of covalent binding of piperacillin to HSA (1-10 days) in both allergic and tolerant patients was ≥ 2.6%, which is required to activate T-cells *in vitro*. Furthermore, the degree of Lysine 541 modification by piperacillin *in vitro* was dose-dependent. Despite these important findings, it is still unclear why only certain individuals progress to develop allergic reactions, but concomitant presence of genetic factors (described above) may be important.

**Future perspective**

HLA alleles are now well-documented risk factors for allergic drug reactions, and the role of HLA supertypes is an emerging possibility. As the only drug confirmed to activate T-cells via the altered peptide model, a great deal of work continues to further investigate ABC hypersensitivity. State-of-the-art mass spectrometry techniques to naturally elute the peptides expressed by HLA alleles in the presence of drug are now available and may be used to define the antigenic source of T-cell activation [103]. While such peptides may be self-derived, Lucas *et al* described ABC-responsive T-cells in all drug-naïve donors who expressed HLA-B\*57:01, and that specific HLA-B\*57:01-restricted epitopes originating from a viral antigen mounted cross-reactive T-cell responses to ABC-dependent synthetic epitopes. Thus, it is possible that the eluted peptides will be non-self and the response to ABC is at least partly directed by cross-reactivity with HLA-B\*57:01-presented viral antigenic peptides [104].

It is clear that HLA associations do not account for the entire risk and many patients who carry risk HLA alleles do not develop the hypersensitivity reaction. It is thus probable that these reactions are a summative effect of multiple susceptibility factors. Further work on dysregulated co-signalling pathways and Treg function is of particular interest. SNPs in both are associated with autoimmune disease and so it is conceivable that they similarly teeter the immune system on a knife edge between tolerance and drug allergy. This balance will be difficult to define as subtle changes in a few pathways may infer drastically different immune thresholds, and their role in predisposition to allergy may require the concomitant presence of other predisposing factors including the relevant HLA allele. Importantly, immune regulation is also epigenetically manipulated by environmental factors and warrants further investigation. Finally, transcriptomic analysis has previously provided valuable insight into toxicity and mechanisms of action of drugs [105]. Gene expression analysis of peripheral blood mononuclear cells and tissue biopsies from patients with allergic drug reactions compared with tolerant patients and healthy individuals will also be important in furthering of our understanding of the important signalling events that regulate these reactions.

**Conclusion**

Access to samples from allergic and tolerant patients will be key in identifying the critical parameters that predispose to immune reactions with a given drug. This will require much larger numbers than have been studied to date because of lower genetic effect sizes, and likelihood of epistatic effects between different genetic factors. This should be accompanied by phenotyping of patients, not only in relation to clinical phenotype, but also the immunological phenotype with respect to the presence and type of drug antigen-responsive T-cells. The future development of *in vitro* screening platforms that can incorporate multiple key risk factors will be vital to the prediction of whether newly developed drugs have the potential to cause drug allergy.

**Key points**

1. Drug-induced allergic reactions have complex pathomechanisms that are dependent on multiple susceptibility factors linked to the drug molecule, host (genetic and non-genetic), underlying disease and environment.
2. The increasing numbers of risk HLA alleles associated with allergic drug reactions suggests a critical role for the adaptive immune system.
3. An in-depth understanding of susceptibility factors of allergic drug reactions will be essential for the design of predictive/screening assays important for safety assessment during preclinical drug development.

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**Figure 1.** Schematic summarising the genetic and non-genetic factors that may be involved in predisposing individuals to drug hypersensitivity.