

REVIEW ARTICLE

Contributions of pharmacogenetics and transcriptomics to the understanding of the hypersensitivity drug reactions

T. D. Fernandez¹, C. Mayorga¹, J. L. Guéant^{2,3}, M. Blanca⁴ & J. A. Cornejo-García¹

¹Research Laboratory, Malaga General Hospital-IBIMA, Malaga, Spain; ²Faculty of Medicine, INSERM U-954, Nutrition-Génétique et exposition aux risques environnementaux, University of Nancy, Vandoeuvre-les-Nancy; ³University Hospital Center of Nancy, Vandoeuvre-les-Nancy, France; ⁴Allergy Service, Malaga General Hospital, Malaga, Spain

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Correspondence

Miguel Blanca, Allergy Service,
Malaga General Hospital-IBIMA, Pl. Hospital
Civil, 29009 Malaga, Spain.
Tel.: +34 951290224
Fax: +34 951290302
E-mail: mblancago@gmail.com

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Abstract

Hypersensitivity drug reactions (HDRs) represent a large and important health problem, affecting many patients and leading to a variety of clinical entities, some of which can be life-threatening. The culprit drugs include commonly used medications including antibiotics and NSAIDs. Nontherapeutic agents, such as contrast media, are also involved. Because the pathophysiological mechanisms are not well known and the current diagnostic procedures are somewhat insufficient, new approaches are needed for understanding the complexity of HDRs.

Histochemical and molecular biology studies have enabled us to classify these reactions more precisely. Pharmacogenetics has led to the identification of several genes, involved mainly in T-cell-dependent responses, with a number of markers being replicated in different studies. These markers are now being considered as potential targets for reducing the number of HDRs. Transcriptomic approaches have also been used to investigate HDRs by identifying genes that show different patterns of expression in a number of clinical entities. This information can be of value for further elucidation of the mechanisms involved. Although first studies were performed using RT-PCR analysis to monitor the acute phase of the reaction, nowadays high-density expression platforms represent a more integrative way for providing a complete view of gene expression.

By combining a detailed and precise clinical description with information obtained by these approaches, we will obtain a better understanding and management of patients with HDRs.

Adverse drug reactions are defined by the World Health Organization as any noxious, unintended, and undesired

effect of a drug that occurs at doses used for prevention, diagnosis, or treatment (1). Hypersensitivity drug reactions (HDRs) account for 6–10% of all adverse drug reactions (2) and represent at least 5% of hospital admissions (3). Hypersensitivity drug reactions are a health problem of great concern, affecting a large number of patients of all ages, and are neither detected nor predicted before postmarketing surveillance, overloading the public health system (2, 3). Hypersensitivity drug reactions are unpredictable and consist of a number of clinical entities varying in manifestations and severity, some of which can be life-threatening (4). These clinical entities are not pathognomonic of drugs and can be induced by other agents such as viruses or even be associated with autoimmune phenomena (5).

Hypersensitivity drug reactions are unavoidable and linked to the intrinsic properties of the drugs and the genetic

Abbreviations

BLs, beta-lactams; CBZ, carbamazepine; DIHS/DRESS, drug-induced hypersensitivity syndrome or drug rash with eosinophilia with systemic symptoms; DILI, drug-induced liver injury; HDRs, hypersensitivity drug reactions; HLA, human leukocyte antigen; IgE, immunoglobulin E; IHC, immunohistochemistry; IR, immediate reactions; MPE, maculopapular exanthema; NIR, nonimmediate reactions; NSAIDs, nonsteroidal anti-inflammatory drugs; PBMcs, peripheral blood mononuclear cells; PMP, phenoxymethylpenicillin; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RNAseq, RNA sequencing; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis complex; SNPs, single nucleotide polymorphisms.

predisposition of the patient. In many instances, there is no therapeutic alternative; therefore, a very accurate diagnosis is necessary to identify the drug as the causative agent. Because the pathophysiological mechanisms are not well known, the current diagnostic procedures are somewhat insufficient compared with other processes such as cancer or viral infections. In fact in HDRs, both an under- and an over-reporting exist (6).

The patterns of HDRs vary over the years and are due to changes in drug consumption, cumulative exposure, the introduction of new drugs, the establishment of new indications (7, 8) and possibly others factors. In this context, HDRs cannot be considered a static process, but a dynamic one. This has been clearly demonstrated for beta-lactam (BL) antibiotics (9) and nonsteroidal anti-inflammatory drugs (NSAIDs) (6).

In this review, we will summarize the current pharmacogenetics and transcriptomics approaches that can be used to achieve a better understanding and diagnosis of HDRs.

Present knowledge

Although the number of clinical entities induced by drugs is limited, it is bigger and more diverse than that induced by protein allergens and is due to a large number of drugs that cover all therapeutic agents available as well as biological products, including vaccines and antibodies (10). This is due to their intrinsic properties, such as how they are metabolized and bind to proteins. Some examples of the most representative drugs are given below.

The best studied model of HDRs has been penicillin allergy, and with the development of new BL structures through the years, this group has become more complex in terms of the number of drugs involved, differences in the chemical structures, and the clinical entities induced (9). Although BLs are the drugs that most frequently elicit immune-mediated HDRs, the diagnosis of BL allergy is not correctly addressed, being both over- and underestimated (6). In fact, many mild reactions attributed to BLs are not HDRs, and a substantial number of severe reactions are not sufficiently evaluated. This misdiagnosis can lead to the use of alternative antibiotics with the risk of more adverse effects and the induction of bacterial resistance. Moreover, alternative medications are usually more expensive with an estimated increased cost of 273€/patient/day (11). Therefore, accurate diagnosis is critical not only to improve patient safety but also to reduce the cost to the public health systems.

In the past, immunoglobulin E (IgE)-mediated reactions to BLs have been diagnosed by skin testing using major and minor determinants of benzylpenicillin with a reasonable sensitivity (9). As benzylpenicillin is no longer the most highly prescribed drug, many other BLs are now involved in IgE-mediated reactions, with amoxicillin being the most important. For patients who are selective responders to amoxicillin, the sensitivity of the test is not higher than 60%, and for a large proportion of cases, drug provocation is required for diagnosis (12). This problem has become more complex with

the inclusion of clavulanic acid, and we still do not know all the antigenic determinants that are generated when this drug binds to endogenous protein carriers. The problem is even more complicated when we consider T-cell-dependent responses. In the late 1980s, the reported sensitivity of intra-dermal/patch testing for the diagnosis was around 70%; however, recent publications indicate that it is below 20% for both child and adult populations (12, 13).

Nowadays quinolones are the second most important HDR-inducing anti-infection agent after BLs. However, it is still unclear whether these drugs induce mediator release in immediate responses through an IgE-dependent mechanism or by other means. Recent studies suggest that for some patients, these drugs can be strong histamine releasers (14).

The picture becomes more complex when considering T-cell reactions because there are more candidate drugs able to induce HDRs, and clinical entities are more heterogeneous in both their presentation and severity. Moreover, these entities can be induced by other confounding factors, such as viruses. In fact, many patients with exanthematic reactions evaluated in specialized clinics have good tolerance to the suspected causal drug, indicating that this was not the culprit and that the cofactor involved was not identified (15).

Nonsteroidal anti-inflammatory drugs represent the most important group of drugs involved in HDRs. In a very high proportion of these cases, these reactions are not triggered by a specific immunological mechanism, instead they are caused by the release of inflammatory mediators such histamine, prostaglandins, and leukotrienes through processes that are not yet completely understood (16). In the case of aspirin-induced asthma, the mechanism has been associated with the arachidonic acid metabolic pathway, but in the case of NSAID-induced acute urticaria, it is still unknown.

A fourth group of chemical compounds that are not therapeutic agents by themselves comprises radiologic contrast media, mostly organic iodine derivatives. These drugs can be involved in both immediate and nonimmediate reactions, but in the first case, it is not clear whether IgE or other mechanisms induce the release of histamine and other mediators. The sensitivity of the methods is not optimal, and for nonimmediate T-cell-dependent responses, skin test sensitivity is also very low as well as information on cross-reactivity between different compounds (17).

Overall, we are dealing with drugs that can induce either immunological reactions by specific IgE antibodies (18) or T cells (19, 20) (such as in BL hypersensitivity), or nonimmunological reactions (such as cross-reactions to NSAIDs) (6, 8), with overlapping time intervals and clinical manifestations such as urticaria-angioedema. Experimental models do not exist, and in spite of the progress achieved in pharmacology and molecular biology, more research is needed to understand the pathophysiological mechanisms underlying these reactions and to improve diagnoses. The approaches we describe below can be useful for both of these purposes. Although the understanding of the underlying mechanisms involved in HDRs may require input from other disciplines, this review will focus on pharmacogenetics and transcriptomics.

Contribution of immunohistochemistry

Immunohistochemistry (IHC) can be a useful tool for evaluating and monitoring HDRs (5, 21). Gell and Coombs established four categories for the classification of HDRs, called types I–IV (22). The most important are type I reactions, which are induced by the formation of IgE antibodies that bind to the high-affinity IgE receptors on mast cells and basophilic leukocytes (immediate reactions, IR), and type IV or T-cell reactions (nonimmediate reactions, NIR). Given that T cells are able to orchestrate different forms of inflammation, resulting in different clinical entities, type IV reactions have been further divided into IVa, IVb, IVc, and IVd (23). Immunohistochemistry can allow us to analyze mast cell activation in immediate reactions (IR), whereas for NIR, it can be used to discriminate between different entities and even between HDRs and viral infections (24). In patients with exanthema and hepatitis, the liver cell destruction by cytotoxic T cells may be the most dangerous event, but for the diagnosis of drug allergy, the presence of an eosinophilic infiltrate in skin biopsies may be sufficient (23).

However, IHC cannot provide information about the culprit drug or the molecular mechanisms underlying the different clinical entities (Fig. 1).

Pharmacogenetics in HDRs

IgE-mediated reactions

Beta-lactams are the most important group of drugs that induce HDRs through an IgE-dependent mechanism (7). Genetic studies on BL IR have followed the candidate gene approach, analyzing mainly the IgE receptor, IL-4, and IL-13 because of their important regulatory role in allergy and

atopy. In the case of the IgE receptor, the E237G variant of the *FcεRIβ* gene has been shown to be related to the development of penicillin allergy, probably through the production of specific IgE antibodies (25). Genetic variants in *STAT6*, a cardinal transcription factor for IL-4 signaling, have also been described in penicillin allergy, but their potential role in the specific response needs further clarification (26).

Concerning cytokines, different associations have been found for single nucleotide polymorphisms (SNPs) in *IL-4* and *IL-13*, in both Asian and European populations. Specifically, the allele Q576 in the α -chain of IL-4 receptor was related to increased serum levels of specific IgE antibodies to benzylpenicilloyl, phenoxymethylpenicillanyl (PMP), or ampicillanyl determinants in Chinese patients with penicillin allergy (27). The same group found significant differences between *IL-4-IL-13*-SNP3 and *IL-4-IL-13*-SNP4 genotypes for patients with positive specific IgE values to benzylpenicillanyl determinant (28).

We have recently investigated the associations between SNPs in *IL-13* (-1055/-1111 C>T and R130Q), *IL-4RA* (I50V, S478P, and Q551R), and *TNFα* (-308G>A) in Italian patients with IR to BLs (29, 30). We found that the combinations of the minor allele of *IL-13* polymorphisms with any of the predominant homozygous genotypes of the 3 analyzed SNPs in *IL-4RA* were more significantly associated with the risk of BL allergy and with total serum IgE levels than any polymorphism considered alone. Thus, we showed the importance of gene–gene interactions between *IL-13* and *IL-4RA* SNPs in IR to BLs (29). Homozygous patients for the less frequent allele of the studied *TNFα* polymorphism showed higher levels of specific IgE than the other two genotypes (30). Regarding the possible importance of the atopic status in these reactions, we have shown that determinants of

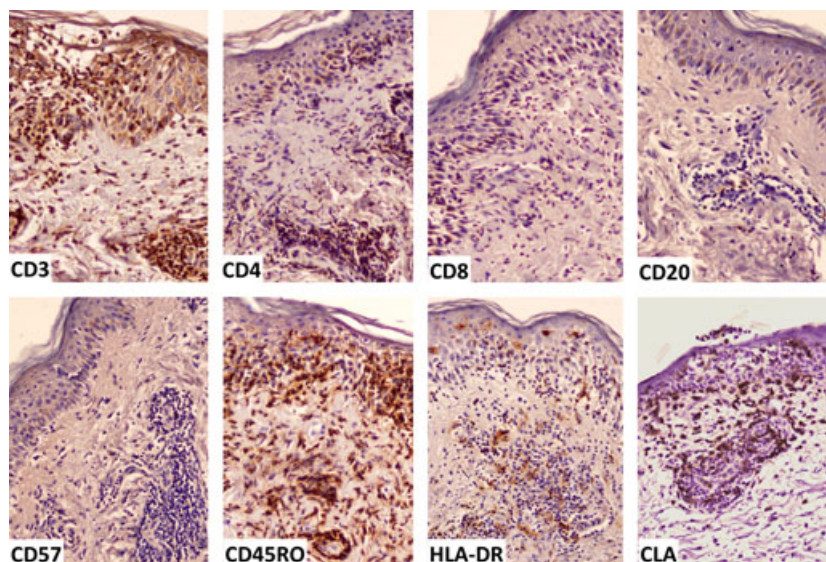


Figure 1 Immunohistochemistry staining of a hypersensitivity drug reaction (HDR) with cutaneous symptoms. The images show the

presence of different cell populations in the affected skin from a patient suffering an HDR.

atopy, total IgE, specific IgE against mites, and *IL-4RA* polymorphisms are predictors of IR in a Spanish population (31). This study represents the largest published series of patients with IR and opens new insights into the mechanisms of BL allergy (31).

Although the potential implication of genetic variations in the polymorphic human leukocyte antigen (HLA) system has been of less interest (32), the HLA-DR7 allele was associated with IR to penicillin in Chinese patients and the DR4 allele with specific response to PMP determinant (33).

T-cell-mediated reactions

Nonimmediate reactions can be classified according to the specific triggering subpopulation into type IVa (Th1 cytokine pattern, producing mainly TNF- α and IFN- γ), IVb (Th2 cells, producing IL-5 and IL-4/IL-13), type IVc (cytotoxic CD4 and CD8 cells, producing perforin/granzyme-B), and type IVd (T cells producing CXCL8 and GM-CSF) (23). However, independently of the specific immune reactant, it is well known that the HLA system plays a crucial role in the presentation of processed hapten–protein complexes to T cells and in the elicitation of delayed HDRs (19). Taking into account the specificity of these reactions, it is not surprising that certain HLA alleles have been associated with the immunological response to drugs. Genetic predisposition to delayed HDRs has been described mainly for particular HLA alleles (34), leading regulatory agencies to recommend prospective genotyping before beginning drug treatment in some cases, in order to reduce the risk of developing HDRs (35).

The three main groups of culprit drugs for which a solid body of knowledge exists supporting the association between a particular HLA allele and HDRs are the anticonvulsant carbamazepine (CBZ), the antihyperuricemic drug allopurinol, and abacavir, a protease inhibitor. The two most severe clinical entities induced by these drugs are Stevens–Johnson syndrome/toxic epidermal necrolysis complex (SJS/TEN) and drug-induced hypersensitivity syndrome or drug rash with eosinophilia with systemic symptoms (DIHS/DRESS). The former is characterized by a different degree of detachment of the epidermis and mucosal epithelium destruction often associated with organ involvement (20); in the latter, skin rash is accompanied by fever, hepatitis and/or other organ involvement, lymphadenopathy, and hematological disorders (hypereosinophilia, leukocytosis, and atypical lymphocytosis) (36). Another potentially serious idiosyncratic reaction induced by medicines is drug-induced liver injury (DILI), for which the antimicrobial agent flucoxacin has been recognized as one of the most common causes (37).

In 2004, Chung et al. (38) reported that the HLA-B*1502 was present in all CBZ-induced patients with SJS, in only 3% of CBZ-tolerant patients, and in 9% of healthy individuals previously not exposed to the drug. Other studies confirmed these associations in an Asian population with CBZ-induced SJS/TEN, but not with mild reactions or DRESS (39). Nevertheless, the involvement of this allele in SJS is far from universal. Thus, no HLA-B*1502 carriers

were detected in a group of patients with SJS and controls in Japan (40), and a recently published paper carried out in Europeans also failed to detect this association (41). This could be due to the very low prevalence of the HLA-B*1502 allele in Japanese and Caucasian populations. However, in Europeans, the HLA-A*3101 has been significantly associated with DIHS/DRESS, maculopapular exanthema (MPE), and SJS/TEN (42).

Another HLA allele associated with severe HDRs is HLA-B*5801, which was present in all Han-Chinese patients with allopurinol-induced SJS that were included in a recently published study (43). Interestingly, this marker was also associated in Europeans (41). The role of HLA-B*5801 in allopurinol-induced SJS has been recently reviewed (44).

There is substantial evidence linking the presence of the HLA-B*57:01 genotype with abacavir hypersensitivity. The presence of this allele is predictive not only of clinically diagnosed abacavir HDRs (35, 45) but also of immunologically confirmed reactions (patch test) (35, 46). Prospective screening of HLA-B*57:01 reduces the incidence of both clinically diagnosed and patch-test-confirmed HDRs to abacavir (35, 46).

Although acetaminophen has been the drug most commonly associated with hepatotoxicity, genetic association data exist mainly for antibiotics. For example, a recent paper on flucoxacin-induced DILI showed an association peak in the HLA region with the strongest association for a SNP in complete linkage disequilibrium with HLA-B*57:01 (47). The importance of both HLA class I and class II alleles has been highlighted in a collaborative work that included patients suffering from amoxicillin–clavulanate-induced DILI, although their utility as predictive or diagnostic biomarkers is limited (48).

Transcriptomics

Transcriptomics can be defined as the quantitative study of all genes expressed in a given biological state. It is often used to compare gene expression between different groups of samples (such as diseased and nondiseased tissues) (49), to provide a catalog of genes that show altered expression, to discriminate samples coming from different manifestations of the same pathology (50), to discover new subtypes in a known pathology (51), or to find markers that allow us to make a prognosis for the evolution of a disease (52). These data can be used to identify individual genes or to create a global profile or ‘signature’ composed of multiple expression changes associated with disease or a particular cell type or cell stage. These signatures can be connected to functions using functional annotation with ontologies (53). There are different strategies available to perform transcriptomic studies. Classically, four methods have been used to quantify transcriptional activity: Northern blotting, providing information about mRNA size, alternative splicing, and the integrity of RNA samples; *in situ* hybridization, which allows localization of transcripts in specific cells within a tissue (54); RNase protection assays, which are useful for mapping transcript initiation and termination sites and intron/exon boundaries, and for discriminating between related mRNAs

of similar size (55). However, these three methods show relatively low sensitivity (56) compared with reverse-transcription polymerase chain reaction (57), an *in vitro* method for enzymatically amplifying defined sequences of mRNA that permits the analysis of different samples in the same experiment (Fig. 2).

Another common approach is the use of gene expression microarrays. This technology permits the analysis of thousands of transcripts in one single assay (58). There is little doubt that the appearance of microarrays represented a change of paradigm in the study of the transcriptome. In particular, this technique has allowed us to take a fully quantitative measurement of gene expression, whereas previous techniques have been semiquantitative. However, this technology has several limitations that need to be taken into

account: the dependence on existing knowledge about genome sequences, which limits the identification of novel mRNAs (probes for transcripts that are absent from databases cannot be designed); the high background signal (noise) mainly due to cross- or nonspecific hybridization (this can make it hard to detect lowly expressed genes above background); probe saturation, making it difficult to quantify highly expressed genes; and the complex normalizations required due to the indirect measurement of gene expression (scanned intensity of fluorescent hybridized probes) (59). Altogether, this makes the technique a valuable resource, but with certain limitations. As more transcriptional information becomes available and the technology improves, arrays will become more complete and ultimately more informative (60).

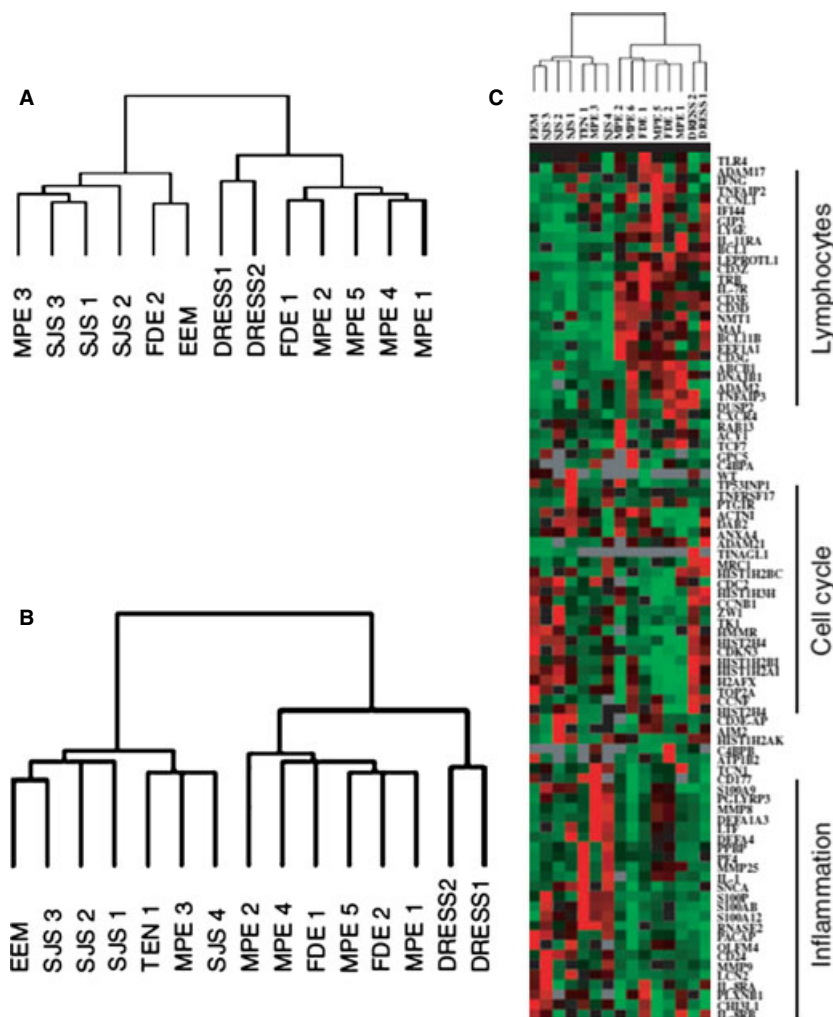


Figure 2 Unsupervised hierarchical clustering analysis of microarray and quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) data from selected genes showing that different clinical manifestations of hypersensitivity drug reactions (HDRs) are clustered on different branches. (A) Dendrogram for cluster analysis of microarray data. (B) Dendrogram for cluster analysis of qRT-PCR data. (C) Cluster analysis of qRT-PCR data; each column represents a different patient. (Reproduced from Bellon T. et al. *British Journal of Dermatology* 2010; 162: 1014–1022.)

In addition to microarrays, a relatively recent high-throughput technology for the quantification of gene expression is RNA sequencing (RNAseq) (59). Like microarrays, this approach allows us to quantify the abundance of all known transcripts expressed in a set of experimental samples. However, it also has a number of advantages over hybridization-based approaches: it does not depend on previous knowledge of the transcriptome and can reveal the precise boundaries of transcripts to single base precision; it can also yield information about exon junctions allowing the study of complex transcription units; it suffers much less from background noise and upper detection thresholds, allowing us to quantify a much wider range of expression values than is possible with microarrays (60).

Transcriptomics in HDRs

Recent work has highlighted the potential utility of transcriptomics in unraveling the underlying mechanisms related to atopy and allergy (61, 62).

Initial transcriptomic studies on HDRs were carried out using RT-PCR analysis to monitor the acute phase of the reaction, describing a polarized pattern of cytokine and transcription factor expression depending on the mechanism involved (63). In IgE-mediated reactions, an upregulation of IL-4 and GATA-3 was observed, alongside a downregulation of IFN- γ and T-bet that normalized as the reaction subsided (Th2 response) (63, 64). In T-cell-mediated reactions, an increase in IFN- γ , IL-12, and T-bet was found, alongside a downregulation of IL-4 and GATA-3, indicative of a Th1 response (63, 64). However, a Th2 pattern with eosinophilia has also been described in some clinical entities mediated by a T-cell mechanism (65, 66).

Other studies have investigated gene expression in the skin, the organ most commonly affected in HDRs (21). A number of studies have demonstrated that the T cells present in the cellular infiltrate are activated and express the skin-homing receptor CLA, as well as cytotoxic markers such as perforin and granzyme-B; this expression correlates with reaction severity (67, 68). Detailed studies carried out in patients with MPE have shown that skin-homing receptors were increased in both the skin and mononuclear cells, whereas the corresponding chemokine ligands were expressed in the skin only (69). These studies also showed an increase in CCR6 and CCR10, as well as their corresponding ligands CCL20 and CCL27 (responsible for skin homing) (67). An increase was also observed for CXCR3 and its ligands CXCL9 and CXCR10, related to the Th1 response (69). In acute generalized exanthematic pustulosis, a skin infiltrate of neutrophils attracted by CD4 lymphocyte-produced IL-8 has been observed (70). In more severe reactions like SJS/TEN, the massive apoptosis of keratinocytes induced by cytotoxic CD8 has been described (71).

These reactions can be studied in more detail through the use of gene expression microarrays (50). In a recent work from our group, we have studied gene expression profiles in peripheral blood mononuclear cells (PBMCs), both during the acute phase and upon resolution, for different types of

HDRs. Hierarchical clustering of array data from these genes arranged the patients into two main groups, nonbullous diseases and bullous reactions with mucocutaneous involvement (more severe) (50). Two genes encoding proteins involved in cell cycle and cell growth (HIST1H2AI and H4F2) were found to be upregulated during the acute phase. Additionally, their expression levels were correlated with the severity of the disease (50). Several alarmins, endogenous molecules that signal tissue and cell damage, have been shown to be upregulated during the acute phase of HDRs with cutaneous involvement (50). Similarly, some alarmin transcript complexes have been previously detected in skin biopsies from patients with SJS/TEN by others (72).

The analysis of the transcriptome of PBMCs is a promising tool for the study of the mechanisms underlying DILI, the most commonly identifiable cause of acute liver failure, whose diagnosis is hindered by the limited utility of traditional clinical chemistries (73). In fact, a recent study in a rat model was able to detect the presence and severity of toxic responses in the liver based on gene expression (74). It also showed that different hepatotoxicants led to different patterns in gene expression (74). Thus, blood gene expression markers may serve as surrogate biomarkers to non-invasively detect as well as distinguish hepatic toxicity (75). In another study, the transcriptomic analysis of human samples was used to distinguish between acetaminophen-intoxicated patients and healthy individuals (76). More recently, it has been reported that only 48 h after exposure to acetaminophen, treated subjects showed a marked downregulation of mitochondrial genes, most prominently those involved in the oxidative phosphorylation pathway, which was not observed in subjects taking placebo (77). The magnitude of downregulation was positively correlated with the percentage of the drug converted to its reactive imide metabolite (77).

Major challenges in HDR transcriptomics and genomics

One of the most important issues for transcriptomic studies of HDRs is the acute phase definition, that is, the time elapsed between drug intake and/or the appearance of clinical symptoms and the collection of biological samples. In the case of PBMCs, it is possible to obtain samples at different time points; however, this can be more difficult when working with skin or liver tissues.

Another critical requirement is an accurate and extensive description of the clinical phenotype for every HDR. For example, even though SJS and TEN belong to the same spectra of reactions, they represent different levels of severity, and as such, some differences could be expected from the point of view of transcriptomics and genomics. To address this issue, a considerable effort is being undertaken by experts who are now reviewing and debating current terminology and diagnostic criteria for a better definition of drug response phenotypes. This is necessary to advance research, not only in pharmacogenetics and transcriptomics, but also for immunological, mechanistic, and epidemiological purposes (78).

Concluding remarks

A significant proportion of subjects with adverse drug reactions develop hypersensitivity reactions with the participation of specific immunological mechanisms or the participation of other mechanisms where no immunological recognition is involved. These lead to a series of reactions that affect the skin, the cardiovascular system, or can be organ specific. To fully understand these reactions and to develop diagnostic tools, a mechanistic approach must be employed. More studies including not only pharmacogenetics but also transcriptomic analysis will be necessary to achieve this goal.

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Author contributions

TDF, CM, JLG, MB, and JACG have equally contributed in the redaction and correction of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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