



Genetic basis of hypersensitivity reactions to nonsteroidal anti-inflammatory drugs

Francisca Gómez^a, James R. Perkins^b, Elena García-Martín^c
Gabriela Canto^d, and José A. Cornejo-García^b

Purpose of review

NSAIDs are the main triggers of hypersensitivity reactions to drugs. However, the full genetic and molecular basis of these reactions has yet to be uncovered. In this article, we have summarized research from recent years into the effects of genetic variants on the different clinical entities induced by NSAID hypersensitivity, focusing on prostaglandin and leukotriene-related genes as well as others beyond the arachidonic acid pathway.

Recent findings

We introduce recent contributions of high-throughput approaches including genome-wide association studies as well as available information from epigenetics and next-generation sequencing. Finally, we give our thoughts on future directions in this field, including the scope for bioinformatics and systems biology and the need for clear patient phenotyping.

Summary

The full genetic and molecular basis of clinical entities induced by NSAIDs hypersensitivity has yet to be uncovered, and despite commendable efforts over recent years, no clinically proven genetic markers currently exist for these disorders. It is clear that we will continue to find more about these reactions in the coming years, concurrently with improvements in technology and experimental techniques, and a precise definition of different phenotypes.

Keywords

candidate gene approach, genome-wide association studies, NSAIDs-exacerbated respiratory disease, NSAIDs-induced urticaria/angioedema

INTRODUCTION

The increase in the prevalence of allergic diseases worldwide [1] also includes hypersensitivity drug reactions (HDRs), with special emphasis in nonsteroidal anti-inflammatory drugs (NSAIDs) [2,3^{***}]. NSAIDs, the most commonly used drugs worldwide, are the main triggers of HDRs [2,3^{***}]. HDRs to NSAIDs can be mediated by specific immunological mechanisms or by others wherein vasoactive mediators are released through pathway activation in absence of specific immunological recognition [4,5]. In the first group (selective reactions), symptoms appear only after the intake of a single NSAID/NSAIDs from the same chemical group with patients tolerating chemically unrelated drugs [6]. Reactions can be further classified into immunoglobulin E (IgE) or T-cell mediated [4,6]. The second group of reactions (cross-intolerance) is more common. Symptoms are induced by at least two chemically unrelated NSAIDs [4,5]. Alterations of arachidonic acid metabolism through

cyclooxygenase (COX)-1 inhibition are thought to have a central role [7]; however, current data suggest the participation of additional mechanisms [8,9].

Three main clinical entities have been described in cross-intolerance: NSAIDs-exacerbated respiratory disease (NERD) in patients with an underlying chronic airway respiratory disease (asthma/rhinosinusitis/nasal polyps), previously aspirin-induced asthma, or aspirin (ASA)-exacerbated respiratory disease (AERD); NSAIDs-exacerbated cutaneous disease

^aAllergy Unit, ^bResearch Laboratory, IBIMA, Regional University Hospital of Malaga, UMA, Malaga, ^cDepartment of Pharmacology, University of Extremadura, Cáceres and ^dAllergy Service, Infanta Leonor Hospital, Madrid, Spain

Correspondence to José A. Cornejo-García, Research Laboratory, IBIMA, Hospital Civil, Plaza del Hospital Civil s/n, Pabellón 5, Sótano, 29009 Málaga, Spain. E-mail: josea.cornejo@ibima.eu

Curr Opin Allergy Clin Immunol 2015, 15:285–293

DOI:10.1097/ACI.0000000000000178

KEY POINTS

- NSAIDs are the main elicitors of hypersensitivity reactions to drugs.
- The study of the genetic basis of clinical entities induced by NSAIDs hypersensitivity needs a more precise phenotype definition.
- Most genetic association studies have been conducted in patients with NERD in spite of NIUA being the most frequent clinical entity.
- Few high-throughput data sets exist on NSAIDs hypersensitivity that hampers the molecular characterization of underlying mechanisms.

(NECD) in patients with a history of chronic spontaneous urticaria; and NSAIDs-induced urticaria/angioedema (NIUA) in otherwise healthy individuals (without history of chronic spontaneous urticaria) [4]. In a number of patients, reactions include both respiratory and skin involvement (blended reactions) [5], which have not been analyzed in detail in the current classification from the European Academy of Allergy and Clinical Immunology [4]. Although NERD is the most studied model [10^{***}], NIUA is the most common clinical entity induced by HDRs [2,3^{***}] and has been defined as a consistent entity that does not evolve to NECD [11], as supported by other authors [12].

Although a family history of NSAIDs-HDRs has been reported in 6% of patients [13], supporting

some genetic influence, there is a lack of studies addressing this point. However, it has been shown that NIUA aggregates in families inheriting the minor allele of the -444A>C polymorphism in the leukotriene C4 synthase (*LTC4S*) gene, although segregation did not follow a clear Mendelian pattern [14]. A case of two homozygous twins with the same NSAIDs-HDRs has recently been reported [15^{*}]. However, NSAIDs hypersensitivity is expected to result from the interaction of different factors as occurs in other complex diseases (Fig. 1).

Despite the recent increase in interest in NIUA [16,17^{*},18], most information comes from NERD [19,20]. However, many studies [19–21] have included limited numbers of individuals, often without replication, largely in Asian populations. Overall, there is a lack of consistent genetic markers for HDRs to NSAIDs.

As NSAIDs block COX-1 and shunt the metabolism of arachidonic acid from prostaglandins to leukotrienes (CysLT) synthesis, most genetic association studies have focused on polymorphisms in genes from this pathway (candidate gene approach) (Fig. 2). Other studies have analyzed the immunoglobulin E receptor, cytokines and histamine homeostasis-related genes. Although most NSAIDs-HDRs are not thought to be immunologically mediated, several studies have also found associations with the human leukocyte antigen (HLA) system. Finally, despite the relevance of NSAIDs-HDRs, few genome-wide association studies (GWAS) are available, hampering the identification of other mechanisms.

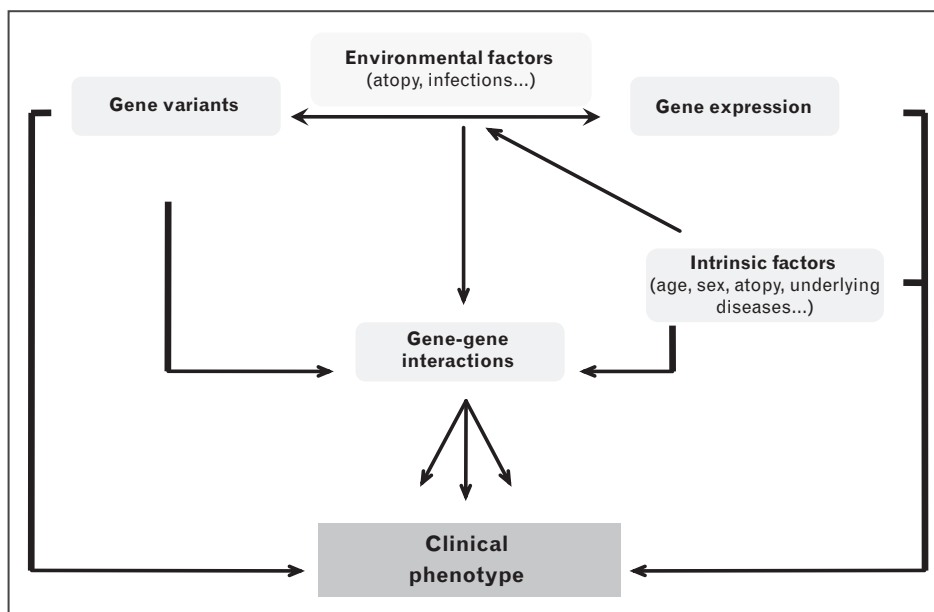


FIGURE 1. Potential factors involved in the pathogenesis of NSAIDs hypersensitivity.

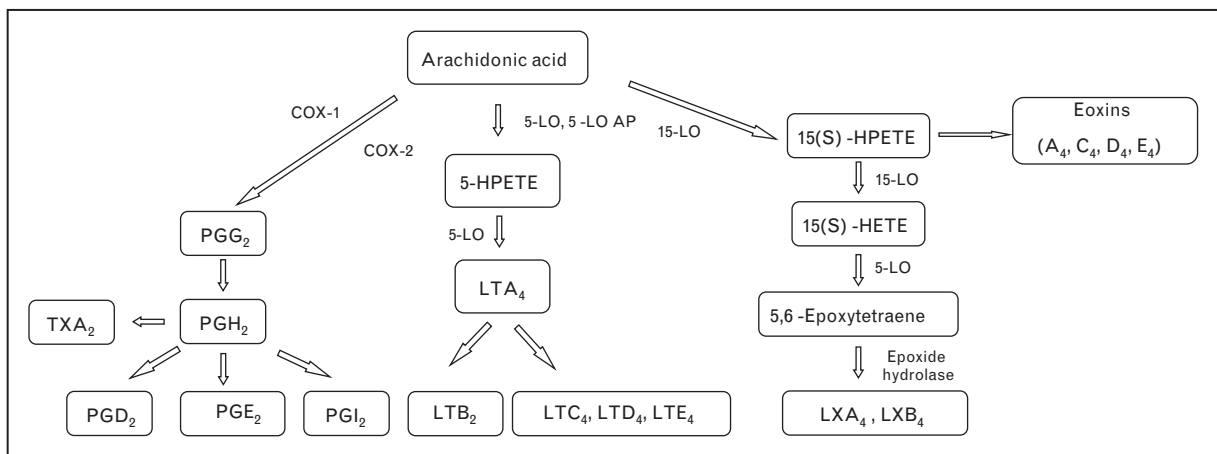


FIGURE 2. Arachidonic acid metabolism. COX, cyclooxygenase.

CANDIDATE GENE APPROACH

Arachidonic acid pathways

Initial genetic research on NSAIDs-HDRs focused on *LTC4S*, a key enzyme in arachidonic acid metabolism. The frequency of the minor allele (MAF) of *LTC4S* –444A>C polymorphism (rs730012) was higher in patients with NERD than in ASA-tolerant asthmatic (ATA) patients and healthy individuals [22], corresponding to increased *LTC4S* expression in peripheral eosinophils [22]. NERD patients have also shown increased *LTC4S* mRNA in bronchial biopsies [23,24]. A mouse model overexpressing *LTC4S* has shown increased production of T-helper type 2 cytokines in bronchoalveolar lavage fluid and an increased production of *LTC4* by mast cells, eosinophils, and macrophages even in the absence of NSAIDs, reproducing key aspects of NERD [25]. However, association with rs730012 and its functional consequences could not be replicated in American [26] or Asian populations [27,28]. In NSAIDs-HDRs with cutaneous involvement, MAF of rs730012 was also higher in a Polish population of patients with NECD [29]. MAF was also higher in urticaria patients compared with those with a mixed reaction pattern (both skin and respiratory airways involvement) in a Venezuelan population [30], but not in Spanish patients with NSAIDs-induced periorbital angioedema [31]. However, these studies included relatively small numbers of cases. We did not find any association between *LTC4S* –444A>C polymorphism and NIUA in a Spanish study [16] that included the largest number of such patients published to date.

Concerning arachidonate 5-lipoxygenase (*ALOX5*), a particular haplotype comprising four single nucleotide polymorphisms (SNPs) in the promoter region, was more frequent in Korean patients with NERD [28]. However, no evidence was found for

rs1132340 (*ALOX5*-activating protein), which we found in patients with NIUA [16]. Another study [18] of Spanish patients with NIUA analyzed the rs4948672 polymorphism in *ALOX5*; however, results were not significant after multiple testing correction. This study [18] also included a large population of patients with selective NSAID-induced urticaria and found that three SNPs in *ALOX5* were nominally associated. We have found a significant association between *ALOX15* rs7220870 (–272C>A) and NIUA in two independent Spanish populations [16], although different results were found in Korean patients [32].

Thromboxane (TBX) A1 synthase (*TBXAS1*) catalyzes the formation of TBXA₂, an eicosanoid responsible for platelet aggregation, bronchoconstriction, and bronchial hyperresponsiveness through its interaction with the G protein-coupled TBXA₂ receptor (*TBXA2R*) [33]. Vidal *et al.* [18] showed, for the first time, an association of a *TBXAS1* polymorphism (rs6962291) in patients with NIUA. The protective role of this variant, which leads to a reduction in catalytic activity, was also reported in NERD [34].

The receptors of arachidonic acid pathway products also appear to have a role in NSAIDs-HDRs [33]. Significant associations with NERD have been found for prostaglandin receptors *PTGER1*–4 and *PTGIR* [35,36]. We have also found associations with NIUA for two SNPs in *PGE1R* (rs3810253 and rs3810255) and rs1254598 in *PTGER2* [16], which were not found in East Asian patients with NERD [35]. A recent study [37] found the MAF of *PTGER4* –1254G>A to be higher in patients with NECD compared with controls. The minor allele showed reduced luciferase activity and decreased *PTGDR4* expression *in vitro* [37]. A polymorphism in *PGDR* (rs8004654) was shown to be significantly associated with NIUA in two independent Spanish populations, as it was in an American asthma study [38].

Three SNPs in the promoter of CysLT receptor 1 gene (*CYSLTR1*) (–634C>T, –475A>C and –336A>G), which affect its expression, have been associated with NERD by Kim *et al.* [39] in Koreans. We have also found a synonymous SNP in *CYSLTR1* (rs320995) to be associated with NIUA [16]. However, this polymorphism has been inconsistently associated with NERD, asthma and lung function in different studies [39–42], as well as urinary LTE4 in asthmatic patients [43]. Interestingly, this variant is in strong linkage disequilibrium with the promoter polymorphisms analyzed by Kim *et al.* [39] that affect gene transcription. Concerning *CYSLTR2*, some SNPs that affect its expression have been associated with NERD [44]. However, no *CYSLTR2* SNPs have been found to be associated with NIUA [16].

A significant association of the –4684T>C polymorphism on the promoter of *TBXA2R* in NIUA has recently been reported compared with controls but not to NECD [45]. However, associations have not been found for other *TBXA2R* SNPs in Spain [18]. Finally, MAF of *TBXA2R* +795T>C was found to be higher in NERD than ATA [46].

Immunoglobulin E receptor, histamine homeostasis-related genes, and cytokines

Immunoglobulin E high-affinity receptor (FcεRI) is a main player in mast cells/basophil activation and inflammatory mediators release, including histamine, cytokines, and leukotrienes [47]. FcεRI is a tetramer, comprising a ligand-binding α-chain, a signal-amplifying β-chain, and a γ-chain dimer involved in signal transduction. An association has been reported for *FCER1G* –237A>G in NERD, but this was not statistically significant after multiple testing corrections [48]. Another study [49] from the same group found no association for a number of SNPs in FcεRI, although *FCER1B* E237G and *FCER1G* –237A>G were associated with atopy within patients with NECD. MAF of *FCER1A* –344C>T was significantly higher in NECD compared with patients having chronic urticaria and good tolerance to ASA and healthy individuals, and was also significantly associated with total serum IgE [50]. However, a recent study [51], albeit of a small sample size, could not replicate these results.

Variants in histamine homeostasis genes may also play a role in NSAID-HDRs given the role of this biogenic amine [52–54]. Variability in the genes related to histamine homeostasis has been implicated in allergic disorders [55,56]. The histamine N-methyltransferase 939A>G polymorphism has been shown to be associated with NECD, potentially decreasing mRNA stability, causing reduced enzyme

activity and increased cellular histamine [57]. A recent study [58] from our group could not find associations for common variants in three histamine receptors with NSAIDs hypersensitivity. Nevertheless, we found that the missense polymorphism rs10156191 (Thr16Met) in diamine oxidase, which causes impaired metabolism of circulating histamine, was associated with NIUA, NERD, and a mixed reaction pattern [59]. Adenosine is a purine nucleotide that may influence mast cell activation and histamine release [60]. A high-transcript haplotype comprising two SNPs in the promoter of adenosine receptor A3 (–1050G>T and –564C>T) was more frequent in patients with urticaria induced by ASA compared with healthy individuals and basophil histamine release was also increased in TC carriers [61]. However, the authors did not differentiate between NIUA and NECD [61].

Interleukin-4 is a key cytokine involved in basophil/mast cell activation. A higher MAF of –589T>C in patients with NERD than ATA has been described, with a gene dose-dependent decline in forced expiratory volume in one second (FEV1) after challenge [62]. Homozygous genotypes for the common allele of the –1111C>T polymorphism in IL-13, a cytokine which shares signaling pathways and the α-chain of the IL4 receptor [63], have been shown to be less frequent in NERD than in ATA, and FEV1 was lower when compared with carriers of the minor allele [64]. Although no statistically significant differences could be found for two promoter SNPs in *IL-13* (–1510 A>C and –1055C>T) or the non-synonymous Arg110Gln (110G>A) polymorphisms between NERD, ATA, and healthy controls in a Korean population, homozygous patients with NERD for the common allele showed a significantly higher peripheral eosinophils count and higher serum eotaxin-1 level compared with homozygotes for the minor allele [65]. No association between these SNPs and NECD were found in a previous study [66] from the same group.

IL-5 and its receptor (IL-5R) influence eosinophil activation, a key feature of NERD. Patients with NERD having a homozygous genotype for the minor allele of the –5993G>A polymorphism in the α-chain of *IL-5R* have higher serum levels of specific IgE to staphylococcal enterotoxin A [67].

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine involved in various diseases. The minor allele of the –308 G>A SNP has been associated with ASA-HDRs in patients with chronic rhinosinusitis and nasal polyposis in a Hungarian population [68], although it was not associated with ASA-induced urticaria in Korean patients [69]. In the latter study [69], two SNPs in the promoter of *TNFA* (–1031T>C and –863C>A) were marginally

associated with NIUA when compared with healthy controls although no differences were found between NIUA and NECD. IL-10 is a cytokine with pleiotropic effects in immunoregulation and inflammation, whose levels have been shown to decrease in patients with NERD after ASA desensitization [70]. The *IL-10* polymorphism –819 T>C has shown association with NERD in a Korean population [71].

A polymorphism in IL-18 (–607A>C), a cytokine involved in acute inflammation through mast cell activation, has been associated with NIUA, and carriers of the minor allele along with the common allele of –137G>C have shown high transcription activity and neutrophil chemotactic activity [72].

Another proinflammatory cytokine is IL-17, which can stimulate COX-2 expression [73]. MAFs of three SNPs on the promoter of IL-17 receptor α -chain (–1075 A>G, –947 A>G, –50C>T) were lower in NERD patients than in ATA [74]. Interestingly, peripheral blood monocytes and mononuclear cells IL-17 receptor α -chain protein expression and mRNA levels were significantly lower in homozygote individuals for the minor allele, suggesting that these SNPs could decrease NERD risk by diminishing its expression [74]. A higher frequency of homozygotes for the common allele of *IL-17A* –737C>T was found in patients with NERD compared with ATA; these individuals showed lower peripheral eosinophil counts compared with arachidonic acid carriers [64]. Associations with other eosinophils-related molecules have been found in NERD for chemokine CC motif receptor 3 [57] and prostaglandin D2 receptor 2 [75].

HLA system

Although cross-intolerance to NSAIDs is not considered immunologically mediated, some *HLA* alleles associations have been reported. A seminal Polish study [76] found the frequency of DPB1*0301 to be higher in patients with NERD compared with ATA and healthy controls, whereas DPB1*0401 was lower. Similar results for DPB1*0301 in NERD were found in a Korean population [77], wherein this allele might also predict leukotriene receptor antagonist responsiveness [78]. Gene–gene interactions between a *TNFA* promoter polymorphism and HLA DPB1*0301 may also increase susceptibility to NERD [79]. The HLA DRB1*1302-DQB1*0609-DPB1*0201 haplotype has also been suggested as a marker for aspirin-induced urticaria by the same group although NIUA and NECD were not analyzed independently [80]. No association between polymorphisms in *CD55*, a key protein in self-recognition and autoimmunity and a central

component of the complement system, and NERD were found in a recent study [81].

MISCELLANEOUS ASSOCIATIONS

A number of genetic associations with different genes beyond the arachidonic acid pathway and the immune system have been reported over recent years. Rs17048175 in dipeptidyl-peptidase 10 (*DPP10*), a protein associated with asthma susceptibility, has been recently associated with NERD and a strong correlation between serum DPP10 and 15-hydroxyeicosatetraenoic acid levels was also reported [82[¶]].

The polymorphism 9246G>C and a particular haplotype of acetyl coenzyme A-dependent *N*-acetyltransferase, an enzyme involved in CysLTs inactivation, was associated with NERD in Korean patients [83]. NSAIDs are mainly metabolized by cytochrome P450 (CYP)2C9 *in vitro* and two polymorphisms in this gene with functional effects have been recently explored, finding that MAF of 681G>A and 636G>A were higher in patients with NERD compared with ATA [84]. Functional *N*-acetyltransferase and *CYP2C* polymorphisms have recently been analyzed in relation to metamizole metabolic profile [85^{¶¶}]. Associations with NERD have been also reported for SNPs in formyl peptide receptor 2, a high-affinity ligand receptor for lipoxins [86], heat shock protein 70 [87], and members of the rat sarcoma viral (RAS) oncogene family [88].

GENOME-WIDE ASSOCIATION STUDIES APPROACH

GWAS have been extensively used to identify new genes and pathways involved in common complex diseases by employing a free hypothesis approach. However, few studies have used this method to disentangle the genetic basis of NSAIDs hypersensitivity. The first GWAS was conducted in a Korean population of NERD patients [89]. The most significant associations were found for SNPs in centrosomal protein of 68 kDa (*CEP68*), involved in centrosome cohesion and epidermal growth factor signaling, and the nonsynonymous polymorphism rs7572857 (Gly74Ser) was associated with the FEV1 decline [89]. We have recently analyzed 53 *CEP68* common variants in a Spanish population of NSAIDs hypersensitivity patients including NIUA, NERD, and blended reactions [89]. Seventeen SNPs were associated with NIUA, including the Gly74Ser variant. Although not remaining significant after multiple testing corrections, eight of these variants were also associated with NERD and blended reactions [89]. These results suggest that *CEP68* variants

may play a key role in the development of various manifestations of NSAIDs hypersensitivity.

In another GWAS, these authors found the most significant association with NERD susceptibility for *HLA-DPB1* rs1042151 (Met105Val) that also showed a gene dose effect on FEV1 decrease after ASA challenge [90]. In-silico analysis suggested that this SNP could act as a *cis*-regulator for *HLA-DPB1* and be located in an exonic splicing enhancer region [90]. More recently, another polymorphism in *HLA-DPB1* (rs3128965), which is in perfect linkage disequilibrium with rs3128965, has been associated with NERD in Koreans, and with FEV1 reduction after challenge as well as with leukotriene receptor antagonist requirements [91].

Concerning NIUA, we have recently conducted a GWAS using both Spanish and Han Chinese patients [92]. We obtained suggestive associations for three clusters in the Spanish population (*RIMS1*, *BICC1*, and *RAD51L1*) and one region in the Han Chinese population (*ABI3BP*). The majority of the regions that showed suggestive associations after meta-analysis are related to Ca^{2+} , cAMP, and/or P53 signaling pathways [92].

BEYOND CANDIDATE GENE AND GENOME-WIDE ASSOCIATION STUDIES APPROACHES

High-throughput technologies have been used for not only GWAS purposes but also epigenetic, gene expression, and deep sequencing studies, which can be useful for disentangling the genetic mechanisms involved in NSAIDs hypersensitivity. In a recent genome-wide methylation study [93] on nasal polyps, a differential pattern was found in NERD compared with ATA, with *PGD synthase*, *ALOX5AP*, and *LTB4R* showing hypomethylation and *PGE synthase* hypermethylation. Eleven other pathways were shown to be differentially methylated, including some related to asthma, T-cell receptor signaling pathway, cytokine–cytokine receptor interactions, and leukocyte transendothelial migration [93].

A set of two gene markers identified from a microarray gene expression analysis of PBMCs has been recently proposed to discriminate NERD from ATA: connector enhancer of kinase suppressor of RAS3 (*CNKS3*) and spectrin β nonerythrocytic 2 (*SPTBN2*) [94], although their role in NSAIDs hypersensitivity needs further investigation.

More recently, exome sequencing has been used to identify new variants associated with NSAIDs hypersensitivity [95^{***}]. In this study, three of the top 20 SNPs were located in *HLA-DPB1*: exm537513, exm537522, and exm537523. The first one represents a novel SNP associated with NERD. The

exm537522 is the same as *HLA-DPB1* rs1042151 from the previous GWAS by the same authors [90].

CONCLUSION

Despite great interest in recent years in the genetics of NSAIDs hypersensitivity, there is still a lack of generalizable predictive genetic markers. However, a set of eight SNPs has been proposed for discriminating NERD from ATA with a sensitivity and specificity of 78 and 88%, respectively [96]. The same group recently developed other set by summing risk scores of 14 SNPs, with a sensitivity and specificity of 64.7 and 85.0%, respectively, and 42.1% positive and 93.4% negative predictive values [97]. However, further validation in other populations is required.

For further studies, bioinformatics tools may also be of interest to analyze how genetic variants affect gene expression in normal and pathologic states. We have recently used such an approach to analyze the putative function of genomic variants, taken from 1000 Genomes Project data, in the 5' upstream regions of *COX-1* and *COX-2* by measuring their influence on transcription factor binding and gene expression [98^{*}]. Other different SNPs with functional effects in these genes exist [99] and next-generation sequencing studies of these genes in patients with NSAIDs hypersensitivity are ongoing. Systems biology represents a powerful way to integrate the vast quantity of data from different studies [100,101] to shed new light on the pathognomonic mechanisms underlying drug hypersensitivity. There are now many examples that have integrated high-throughput technology-derived data using systems biology approaches to elucidate the functional networks involved in various diseases including NSAIDs hypersensitivity [102^{**}].

In addition to a more detailed definition of blended reactions, another key point in NSAIDs hypersensitivity is the role of atopy that has been highlighted by different authors [5,103,104]. We must elucidate whether this is because of a genetic link [48,50], some effect of allergens on COX-1 activity [105], or to the participation of an innate immune response [106], among other possibilities.

Acknowledgements

None.

Financial support and sponsorship

J.A. Cornejo-García is a researcher from the Miguel Servet Program (Ref CP14/00034), and J.R. Perkins from the Sara Borrell Program (Ref CD14/00242) (Carlos III National Health Institute, Spanish Ministry of Economy and Competitiveness).

This work has been supported by grants from the Carlos III Institute RD12/0013 (RIRAAF network), FIS PI12/02247, and FIS PI13/02598; Marie Curie (IAPP 7th Framework Program Mr. SymBioMath, no. 324554); and the Health Government of Andalusia (PI-0279-2012).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Papadopoulos NG, Agache I, Bavbek S, *et al.* Research needs in allergy: an EAACI position paper, in collaboration with EFA. *Clin Transl Allergy* 2012; 2:21.
2. Dona I, Blanca-Lopez N, Torres MJ, *et al.* Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J Investig Allergol Clin Immunol* 2012; 22:363–371.
3. Dona I, Barrionuevo E, Blanca-Lopez N, *et al.* Trends in hypersensitivity drug reactions: more drugs, more response patterns, more heterogeneity. *J Investig Allergol Clin Immunol* 2014; 24:143–153.
- A recent review analyzing drug hypersensitivity patterns, including those triggered by selective mechanisms and cross-intolerance to NSAIDs.
4. Kowalski ML, Asero R, Bavbek S, *et al.* Classification and practical approach to the diagnosis and management of hypersensitivity to nonsteroidal anti-inflammatory drugs. *Allergy* 2013; 68:1219–1232.
5. Dona I, Blanca-Lopez N, Cornejo-Garcia JA, *et al.* Characteristics of subjects experiencing hypersensitivity to nonsteroidal anti-inflammatory drugs: patterns of response. *Clin Exp Allergy* 2011; 41:86–95.
6. Canto MG, Andreu I, Fernandez J, Blanca M. Selective immediate hypersensitivity reactions to NSAIDs. *Curr Opin Allergy Clin Immunol* 2009; 9:293–297.
7. Stevenson DD, Szczeklik A. Clinical and pathologic perspectives on aspirin sensitivity and asthma. *J Allergy Clin Immunol* 2006; 118:773–786.
8. Campo P, Ayuso P, Salas M, *et al.* Mediator release after nasal aspirin provocation supports different phenotypes in subjects with hypersensitivity reactions to NSAIDs. *Allergy* 2013; 68:1001–1007.
9. Steinke JW, Liu L, Huyett P, *et al.* Prominent role of IFN-gamma in patients with aspirin-exacerbated respiratory disease. *J Allergy Clin Immunol* 2013; 132:856–865.
10. Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: a meta-analysis of the literature. *J Allergy Clin Immunol* 2014; 135:676–681.
- A very recent study analyzing the prevalence of aspirin-exacerbated respiratory disease (NSAIDs-exacerbated respiratory disease according to the last EAACI position paper).
11. Dona I, Blanca-Lopez N, Torres MJ, *et al.* NSAID-induced urticaria/angioedema does not evolve into chronic urticaria: a 12-year follow-up study. *Allergy* 2014; 69:438–444.
12. Asero R. Multiple nonsteroidal anti-inflammatory drug-induced cutaneous disease: what differentiates patients with and without underlying chronic spontaneous urticaria? *Int Arch Allergy Immunol* 2014; 163:114–118.
13. Szczeklik A, Nizankowska E, Duplaga M. Natural history of aspirin-induced asthma. *AIANE Investigators. European Network on aspirin-induced asthma. Eur Respir J* 2000; 16:432–436.
14. Mastalerz L, Setkowicz M, Sanak M, *et al.* Familial aggregation of aspirin-induced urticaria and leukotriene C synthase allelic variant. *Br J Dermatol* 2006; 154:256–260.
15. Caimmi SM, Manca E, Caimmi D, *et al.* NSAID hypersensitivity in twins. *Pediatr Allergy Immunol* 2014; 25:828–829.
- The authors presented a case of two twins suffering from the same type of hypersensitivity to NSAIDs, supporting a genetic influence in these reactions.
16. Cornejo-Garcia JA, Jagemann LR, Blanca-Lopez N, *et al.* Genetic variants of the arachidonic acid pathway in nonsteroidal anti-inflammatory drug-induced acute urticaria. *Clin Exp Allergy* 2012; 42:1772–1781.
17. Cornejo-Garcia JA, Flores C, Plaza-Seron MC, *et al.* Variants of CEP68 gene are associated with acute urticaria/angioedema induced by multiple nonsteroidal anti-inflammatory drugs. *PLoS One* 2014; 9:e90966.
- We examined 56 SNPs in CEP68, including the nonsynonymous Gly74Ser variant previously associated with NERD in Korea.

18. Vidal C, Porras-Hurtado L, Cruz R, *et al.* Association of thromboxane A1 synthase (TBXAS1) gene polymorphism with acute urticaria induced by nonsteroidal anti-inflammatory drugs. *J Allergy Clin Immunol* 2013; 132:989–991.
19. Kim SH, Sanak M, Park HS. Genetics of hypersensitivity to aspirin and nonsteroidal anti-inflammatory drugs. *Immunol Allergy Clin North Am* 2013; 33:177–194.
20. Shrestha Palikhe N, Kim SH, Jin HJ, *et al.* Genetic mechanisms in aspirin-exacerbated respiratory disease. *J Allergy (Cairo)* 2012; 79:4890.
21. Gueant JL, Gueant-Rodriguez RM, Gastin IA, *et al.* Pharmacogenetic determinants of immediate and delayed reactions of drug hypersensitivity. *Curr Pharm Des* 2008; 14:2770–2777.
22. Sanak M, Pierzchalska M, Bazan-Socha S, Szczeklik A. Enhanced expression of the leukotriene C(4) synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000; 23:290–296.
23. Sampson AP, Cowburn AS, Sladek K, *et al.* Profound overexpression of leukotriene C4 synthase in bronchial biopsies from aspirin-intolerant asthmatic patients. *Int Arch Allergy Immunol* 1997; 113:355–357.
24. Cowburn AS, Sladek K, Soja J, *et al.* Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998; 101:834–846.
25. Hirata H, Arima M, Fukushima Y, *et al.* Over-expression of the LTC4 synthase gene in mice reproduces human aspirin-induced asthma. *Clin Exp Allergy* 2011; 41:1133–1142.
26. Van Sambeek R, Stevenson DD, Baldasaro M, *et al.* 5' flanking region polymorphism of the gene encoding leukotriene C4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J Allergy Clin Immunol* 2000; 106 (1 Pt 1):72–76.
27. Kawagishi Y, Mita H, Taniguchi M, *et al.* Leukotriene C4 synthase promoter polymorphism in Japanese patients with aspirin-induced asthma. *J Allergy Clin Immunol* 2002; 109:936–942.
28. Choi JH, Park HS, Oh HB, *et al.* Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase. *Hum Genet* 2004; 114:337–344.
29. Mastalerz L, Setkowicz M, Sanak M, Szczeklik A. Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. *J Allergy Clin Immunol* 2004; 113:771–775.
30. Sanchez-Borges M, Acevedo N, Vergara C, *et al.* The A-444C polymorphism in the leukotriene C4 synthase gene is associated with aspirin-induced urticaria. *J Investig Allergol Clin Immunol* 2009; 19:375–382.
31. Torres-Galvan MJ, Ortega N, Sanchez-Garcia F, *et al.* LTC4-synthase A-444C polymorphism: lack of association with NSAID-induced isolated periorbital angioedema in a Spanish population. *Ann Allergy Asthma Immunol* 2001; 87:506–510.
32. Lee HY, Kim SH, Ye YM, *et al.* Lack of association of ALOX12 and ALOX15 polymorphisms with aspirin-exacerbated respiratory disease in Korean patients. *Ann Allergy Asthma Immunol* 2009; 103:84–86.
33. Honda T, Kabashima K. Prostanoids in allergy. *Allergol Int* 2015; 64:11–16.
34. Oh SH, Kim YH, Park SM, *et al.* Association analysis of thromboxane A synthase 1 gene polymorphisms with aspirin intolerance in asthmatic patients. *Pharmacogenomics* 2011; 12:351–363.
35. Kim SH, Kim YK, Park HW, *et al.* Association between polymorphisms in prostanoid receptor genes and aspirin-intolerant asthma. *Pharmacogenet Genomics* 2007; 17:295–304.
36. Park BL, Park SM, Park JS, *et al.* Association of PTGER gene family polymorphisms with aspirin intolerant asthma in Korean asthmatics. *BMB Rep* 2010; 43:445–449.
37. Palikhe NS, Sin HJ, Kim SH, *et al.* Genetic variability of prostaglandin E2 receptor subtype EP4 gene in aspirin-intolerant chronic urticaria. *J Hum Genet* 2012; 57:494–499.
38. Oguma T, Palmer LJ, Birben E, *et al.* Role of prostanoid DP receptor variants in susceptibility to asthma. *N Engl J Med* 2004; 351:1752–1763.
39. Kim SH, Oh JM, Kim YS, *et al.* Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. *Clin Exp Allergy* 2006; 36:433–439.
40. Arriba-Mendez S, Sanz C, Isidoro-Garcia M, *et al.* Analysis of 927T > C CYSLTR1 and –444A > C LTC4S polymorphisms in children with asthma. *Allergol Immunopathol (Madr)* 2008; 36:259–263.
41. Hong X, Zhou H, Tsai HJ, *et al.* Cysteinyl leukotriene receptor 1 gene variation and risk of asthma. *Eur Respir J* 2009; 33:42–48.
42. Arriba-Mendez S, Sanz C, Isidoro-Garcia M, *et al.* 927T>C polymorphism of the cysteinyl-leukotriene type-1 receptor (CYSLTR1) gene in children with asthma and atopic dermatitis. *Pediatr Allergy Immunol* 2006; 17:323–328.
43. Bizzintino JA, Khoo SK, Zhang G, *et al.* Leukotriene pathway polymorphisms are associated with altered cysteinyl leukotriene production in children with acute asthma. *Prostaglandins Leukot Essent Fatty Acids* 2009; 81:9–15.
44. Park JS, Chang HS, Park CS, *et al.* Association analysis of cysteinyl-leukotriene receptor 2 (CYSLTR2) polymorphisms with aspirin intolerance in asthmatics. *Pharmacogenet Genomics* 2005; 15:483–492.
45. Palikhe NS, Kim SH, Lee HY, *et al.* Association of thromboxane A2 receptor (TBXA2R) gene polymorphism in patients with aspirin-intolerant acute urticaria. *Clin Exp Allergy* 2011; 41:179–185.

46. Kohyama K, Hashimoto M, Abe S, *et al*. Thromboxane A2 receptor +795T>C and chemoattractant receptor-homologous molecule expressed on Th2 cells -466T>C gene polymorphisms in patients with aspirin-exacerbated respiratory disease. *Mol Med Rep* 2012; 5:477–482.
 47. Dema B, Suzuki R, Rivera J. Rethinking the role of immunoglobulin E and its high-affinity receptor: new insights into allergy and beyond. *Int Arch Allergy Immunol* 2014; 164:271–279.
 48. Palikhe NS, Kim SH, Cho BY, *et al*. Association of three sets of high-affinity IgE receptor (FcεpsilonR1) polymorphisms with aspirin-intolerant asthma. *Respir Med* 2008; 102:1132–1139.
 49. Palikhe N, Kim SH, Yang EM, *et al*. Analysis of high-affinity IgE receptor (FcεpsilonR1) polymorphisms in patients with aspirin-intolerant chronic urticaria. *Allergy Asthma Proc* 2008; 29:250–257.
 50. Bae JS, Kim SH, Ye YM, *et al*. Significant association of FcεpsilonR1alpha promoter polymorphisms with aspirin-intolerant chronic urticaria. *J Allergy Clin Immunol* 2007; 119:449–456.
 51. Hsieh CW, Lee JW, Liao EC, Tsai JJ. A disease marker for aspirin-induced chronic urticaria. *Int J Mol Sci* 2014; 15:12591–12603.
 52. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. *Nat Rev Immunol* 2014; 14:478–494.
 53. Garcia-Martin E, Ayuso P, Martinez C, *et al*. Histamine pharmacogenomics. *Pharmacogenomics* 2009; 10:867–883.
 54. Cornejo-Garcia JA, Blanca-Lopez N, Dona I, *et al*. Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs. *Curr Drug Metab* 2009; 10:971–980.
 55. Garcia-Martin E, Garcia-Menaya J, Sanchez B, *et al*. Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis. *Clin Exp Allergy* 2007; 37:1175–1182.
 56. Gervasini G, Agundez JA, Garcia-Menaya J, *et al*. Variability of the L-histidine decarboxylase gene in allergic rhinitis. *Allergy* 2010; 65:1576–1584.
 57. Kim SH, Kang YM, Kim SH, *et al*. Histamine N-methyltransferase 939A>G polymorphism affects mRNA stability in patients with acetylsalicylic acid-intolerant chronic urticaria. *Allergy* 2009; 64:213–221.
 58. Ayuso P, Blanca M, Cornejo-Garcia JA, *et al*. Variability in histamine receptor genes H1RH1, H1RH2 and H1RH4 in patients with hypersensitivity to NSAIDs. *Pharmacogenomics* 2013; 14:1871–1878.
 59. Agundez JA, Ayuso P, Cornejo-Garcia JA, *et al*. The diamine oxidase gene is associated with hypersensitivity response to nonsteroidal anti-inflammatory drugs. *PLoS One* 2012; 7:e47571.
 60. Yip KH, Lau HY, Wise H. Reciprocal modulation of anti-IgE induced histamine release from human mast cells by A(1) and A(2B) adenosine receptors. *Br J Pharmacol* 2011; 164:807–819.
 61. Kim SH, Nam EJ, Kim YK, *et al*. Functional variability of the adenosine A3 receptor (ADORA3) gene polymorphism in aspirin-induced urticaria. *Br J Dermatol* 2010; 163:977–985.
 62. Kim BS, Park SM, Uhm TG, *et al*. Effect of single nucleotide polymorphisms within the interleukin-4 promoter on aspirin intolerance in asthmatics and interleukin-4 promoter activity. *Pharmacogenet Genomics* 2010; 20:748–758.
 63. Erle DJ, Sheppard D. The cell biology of asthma. *J Cell Biol* 2014; 205:621–631.
 64. Kohyama K, Abe S, Kodaira K, *et al*. IL-13 and IL-17A gene polymorphisms in Japanese patients with aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol* 2011; 107:510–516.
 65. Palikhe NS, Kim SH, Cho BY, *et al*. IL-13 gene polymorphisms are associated with rhinosinusitis and eosinophilic inflammation in aspirin intolerant asthma. *Allergy Asthma Immunol Res* 2010; 2:134–140.
 66. Palikhe NS, Kim SH, Choi GS, *et al*. No evidence of association between interleukin-13 gene polymorphism in aspirin intolerant chronic urticaria. *Allergy Asthma Immunol Res* 2009; 1:36–40.
 67. Losol P, Kim SH, Shin YS, *et al*. A genetic effect of IL-5 receptor alpha polymorphism in patients with aspirin-exacerbated respiratory disease. *Exp Mol Med* 2013; 45:e14.
 68. Szabo K, Kiricsi A, Revesz M, *et al*. The -308 G>A SNP of TNFA is a factor predisposing to chronic rhinosinusitis associated with nasal polyposis in aspirin-sensitive Hungarian individuals: conclusions of a genetic study with multiple stratifications. *Int Immunol* 2013; 25:383–388.
 69. Choi JH, Kim SH, Cho BY, *et al*. Association of TNF-alpha promoter polymorphisms with aspirin-induced urticaria. *J Clin Pharm Ther* 2009; 34:231–238.
 70. Aksu K, Kurt E, Alatas O, Gulbas Z. Effect of aspirin desensitization on T-cell cytokines and plasma lipoxins in aspirin-exacerbated respiratory disease. *Allergy Asthma Proc* 2014; 35:148–155.
 71. Palikhe NS, Kim SH, Jin HJ, *et al*. Association of interleukin 10 promoter polymorphism at -819 T>C with aspirin-induced urticaria in a Korean population. *Ann Allergy Asthma Immunol* 2011; 107:544–546.
 72. Kim SH, Son JK, Yang EM, *et al*. A functional promoter polymorphism of the human IL18 gene is associated with aspirin-induced urticaria. *Br J Dermatol* 2011; 165:976–984.
 73. Li Q, Liu L, Zhang Q, *et al*. Interleukin-17 indirectly promotes M2 macrophage differentiation through stimulation of COX-2/PGE2 pathway in the cancer cells. *Cancer Res Treat* 2014; 46:297–306.
 74. Park JS, Park BL, Kim MO, *et al*. Association of single nucleotide polymorphisms on interleukin 17 receptor A (IL17RA) gene with aspirin hypersensitivity in asthmatics. *Hum Immunol* 2013; 74:598–606.
 75. Palikhe NS, Kim SH, Cho BY, *et al*. Genetic variability in CRTH2 polymorphism increases eotaxin-2 levels in patients with aspirin exacerbated respiratory disease. *Allergy* 2010; 65:338–346.
 76. Dekker JW, Nizankowska E, Schmitz-Schumann M, *et al*. Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes. *Clin Exp Allergy* 1997; 27:574–577.
 77. Choi JH, Lee KW, Oh HB, *et al*. HLA association in aspirin-intolerant asthma: DPB1*0301 as a strong marker in a Korean population. *J Allergy Clin Immunol* 2004; 113:562–564.
 78. Park HS, Kim SH, Sampson AP, *et al*. The HLA-DPB1*0301 marker might predict the requirement for leukotriene receptor antagonist in patients with aspirin-intolerant asthma. *J Allergy Clin Immunol* 2004; 114:688–689.
 79. Kim SH, Ye YM, Lee SK, *et al*. Association of TNF-alpha genetic polymorphism with HLA DPB1*0301. *Clin Exp Allergy* 2006; 36:1247–1253.
 80. Kim SH, Choi JH, Lee KW, *et al*. The human leucocyte antigen-DRB1*1302-DQB1*0609-DPB1*0201 haplotype may be a strong genetic marker for aspirin-induced urticaria. *Clin Exp Allergy* 2005; 35:339–344.
 81. Lee JS, Bae JS, Kim JH, *et al*. CD55 polymorphisms and risk of aspirin-exacerbated respiratory disease. *Mol Med Rep* 2012; 6:1087–1092.
 82. Kim SH, Choi H, Yoon MG, *et al*. Dipeptidyl-peptidase 10 as a genetic biomarker for the aspirin-exacerbated respiratory disease phenotype. *Ann Allergy Asthma Immunol* 2015; 114:208–213.
- The authors suggested that DPP10, previously associated with asthma susceptibility and with some effect in lung function, may be a genetic biomarker for NERD.
83. Kim JM, Park BL, Park SM, *et al*. Association analysis of N-acetyl transferase-2 polymorphisms with aspirin intolerance among asthmatics. *Pharmacogenomics* 2010; 11:951–958.
 84. Kohyama K, Abe S, Kodaira K, *et al*. Polymorphisms of the CYP2C19 gene in Japanese patients with aspirin-exacerbated respiratory disease. *J Allergy Clin Immunol* 2011; 128:1117–1120.
 85. Martinez C, Andreu I, Amo G, *et al*. Gender and functional CYP2C and NAT2 polymorphisms determine the metabolic profile of metamizole. *Biochem Pharmacol* 2014; 92:457–466.
- This study identified phenotypic and genetic factors associated with metamizole biotransformation and toxicity, which may be of interest for analyzing drug response, including hypersensitivity reactions.
86. Kim HJ, Cho SH, Park JS, *et al*. Association analysis of formyl peptide receptor 2 (FPR2) polymorphisms and aspirin exacerbated respiratory diseases. *J Hum Genet* 2012; 57:247–253.
 87. Kikuchi K, Abe S, Kodaira K, *et al*. Heat shock protein 70 gene polymorphisms in Japanese patients with aspirin-exacerbated respiratory disease. *J Invest Med* 2013; 61:708–714.
 88. Park JS, Heo JS, Chang HS, *et al*. Association analysis of member RAS oncogene family gene polymorphisms with aspirin intolerance in asthmatic patients. *DNA Cell Biol* 2014; 33:155–161.
 89. Kim JH, Park BL, Cheong HS, *et al*. Genome-wide and follow-up studies identify CEP68 gene variants associated with risk of aspirin-intolerant asthma. *PLoS One* 2010; 5:e13818.
 90. Park BL, Kim TH, Kim JH, *et al*. Genome-wide association study of aspirin-exacerbated respiratory disease in a Korean population. *Hum Genet* 2013; 132:313–321.
 91. Kim SH, Cho BY, Choi H, *et al*. The SNP rs3128965 of HLA-DPB1 as a genetic marker of the AERD phenotype. *PLoS One* 2014; 9:e111220.
 92. Cornejo-Garcia JA, Liou LB, Blanca-Lopez N, *et al*. Genome-wide association study in NSAID-induced acute urticaria/angioedema in Spanish and Han Chinese populations. *Pharmacogenomics* 2013; 14:1857–1869.
 93. Cheong HS, Park SM, Kim MO, *et al*. Genome-wide methylation profile of nasal polyps: relation to aspirin hypersensitivity in asthmatics. *Allergy* 2011; 66:637–644.
 94. Shin S, Park JS, Kim YJ, *et al*. Differential gene expression profile in PBMCs from subjects with AERD and ATA: a gene marker for AERD. *Mol Genet Genomics* 2012; 287:361–371.
 95. Shin SW, Park BL, Chang H, *et al*. Exonic variants associated with development of aspirin exacerbated respiratory diseases. *PLoS One* 2014; 9:e111887.
- A very interesting study analyzing the role of exonic variants in NERD. The authors proposed a combination model of seven SNPs as a useful genetic marker combination for predicting NERD.
96. Shin SW, Park J, Kim YJ, *et al*. A highly sensitive and specific genetic marker to diagnose aspirin-exacerbated respiratory disease using a genome-wide association study. *DNA Cell Biol* 2012; 31:1604–1609.
 97. Chang HS, Shin SW, Lee TH, *et al*. Development of a genetic marker set to diagnose aspirin-exacerbated respiratory disease in a genome-wide association study. *Pharmacogenomics J* 2015; doi: 10.1038/tpj.2014.78. [Epub ahead of print]
 98. Agundez JA, Gonzalez-Alvarez DL, Vega-Rodriguez MA, *et al*. Gene variants and haplotypes modifying transcription factor binding sites in the human cyclooxygenase 1 and 2 (PTGS1 and PTGS2) genes. *Curr Drug Metab* 2014; 15:182–195.
- A good example for addressing how bioinformatic tools can help to identify functional SNPs in COX-1 and 2.

99. Agundez JA, Blanca M, Cornejo-García JA, García-Martin E. Pharmacogenomics of cyclooxygenases. *Pharmacogenomics* 2015; 16:501–522.
 100. Perkins JR, Barrionuevo E, Ranea JA, *et al.* Systems biology approaches to enhance our understanding of drug hypersensitivity reactions. *Clin Exp Allergy* 2014; 44:1461–1472.
 101. Perkins JR, Ayuso P, Cornejo-García JA, Ranea JA. The study of severe cutaneous drug hypersensitivity reactions from a systems biology perspective. *Curr Opin Allergy Clin Immunol* 2014; 14:301–306.
 102. Perkins JR, Sanak M, Canto G, *et al.* Unravelling adverse reactions to NSAIDs ■■ using systems biology. *Trends Pharmacol Sci* 2015; 36:172–180.
- A recent study that showed the potential use of systems biology to disentangle the underlying mechanisms of NSAIDs hypersensitivity by analyzing current data sets.
103. Sanchez-Borges M, Capriles-Hulett A. Atopy is a risk factor for nonsteroidal anti-inflammatory drug sensitivity. *Ann Allergy Asthma Immunol* 2000; 84:101–106.
 104. Sanchez-Borges M, Acevedo N, Caraballo L, *et al.* Increased total and mite-specific immunoglobulin E in patients with aspirin-induced urticaria and angioedema. *J Investig Allergol Clin Immunol* 2010; 20:139–145.
 105. Barrett NA, Maekawa A, Rahman OM, *et al.* Dectin-2 recognition of house dust mite triggers cysteinyl leukotriene generation by dendritic cells. *J Immunol* 2009; 182:1119–1128.
 106. Trompette A, Divanovic S, Visintin A, *et al.* Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009; 457:585–588.