

Unravelling adverse reactions to NSAIDs using systems biology

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We introduce the reader to systems biology, using adverse drug reactions (ADRs), specifically hypersensitivity reactions to multiple non-steroidal anti-inflammatory drugs (NSAIDs), as a model. To disentangle the different processes that contribute to these reactions – from drug intake to the appearance of symptoms – it will be necessary to create high-throughput datasets. Just as crucial will be the use of systems biology to integrate and make sense of them. We review previous work using systems biology to study related pathologies such as asthma/allergy, and NSAID metabolism. We show examples of their application to NSAIDs-hypersensitivity using current datasets. We describe breakthroughs in high-throughput technology and speculate on their use to improve our understanding of this and other drug-induced pathologies.

Introduction

Systems biology is gaining traction as a method to piece together the large datasets produced by high-throughput technologies such as microarrays and next-generation sequencing. It has been applied to the study of immunology and allergy, particularly as they relate to asthma [1–3] and allergen sensitisation [4]. Here we describe systems biology and its potential utility for the study of ADRs, focussing on hypersensitivity reactions to NSAIDs, giving examples of how it has been used to study arachidonic acid-related pathways and related pathologies. We describe what is currently known about the underlying mechanisms, highlighting the phenotypic and genetic complexity. We then present examples of how systems biology techniques can integrate high-throughput NSAIDs-hypersensitivity drug reaction (HDR) datasets to uncover putative new mechanisms. Drawing on these, we speculate on future directions for the field in light of emerging technologies, the type of data they generate, and how this data can be fully

exploited. Finally, we provide some remarks about how we expect the field to develop over the next few years.

The study of NSAIDs-hypersensitivity will benefit strongly from systems biology analysis due to its complexity in terms of clinical presentations and underlying mechanisms [5,6]. The utility and widespread use of NSAIDs, along with the potentially life threatening reactions they can cause, warrant such investigation and the generation of datasets [7].

Systems biology

We define systems biology as an approach to integrate various datasets, encompassing data mining, bioinformatics, and network biology techniques, with the overarching aim to model the relationships between different entities (e.g., genes, metabolites, medicines, and diseases [8,9]). This fits in with the descriptive, data-driven approach of Arnon Arazi and colleagues [10]. They also proposed a ‘hypothesis-based modelling’ definition for systems biology, in which expert knowledge and domain-specific measurements are combined to produce a mathematical model, from which hypotheses can be tested and compared to empirical data [10]. Below we give some examples of these two approaches.

Data-driven systems biology

Data-driven systems biology is the analysis of large datasets with no precise hypothesis in mind, although some knowledge of the processes being studied is necessary to guide the approach [11]. Data often comes from high-throughput experiments, such as RNA-seq experiments that compare gene expression between tissue samples from patients and controls, or animals in which a specific disease model has been invoked. Further data can also be obtained from databases such as Online Mendelian Inheritance in Man (OMIM) (<http://www.omim.org/>) [12], which contains genes associated with various Mendelian and complex diseases, or the Gene Ontology database (<http://geneontology.org/>) [13], which provides annotation on gene function. The latter is particularly useful to search for the overrepresentation of genes involved in a particular function/process in a list of genes; such lists often arise in systems biology experiments.

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An example of such an approach is provided by Hwang *et al.* [2] who obtained asthma-associated genes from OMIM and genes that were differentially expressed in asthma from the Gene Expression Omnibus database [14], a repository of gene expression datasets. They used these genes to build an asthma-related protein interaction network, where each gene is a network node, and edges were drawn between these nodes if the proteins corresponding to these genes interact (according to the Human Protein Reference Database <http://www.hprd.org/> [15]). The idea is that genes with a high connectivity to other nodes in the network are crucial to its functioning and thus potential targets for the study of asthma. The networks were assessed visually and used to find highly connected genes by calculating various metrics for each gene (e.g., through network degree, which is the number of interactions in the network in which a given gene is involved). By using annotation data from the gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to elucidate the function of the interaction partners of these important genes, the authors found putative novel asthma genes such as *GNB2L1* (guanine nucleotide binding protein, beta polypeptide 2-like 1) [13,16].

A similar example is provided by Renkonen *et al.* who performed a study of allergy using a protein interaction network, built by combining protein–protein interactions involving allergy-related genes taken from genetic studies [17]. Proteins were included in the network, along with their interaction partners, if they had at least two interactions. Expression data obtained from nasal epithelial cells of birch pollen-allergic patients was used to validate the network: almost a tenth of the genes in the network showed differential expression. The function of the genes in the network was analysed and found to be enriched in protein kinases and genes involved in virus–host interactions. This study highlights how network analysis has the potential to give novel insights into the processes underpinning a given pathology.

Hypothesis-based systems biology

One such application of the ‘hypothesis-based modelling’ approach has been to investigate the antitumour effects of NSAIDs by modelling their effects on various cancer hallmarks [18]. The authors used high-throughput gene expression data, drug response information, data-mining, and statistical methodology to model a system and make testable predictions. They built a complex model, dubbed the ‘NSAID model’, by combining pathway data from various sources. More specifically, they took the cyclooxygenase (COX) pathway (the target of NSAIDs), which begins with arachidonic acid and results in a number of prostaglandin molecules. They then took the signalling pathways invoked by the binding of these prostaglandins to their receptors, and further data on the downstream signalling pathways. They used an *in silico* methodology, flux-comparative-analysis, to analyse the effects of NSAIDs on tumour progression by propagating input data throughout the network to establish a flux value for each of its components. This input data was gene expression data from a control state (tumour cells) and a perturbation state (tumour cells plus intervention, specifically a COX-2 inhibitor); the effects on

cancer progression were defined by ‘cancer hallmarks’ – the flux of specific network components that served biomarkers for cancer-related processes: angiogenesis, evasion of apoptosis, proliferation, and tissue invasion. They identified potential targets and biomarkers for further study. Such an approach could be adapted to the study of ADRs, for example, to compare samples from someone with NSAIDs-hypersensitivity to a control patient following drug intake, by changing the outcome measurements (the cancer hallmarks) to other systems, such as basophil activation or lipid profiles in urine/blood plasma [92]. This could inform us about potential differences between different NSAID drugs in terms of signalling activation, and crucially could provide clues about downstream signalling pathways affected in NSAIDs-hypersensitive patients. Examples of systems biology approaches to study the immune system can be found in recent reviews [10,11,19].

NSAIDs adverse reactions and hypersensitivity

ADRs are noxious unintended responses to a drug occurring at therapeutic doses [20]. They can be classified as predictable, dosage-related side effects (Type A), and unpredictable, non-dose-related reactions in susceptible individuals (Type B) [21]. The latter includes HDRs, an important health problem due to the large number of patients affected and difficulties in their identification [22].

NSAIDs are a group of compounds with heterogeneous chemical structures that have high efficacy for the treatment of pain and inflammation [23]. However, they are also associated with a wide variety of adverse reactions, being the leading cause of HDRs [24]. They are caused by the release of histamine or other vasoactive mediators and can be loosely grouped into two categories: immune-mediated and non-immune-mediated [6]. The former are induced by a single NSAID (selective reactions), and involve specific IgE antibodies or T cells; the patient will show tolerance to NSAIDs from different chemical groups [6,25]. By contrast, non-immune mediated reactions are induced by NSAIDs with different chemical structures (cross-intolerance). Their underlying proposed mechanism is through COX-1 inhibition, leading to a deviation in the arachidonic acid metabolic pathways from the synthesis of prostaglandins to cysteinyl-leukotrienes (CysLTs) [6,23,26]. NSAIDs-exacerbated respiratory disease (NERD) has been more highly studied, however NSAIDs-induced urticaria/angioedema (NIUA) is now considered the most frequent clinical entity induced by HDRs [24,27].

Although the risk factors associated with NIUA/NERD have not been clearly established, atopy could be key. Originally described over 40 years ago [28,29], recent studies have reported associations between NIUA patients and house dust mite allergy [30–33]. We recently found that atopy was present in 60% of cases with NIUA/NERD [27]. Moreover, the allergens involved differed depending on the clinical entity: individuals with NIUA showed a higher frequency of positive skin tests to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* than those with NERD [27].

The factors underlying the association between atopy and NIUA/NERD are unknown. Atopic diseases (eczema/rhinitis/asthma) are mediated by specific IgE antibodies,

whereas NIUA/NERD are related to COX-1 inhibition and the release of CysLTs (LTC4, LTD4, and LTE4) [34]. However, genetic variants in the IgE receptor have been associated with NIUA/NERD [35–37]. Leukotriene C4 synthase (LTC4S), a key enzyme in CysLT synthesis, is located on chromosome 5q35 [38,39], in close proximity to the cluster of genes for cytokines orchestrating allergic inflammation (*IL3*, *IL4*, *IL5*, *IL9*, *IL13*, and *CSF2*) [40]. In addition, *CYSLTR2*, one of the two CysLT receptors identified in humans, maps to chromosome 13q14 [41], in consistent linkage with atopy and total serum IgE concentrations [42]. Furthermore, variants in both *LTC4S* and *CYSLTR2* have been shown to be associated with atopic diseases [43–46].

In addition to its genetic association with atopy, IL4, a key cytokine in IgE synthesis, induces the expression of LTC4S in mast cells [47] and CysLTR1 in several cell types [48,49]. The effect of *D. pteronyssinus* and *D. farinae* allergens on COX-1, the release of CysLTs [50], and the elicitation of an innate immune response through the stimulation of dendritic cells and cytokine production [51] or toll-like receptor pathway activation [52] may also contribute.

Current high-throughput NSAIDs-hypersensitivity data

Several high-throughput studies related to NSAIDs-hypersensitivity have been performed. At the genetic level, genome-wide association studies (GWAS) have been performed using SNP arrays for Spanish and Han Chinese [53], and Korean populations [54,55]. Gene expression data also exists: microarray experiments profiling expression in peripheral blood mononuclear cells and nasal polyps between NERD patients and control subjects [56,57]. At the epigenetic level, microarrays and bisulfite technology have been used to measure genomic DNA methylation in nasal polyps of NERD patients [58]. Smaller scale candidate studies have also found genetic associations with NSAIDs-HDRs (reviewed in [59,60]). Below we provide examples of how these data could be integrated.

Applications of systems biology to NSAIDs-hypersensitivity analysis

Uncovering mutations in noncoding genomic regions

Approximately 88% of disease-associated SNPs found by recent GWAS are found in intergenic/intronic, noncoding genomic regions [61]. These regions, once assumed to be of little functional importance, are sparsely annotated compared with exons. However, this view has changed dramatically in recent years, and a number of functions can now be ascribed to variations in these regions [62], as well as a strong contribution to disease through various mechanisms [63]. A recent large-scale collaborative effort, the ENCODE project, used various high-throughput technologies to probe the entire genome for evidence of potential function, including areas of active transcription, transcription factor (TF) binding, open chromatin, and epigenetic modification [64]. Although this data was produced using cell lines and the regions found are not necessarily physiologically relevant in all tissues, nevertheless it can provide clues that allow us to better understand the impact of NSAIDs-HDR-associated variants: for example, the variants might map to TF binding sites, in which case we can

hypothesise that they may affect TF binding affinity, leading to altered gene expression. Similarly, the allele could map to an area involved in histone modification, affecting chromatin state and thus gene regulation. Figure 1 presents an example of such variants, the SNPs rs849530 and rs861078, which showed suggestive associations with NERD according to an initial GWAS; this association was also found in a subsequent follow-up study in a separate group of patients [54]. These SNPs are found in an intronic region of the gene *NRP2* (neuropilin 2; Figure 1, left). Interestingly, this gene also shows threefold decreased expression in peripheral blood mononuclear cells of NERD patients compared to aspirin-tolerant asthmatic patients [56] (Figure 1, right). The associated SNPs are close to a genomic region containing a number of potential regulatory elements, including areas of open chromatin, methylation, histone acetylation, and a potential CpG island. Taken together, these data suggest that the SNPs might be in linkage disequilibrium with a functional SNP that affects some regulatory event, such as protein–DNA binding, and through this process the regulation of gene expression. Protein–RNA binding may also be affected by these SNPs: they are found in intronic regions, which are transcribed and can be targeted by RNA binding proteins in order to control alternative splicing [62,65]. If these variants map to regions targeted by RNA-binding proteins (RNA recognition elements) and disrupt their binding, this may lead to a dysregulation of splicing, producing alternative splice variants. This in turn can lead to a different function for the encoded protein, assuming it is even translated, resulting in various downstream effects. MicroRNAs (miRNAs) can also bind transcribed RNA and altered miRNA binding sites have also been linked to disease [66]. Further analysis, for example, by examining other tissues and using recent, higher resolution sequencing technologies as described in Box 1, is required to determine the precise function of the variant. By combining this SNP with data from efforts such as the 1000 Genome Project, a recent and ongoing study to uncover genetic variation in different populations through the whole genome sequencing of many individuals [67], it may be possible to impute further variants [68], providing new clues to help us understand its contribution to NSAIDs-hypersensitivity.

Using graphical models to visualise relationships between genes

As mentioned above, a strong and intriguing association has been reported between NSAIDs-hypersensitivity and atopy [27]. Graphical models can be used to visualise known relationships between the genes involved in these conditions, such as interactions or coexpression [69]. We constructed a protein interaction network from public resources [70,71] to explore NSAIDs-HDR-related genes, adapting the methodology used by Renkonen *et al.* [17] described above. In our network, the nodes represent proteins and edges represent interactions. These include not only direct physical interactions taken from iRefindex [71], but also functional relationships as defined by the STRING resource [70]. This network was then filtered using genes associated with NERD and NIUA, taken from recent reviews by Kim *et al.* [59] and Park *et al.* [60]. These

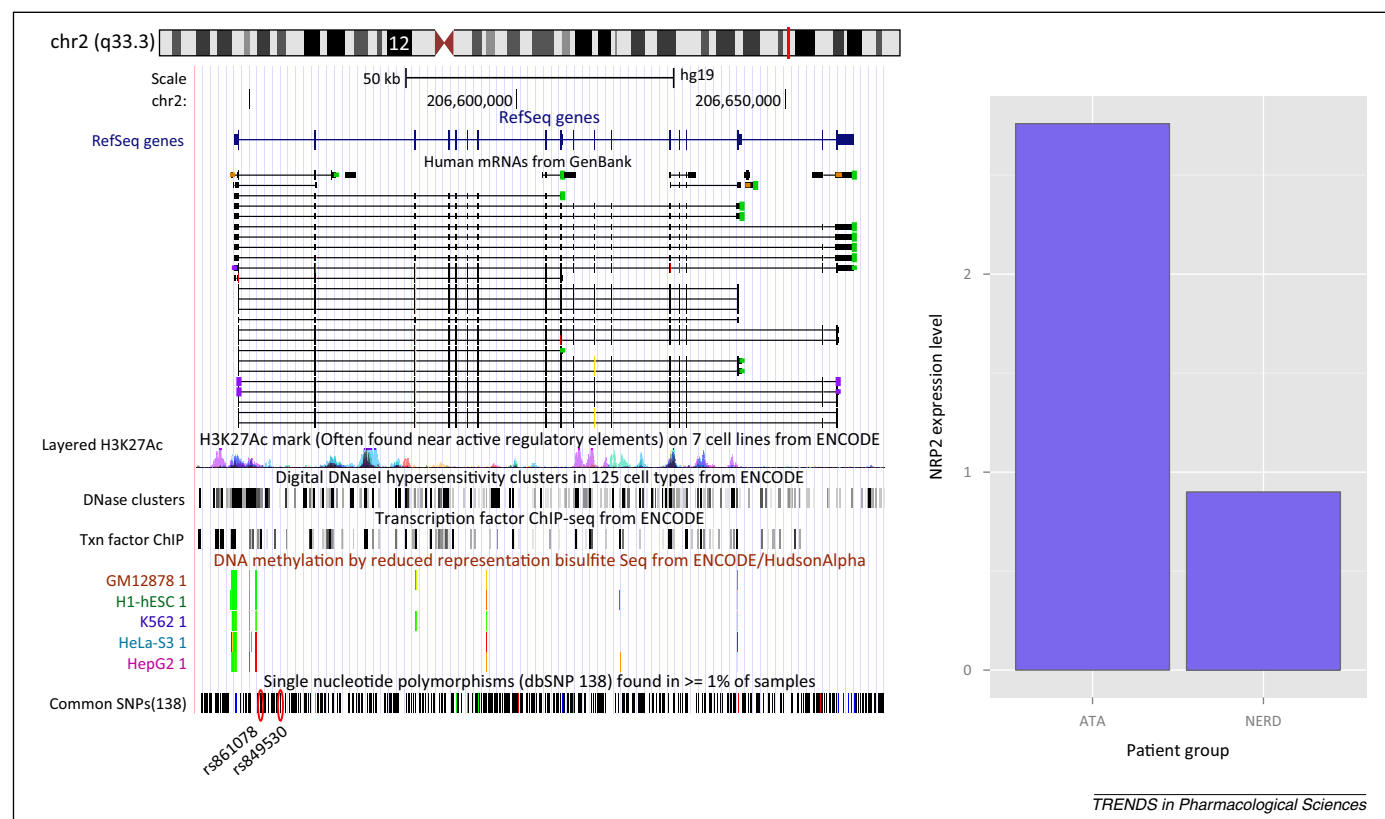


Figure 1. Genetic variation in the *NRP2* gene and changes in expression. Left: the position of *NRP2* in the genome, the different transcripts it encodes (black), and the exon/intron structure of the gene (blue). Below this are a number of graphics showing potential regulatory regions including acetylation marks (titled H3K27Ac), areas of open chromatin (DNase clusters), transcription factor (TF) binding (Txn Factor ChIP), and areas of methylation (DNA methylation). This data is obtained from the ENCODE project. This is followed by the position of known SNPs in this region. Those circled in red represent SNPs associated with the risk of NERD according to Park *et al.* [54]. SNPs from this study were mapped to the Hg18 genome in order to use the latest genome annotation (Ensembl 73). Right: expression levels of *NRP2* in peripheral blood between NERD sufferers and aspirin-tolerant controls. *P* (t-test): 0.0054; multiple testing adjusted *P* value: 0.0320. Expression levels are shown in arbitrary units derived from background-corrected probe intensity values, averaged across biological replicates. Abbreviations: ATA, aspirin-tolerant asthmatics; NERD, NSAIDs-exacerbated respiratory disease; *NRP2*, neuropilin 2; NSAID, non-steroidal anti-inflammatory drug. Data taken from Shin *et al.* [56]. The figure was created in Inkscape using graphics created using R and the UCSC (University of California, Santa Cruz) genome browser [84].

genes were used to construct the interaction network shown in Figure 2A. This was then used to explore the overlap between NSAIDs-HDR and atopy. For this we took genes associated with high levels of IgE according to a recent GWAS [72] and added them to our NSAIDs-HDR interaction network if they interacted with at least one known NSAIDs-HDR gene (Figure 2B). Thus we can

Box 1. Recent developments in sequencing and related technologies

The price of sequencing is declining rapidly. Besides genome sequencing to look for genetic variants associated with disease, a wealth of other applications are available, including transcriptional profiling (RNA-seq) [93], allowing the detection of alternative splicing, as shown in Figure 3A in main text. Other applications include methylation (bisulfite sequencing) [94], TF binding sites (ChIP-seq) [95], and other regulatory elements (FAIRE-Seq/DNase-Seq) [96], protein–RNA interactions (HITS-CLIP/PAR-Seq/RIP-Seq/CLIP-Seq) [97,98], and sequencing of B cell/T cell receptor repertoires (immuno-seq/Ig-seq) [99]. See [100] for a recent review on sequencing applications.

Non-sequencing-based technologies include yeast-1-hybrid, a method to identify which proteins bind a given promoter sequence [101], microfluidic ‘lab on a chip’ technology that parallelise various laboratory procedures [102], and systems microscopy approaches using image analysis and other techniques to analyse the behaviour of individual cells [103].

visually interpret the atopy GWAS results in terms of overlap with NSAIDs-HDR related genes: for example, we see that *IL4R*, associated with atopy but not HDR-hypersensitivity in the network, is connected to *ADAM33* (disintegrin and metalloproteinase domain-containing protein 33) and *MS4A2* (high affinity immunoglobulin epsilon receptor subunit beta), which are associated with HDR but not atopy. Moreover, *IL4*, encoding the ligand for *IL4R* is associated with both entities. Viewing the data in this way allows us to hypothesise potential mechanisms underlying the observed relationship between the entities, such as disruptions in *IL4*-related signalling. Such interpretations would be more difficult with separate lists of genes only. In the case of larger networks where visual interpretation is difficult, network metrics can be used to analyse the topological properties of the network. For example, network degree can be used to find highly connected genes, which often prove to be essential for network function and signalling. Another way to exploit network topology is through clustering algorithms. These break a network into smaller components of interconnected nodes, aiding visual interpretation. Clustering can be combined with gene enrichment analysis to find modules of interconnected genes contributing to a specific cellular process. Figure 2C shows the same network, but with additional nodes added. These nodes represent other genes that are highly connected to

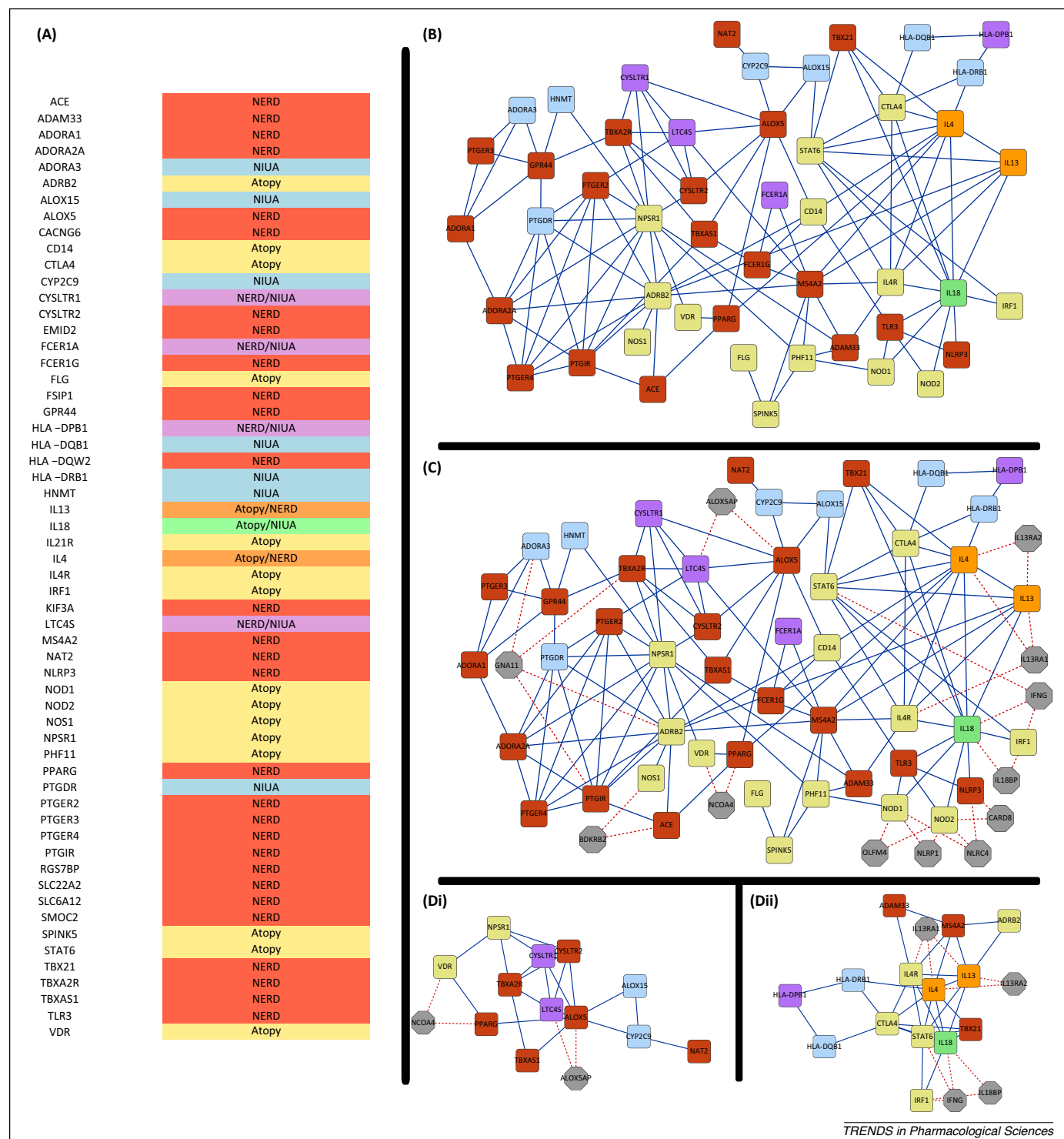


Figure 2. Protein interaction network derived from NSAIDs-hypersensitivity related genes and protein interaction/association data. **(A)** Genes associated with NIUA, NERD, and atopy. NERD and NIUA genes were taken from reviews by Kim *et al.* and Park *et al.* [59,60]; atopy genes were taken from the GWAS of Weidinger *et al.* [72], who looked at the association with elevated IgE levels, using this as a proxy for atopy. **(B)** These associated genes are displayed as nodes in a network, where connections between nodes represent protein-protein interactions or functional relationships, according to the STRING resource [70]. Node colours correspond to the disease entities in (A). **(C)** Extra genes (grey) that were highly connected to NIUA/NERD/atopy-associated genes but not highly connected to non-network genes were added to the network. **(D)** The network (including the additional genes) was divided into sub-networks using a clustering algorithm (EAGLE, [104]) to find groups of highly interconnected genes. Two of these clusters are shown in (Di) and (Dii), representing nuclear and neuropeptide S receptors interacting with CysLT-related genes and immunosignalling genes interacting with the high-affinity IgE receptor β -subunit gene (MS4A2). Abbreviations: CysLT, cysteinyl-leukotriene; GWAS, genome-wide association study; NERD, NSAIDs-exacerbated respiratory disease; NIUA, NSAIDs-induced urticaria/angioedema; NSAID, non-steroidal anti-inflammatory drug. The network figures were created in Inkscape using graphics generated with R and Cytoscape [83].

the annotated network genes (that is, they form many interactions with them), but less well connected to genes outside the network, and may therefore be involved in similar processes to the network genes. One such gene is

BDKRB2 (bradykinin receptor B2), which interacts with both NERD and atopy-related genes and plays an important role in the release of arachidonic acid [73], prostaglandin, and CysLTs [74], pointing to the utility of

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using networks to search for potentially related genes. **Figure 2D** shows two clusters found within the network, which was separated into clusters using EAGLE [70]. **Figure 2Di** shows a cluster containing a majority of NSAIDs-hypersensitivity-associated genes, in particular those related to leukotriene production. There is a strong overlap with atopy-associated genes, including *NPSR1* (neuropeptide S receptor 1), which has network associations with CysLT and thromboxane receptors. **Figure 2Dii** shows primarily atopy-related genes including cytokines and TFs. There are also some NSAIDs-hypersensitivity associated genes, including *TBX21* (T-box 21), which has been shown to be related to Th1/Th2 profiles in drug-induced allergies [75] and *MS4A2*, involved in IgE binding [76]. By presenting the data in this way, the researcher is able to visualise the relationships between genes and clusters and can focus on data interpretation and looking for novel genes that may link atopy and NSAIDs-hypersensitivity.

Future directions

We would like to draw attention to the recent explosion in high-throughput technology (**Box 1**). Potential uses of these technologies to obtain a more complete understanding of NSAIDs-hypersensitivity are detailed below.

The relationship between genetics and epigenetics

A number of recent papers have investigated the role of both genetics and epigenetics on TF binding [77–79]. They

show that a genomic variant at a specific genomic position leads to TF binding under certain circumstances, resulting in epigenetic chromatin modifications, affecting the DNA histone tail and altering the expression of nearby genes [80]. Different alleles at this region prevent the TF from binding, leaving expression unaffected. Closer to the field of allergy, previous work investigating the asthma-associated 17q12-21 chromosomal region showed that the effect of SNPs on gene activity can be masked by methylation [81]. Such studies indicate that interplay between genetic polymorphisms, sensitisation events, and epigenetics can affect gene expression and ultimately clinical phenotype. This idea is shown in **Figure 3B**. Therefore, to investigate NSAIDs-hypersensitivity, we suggest combining ChIP-seq, RNA-seq, bisulfite sequencing, and genome sequencing to obtain a high coverage and high-resolution picture of structural and functional genomics. For example, by combining genomics data on variants in promoter regions with ChIP-seq data on TF binding for the same individuals, we can search for variants that affect the TF binding affinity. By including gene expression data, we obtain information on the effects of the different binding patterns in terms of regulation. However, this effect will only manifest in the population if the chromatin in which it is located is accessible. Thus data on epigenetic modifications in this region arising from environmental interactions might be able to explain the incomplete penetrance of the variant – they may be necessary to allow the hypersensitivity to

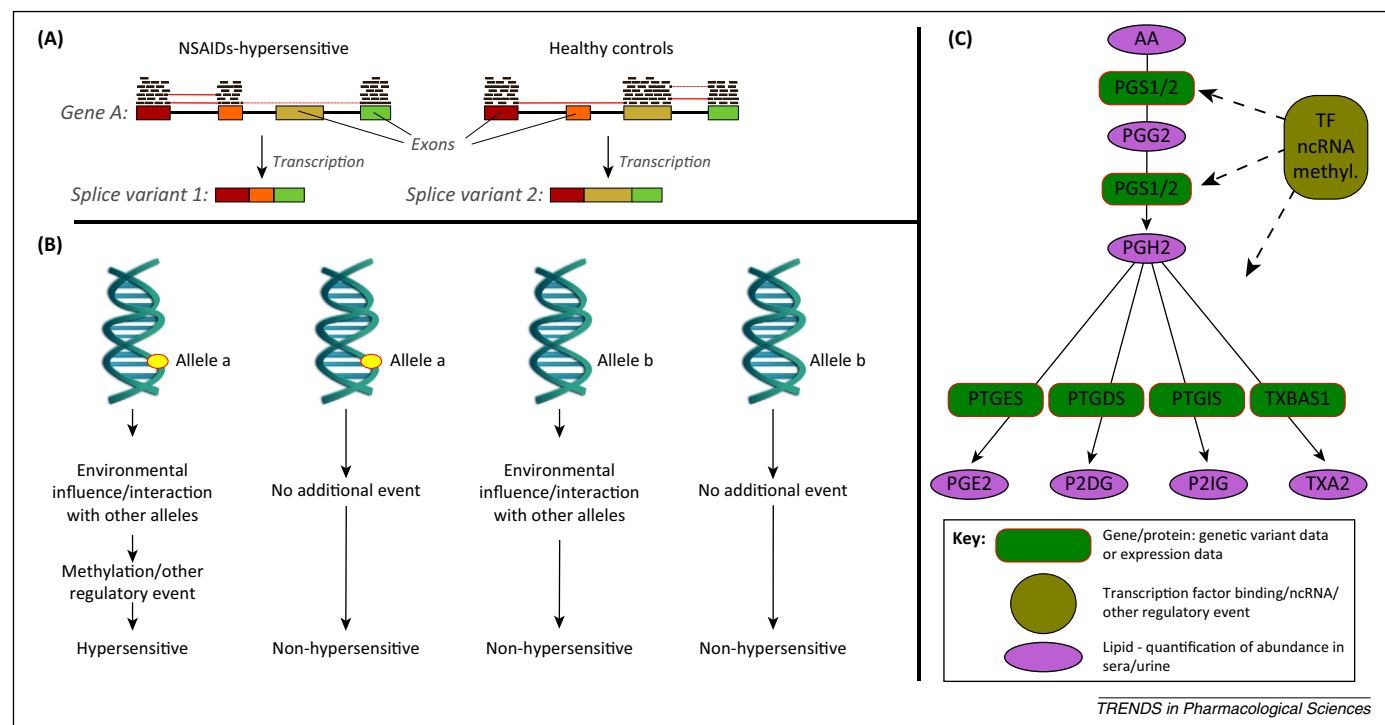


Figure 3. Potential future applications of high-throughput data and systems biology to NSAIDs-hypersensitivity. **(A)** Applications of next-generation sequencing to the quantification of gene expression and alternative splicing. A gene is shown as coloured boxes representing exons, connected by black lines that represent intronic regions. The lines above the genes represent reads of sequenced mRNA from a patient sample. More reads above an exon represents a higher transcription level in the sample. Lines spanning two exons represent mRNA reads that spanned a splice junction. Two samples are shown, for tissue from an NSAIDs-hypersensitive patient and a healthy individual. Exon expression varies between these samples, suggesting alternative splicing. **(B)** A potential model for the influence of genetics and environmental effects on patient phenotype, showing how a genetic predisposition and an environmental effect may lead to some regulatory change making an individual susceptible to NSAIDs-HDRs. **(C)** A pathway model that could be used to integrate diverse data sources in order to elucidate regulatory changes involved in NSAIDs-HDRs. The olive green box contains non-exhausted potential factors that might influence the network by affecting gene expression, however further datasets are required to elucidate their roles. Abbreviations: AA, arachidonic acid; HDR, hypersensitivity drug reaction; methyl, methylation; ncRNA, non-coding RNA; NSAID, non-steroidal anti-inflammatory drug; TF, transcription factor. The figure was created using Inkscape.

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develop, but require additional events involving further regulatory processes. The importance of a well-defined, precise phenotype and detailed clinical history to these experiments cannot be overstated, given the strong environmental influence and heterogeneous clinical entities in NSAIDs-HDRs [5,6]. On a technical note, there are various ways to combine different data sources, in our experience the GenomicRanges package for the R programming language allows genomic regions – which can be used to represent genes, genomic variants, ChIP-seq peaks regions of epigenetic modification, as well as quantitative data such as transcribed regions – to be stored and compared in an efficient way. Keeping the data in similar formats makes searching for overlaps easy (e.g., a SNP in an RNA recognition element can be cross-referenced with RNA-seq data quantifying exon-level expression). However, most datasets will require pre-processing steps beforehand and many considerations to take into account including statistical analysis and computational requirements, such as data storage and processing. Various tools are also available to help with these issues such as BioMet Toolbox [82], as well as commercial tools such as OmicsOffice or IPA, which do not require programming knowledge. Visualisation of results can be achieved using tools such as UCSC (University of California, Santa Cruz) Genome Browser or Cytoscape [83,84].

Other approaches might include profiling methylation state change over time and how this correlates with phenotypic information, such as atopy-related traits and NSAIDs-HDR status. This could be combined with cell sorting to explore the methylation patterns of distinct cell types. This is likely to be important to detect methylation in a small subset of cells [85]. Hypermethylation of prostaglandin E (PGE) synthase, accompanied by hypomethylation of other arachidonic-acid related genes, has been found in nasal polyps from NERD sufferers compared to controls, suggesting that environmental influences can affect NSAIDs-HDRs by altering gene expression in arachidonic acid pathway genes that are mediated by epigenetic events. This may lead to altered prostanoid/leukotriene profiles, potentially measured using the lipidomic techniques described below. More details on the relationship between epigenetics and altered responses to drugs are given in a recent review [86], which highlights the role of epigenetics modifications that affect the expression of genes encoding proteins involved in drug processing as well as modifications affecting drug targets themselves. The technology promises great advances in biomedicine in the next few years.

Lipidomics

A recent review by Fanning and Boyce described various lipid mediators in terms of their involvement in asthma and allergic diseases, highlighting the changes in levels of certain lipids in NERD patients [87]. Lipidomics, the systematic study of lipids in a given sample, has been growing rapidly in the past few years, due to technological advances in mass spectrometry and increased recognition of the importance of lipids in signalling [88]. For example, it has been used to investigate the genetic control of sphingolipid metabolism [89] and the influence of lipid

desaturase on polyunsaturated acids [90]. Lipidomics data could be used to investigate the effects of variants in arachidonic acid pathway genes associated with NIUA and NERD, as described above [26]. This would require genotype, expression, and lipid data per individual in order to investigate associations between genetic variants and expression/lipid levels, as is performed for the measurement of quantitative trait loci (QTL) [62]. However, this field is still young and problems such as distinguishing closely related lipids limit its ability to measure the full lipidome [88].

Pathway modelling and data integration

The ultimate aim of systems biology is not only to generate diverse datasets but also to model key biological processes to better understand them [10]. This requires the development of a model that can integrate multi-scale molecular interactions, such as at the metabolic, proteomic, and genomic level, as shown in Figure 3C. Unlike the interaction network in Figure 2, which is constructed using all available data to discover novel relationships, the pathway in Figure 3C has been designed using previous knowledge about arachidonic acid pathways. By integrating information on the rates of the related enzymatic reactions, quantitative lipid data, gene and protein expression, TF binding, and genetic variation, we can develop hypotheses to explain the model. From this we can obtain a better idea of the effects of genetic variation and predict the effects of perturbing the system. Similar work has been carried out for NSAID downstream signalling pathways, as described earlier, HIV infection, and more [10,18,91].

Concluding remarks

Recent years have seen numerous breakthroughs in our understanding of NSAIDs-HDRs. Many recent studies have pointed towards putative new mechanisms, such as calcium signalling [53] and the participation of the adaptive immune system [54]. It is clear that this complex web of genetic and environmental threads is more tangled than previously thought. In order to fully understand these reactions, it is crucial that we start approaching them from several angles including genomics, epigenetics, lipidomics, proteomics, and gene expression. To do so, we must start generating datasets. Systems biology efforts will be necessary as a framework to put these data together and unravel the variety of processes and aetiologies, as well as differences between populations, granting us a deeper understanding of NSAIDs-HDRs and ADRs (Box 2).

Box 2. Outstanding questions

- What additional sensitising factors are required in addition to genetic variants for the development of NSAIDs-hypersensitivity?
- Why do the reactions produce such different clinical symptoms in susceptible individuals?
- How can we explain the link with atopy? What mechanisms are involved and how do they lead to the release of inflammatory mediators?
- Can we use recent sequencing technology to build biomarkers based on gene/protein expression and genetic/epigenetic markers?

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