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**Systems biology approaches to enhance our understanding of
drug hypersensitivity reactions**

Short title: Systems biology and drug hypersensitivity

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Abbreviations

ADRs: adverse drug reactions

AERD: aspirin-exacerbated respiratory disease (now known as NERD)

AGEP: acute generalised exanthematous pustulosis

AIA: aspirin-intolerant asthma (now known as NERD)

BLs: beta-lactam antibiotics

COX: cyclooxygenase

DILI: drug induced liver injury

DRESS: drug rash with eosinophilia and systemic symptoms

eQTL: expression-quantitative trait loci

GWAS: genome wide association study

HDRs: hypersensitivity drug reactions

IRs: immediate reactions

LTs: leukotrienes

NECD: NSAIDs-exacerbated cutaneous disease

NERD: NSAIDs-exacerbated respiratory disease

NIRs: non-immediate reactions

NIUA: NSAIDs-induced urticaria/angioedema

NSAIDs: non-steroidal anti-inflammatory drugs

PGs: prostaglandins

SJS: Stevens-Johnson syndrome

TEN: toxic epidermal necrolysis

Abstract

Hypersensitivity drug reactions (HDRs) encompass a wide spectrum of unpredictable clinical entities. They represent an important health problem, affecting people of all ages, and lead to a large strain on the public health system. Here, we summarise experiments that use high throughput genomics technologies to investigate HDRs. We also introduce the field of systems biology as a relatively recent discipline concerned with the integration and analysis of high throughput datasets such as DNA microarrays and next generation sequencing data. We describe previous studies that have applied systems biology techniques to related fields such as allergy and asthma. Finally, we present a number of potential applications of systems biology to the study of HDRs, in order to make the reader aware of the types of analyses that can be performed and the insights that can be gained through their application.

Introduction

Adverse drug reactions (ADRs) encompass noxious and unintended responses to a drug at therapeutic doses (1). They can be classified as Type A: predictable, drug-related side effects related to dosage, and Type B: unpredictable, non-dose related reactions that appear only in susceptible individuals (2). The latter includes hypersensitivity drug reactions (HDRs), which represent a health problem of great concern, due to the large number of patients affected and the difficulties in their identification (3–5).

HDRs may be further divided into immunologic and non- immunologic reactions depending on the participation of the immune system (6). Beta-lactam antibiotics (BLs) are the most frequent cause of immunologic reactions (7,8); non-steroidal anti-inflammatory drugs (NSAIDs) are the leading cause of non-immunologic HDRs (9). Immunologic reactions can be further

classified as immediate, IgE mediated allergic, (IR) or non-immediate (NIR) based on the time elapsed between drug intake and appearance of clinical symptoms. IRs occur within the first hour and are mediated by specific IgE antibodies, typically leading to urticaria/angioedema and/or anaphylaxis. NIRs appear more than 24 hours after drug intake (10) and include reactions mediated by T cells (11). IgG antibodies, involved in hematologic reactions like thrombocytopenia or hemolytic anemia, appear not to be involved in NIRs (8). NIRs can lead to a variety of clinical manifestations, ranging from mild, such as urticaria and maculopapular exanthema, to more severe reactions such as bullous exanthems, e.g. Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN), organ-specific reactions such as drug induced liver injury (DILI), and systemic reactions such as drug rash with eosinophilia and systemic symptoms (DRESS) (7,8,12,13).

Non-allergic reactions include HDRs to NSAIDs, the most consumed medicines for the treatment of pain and inflammation (14,15). Although they can trigger reactions mediated by specific IgE or T cells, the most frequent type of reaction they induce are non-immune mediated (9,16,17). Full details of the biological mechanisms underlying these reactions have not yet been uncovered, although it is thought that pathways related to arachidonic acid metabolism are involved (18). The immune mediated reactions are elicited by a single NSAID, whilst the non-immune reactions can be induced by any one of a number of chemically distinct COX-1 inhibitors (9).

In this work we introduce the field of systems biology, providing the reader with a practical definition for the study of HDRs. We also introduce the topic of high throughput datasets, these are produced by technologies that assay a large number of biological molecules in parallel, such as gene expression microarrays that measure the expression of tens of

thousands of transcripts in a given biological sample. As we will see, much of systems biology is related to the analysis of these datasets. We present examples where systems biology approaches have been applied to the study of the immune system and suggest ways by which such methods could be used to further our understanding of HDRs. We focus on reactions caused by BLs and NSAIDs because 1) taken together they are responsible for the majority of HDRs (16), 2) they lead to a wide spectrum of pathologies (16,19) and 3) they are thought to be caused by different, complex biological processes (11,20). Many of the techniques presented here are also applicable to the study of the reactions induced by other drugs, such as contrast media and sulphonamides. The cost of DNA sequencing and related techniques is declining (21). As a result, more high-throughput datasets in need of interpretation are being produced. It is our view that systems biology, along with related fields such as bioinformatics, systems immunology and immunoinformatics, will become increasingly important for the study of drug hypersensitivity and allergy in the next few years. These concepts and related topics are further explained in Table 1, which provides definitions/summaries for all systems biology related terms used in this work.

Systems biology in immunology

Controversy exists over exactly what constitutes systems biology. It is said that if you ask five systems biologists to define the subject you will get 10 replies (22). Almost all biological studies involve a system – even the functional study of a single gene, e.g. a knockout study, examines the effect of that gene on a system. However the difference lies in the focus. Most of our current understanding of the immune system comes from a reductionist approach, analysing the different molecules and cell types involved in order to provide a parts list of the different players and their functions. In contrast, the aim of systems biology is to study the interactions between these players and to discover further information about the processes

involved. Data on these players are often derived from high-throughput experiments that quantitatively measure a large number of molecules in a given sample or through the integration of data from smaller scale studies.

However this definition is rather abstract. For the purpose of this review we take a pragmatic approach and define systems biology as the systematic measurement and characterisation of biological molecules relating to a process or system of interest. This encompasses the management and integration of high-throughput system-wide datasets and their usage to model biological processes. For example, interactions between biological entities (such as protein-protein and/or gene co-expression) can be represented as a network. These entities can also represent associations between diseases or metabolites (23,24). By focussing on the interactions rather than the individual components, one can discover additional properties that could not be predicted using the reductionist approach (25). The term network medicine has recently been coined, to describe a field that combines systems biology with network approaches to study human diseases (26,27).

The integrated approach offered by systems biology is especially suited to complex diseases that are likely to be mediated by a number of genes and their interaction with the environment. It has been applied to various problems related to immunity and led to new insights. In fact, the last few years have seen the development of a new discipline within the field of immunology, “systems immunology” (28,29). To date, a plethora of articles have been published that explore the use of systems immunology-based approaches to further our understanding of the innate immune system (30–32), vaccine design (33), infection (34–37) and auto-immune diseases (38–40). In fact, 2013 saw an inaugural EMBO workshop devoted to the study of complex systems in immunology, demonstrating the rapid growth of the field (41).

Our aim is not to describe all work in the field of systems immunology, but to present notable studies that use data, techniques and ideas that might be applicable to the study of HDRs. Of particular interest is the ImmGen Project, an international collaborative study that aims to profile the gene expression of all mouse lymphoid and myeloid cells, in various tissues, developmental stages and other states (42,43). These data are used for computational network reconstruction, in order to elucidate transcriptional regulation for different cell types and to describe variation in gene expression (43,44). Similar, less comprehensive data also exists for human haematopoietic cell lines (45). Other efforts to create and catalogue high throughput information relating to the immune system include RefDIC (46), ImmPort (47), innateDB (48) and the innate immune database (49). Returning to the field of allergy, AllergenOnline (50) is a long-running database containing protein sequences of potential allergens. A similar database, Allergome (51), contains data on allergens, their putative biological mechanisms, and the pathologies they can lead to.

However, the area of allergy has received little attention in terms of systems-based analysis. Most studies have focussed on asthma and allergic rhinitis. Of note is the work of Renkonen et al., (52) who compiled a list of genes related to acute allergic diseases based on literature searching and manual curation. These were then used to search public protein-protein interaction databases, in order to build an interaction network, which was then analysed in terms of network topology and the functional groups to which the genes belong. More details on network topology are given in Table 1. Similar work has been performed using asthma related expression and single nucleotide polymorphism (SNP) data (53–55). A recent study used RNA interference to screen genes and find proteins involved in MHC II related pathways, in order to improve our knowledge about the mechanisms involved in antigen presentation (24). To the best of our knowledge, no study to date has been made that employs

systems biology-based techniques to investigate drug hypersensitivity. As we shall see in the next section the complexity of HDRs in terms of clinical presentation and underlying mechanisms makes it a problem well suited to systems biology.

The complexity of HDRs

Pathomechanisms

As mentioned in the introduction, HDRs encompass a wide spectrum of pathologies. They cover all four groups in the Gell and Coombs classification, which categorises allergic reactions according to the immunological effector mechanism (56). Thus they might be mediated by IgE antibodies bound to basophils and mast cells (Type I), commonly leading to anaphylaxis and urticaria; complement-fixing IgG or IgM antibodies (Types II and III), causing haemolytic anaemia and serum sickness; or associated with the activation of T cells, monocytes, macrophages, neutrophils or eosinophils (Type IV), leading to delayed reactions (11). Type IV reactions can be further divided into Type IVa, involving Th1 cells and a high level of IFN- γ /TNF- α secretion, thought to involve monocyte/macrophage activation and leading to contact dermatitis; Type IVb, mediated by eosinophils and involving Th2 cytokine production (IL-4/IL-5/IL-13), also manifesting as contact dermatitis; Type IVc, mediated by cytotoxic CD4 and CD8 T cells and manifesting as maculopapular and bullous exanthems; finally, Type IVd reactions are triggered by neutrophils and associated with high levels of CXCL-8/GM-CSF production by T cells (11).

In addition, an important proportion of HDRs are not induced by immunological mechanisms, and fall outside of the Gell and Coombs classification. The most prevalent of these are HDRs to NSAIDs, which were originally classified based on the clinical manifestation,

the concurrence of any underlying disease, and the cross-reactivity with other cyclooxygenase (COX)-1 inhibitors (57). Some patients develop clinical symptoms after the intake of a specific NSAID, but show tolerance to other NSAIDs with distinct chemical structures. In this case the reaction is considered allergic. Clinical entities induced by allergic reactions to NSAIDs can show an acute (immediate to several hours after exposure) or delayed onset (usually more than 24 hours). The first group includes IgE-mediated reactions like urticaria and/or angioedema and anaphylaxis and are now referred to as “single NSAID-induced urticaria/angioedema or anaphylaxis”. This is distinct from “single NSAID-induced delayed reactions”, which encompass a heterogeneous set of conditions including fixed drug eruption, acute generalised exanthematous pustulosis (AGEP), DRESS, and SJS/TEN (58). When patients present symptoms induced by various, chemically unrelated NSAIDs the mechanism is not immunological and this type of hypersensitivity is commonly described as cross-reactive (19). The skin is the organ most often affected, presenting as either NSAIDs-exacerbated cutaneous disease (NECD) or NSAIDs-induced urticaria/angioedema (NIUA), depending on whether the patient had a previous history of spontaneous urticaria. Some asthmatic patients may suffer from an exacerbation of their pathology after NSAIDs intake, a condition recently termed NSAIDs-exacerbated respiratory disease (NERD) (19), previously known as aspirin-exacerbated respiratory disease (AERD).

Mediators

The clinical entities induced by HDRs result from a broad combination of different inflammatory mediators released by a variety of cell types. The acute phase of immediate reactions is triggered by basophils/mast cells degranulation, the synthesis of cytokines, chemokines and growth factors, as well as the generation of lipid-derived mediators (59). Tryptase, a major component of these granules, and histamine are the main mediators in

these reactions (60). Tryptase can be measured in peripheral blood, whilst histamine can be measured in the peripheral blood or urine as N-methyl histamine metabolite (61,62). Whereas histamine peaks for only a few minutes after the acute phase in peripheral blood, tryptase peaks between 2-4 hours. Both mediators return to basal levels after 24 hours (61,62).

As mentioned above, Th1 and Th2 cytokines are important for non-immediate type IVa and IVb reactions. In addition, increases in the levels of the pro-inflammatory cytokines TNF- α , IFN- γ and IL-6 have been found in patients with TEN (63). A recent study found an increase in pro and anti-inflammatory cytokines, including TNF- α , IL-10, IL-6, IL-1RA and GM-CSF, during the acute phase in TEN when compared to erythema multiforme, maculopaular exanthema, SJS and DRESS, followed by a decrease of these cytokines, as well as IFN- γ , when the reaction subsides (64). The increase of anti-inflammatory cytokines such as IL-10 and IL-1RA may represent a mechanism to counteract the effects of pro-inflammatory cytokines and epidermal cell death, which is severe in TEN but not in the other diseases (64). The cause of epidermal cell death in TEN is through apoptosis of keratinocytes with granulysin playing a key role, although other molecules such as Fas ligand and perforin/granzyme B are also involved (65). Recently IL-17, which induces the synthesis of pro-inflammatory cytokines such as IL-6 and TNF- α , has also been implicated in the pathogenesis of SJS/TEN (66).

Non-allergic reactions to NSAIDs have been associated with the capacity to inhibit the COX-1 isoform in susceptible individuals. This inhibition suppresses the production of prostaglandin (PG)E₂, and shunts the metabolism of arachidonic acid to the synthesis of cysteinyl-leukotrienes (LTs) (including LTC₄, LTD₄ and LTE₄) by 5-lipoxygenase (5-LO) and leukotriene C₄ synthase (LTC₄S), which is consequently over-expressed in basophils, mast cells, and eosinophils (20,58). Increased basal levels of LTE₄ have been described in patients with aspirin-

intolerant asthma (AIA, now known as NERD) in urine, nasal washes, bronchoalveolar lavage and exhaled lung condensates after aspirin exposure (67–70). Although this mechanism has also been suggested in the case of skin involvement (NIUA) (71) recent results indicate that other pathways may be involved (72). The complexity of the etiopathogenesis of these reactions is highlighted by recent studies showing no differences in PGE2 or 5-LO product levels between AIA and asthmatic patients with good tolerance to aspirin (73,74) and an overproduction of PGD2 during bronchoconstriction in AIA (73). It is becoming clear that clinical phenotypes in HDRs are more complex than previously thought (17,75).

High-throughput HDR studies and datasets

Immune mediated HDRs

As mentioned previously, immune-mediated HDRs vary greatly in terms of manifestation, severity, time of onset and underlying mechanisms. Most high-throughput studies have focussed on the most severe pathologies, in particular SJS and TEN, and a number of associated SNPs have been reported, including many in the HLA region (76–79). However, one study did not find any associated SNPs (80) and another found an association of PGE2 receptor 3 (PTGER3) (81). Some progress has been made in characterising gene expression of peripheral blood mononuclear cells in immune-mediated HDRs, of note is the work of Bellon et al., (82) who found the upregulation of a number of inflammatory mediators, endogenous damage-associated molecular patterns and alarmins in patients with severe bullous diseases, as well as a down-regulation of some T cell specific genes. More studies are needed to further elucidate these mechanisms, although progress is hampered by difficulties in obtaining samples during the acute phase of the reaction. It is also critical to characterize the phenotype of the reactions as accurately as possible prior to undertaking genetic studies. Given the severity of these reactions, further research is essential.

Non-immune mediated HDRs to NSAIDs

As mentioned previously, a number of genes belonging to the arachidonic acid metabolism and related pathways have been implicated in HDRs to NSAIDs (18). However, recent studies have revealed important molecules belonging to other pathways also associated with these reactions (11,83,84). A full understanding of the precise biological mechanisms involved remains elusive (85). In the last few years several high-throughput studies making use of – omics technologies have attempted to shed light on this problem, by measuring thousands of genes in parallel in terms of genetics (11,83,84,86), gene expression (87) and methylation (88). These concepts are further described in Table 1. These studies are important because they allow us to find novel genes and pathways involved in NSAID-hypersensitivity without *a priori* selection of the genes/molecules to be studied. These studies are also important because they may help in the search for a genetic signature, and ultimately the development of a predictive genetic test (86). This is an important task, given the time, cost and risk involved in determining whether patients are NSAID hypersensitive using current methods. However this research is still in its infancy and more datasets are needed, with more patients from a greater range of populations and crucially more precise and consistent phenotypes (17,58). Additionally, datasets should be combined in order to leverage more information from the available data. Examples of how datasets may be combined are given in Figures 1 and 2. It is important to fully understand the experiments being compared, in terms of the platforms used and their limitations. For example, not all -omics technologies probe the entire genome so it is important to ensure you are comparing equivalent sets of genes. It is also essential when using patient data to ensure that cases and controls are suitable, and directly comparable between datasets. All data should be made publicly available from appropriate databases. This will enable other researchers to perform meta-analyses and other integrative studies, increasing the impact of the original studies and allowing additional information to be gleaned.

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These high throughput datasets are very important in terms of highlighting the complexity of HDRs and providing new clues for future research. They allow the generation of new hypotheses: in contrast to previous studies investigating selected candidate genes they provide an unbiased approach to study the pathology. However, they also create new challenges. The discovery of genes with no previously known connection to the pathways involved in HDRs complicates our understanding. Systems biology therefore represents a way by which we can look for links between putative novel genes found using these methods and canonical HDR related pathways. We will now present some examples of how systems biology can be used to improve our understanding of HDRs.

Potential applications of systems biology approaches to the study of HDRs

The use of protein interaction networks to study relationships between genes

This approach entails the retrieval of protein interaction and association data from various sources and their integration in order to produce a network. Such data may consist of direct interactions or co-complex membership obtained through experimental techniques such as yeast two-hybrid or tandem affinity purification followed by mass spectrometry (89,90) (Table 1). Additionally, there are a number of methods that predict associations between genes based on evolutionary history, co-expression across various conditions, text mining and structural information (91,92). Association data are then used to build a network in which genes are represented as nodes and interactions/associations as edges. One way to analyse such a network is through cluster discovery methods. A cluster refers to a group of genes within the network, where the genes within the group are more closely connected to each other than to genes outside (26). Thus, if a cluster is highly enriched in genes related to a phenotype of interest (for example, associated with HDRs) then other genes in the cluster may

represent interesting targets for future study. Similarly, the topology of the network could be exploited to prioritise candidate genes based on their closeness to HDR related genes (26). A gene that interacts with many genes in the network, regardless of involvement in HDRs (for example general regulatory genes such as ubiquitin), will be less informative than a gene that interacts exclusively with HDR-related genes, and this must be taken into account when calculating closeness. There are currently a number of methods available for this (92,93).

Figure 1 shows the application of network-based analysis to the study of NSAID hypersensitivity. The network was created using interaction data taken from the STRING resource (93) and shows interactions between 42 genes previously reported as associated with NSAID hypersensitivity (85,94). Although most interactions involve genes from the arachidonic acid metabolic pathway, the figure also shows connections with both the innate and adaptive immune system. It should be noted that not all genes have necessarily been assayed for all clinical entities induced by NSAID hypersensitivity.

Combining genome wide association study (GWAS) and ENCODE data

Candidate approaches for disease gene association have focused on a limited number of SNPs with a potential effect on proteins. However, most disease-associated SNPs found using genome wide association studies (GWAS) are in non-exonic regions (95). Further support of the importance of these variants also comes from the ENCODE project, a recent collaborative effort, which found evidence of potential function in the majority of the genome (96). It used a variety of techniques to detect transcribed regions (97), areas of histone modification (98) and transcription factor binding sites (99) (more details in Table 1). By cross-referencing the position of non-exonic SNPs with the areas of the genome identified by the ENCODE project, we can further unravel the mechanisms underlying HDRs. This is particularly powerful when

combined with imputation techniques that allow us to fine-map a given genomic region and thus can help identify the causal gene.

Figure 2 provides an example of how to combine and visualise GWAS and ENCODE data. It shows keratin 75 (*KRT75*) and keratin 6B (*KRT6B*), and the intergenic region between them. A number of SNPs located in this region showed suggestive association with NERD in a Korean GWAS (rs298109, rs4761880, rs1798642, rs1117012, rs400774 and rs597340), these associations were also found in a secondary fine mapping analysis in a separate population (84). By combining the SNP data with ENCODE data on histone methylation and acetylation markings, we can see that, although intergenic, this region is likely to have an important regulatory role. The relationship between keratin and NERD is unclear, however a link between the keratin related protein filaggrin, maintenance of the barrier function of the skin, eczema and allergic asthma has been suggested (100). The connection between these SNPs and gene expression needs further analysis.

Profiling expression changes in HDRs

Several studies have analysed changes in gene expression in HDRs using the candidate gene approach, where a limited number of *a priori* selected genes were measured (101–103). A more systematic approach is high-throughput gene expression measurement, using DNA microarrays or more recently RNA-sequencing. It has been used extensively to characterise expression in myriad cell types under many conditions, including HDRs (104). However, the expression of RNA only represents a portion of the changes occurring at the molecular level. Recent technological improvements have now made it feasible to profile other molecules, mainly proteins, at a systems-wide level.

Mass-spectrometry and immune-cell secretion

Mass-spectrometry techniques for protein identification and quantification have improved in recent years, and now allow us to use label free approaches to quantitatively identify and measure the expression of thousands of proteins in a given sample (105). Recent and exciting work using Toll-like receptor 4-activated macrophages allowed the characterisation of the proteins secreted following immune system stimulation (106). Although the technology is still in its infancy (107), such an approach represents a promising tool to characterise the molecules involved in the immune response in HDRs and perhaps for the development of a potential *in vitro* test for susceptibility.

Single-cell RNA sequencing

Until very recently, transcriptomic experiments have used populations of cells, including heterogeneous tissue— tissue made up of a wide range of different cell types (108)(Table 1), or fluorescence-activated cell sorting derived populations of the same cell type (109). Thus expression measurements reflect average gene expression across populations of cells, with the drawback of losing information on variation between single cells. Recent advances in sequencing and cell-sorting technology now allow us to profile expression at the single cell level (110). This is important since, as shown by the ImmGen Project described above even within established cell types there can be a number of sub-types, which can vary in terms of gene expression as well as function (43). Single cell sequencing would improve our knowledge of these different sub-types and could benefit the study of HDRs by profiling not only gene expression from but also the relative proportions of the different subtypes between different groups of patients.

Combining genetic and expression data

Currently available HDR high throughput datasets have measured gene expression, methylation or genetic mutations (11,83,84,86–88). However they have not combined measurements for a single patient. Expression-quantitative trait loci (eQTL) is concerned with this topic – finding genes whose genetic variation is associated with differential expression. Combining different data sources for the same individual leads to a greater power to correlate expression and genetics/methylation. This can be useful both for generating hypotheses and also to investigate some areas in more detail, such as the SNPs shown in Figure 2.

Concluding remarks

We are in the middle of a genomics data explosion. The cost of generating high-throughput datasets is declining rapidly whilst the number of different aspects of disease biology that can be investigated is increasing (111). These advances are due in the most part to technological improvements and allow us to find novel genes and processes. However, these technological advances must be accompanied by well-planned experimental designs and ultra-precise phenotyping (17,58). Furthermore, we must ensure these datasets are made publicly available so that they can be integrated easily and the maximum information can be obtained. The examples presented here provide ideas of how currently available data can be exploited, as well as related approaches in similar fields that could be applied to the study of HDRs. As more datasets are produced and made available, systems biology will become increasingly important to obtain a complete picture of the biological processes involved in hypersensitivity drug reactions.

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Conflict of interest

The authors declare no conflict of interest.

Tables

Table 1: Definitions of important terms related to the field of systems biology

Term	Definition
Acetylation	The introduction of an acetyl group to a protein. The acetylation and deacetylation of core histone proteins is important for the regulation of gene expression.
Bioinformatics	Although many definitions have been put forward, most consider it as a field of study in which computers are used to analyse biological information. However differences of opinion exist as to its scope – whether it refers to the study of molecular data exclusively, or can be increased to include e.g. ecology or neuroscience data.
DNA microarrays	A technological platform consisting of a solid support to which DNA spots are attached. These DNA spots are used to measure the expression of genes with complementary sequences, or in the case of SNP microarrays, to detect the presence of SNPs in genomic DNA. Thousands of transcripts or hundreds of thousands of SNPs can be measured in parallel for the same sample.
ENCODE	The ENCyclopedia of DNA Elements, a large international project that aims to identify and catalogue all functional elements in the human genome, with a strong focus on areas outside of protein-coding genes (although the impact of these regions on genes is

	also investigated).
Epigenetics	The process by which heritable changes can be brought about through processes not involving a change in DNA sequence. These include methylation and chromatin modification.
Functional genomics	The study of dynamic aspects of the genome, including the expression of gene-transcripts and proteins, and their interactions.
Genetic signature	A group of genes whose expression pattern can be indicative of a specific cellular state and thus may be useful for prognosis or prediction of a response to an intervention.
Genome wide association study (GWAS)	The parallel measurement of thousands to millions of genetic variants (usually, single nucleotide polymorphisms) in the genomes of different individuals in order to look for associations between variants and specific phenotypic traits.
Genomics	The analysis of the function and structure of genomes – the full complement of DNA in a given cell.
High throughput experiments	Experiments that exploit technological advances in order to measure biological parameters in parallel, e.g. measuring the expression of thousands of genes simultaneously in a given tissue sample.
Histone modification	Post translation modification of histone proteins, e.g. through acetylation/methylation/phosphorylation of specific amino acids. Generally involved with the control of transcription.
Immunoinformatics	Also known as computational immunology, this is concerned with applying bioinformatics approaches to study the immune system.
Imputation	The process of replacing missing values. In genetics it is used to infer missing genotypes for a given sample, based on haplotypes from a set of reference genomes.
Interactome	The complete set of interactions occurring within a given cell or tissue, usually represented as a network. Such interactions can be physical interactions between proteins or other molecules, gene-gene interactions, or other types of interactions, such as regulatory.
Mass spectrometry (MS)	A technique to deduce the chemical structure of a given molecule, such as a protein, by ionizing it and then measuring its mass to charge ratio (or that of its peptide components). This mass to charge ratio can then be used to identify the protein.
Methylation	The addition of a methyl group. Can be used to regulate gene expression through the addition of a methyl group to adenine/cytosine DNA. Methylation of histone proteins can also affect gene regulation.
Network medicine	The study of the relationships between genes, phenotypes and diseases.
Network science	“The study of network representations of physical, biological and social phenomena leading to predictive models of these

	phenomena" <i>United States Research Council (112)</i>
Network topology	The layout of a network in terms of the way the connections between different nodes are configured.
Next-generation sequencing (NGS)	High-throughput sequencing methods able to determine the sequence of thousands/millions of "reads" – fragments of nucleic acids – concurrently for a given sample.
-omics	The addition of the suffix "–omics" indicates to the study of a given area in biology in its entirety. For example, proteome refers to the study of all proteins, metabolomics refers to the study of all metabolites and genomics to the study of the DNA/genes in the genome. Although a useful concept some believe has been overused and it has been the subject of some derision.
Predictive genetic test	A test for the diagnosis of predisposition to genetic diseases, based on genetic variation.
Protein-protein interactions	Physical interactions between two or more proteins. Can be permanent, forming a multi-protein complex, or transient, for example during cell signalling. Can be mediated by covalent or non-covalent bonds. More details in (90).
RNA-sequencing (RNA-seq)	The use of next-generation sequencing to quantify the full complement of RNA a given sample. Can be used for gene expression and also for expression arising from outside of exonic areas of the genome.
Single nucleotide polymorphism (SNP)	A variation in DNA sequence between individuals occurring at a single position, occurring in more than 1% of the population
Systems biology	Although there is no single accepted definition, the key concepts are given in the following, taken from the Harvard Medical School and modified slightly: The study of systems of biological molecules/cells/organisms/populations, using quantitative measurement of their behaviour and interactions. These are usually based on systemic measurement technologies such as genomics, bioinformatics and mathematical/computational models to describe and predict their behaviour (https://sysbio.med.harvard.edu).
Systems immunology	A subset of systems biology, aimed at studying the immune system in an integrated manner.

Tandem affinity purification (TAP)	A technique for identifying interacting proteins. A protein is tagged with a "TAP tag". This fusion protein can be considered a "bait" and is expressed in cells where it forms a complex with endogenous proteins. This complex is then isolated and digested, mass spectrometry can subsequently be used to identify the proteins in the complex.
Transcription factor binding site	An area of DNA to which transcription factors can bind, in order to regulate transcription.
Transcriptomics	The study of the full complement of RNA transcripts transcribed in a given cell/tissue, often under different conditions or over a time-course. Can be considered a subset of functional genomics.
Yeast two-hybrid (Y2H)	A technique to detect protein-protein interactions for a given protein. A "bait" protein, fused to the DNA binding domain of a transcription factor binds with a "prey" protein, fused to the activation domain of a transcription factor. This leads to the expression of a reporter gene as an indication that the bait and prey physically interact.

Figure legends

Figure 1: Protein interaction network derived from NSAID hypersensitivity related genes and protein interaction/association data.

A) NSAID hypersensitivity associated genes identified using candidate based genetic studies, created from reviews by Kim et al. and Park et al., (85,94). B) These genes shown in terms of their network associations. Nodes represent genes/proteins; edges between nodes

represent physical protein-protein interactions or functional relationships, found using the STRING resource (91). Network figure produced using cytoscape (113).

Figure 2: The genomic positions of SNPs associated with NERD located 5' of KRT6B and 3' of KRT75.

Consists of 10 horizontal tracks. The first four show the genomic position of the region. This is followed by the exon/intron structures of the genes KRT6B (left) and KRT75 (right). Below this is a track displaying ENCODE data which displays transcription patterns for this region. The next two tracks show histone mark data, for methylation (H3K4Me1) and acetylation (H3K27Ac). These provide evidence of regulatory activity. Following this is the DNase cluster track, showing areas of accessible chromatin, which is also suggestive of regulation. Finally, the known SNPs located in this region are shown. Those circled in red represent those that are associated with the risk of NERD according to Park et al., (84). Figure produced using the Hg18 genome build to enable direct mapping of the associated SNP data from this study.

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