Unravelling adverse reactions to non-steroidal anti-inflammatory drugs using systems biology

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Abstract

Unpredictable adverse drug reactions are non-dose-related reactions that appear only in susceptible individuals. Their biological basis remains a puzzle.

Here we introduce the reader to systems biology using adverse drug reactions, specifically hypersensitivity reactions to multiple NSAIDs, as a model. In order to disentangle the different processes that contribute to these reactions –from drug intake to the appearance of symptoms– it will be necessary to create more high-throughput datasets. Just as crucial as the generation of data will be the use of systems biology techniques to integrate and make sense of it.

We review previous work that has used systems biology to study related pathologies such as asthma and allergy and the metabolism of NSAIDs. We show examples of how they could be applied to NSAIDs-hypersensitivity reactions using currently available datasets. We also describe breakthroughs in high-throughput technologies such as next generation sequencing and speculate on how they could be used to improve our understanding of this and related drug-induced pathologies.

Key words

Systems biology, adverse drug reactions, hypersensitivity drug reactions, NSAIDs-hypersensitivity, functional genomics, omics, high-throughput data, protein-protein interactions, protein interaction networks.

Abbreviations

COX: cyclooxygenase

CysLTs: cysteinyl leukotrienes

DF: *Dermatophagoides farinae*

DP: *Dermatophagoides pteronyssinus*

GWAS: genome-wide association study

HDRs: hypersensitivity drug reactions

NERD: NSAIDs-exacerbated respiratory disease

NIUA: NSAIDs-induced urticaria/angioedema

NSAIDs: non-steroidal anti-inflammatory drugs

Introduction

Systems biology is gaining traction as a method to piece together the large datasets produced by relatively recent high throughput technologies such as microarrays and next generation sequencing. It has been applied to the study of immunology and allergy, with a focus on asthma [1–3] and allergen-sensitisation [4]. However, little attention has been given to adverse drug reactionsIn this work we will describe systems biology and its potential utility for the study of adverse drug reactions, focussing on NSAIDs-hyperesensitivity, giving examples of how it has been used to study arachidonic acid related pathways and related pathologies . We will describe what is currently known about the underlying mechanisms, highlighting the phenotypic and genetic complexity. For a more general discussion of drug hypersensitivity we direct the reader to a recent review[7]. We will then present examples of how systems biology techniques can be used to integrate high throughput NSAIDs-HDR datasets in order to uncover putative new mechanisms. Drawing on these we will speculate on future directions for the field in light of new and emerging technologies, the type of data they will generate and how this data can be fully exploited. Finally we will provide some remarks about how we expect to see the field develop over the next few years, with reference to pharmacogenetics and personalised medicine.

We believe the study of NSAIDs-hypersensitivity will benefit strongly from systems biology analysis due to its complexity in terms of the variety of clinical presentations and underlying mechanisms [8,9]. The utility and wide-spread use of NSAIDs for the treatment of pain and inflammation, along with the potentially

life threatening reactions they can lead to, warrant such investigation and the generation of future datasets [7].

Systems biology

We define systems biology as an approach to integrate various datasets, both high-throughput data produced using -*omics* technologies, as well as the results of smaller scale analyses. This encompasses data mining, bioinformatics and network biology techniques, with the overarching aim to model the relationships between different entities, such as genes, metabolites, medicines and diseases [40,41]. This fits in with the descriptive, data-driven approach of Arazi et al. [42]. This group also proposed a "hypothesis-based modelling" definition for systems biology, where expert knowledge and domain-specific measurements are combined to produce a mathematical model, from which hypotheses can be tested and compared to empirical data [42].

One such application of this latter approach has been to investigate the effects of NSAIDs on cancer [43]. 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 combined the arachidonic acid metabolism pathway with downstream signalling pathways to build a network they dubbed the "NSAID model". By perturbing this model and observing the effects in terms of cancer progression they identified potential targets and biomarkers for further study.

For examples of systems biology approaches applied to the immune system we direct the reader to recent reviews from this journal [46,47] and others [42,44,45].

NSAIDs adverse reactions and hypersensitivity

Adverse drug reactions are noxious and unintended responses to a drug that occur at doses normally used for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function. They can be classified as predictable, drug related side effects related to dosage (Type A), and unpredictable, non-dose-related reactions that appear only in susceptible individuals (Type B). The latter includes hypersensitivity drug reactions (HDRs), which represent an important health problem due to the large number of patients affected and the difficulties in their identification.

NSAIDs are a heterogeneous group of compounds with different chemical structures that have high efficacy for the treatment of pain and inflammatory disorders. However, they are also associated with a wide variety of adverse reactions, and have been shown to be the leading cause of HDRs. Such reactions are caused by the release of histamine or other vasoactive mediators through both immunological and non-immunological (pharmacological) mechanisms. Pyrazolones are the main triggers of the first type, whereas propionic acid derivatives, especially ibuprofen, are the most frequently involved in pharmacological reactions. NSAIDs-HDRs comprise a set of distinct but overlapping pathologies with differences in severity, onset-time and clinical presentation. According to the European Academy of Allergy and Clinical Immunology these reactions can be loosely grouped into two categories: immune-mediated and non-immune-mediated[9]. The former are induced by a single NSAID (selective reactions), and involve specific IgE antibodies or T cells; the patient will show tolerance to other NSAIDs belonging to different chemical

groups [9,10]. In contrast, non-immune mediated reactions are induced by NSAIDs with different chemical structures (cross-intolerance), and represent the most frequent type of NSAIDs HDRs. Their underlying proposed mechanism has been related to cyclooxygenase (COX)-1 inhibition, leading to a deviation in the arachidonic acid metabolic pathways from the synthesis of prostaglandins to cysteinyl-leukotrienes (CysLTs) [9,11,12]. NSAIDs-exacerbated respiratory disease (NERD) has been the most studied model, however NSAIDs-induced urticaria/angioedema (NIUA) is now considered the most frequent clinical entity induced by HDRs [5,6].

Although the risk factors associated with NIUA/NERD have not been clearly established, atopy could play a key role [14]. Although the relationship between atopy and NERD was reported more than 40 years ago [15,16], recently, studies have reported associations between patients with NIUA and house dust mite allergy[17–20]. In a recent study, which included the highest number of patients with HDRs to NSAIDs studied to date, we have found that atopy was present in 60% of cases with NIUA/NERD [5]. Moreover, the allergens involved differed depending on the clinical entity, i.e. individuals with NIUA showed a higher frequency of positive skin tests to *Dermatophagoides pteronyssinus* (DP) and *D. farinae* (DF) than those with NERD [5].

The factors that contribute to the association between atopy and NIUA/NERD are unknown. Atopic diseases (eczema, rhinitis, and asthma) are mediated by specific IgE antibodies whereas NIUA/NERD are thought to be related to COX-1 inhibition and the release of CysLTs (LTC4, LTD4 and LTE4) [21]. However, it is interesting to note that several genetic variants in the IgE receptor have been associated with NIUA/NERD [22–24]. Leukotriene C4 synthase (LTC4S), the main

155 enzyme in CysLT synthesis, is located on chromosome 5q35 [25,26], in close
156 proximity to the cluster of genes for cytokines orchestrating allergic
157 inflammation (*IL3*, *IL4*, *IL5*, *IL9*, *IL13*, and *CSF2* [27]. In addition, *CYSLTR2*, one of
158 the two CysLT receptors identified in humans, maps to chromosome 13q14 [28],
159 which is also in consistent linkage with atopy and total serum IgE concentrations
160 [29]. Furthermore, genetic variants in both *LTC4S* and *CYSLTR2* have been shown
161 to be associated with atopic diseases [30–33].

162 In addition to its genetic association with atopy, IL4, a key cytokine in IgE
163 synthesis, induces the expression of LTC4S in mast cells [34] and CysLTR1 in
164 several cell types [35,36]. The effect of DP and DF allergens on COX-1, the release of
165 CysLTs [37], and the elicitation of an innate immune response through the
166 stimulation of dendritic cells and cytokine production [38] or toll-like receptor
167 pathway activation [39], may also contribute.

168 ***Current high-throughput NSAIDs-hypersensitivity data***

169 Although current data could be considered sparse in comparison with other
170 diseases, there are several high throughput studies related to NSAIDs-
171 hypersensitivity. At the genetic level, a number of genome wide association
172 studies (GWAS) have been performed using SNP arrays for Spanish and Han
173 Chinese [48], and Korean populations [49,50]. Gene expression data also exists in the
174 form ofmicroarray experiments profiling the difference in expression in
175 peripheral blood mononuclear cells and nasal polyps between NERD patients
176 and control subjects [51,52]. At the epigenetic level, microarrays have been used
177 along with bisulphite technology to measure genomic DNA methylation in nasal
178 polyps of NERD patients [53]. In addition, there have been many smaller scale

179 candidate studies that have found genetic associations with NSAIDs-HDRs, these
180 have been extensively reviewed [54,55].

181 In the next section we will provide examples of how these data could be
182 integrated (Figures 1 and 2), followed by ideas for more novel analyses that
183 could be undertaken if more datasets were produced, using sequencing and
184 other technologies. Some of these ideas are represented in Figure 3.

185 ***Applications of systems biology to NSAIDs-hypersensitivity analysis***

186 **Uncovering mutations in non-coding genomic regions**

187 Approximately 88% of disease associated SNPs found by recent GWAS studies
188 were found in intergenic/intronic, non-coding regions of the genome [56]. These
189 regions, once assumed to be of little functional importance, are sparsely
190 annotated in comparison with exons. However, recent work such as the ENCODE
191 project, has found evidence of potential function, and could allow us to better
192 understand the impact of NSAIDs-HDR associated variants [57]. Figure 1 presents
193 an example of such variants – the SNPs rs849530 and rs861078, which showed
194 suggestive associations with NERD according to an initial GWAS; this association
195 was also found in a subsequent follow-up study in a separate group of patients [49].
196 These SNPs are found in an intronic region of the gene neuropilin 2 *(NRP2)* (Fig
197 1, left). Interestingly, this gene also shows three-fold decreased expression in
198 peripheral blood mononuclear cells of NERD patients compared to aspirin-
199 tolerant asthmatic patients [51] (Fig 1, right). The associated SNPs are close to a
200 genomic region containing a number of potential regulatory elements, including
201 areas of open chromatin, methylation, histone acetylation and a potential CpG
202 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. Further analysis, for example examining other tissues and using recent, higher resolution sequencing technologies as described below are required. By combining this SNP with data from the 1000 Genome Project [58], it may be possible to impute further variants [59], 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. Graphical models can be used to known relationships between the genes involved in these conditions, such as interactions or co-expression. As an example, we constructed a protein interaction network from public resources [60,61] to explore NSAIDs-HDR related genes, adapting the methodology used by Renkonen *et al.* [62]. In this network, the nodes represent proteins and edges represent interactions. These include not only direct physical interactions, but also functional relationships as defined by the STRING resource [60]. This network was then filtered using genes associated with NERD and NIUA, taken from recent reviews by Kim et al. [54] and Park et al. [55] as well as genes associated with high levels of IgE according to a recent GWAS [63] (Figure 2A-B). The latter study was used as a proxy for atopy. The network was subset to only include these annotated genes and their associations with each other (Fig 2B). Figure 2C shows the same network, but with additional nodes added. These nodes represent other genes that are highly connected to the annotated network genes. One such gene is BDKRB2, which interacts with both

NERD and atopy related genes and plays an important role in the release of arachidonic acid [64], prostaglandin and CysLTs [65]. Figure 2D shows two clusters found within the network using the STRING resource [60]. 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 neuropeptide S receptor *(NPSR1)*, which has network associations with CysLT and thromboxane receptors. Figure 2Dii shows primarily atopy-related genes including cytokines and transcription factors. There are also some NSAIDs-hypersensitivity associated genes, including T-box transcription factor (TBX21), which has been shown to be related to Th1/Th2 profile in drug induced allergies [66] and membrane spanning 4-domains, subfamily A, member 2 *(MS4A2)*, involved in IgE binding [67]. 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

As well as the above examples showing how to apply systems biology to the analysis of current datasets, we would like to draw attention to the explosion in high-throughput technology in the last few years and suggest examples of how they could be used to shed light on NSAIDs-HDRs.

Available methodology

The price of sequencing has declined rapidly in recent years and continues to do so. Besides genome sequencing to look for genetic variants associated with disease and to predict drug reactions, there is a wealth of other applications

available. These include genome-wide transcription profiling from , including genic, intergenic and intronic regions (RNA-seq) [68], allowing the detection of alternative transcription, as shown in Figure 3A. Other applications include methylation (bisulphite sequencing) [69], transcription factor binding sites (CHiP-seq) [70] and other regulatory elements (FAIRE-Seq/DNase-Seq) [71], protein-RNA interactions (RIP-Seq/CLIP-Seq) [72] and sequencing of B-cell and T-cell receptor repertoires (immuno-seq/Ig-seq) [73]. See [74] 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 [75], microfluidic "lab on a chip" technology that aim to parallelise various laboratory procedures [76] and systems microscopy approaches that use image analysis and other techniques to analyse the behaviour of individual cells over time and under different conditions [77].

We will now propose some potential uses of these technologies in order to obtain a more complete understanding of NSAIDs-hypersensitivity.

The relationship between genetics and epigenetics

A recent slew of papers have investigated the role of both genetics and epigenetics on transcription factor (TF) binding [78–80]. They suggest a certain allele at a given leads to TF binding under certain circumstances, which in turn leads to epigenetic chromatin modifications, affecting the DNA histone tail and altering the expression of nearby genes [81]. Different alleles at this region might not allow the TF to bind, and thus gene expression would not be affected in the same way. Closer to the field of allergy, previous work by Berlivet *et al.*,

investigating the asthma-associated 17q12-21 genomic region showed that the effect of SNPs on gene activity can be masked by methylation [82]. 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, in order to investigate NSAIDs-hypersensitivity we suggest combining ChiP-seq, RNA-seq, bisulphite and genome sequencing, to obtain a high coverage and high-resolution picture of structural and functional genomics and epigenetics related to NSAIDs-hypersensitivity. By combining these data with comprehensive phenotypic information and detailed clinical history, this approach has the potential to help us understand why some of the variants are of low penetrance – they may be necessary to allow the hypersensitivity to develop, but require additional events involving further regulatory process. The importance of a well defined, precise phenotype and detailed clinical history to these experiments cannot be overstated, given the strong environmental influence on these pathologies as well as the heterogeneous clinical entities in NSAIDs-HDRs [8,9]. 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 approach could be combined with cell sorting in order to explore the methylation patterns of distinct cell types. This is likely to be important in order to obtain high sensitivity to detect methylation in a small subset of cells [83]. 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 [84]. However these studies investigated pre-selected targets. The field of lipidomics, the systematic study of lipids in a given sample, has been growing rapidly in the past few years, due in part to technological advances in mass spectrometry and increased recognition of their importance [85]. For example, it has been used to investigate the genetic control of sphingolipid metabolism [86] and the influence of lipid desaturase on polyunasturated acids [87]. This kind of data could be combined with both gene-expression and genetic information to investigate the effects of variants in arachidonic acid pathway genes associated with NIUA and NERD [12], in terms of lipid metabolism and signalling. However, this field is still young and suffers from a number of difficulties, such as distinguishing closely related lipids, limiting the ability of the technology to measure the full complement of the lipidome [85].

Pathway modelling and data integration

The ultimate aim of systems biology is not only to generate diverse data sets but also to model key biological processes in order to better understand them [42]. 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 in order to discover novel relationships, the pathway in Figure 3C has been designed using previous knowledge about

arachidonic acid metabolism. By integrating information on the rates of the related enzymatic reactions, quantitative lipid data, gene and protein expression, transcription factor binding and genetic variation, we can create hypotheses able 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, HIV infection and more [42,43,88].

Concluding remarks

The last few years have seen numerous breakthroughs in our understanding of NSAIDs-HDRs. Many recent studies have pointed towards putative new mechanisms, such as calcium signalling [48] and the participation of the adaptive immune system [49]. It is clear that this complex web of genetic and environmental threads is more tangled than previously thought. In order to understand this pathology in full, it will be necessary to approach it from several angles, including genomics, epigenetics, lipidomics and gene expression. It is crucial we start generating datasets related to these issues. Moreover, systems biology efforts will be necessary to put these data together and explain the results. As well as leading to a better understanding of these pathologies, high throughput measurements could also be combined in order to build a biomarker-based predictive test. This is of particular importance given the high prevalence of NSAIDs-HDRs, the recent advent of the $1000 genome and, despite some recent advances [89], the lack of an appropriate predictive test for this pathology. However care must be taken to ensure that the results can be generalised to different populations.

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612

Figure legends

Figure 1: Genetic variation in the *NRP1* gene and change in expression

The left pane of shows the position of the NRP1 gene in the genome, and the different transcripts it encodes (brick-red) and the exon/intron structure of the gene (blue). Below this are a number of tracks showing potential regulatory regions including acetylation marks (H3K27Ac track) and areas of methylation (DNA methylation and CpG methylation tracks). 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., [49]. SNPs from this study were mapped to the Hg18 genome in order to use the latest genome annotation (Ensembl 73).

Figure 2: Protein interaction network derived from NSAIDs-hypersensitivity related genes and protein interaction/association data.

A) Genes genes associated with NIUA, NERD and atopy. NERD and NIUA genes were taken from reviews by Kim et al. and Park et al., [54,55]; Atopy genes were taken from the GWAS study by Weidinger et al. [63], who looked at association with elevated IgE levels, using this as a proxy for atopy. B) These associated genes are displayed as nodes a network, where connections between nodes represent protein-protein interactions or functional relationships, according to the STRING resource [60]. C) The network was used as a seed to search for other genes that were relatively highly connected to NIUA/NERD/Atopy associated genes. D) The network including the additional genes was clustered, in order to find groups of highly inter-connected genes. Two of these clusters are shown in Figure Di and Dii, representing nuclear and neuropeptide S receptors interacting with cysteinyl

leukotrienes-related genes and immunosignaling genes interacting with the high-affinity IgE receptor β-subunit gene (MS4A2). All network figures were produced using Cytoscape [90].

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 transcription. A gene is shown as coloured boxes and the lines above the boxes represent reads, 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 NSAID hypersensitive patient and a healthy individual. Exon expression varies between these samples, suggesting alternative transcription. 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 NSAID-HDRs. C) Pathway model that could be used to integrate diverse data sources in order to elucidate regulatory changes involved in NSAID HDRs (AA: arachidonic acid; methyl.: methylation; ncRNA: non-coding RNA; TF: transcription factor).

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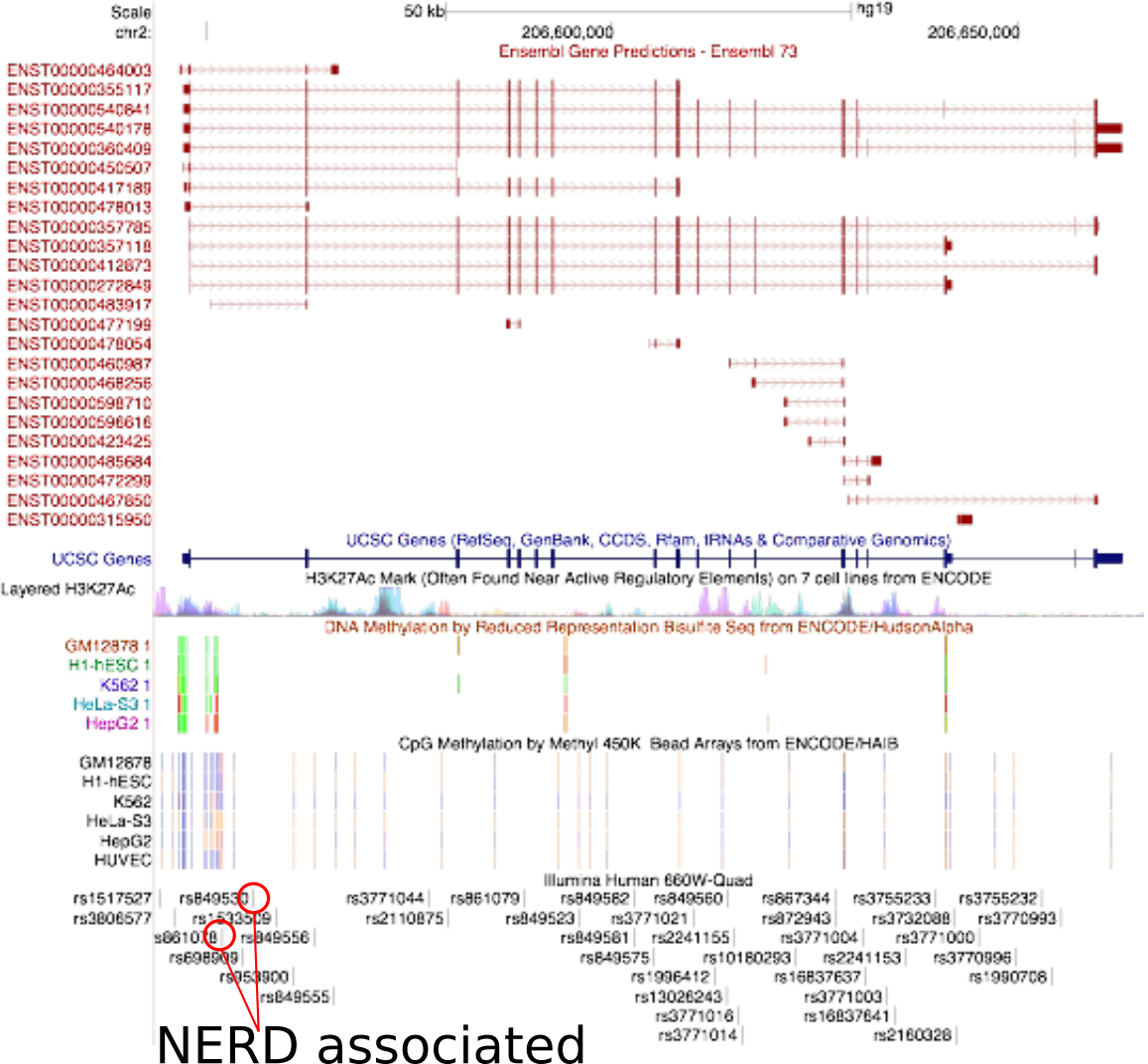
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UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
UCSC Genes
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
Layered H3K27Ac
DNA Methylation by Reduced Representation Bisulfite Seq from ENCODE/HudsonAlpha
GM12878 1
H1-hESC 1
K562 1
HeLa-S3 1
HepG2 1
CpG Methylation by Methyl 450K Bead Arrays from ENCODE/HAIB
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K562
HeLa-S3
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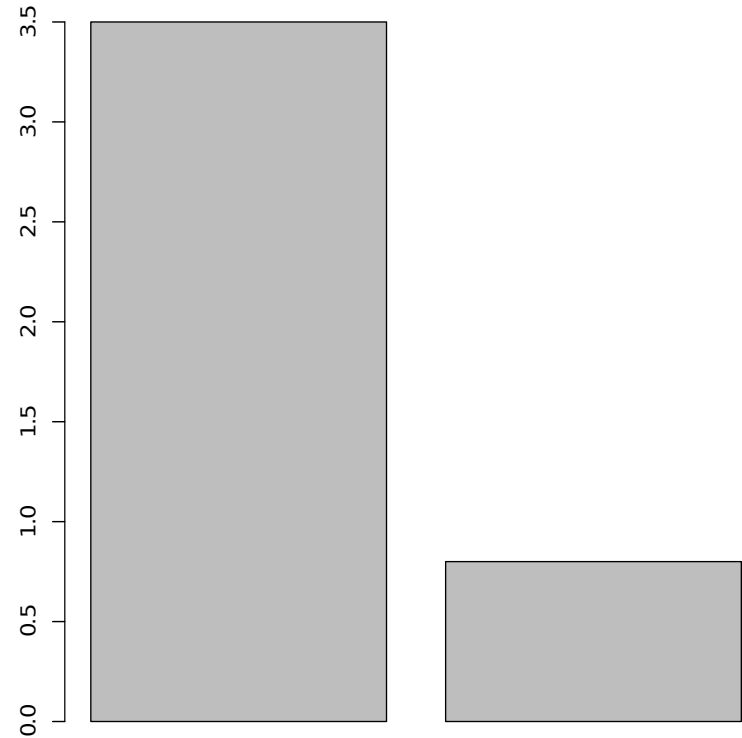
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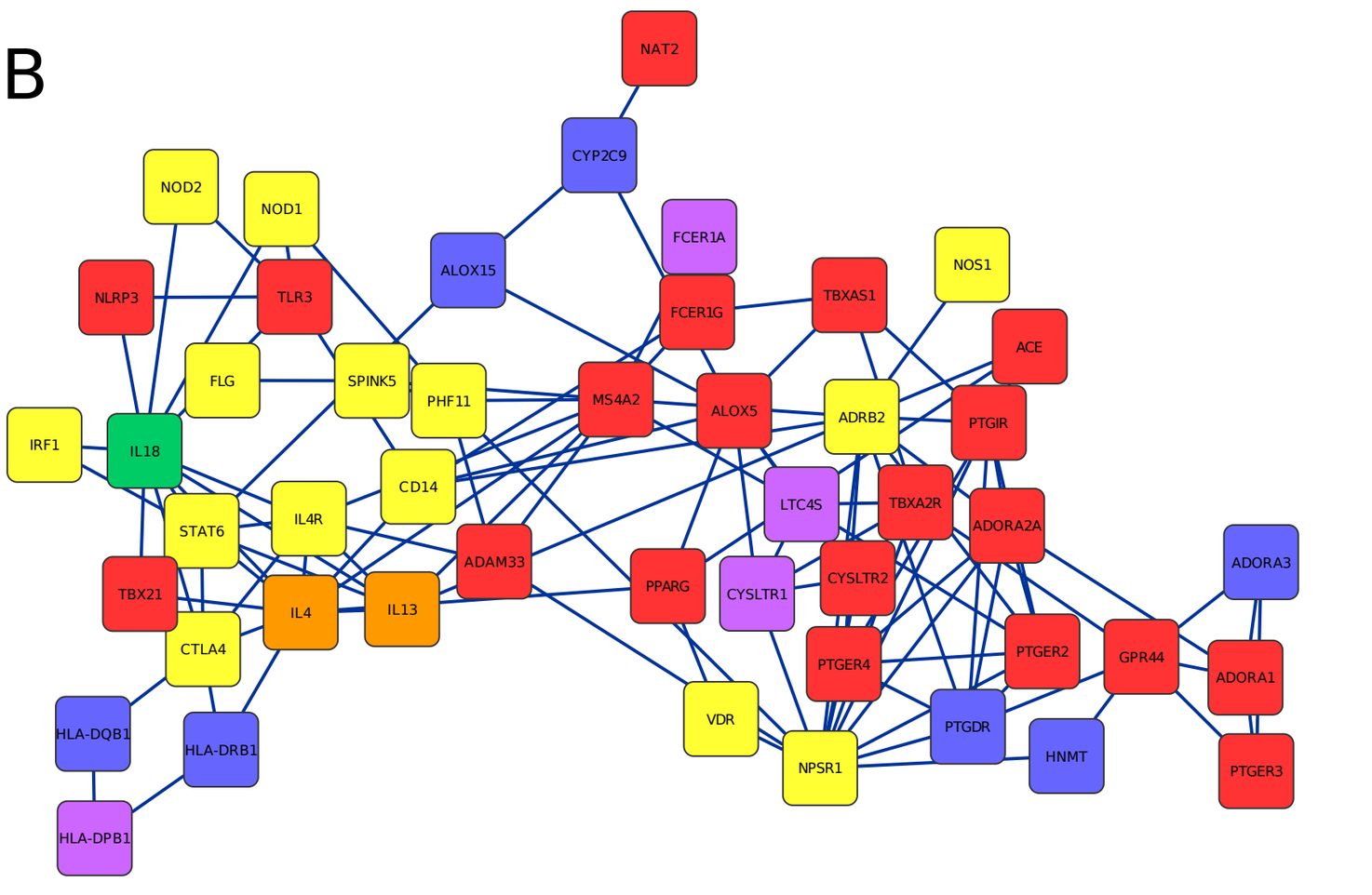
NERD
ATA

1A

ACE	NERD
ADAM33	NERD
ADORA1	NERD
ADORA2A	NERD
ADORA3	NIUA
ADRB2	Atopy
ALOX15	NIUA
ALOX5	NERD
CACNG6	NERD
CD14	Atopy
CTLA4	Atopy
CYP2C9	NIUA
CYSLTR1	NERD/NIUA
CYSLTR2	NERD
EMID2	NERD
FCER1A	NERD/NIUA
FCER1G	NERD
FLG	Atopy
FSIP1	NERD
GPR44	NERD
HLA–DPB1	NERD/NIUA
HLA–DQB1	NIUA
HLA–DQW2	NERD
HLA–DRB1	NIUA
HNMT	NIUA
IL13	Atopy/NERD
IL18	Atopy/NIUA
IL21R	Atopy
IL4	Atopy/NERD
IL4R	Atopy
IRF1	Atopy
KIF3A	NERD
LTC4S	NERD/NIUA
MS4A2	NERD
NAT2	NERD
NLRP3	NERD
NOD1	Atopy
NOD2	Atopy
NOS1	Atopy
NPSR1	Atopy
PHF11	Atopy
PPARG	NERD
PTGDR	NIUA
PTGER2	NERD
PTGER3	NERD
PTGER4	NERD
PTGIR	NERD
RGS7BP	NERD
SLC22A2	NERD
SLC6A12	NERD
SMOC2	NERD
SPINK5	Atopy
STAT6	Atopy
TBX21	NERD
TBXA2R	NERD
TBXAS1	NERD
TLR3	NERD
VDR	Atopy

1B



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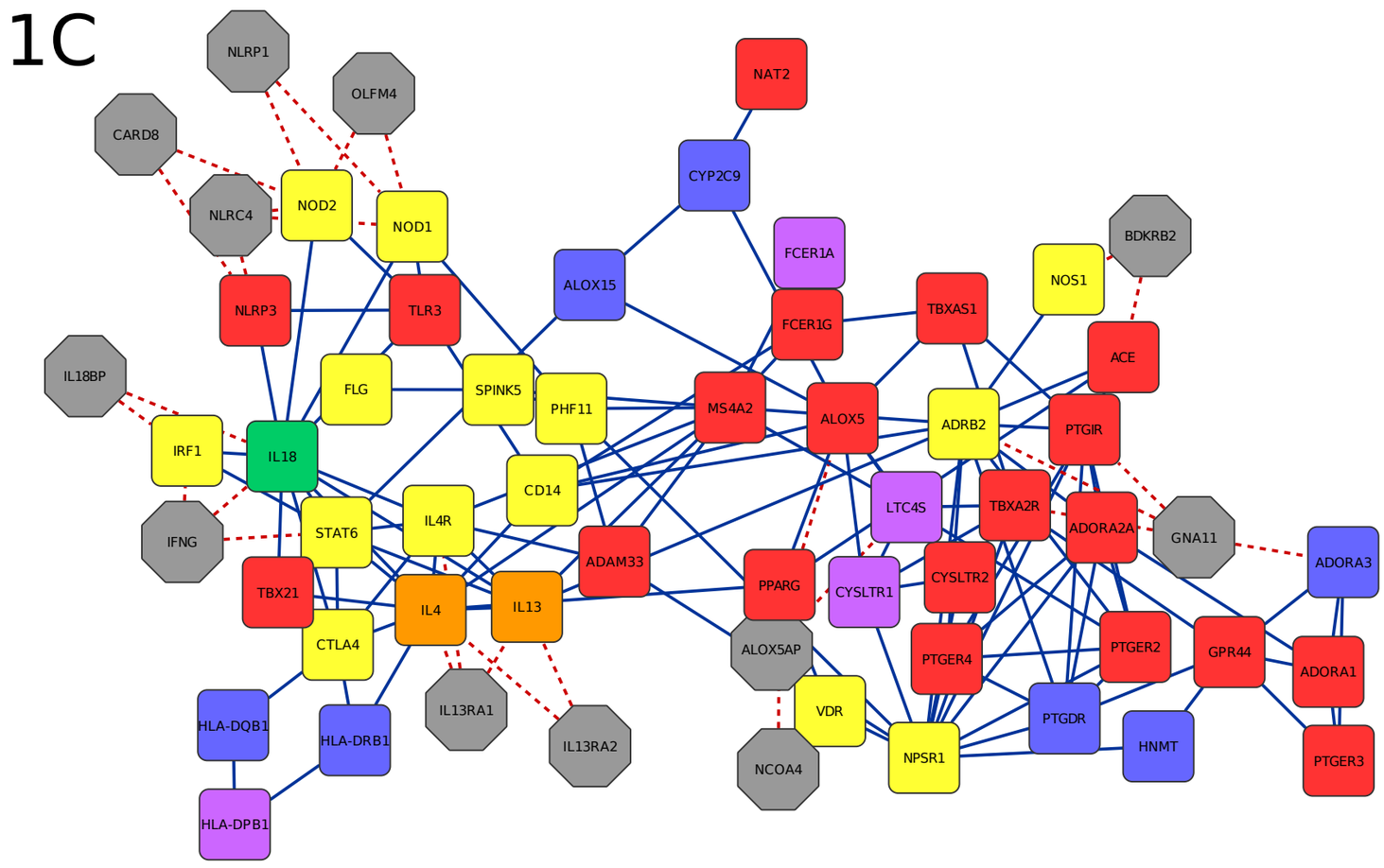

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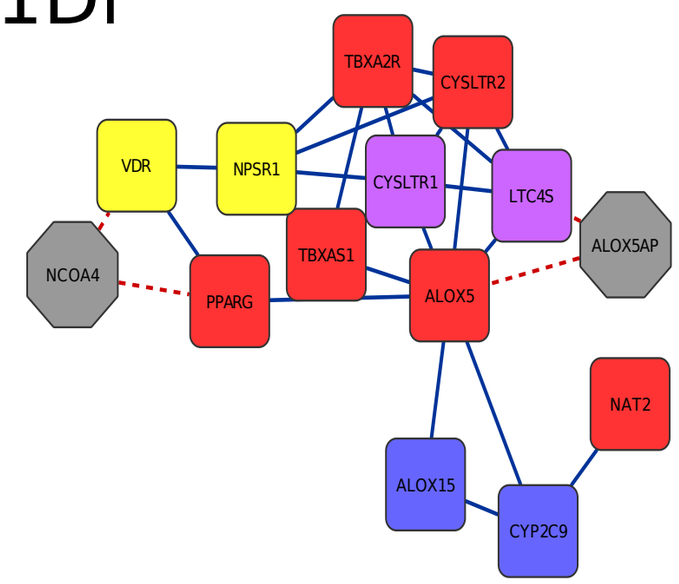
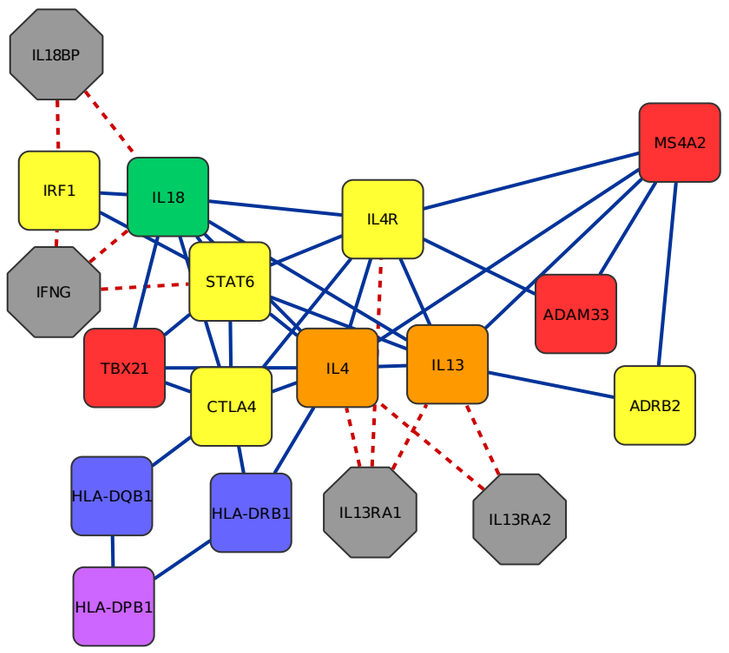
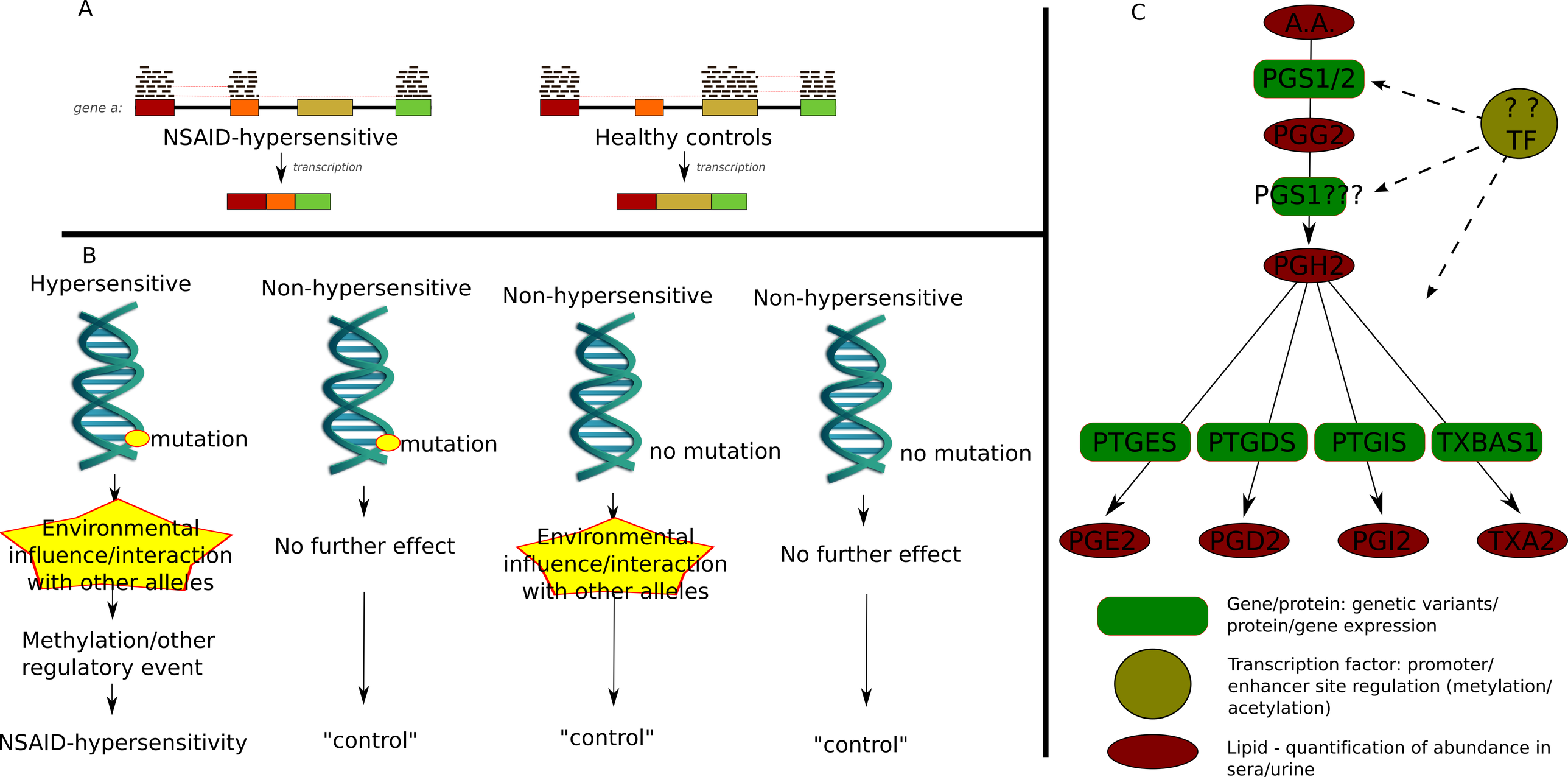


1Dii

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transcription
Healthy controls
transcription
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Environmental influence/interaction with other alleles
Methylation/other regulatory event
NSAID-hypersensitivity
Non-hypersensitive
mutation
No further effect
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Non-hypersensitive
no mutation
Environmental influence/interaction with other alleles
"control"
Non-hypersensitive
no mutation
No further effect
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C
A.A.
PGS1/2
PGG2
PGS1???
PGH2
? ?
TF
PTGES
PTGDS
PTGIS
TXBAS1
PGE2
PGD2
PGI2
TXA2
Gene/protein: genetic variants/ protein/gene expression
Transcription factor: promoter/ enhancer site regulation (metylation/ acetylation)
Lipid - quantification of abundance in sera/urine