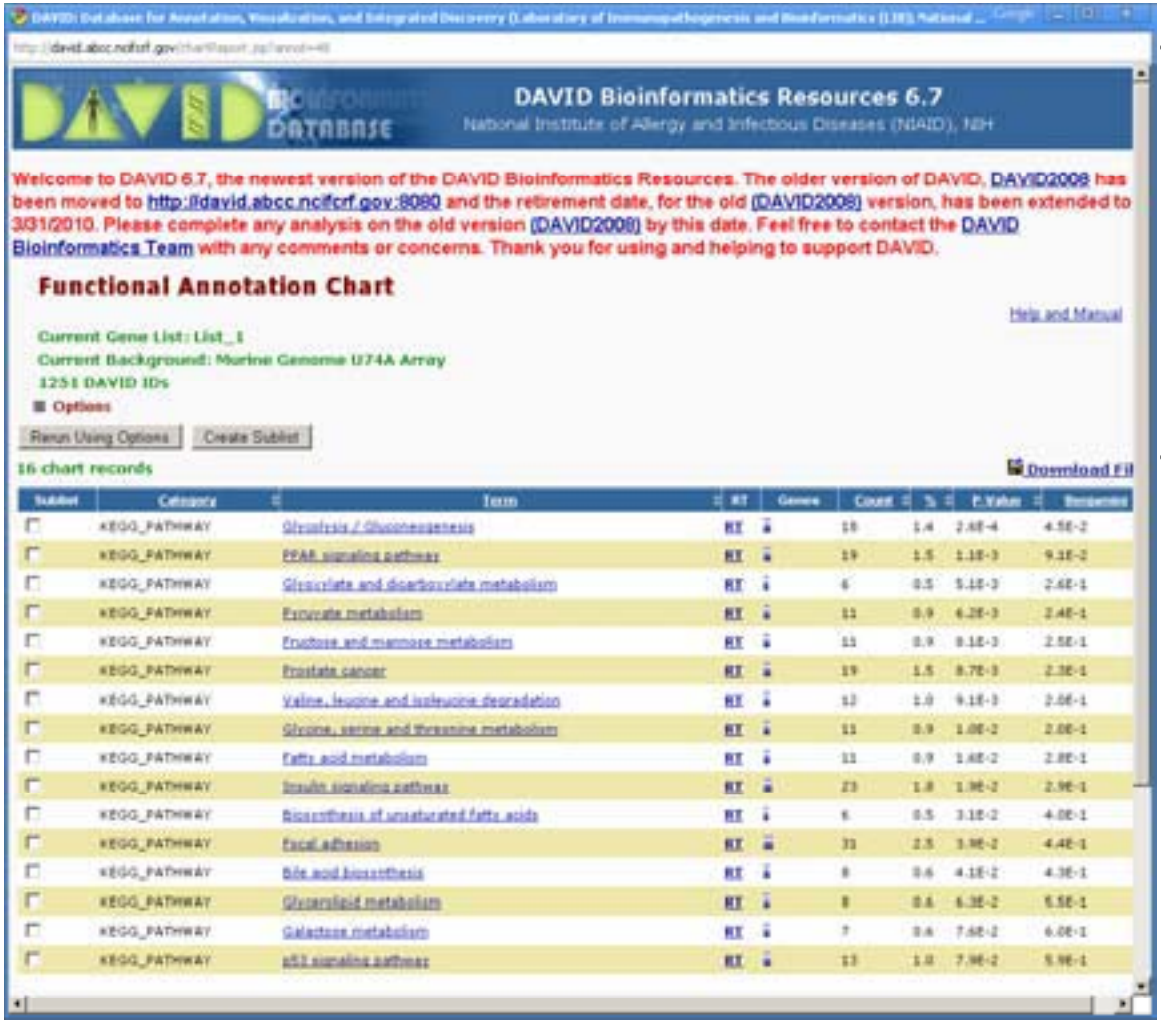
Using Transcriptomics to Identify Pathways

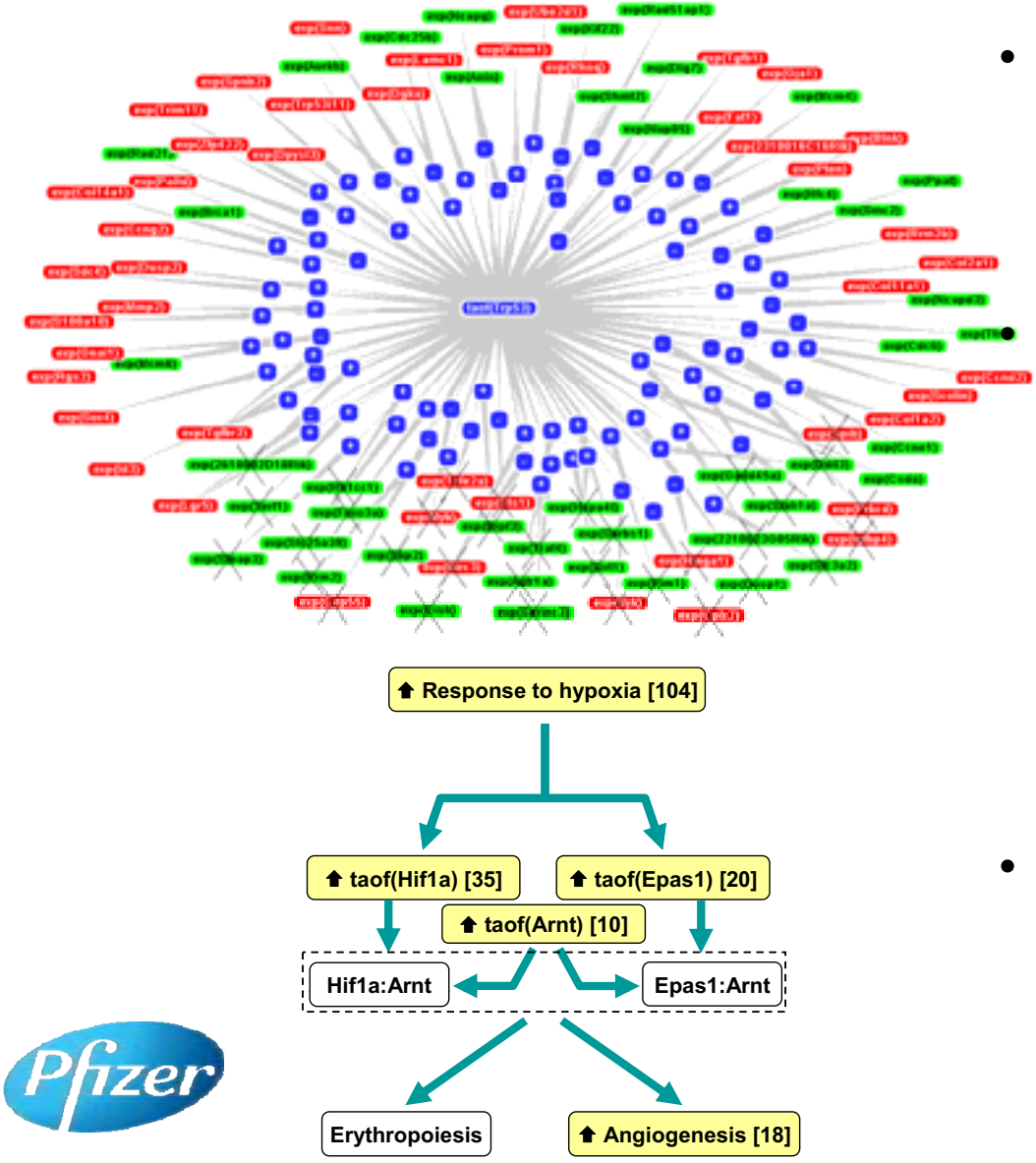


- Same differentially expressed genes from previous troglitazone experiment
- Compound is a PPAR agonist used in type II diabetes

1: Asano T, Wakisaka M, Yoshinari M, Nakamura S, Doi Y, Fujishima M. Troglitazone enhances glycolysis and improves intracellular glucose metabolism in rat mesangial cells. Metabolism. 2000 Mar;49(3):308-13. PubMed PMID: 10726906.

2: Fulgencio JP, Kohl C, Girard J, Pégorier JP. Troglitazone inhibits fatty acid oxidation and esterification, and gluconeogenesis in isolated hepatocytes from starved rats. Diabetes. 1996 Nov;45(11):1556-62. PubMed PMID: 8866561.

Casual Reasoning



- Similar to pathway analyses with directionality of change also considered

Pathways 'hypotheses' generally built from manually curated gene/protein relationships

  - A hypothesis may be activity of a gene
  - … or a more general activity

- Selventa / GenStruct commercial pioneer of the approach however a few public efforts underway

Toxicogenomics Workflow – Informatics Driven

Transcriptomics/Proteomics/Metabolomics

Compound-driven

Experimental design
- Multiple replicates
- Multiple doses
- Multiple timepoints
- Sample pooling

Data generation
- Signal generation
- Normalization

Identification of differentially expressed genes
- May entail conversion of protein or other IDs to gene IDs

Target-driven

Informatic identification of related genes
- Gene in same pathway
- Genes with similar active site
- Genes with similar phenotype

Multi-Gene Analysis

Toxicogenomics / Systems Biology
- *Categorical Analysis*
- *Pathway Analysis*
- *De novo Networks*
- *Casual Reasoning*

Informatic Identification of Related Genes

- Identification of safety risks before any experimental work takes place would yield significant cost savings
  - We're not there yet but we're getting closer
- New drug targets are rarely novel to the literature
  - It is not uncommon for targets to have been mapped to one or more pathways
- Pathway neighbors and / or co-reported genes can be used as related set of genes
  - For co-reported genes, its best to use multiple reports to infer a relationship
- Related genes can then be used with any of the previous informatic analysis techniques

Mouse Mutation Phenotype GeneSets

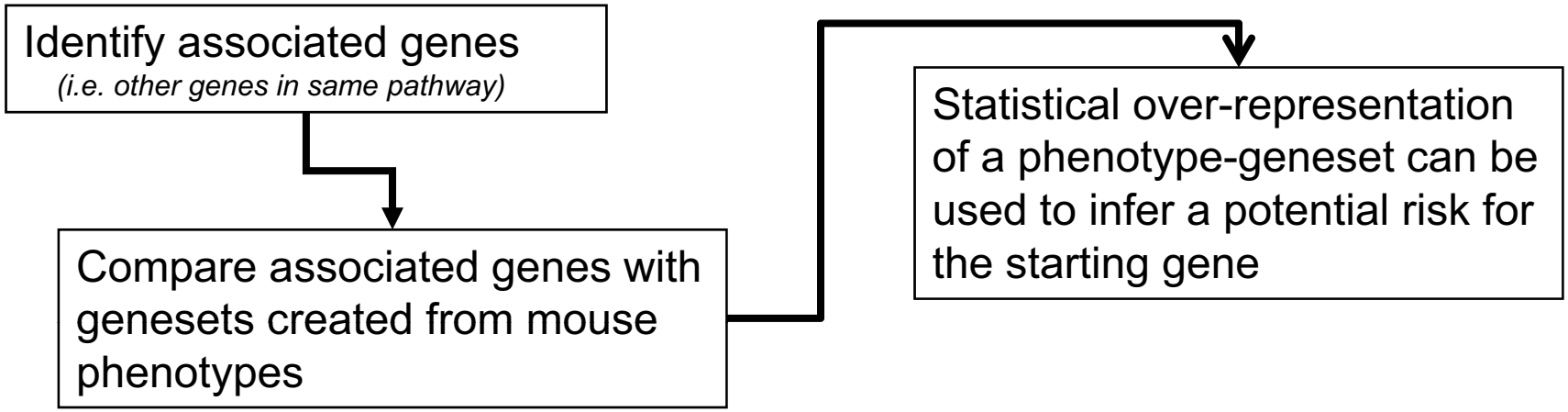
- Mouse Genome Institute at Jackson Labs maintains a large repository of mouse gene mutations
  - MGI uses a controlled vocabulary ontology to characterize the phenotypes displayed by these mutants

- Mouse Phenotypes geneset consists of single phenotypes linked to all genes whose mutation is associated with the phenotype
  - Some animals have double (or more) gene mutations
  - Most mutations are knock-outs but some are knock-ins

- Like siRNA experiments, phenotypes are generally the result of one or two mutations
  - “Clean” phenotypes (not result of multi-gene effects)
  - Whole animal phenotypes versus cell culture phenotypes
  - Analyzing entire set of genes with a single phenotype results in good picture of pathways involved

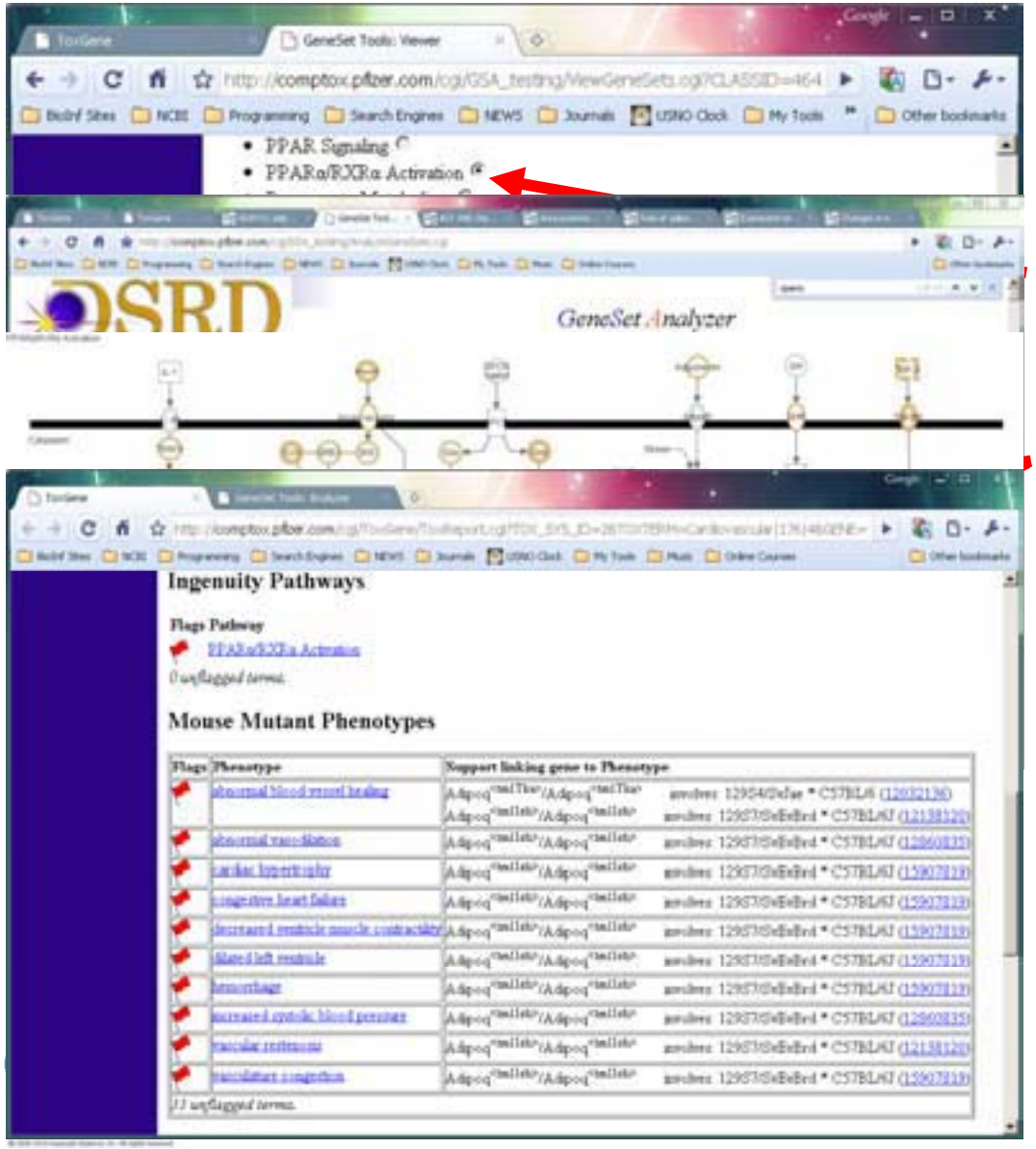
Using Gene Associations to Infer Risk

If A Target's Pathway(s) Are Known …



- Using GeneSet Viewer, you can look for commonality between any two categories
  - *A single pathway can be compared to the* 'Mouse Mutant Phenotypes' gene-sets to identify potential off-target toxicities
  - For compounds that hit multiple targets, protein domain can be compared to the 'Mouse Mutant Phenotypes' gene-sets to identify potential off-target toxicities
    - Ie cardiotox; 'protein tyrosine kinase activity' GO term versus Mouse phenotypes may suggest off-target kinases with a relationship to cardiovascular effects

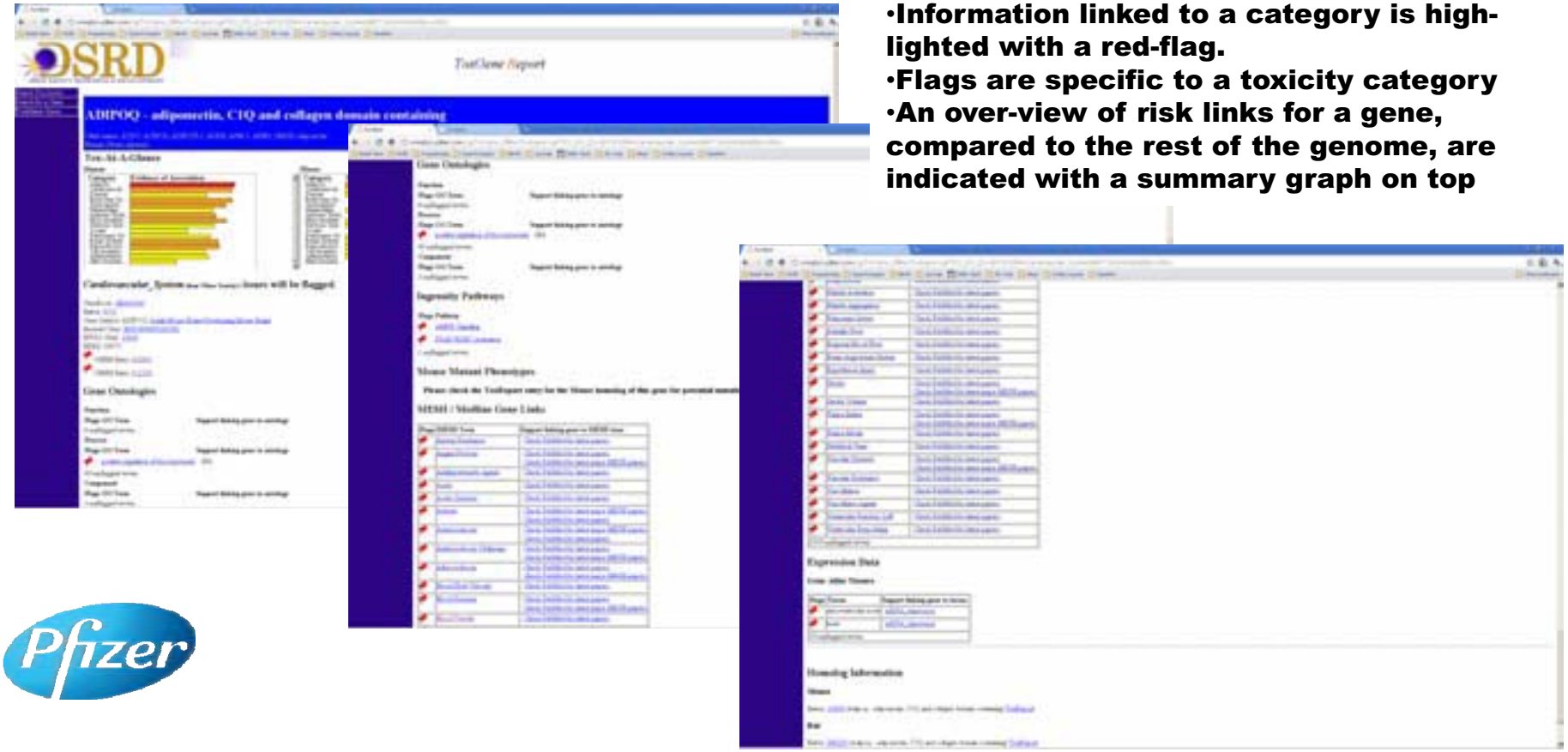
Analysis of Pathway Risk



- Adiponectin is a hormone made exclusively by adipocytes and is thought to play a role in diabetes
- ADIPOQ maps to the 'PPARα/RXRα Activation' pathway
- Pathway analyses indicates pathway play a role in the cardiovascular system
- Mapping the cardiovascular genes back to the pathway shows extensive coverage
- Examination of reveals Adipoq mouse mutants exhibit several cardiovascular phenotypes

ToxReporter – Putting It All Together to Estimate Target Risk

- ToxReporter is a tool bring multiple sources of information related to various areas of risk into a single gene view
- Risk areas are categorized into broad areas of toxicity high-lighting potential areas of concern



•Information linked to a category is high-lighted with a red-flag.
•Flags are specific to a toxicity category
•An over-view of risk links for a gene, compared to the rest of the genome, are indicated with a summary graph on top

Conclusions

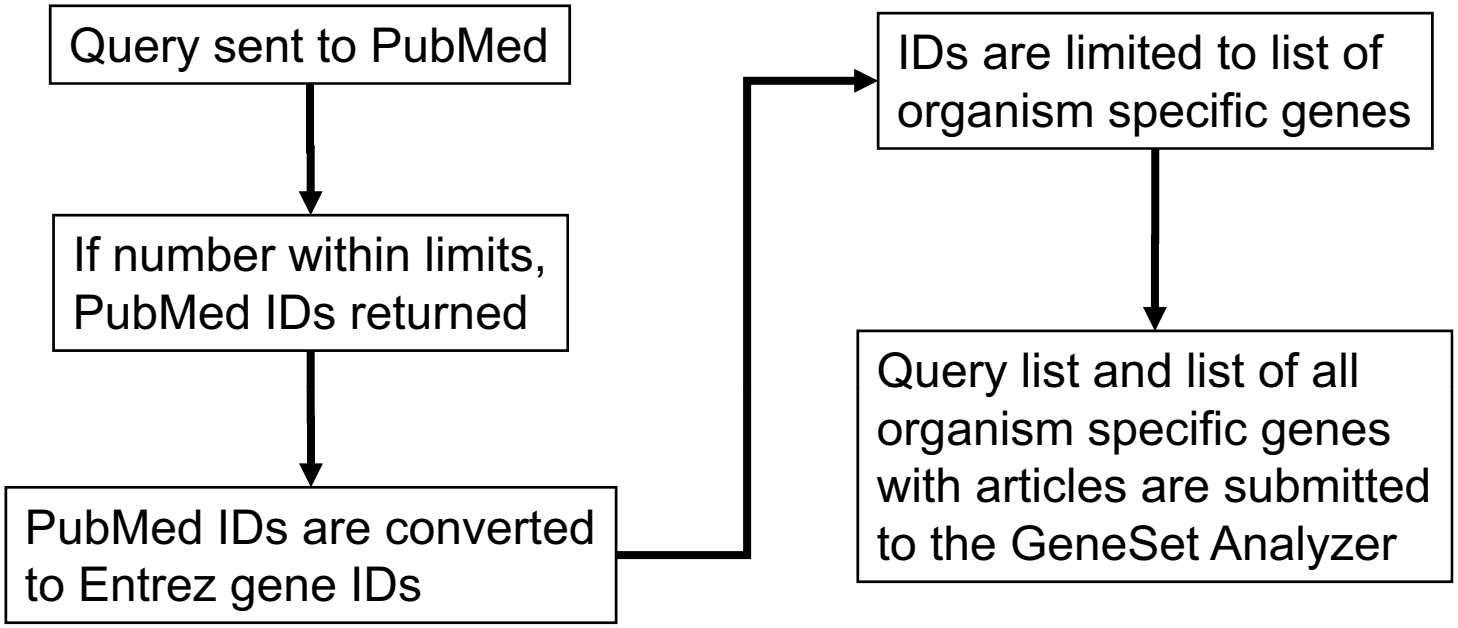
- Toxicogenomic methodologies allow researchers to get a ‘big picture’ view of changes happening in organisms exposed to toxicants and thereby provide in-sight in mechanisms

- Through the use of gene and related gene information, potential toxicities of a new target can be considered at the very earliest stages of drug discovery

- Individual genetic variation plays a significant role in the response of an individual to a compound and pharmacogenomic analyses can provide in-sight into the genes behind this variation

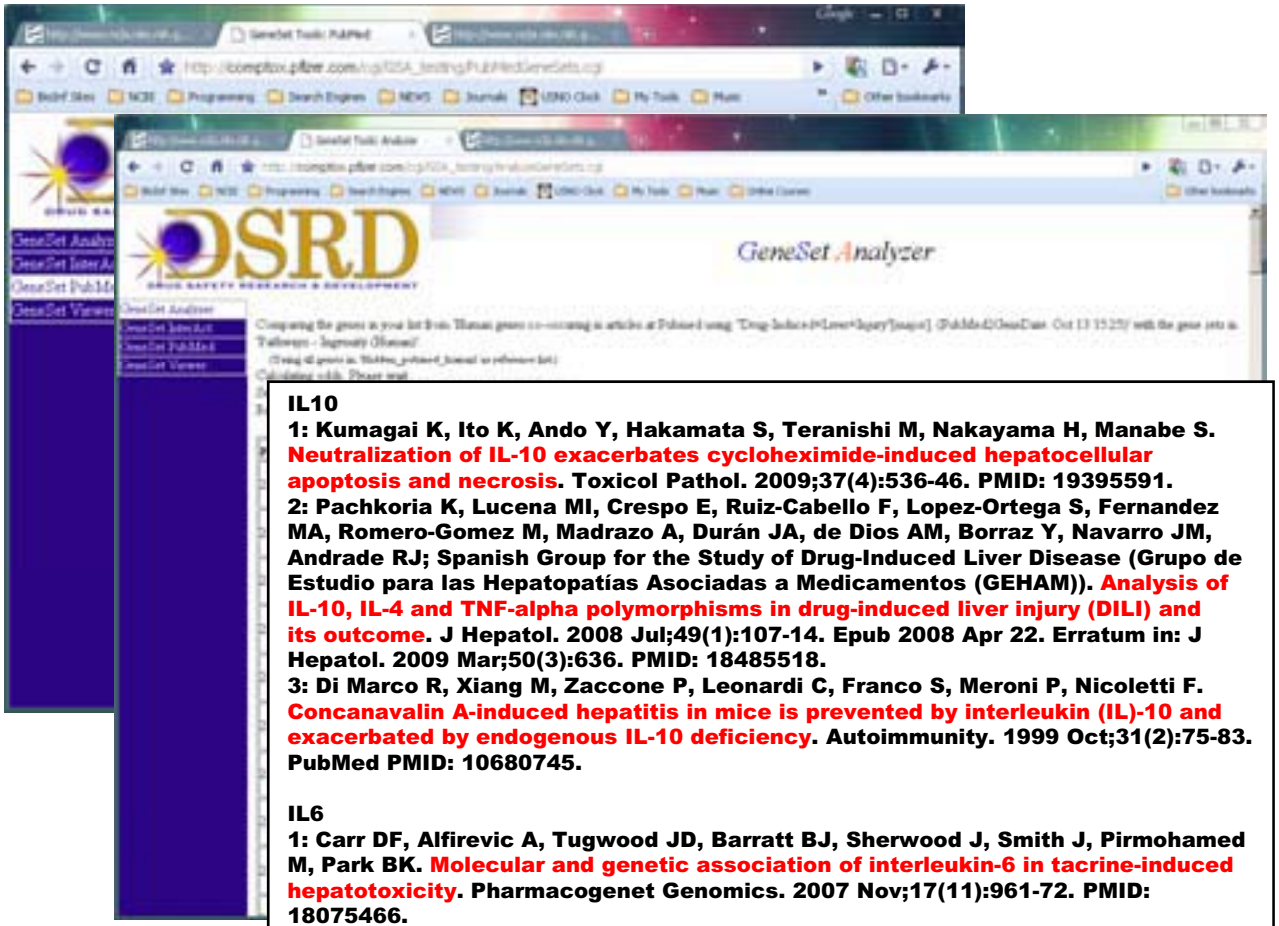
Additional Materials

Search for General Mechnisms of Toxicity



- Using GeneSet PubMed, you can get a general idea of the major pathways being discussed for any disease or toxicity or other area of interest
- Its recommended query be run on PubMed first before submission to the Analyzer to ensure query validity

Pathways Related to Liver Injury



IL10
1: Kumagai K, Ito K, Ando Y, Hakamata S, Teranishi M, Nakayama H, Manabe S. Neutralization of IL-10 exacerbates cycloheximide-induced hepatocellular apoptosis and necrosis. Toxicol Pathol. 2009;37(4):536-46. PMID: 19395591.
2: Pachkoria K, Lucena MI, Crespo E, Ruiz-Cabello F, Lopez-Ortega S, Fernandez MA, Romero-Gomez M, Madrazo A, Durán JA, de Dios AM, Borraz Y, Navarro JM, Andrade RJ; Spanish Group for the Study of Drug-Induced Liver Disease (Grupo de Estudio para las Hepatopatías Asociadas a Medicamentos (GEHAM)). Analysis of IL-10, IL-4 and TNF-alpha polymorphisms in drug-induced liver injury (DILI) and its outcome. J Hepatol. 2008 Jul;49(1):107-14. Epub 2008 Apr 22. Erratum in: J Hepatol. 2009 Mar;50(3):636. PMID: 18485518.
3: Di Marco R, Xiang M, Zaccone P, Leonardi C, Franco S, Meroni P, Nicoletti F. Concanavalin A-induced hepatitis in mice is prevented by interleukin (IL)-10 and exacerbated by endogenous IL-10 deficiency. Autoimmunity. 1999 Oct;31(2):75-83. PubMed PMID: 10680745.

IL6
1: Carr DF, Alfirevic A, Tugwood JD, Barratt BJ, Sherwood J, Smith J, Pirmohamed M, Park BK. Molecular and genetic association of interleukin-6 in tacrine-induced hepatotoxicity. Pharmacogenet Genomics. 2007 Nov;17(11):961-72. PMID: 18075466.
2: Masubuchi Y, Bourdi M, Reilly TP, Graf ML, George JW, Pohl LR. Role of interleukin-6 in hepatic heat shock protein expression and protection against acetaminophen-induced liver disease. Biochem Biophys Res Commun. 2003 Apr 25;304(1):207-12. PMID: 12705907.
3: Kovalovich K, DeAngelis RA, Li W, Furth EE, Ciliberto G, Taub R. Increased toxin-induced liver injury and fibrosis in interleukin-6-deficient mice. Hepatology. 2000 Jan;31(1):149-59. PMID: 10613740.

- Search initiated for all PubMed articles which are annotated as having “Drug Induced Liver Injury” as a major MESH topic
  - 13,210 articles
- Article IDs are mapped to 171 human genes
  - Genes mapped to Ingenuity pathways
- IL10 & IL6 were surprising *(to me)*
  - *Literature confirms links*

Pharmacogenomics

- Any two individuals are 99.9% identical at the nucleotide level
  - ~4M out of 3.2 billion bases
  - (Ignoring epigenetic variation)
- Types of variation
  - SNPs (single nucleotide polymorphisms) account for ~90% of this variation
  - Insertions /deletions
  - Copy number variations
- SNP variation in coding regions can induce functional changes in activity
  - While coding SNPs can directly affect protein activity, non-coding SNPs can have considerable effects on gene regulation

- As evidenced by these age matched animals, small genetic differences can account for considerable phenotypic variation

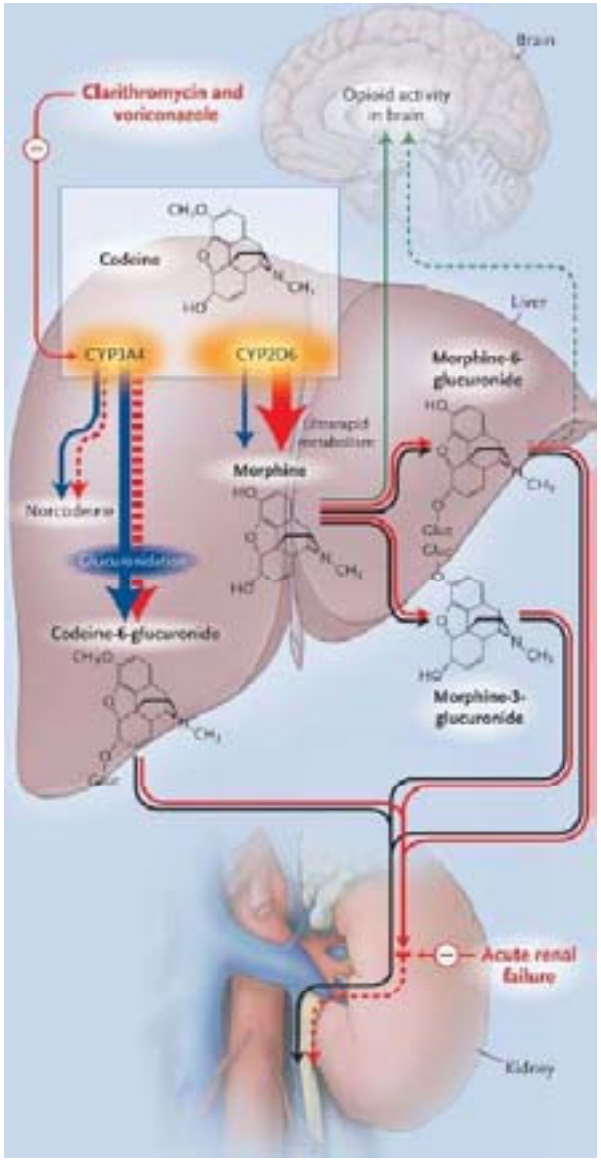
Numeroues Examples Of Pharmacogenomics Effects Are Now In The Literature

- Variations have been reported in drug targets, metabolizing enzymes, drug transporters, and HLA groups
- Most toxicogenetics reports of variation focus on drug metabolizing enzymes rather than drug targets
  - Variation can result in poor metabolism or hyper-metabolism

- FDA web-site indicates that ~10% of all drug labels contain pharmacogenomic information
  - http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm
- PharmGKb database maintains extensive information about pharmacogenomics effects on drug responses

Cyp2D6 and Codeine Metabolism

- Codeine is metabolized in the body to morphine
- One segment of the population have decreased levels of Cyp2D6 which results in poor conversion to morphine
  - Individuals with this allele are poor responders to the analgesic effects of codeine
- A portion of the population have multiple copies of the Cyp2D6 enzyme which results in rapid activation of codeine
  - Individuals with this allele are subject to toxic effects of morphine over-dose



1: Lurcott G. The effects of the genetic absence and inhibition of CYP2D6 on the metabolism of codeine and its derivatives, hydrocodone and oxycodone. Anesth Prog. 1998 Fall;45(4):154-6. Review. PubMed PMID: 10483388; PubMed Central PMCID: PMC2148980.

2: Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P, Desmeules J. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N Engl J Med. 2004 Dec 30;351(27):2827-31. Erratum in: N Engl J Med. 2005 Feb 10;352(6):638. PubMed PMID: 15625333.

Cyp2c19 and Clopidogrel Activity, a Pharmacogenomic Approach

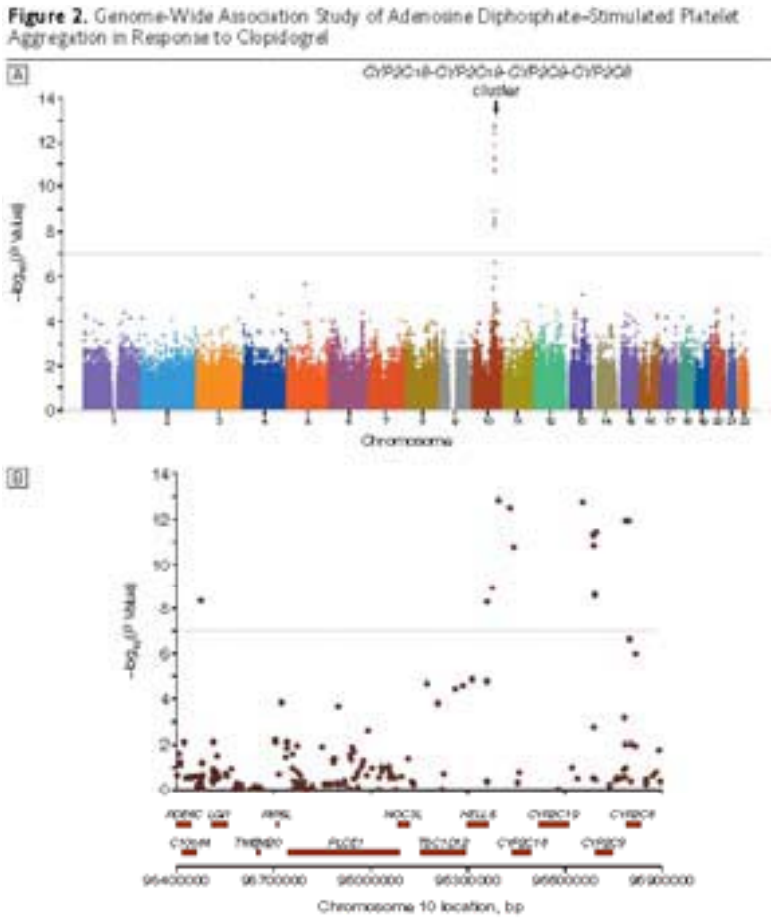


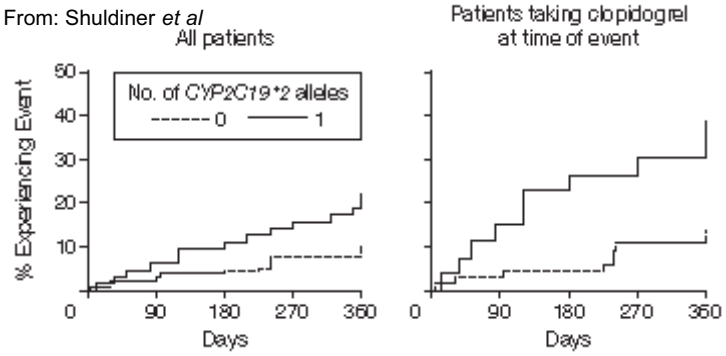
Figure 2. Genome-Wide Association Study of Adenosine Diphosphate–Stimulated Platelet Aggregation in Response to Clopidogrel

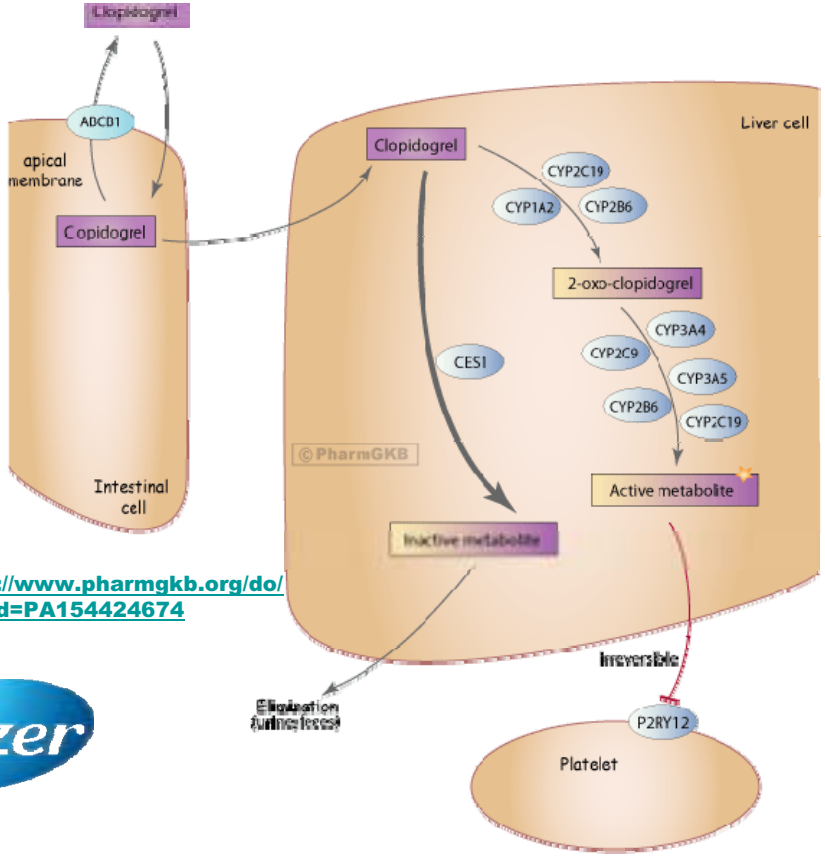
- Clopidogrel (Plavix) inhibits platelet activation and used to treat patients with acute coronary syndromes
- ~20% of patients are non-responders
- Shuldiner *et al* measured platelet aggregation in 429 Amish subjects
- Subjects were also profiled using Affymetrix SNP chips
- Most significant association between SNPs and clopidogrel mapped to a cluster of p450 enyzmes on chromosome 10

1: Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA. 2009 Aug 26;302(8):849-57. PubMed PMID: 19706858.

Cyp2c19 and Clopidogrel Activity, a Pharmacogenomic Approach

From: Shuldiner *et al*





From: http://www.pharmgkb.org/do/serve?objId=PA154424674

- Further mapping indicated that Cyp2C19*2 accounted for ~12% of the clopidogrel response
- Based on 1-year survival data, patients with no copies of the mutant Cyp2C19*2 allele exhibit better survival
- Cyp2C19 is involved in conversion of clopidogrel to the active metabolite

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Acknowledgements

- Pfizer
  - Jon Cook
  - Nigel Greene
  - Anne Ryan

- The Scripps Research Institute & the Florida Funding Corporation
  - Nicholas Tsinoremas

Useful Databases / Tools

- Genes
  - Entrez Gene
    - http://www.ncbi.nlm.nih.gov/gene/
  - JAX Mammalian Phenotype Browser
    - http://www.informatics.jax.org/searches/MP_form.shtml
- Proteomic
  - Human Proteome Initiative (HPI)
    - http://www.expasy.ch/sprot/hpi/
  - International Protein Index (IPI)
    - http://www.ebi.ac.uk/IPI/IPIhelp.html
- Metabolomic / Chemical
  - Comparative Toxicogenomics Database (CTD)
    - http://ctd.mdibl.org/
  - DrugBank
    - http://www.drugbank.ca/
  - Toxin and Toxin Target Database (T3DB)
    - http://www.t3db.org/
  - The Human Metabolome Project
    - http://www.metabolomics.ca/
  - Consortium for Metabonomic Toxicology
    - http://bc-comet.sk.med.ic.ac.uk/

- Pathways
  - KEGG
    - http://www.genome.jp/kegg/kegg2.html
  - Ingenuity
    - http://www.ingenuity.com/
  - Pathguide
    - http://www.pathguide.org/

- Tools
  - DAVID
    - http://david.abcc.ncifcrf.gov/tools.jsp
  - GSEA
    - http://www.broadinstitute.org/gsea/
  - Connectivity Map
    - http://www.broadinstitute.org/cmap/
  - GenStruct
    - http://www.genstruct.com/home.php

- Pharmacogenomics
  - PharmGKb
    - http://www.pharmgkb.org/
  - dbSNP
    - http://www.ncbi.nlm.nih.gov/snp
  - dbVar
    - http://www.ncbi.nlm.nih.gov/dbvar/