Carolina Bioinformatics Research Center

Project 3:
Computational Infrastructure for Systems Toxicology

- David Stotts, Ph.D. (co-P.I.) – computer science, software engineering
- **Ivan Rusyn, M.D., Ph.D. (co-P.I.) – toxicology, genomics**
- Wei Wang, Ph.D. – computer science, data mining
- David Threadgill, Ph.D. – mammalian genetics, genomics
- Additional programmers and students

Ivan Rusyn
Director, Laboratory of Environmental Genomics
Department of Environmental Sciences & Engineering
University of North Carolina
Chapel Hill, NC 27599
919-843-2596
iir@unc.edu

Project 3 objectives

- Develop and implement algorithms that streamline the analysis of multi-dimensional data streams in dose-response assessment and cross-species extrapolation.

- Facilitate the development of an industry-standard workflow for (i) analysis of the -omics data, (ii) linkages to classical indicators of adverse health effects, and (iii) integration with other types of biological information such as genome sequences and genetic differences between species.

- Build web-based, open-source and user-friendly graphical interfaces associated with interoperable computational tools for data analysis that facilitate incorporation of new data streams into basic research and decision-making pipelines (methods from Projects 1 and 2).

- Provide an interdisciplinary computer science resource to the environmental sciences and toxicology community

- Longer-term objectives include new software engineering methods for better execution and maintenance of above, and sharing and disseminating results

A driving biological problem:

- **Toxicogenetic analysis of the genetic susceptibility to toxicant-induced organ injury**

- **The model being used by Drs. Rusyn and Threadgill involves extensive phenotypic, gene expression and metabolomic profiling of xenobiotic-induced organ injury in the large panels of inbred mouse strains**

- **Current data on acetominophen and alcohol on liver**

- **Studies are underway with trichloroethylene and other toxicants on liver, kidney, and other organs**

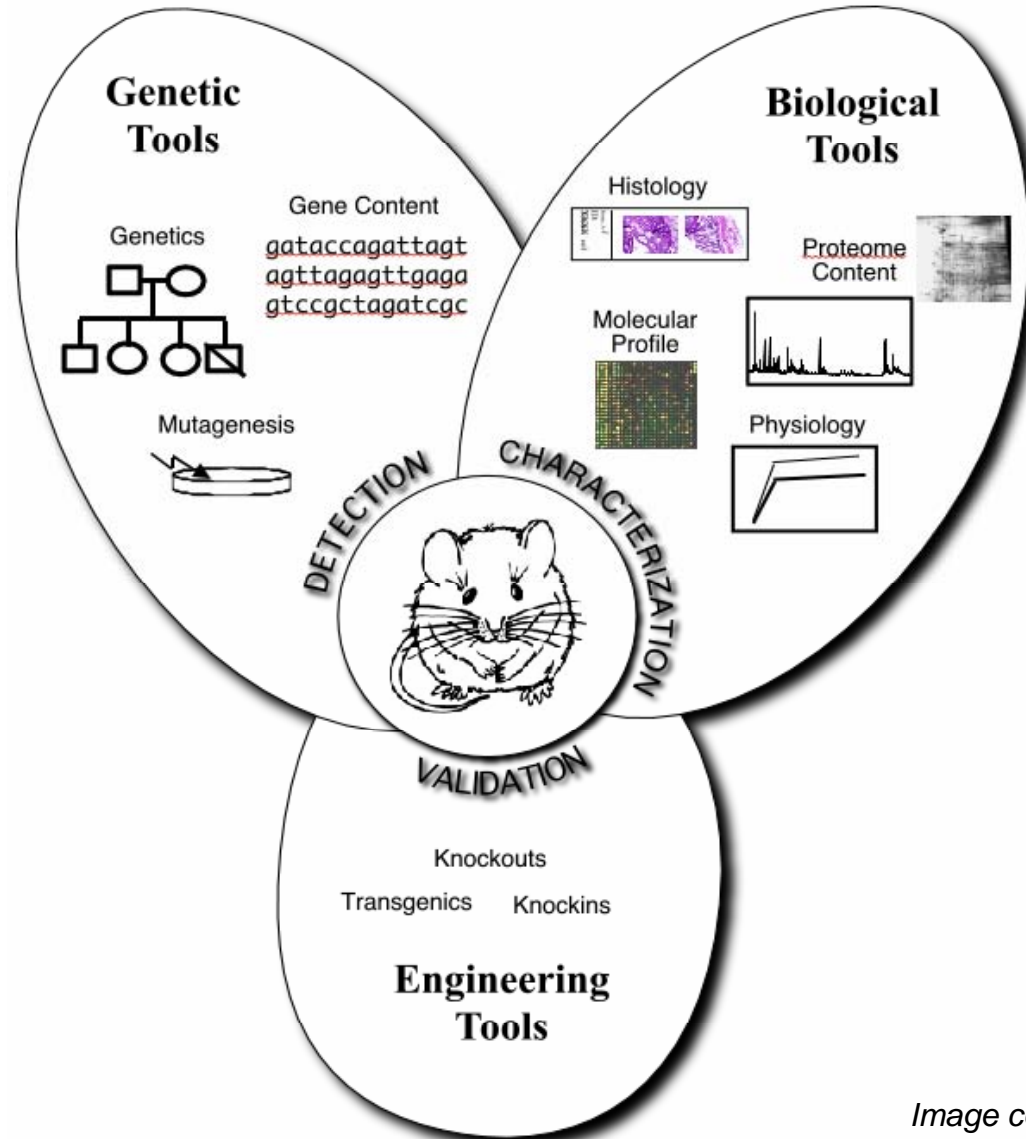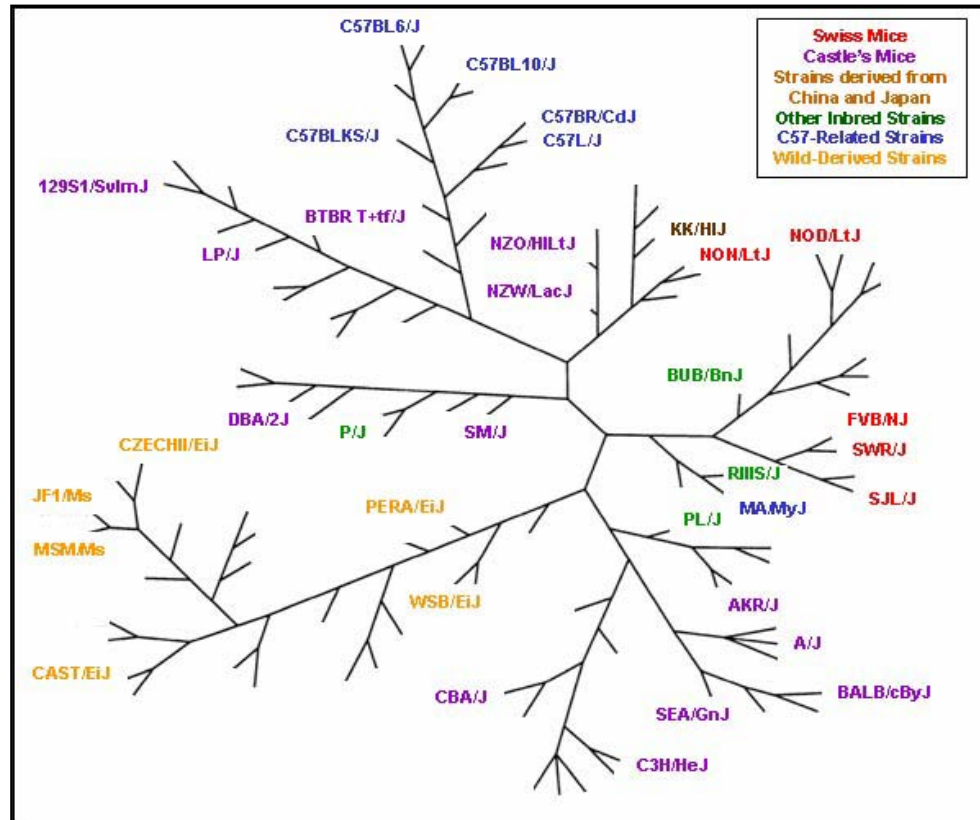# Genotype-Phenotype Interactions in Complex Biological Systems
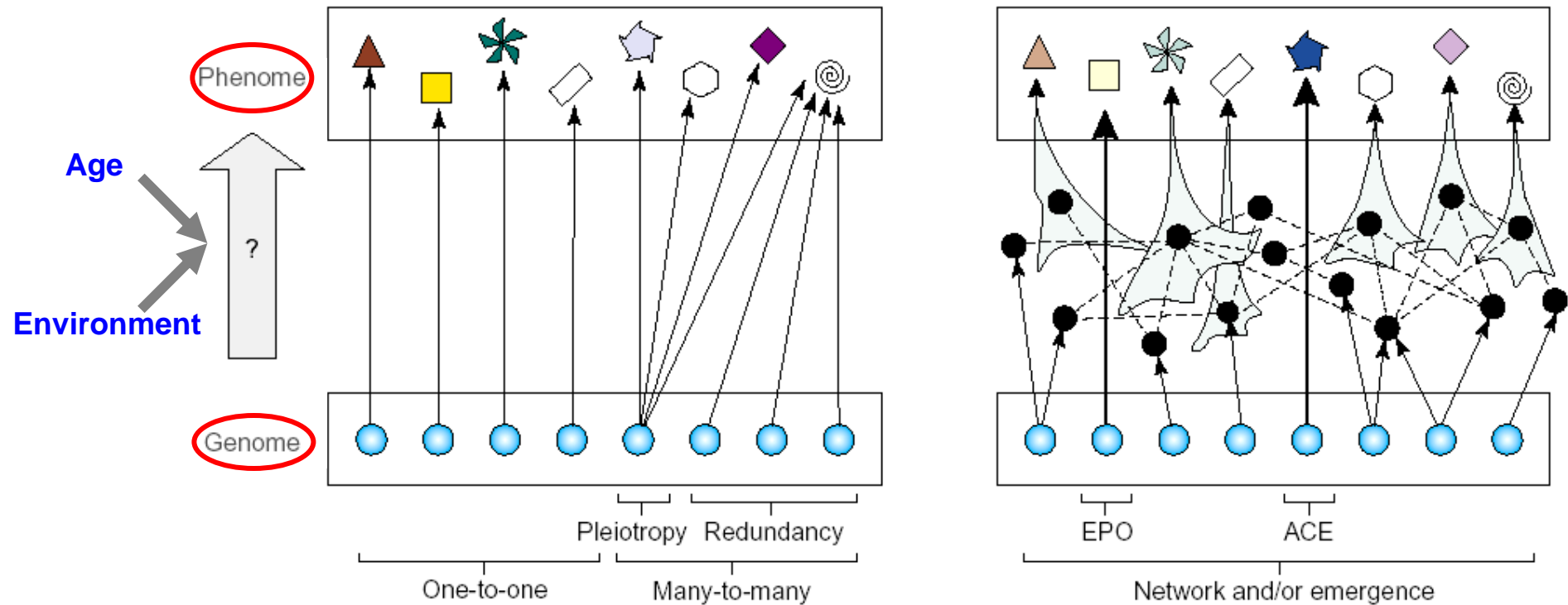
Adapted from: Huang, 2002

Mouse as an Exceptional Model for Studying Genotype-Phenotype Interactions

Image courtesy of D.W. Threadgill

Time-response: 4, 24 and 72 hrs
Dose: APAP 300 mg/kg by gavage

Panel of 36 inbred strains

ENDPOINTS
• Histopathology (liver, kidney)
• Clinical chemistry (ALT, AST, BUN)
• Other toxicity endpoints (survival, GSH, CYPs protein levels, etc.)
• Gene expression profiles
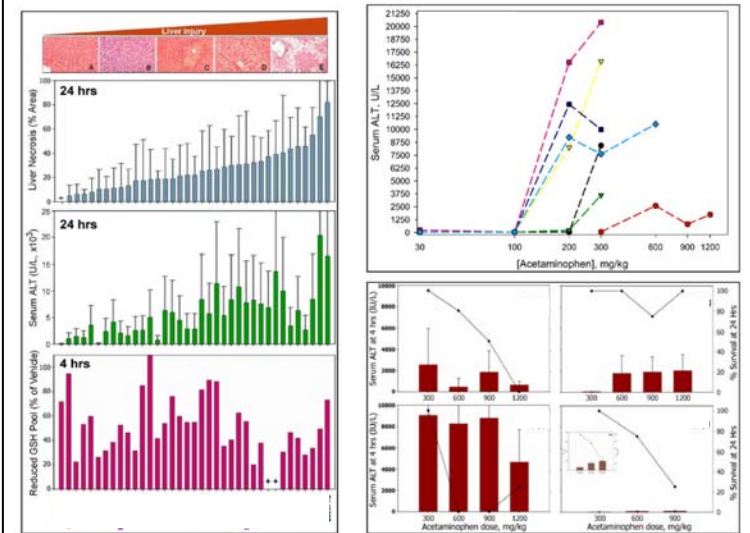• Genotypes (160K SNPs to whole genome sequences)

Strain selection

Dose-response: APAP 30 – 1,200 mg/kg by gavage

Selected strains (Sensitive)   Selected strains (Resistant)

ENDPOINTS
• Histopathology (liver, kidney)
• Survival
• Clinical chemistry (ALT, AST, BUN)
• Other toxicity endpoints (survival, GSH, CYPs proteins, etc.)
• Genotypes (160K SNPs to whole genome sequences)

knowledge

Swiss Mice
Castle's Mice
Strains derived from China and Japan
Other Inbred Strains
C57-Related Strains
Wild-Derived Strains

C57BL6/J
C57BL10/J
C57BR/CdJ
C57L/J
C57BLKS/J
129S1/SvlmJ
BTBR T+tf/J
LP/J
NZO/HILtJ
NZW/LacJ
KK/HIJ
HON/LtJ
NOD/LtJ
BUB/BnJ
FVB/NJ
SWR/J
DBA/2J
P/J
SM/J
CZECHII/EiJ
RIIIS/J
SJL/J
JF1/Ms
PERA/EiJ
PL/J
MA/MyJ
MSM/Ms
WSB/EiJ
AKR/J
A/J
CAST/EiJ
CBA/J
SEA/GnJ
BALB/cByJ
C3H/HeJ

Profiling Liver Toxicity in a Genetically-Diverse Population

"Systems Toxicology" Approach
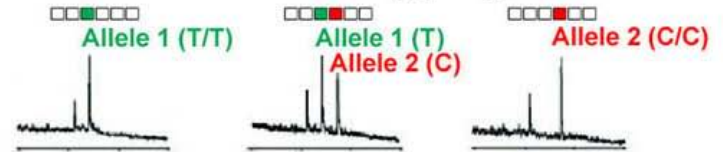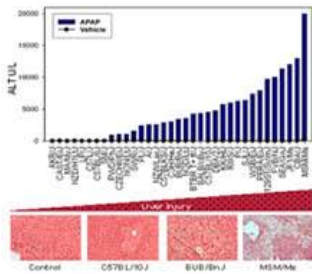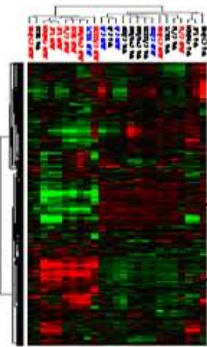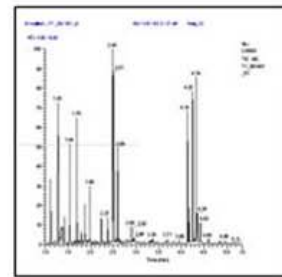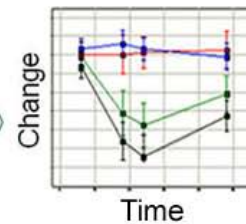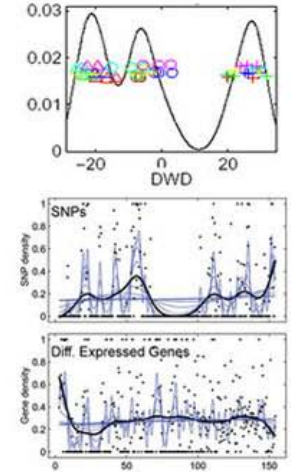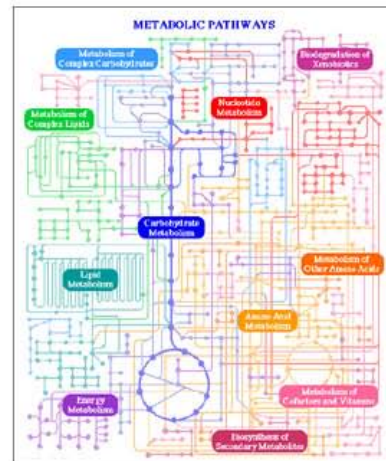
Tylenol may elevate liver enzymes
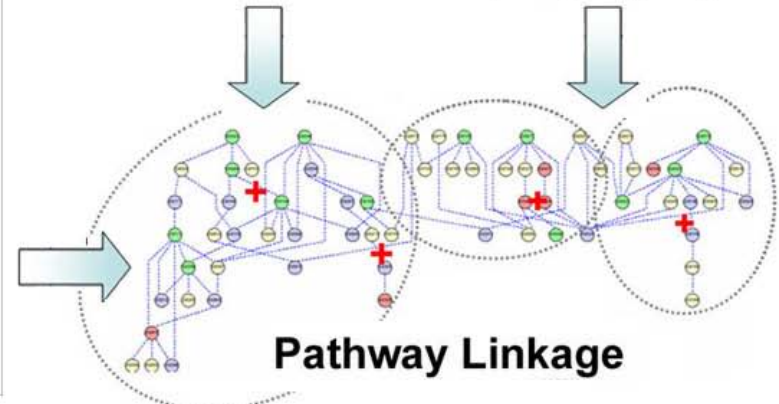
Updated 7/4/2006 7:49 PM ET

E-mail | Save | Print | Reprints & Permissions | Subscribe to stories like this   **RSS**

⊕ Enlarge   USA TODAY

The maximum recommended daily dose of acetaminophen may elevate liver enzymes, a study says.

By Rita Rubin, USA TODAY

CHICAGO — The maximum recommended daily dose of acetaminophen, the pain reliever best known as Tylenol, can cause liver blood tests that suggest the presence of disease, according to a study.

"Several of the subjects actually had (liver enzyme) elevations to the point that any physician would become very alarmed and want to know why," says lead author Paul Watkins of the University of North Carolina-Chapel Hill. Still, Watkins emphasized, "I don't think it means that acetaminophen is dangerous as it's being consumed."

Acetaminophen overdoses can severely damage the liver, but when taken as directed, the drug has a long track record of safety, Watkins notes. In addition, he says, no previous study had ever found that taking the maximum daily recommended dose of 4 grams — or eight Extra-Strength Tylenol — raised liver enzyme levels.

The study, in the *Journal of the American Medical Association*, was financed by Purdue Pharma, which had halted clinical trials of a combination opiate and acetaminophen product because of elevated liver enzyme levels, Watkins says.

Current Concepts of Experimental Acetaminophen Hepatotoxicity

Kaplowitz. Nat Rev Drug Discov. 2005 Jun;4(6):489-99.
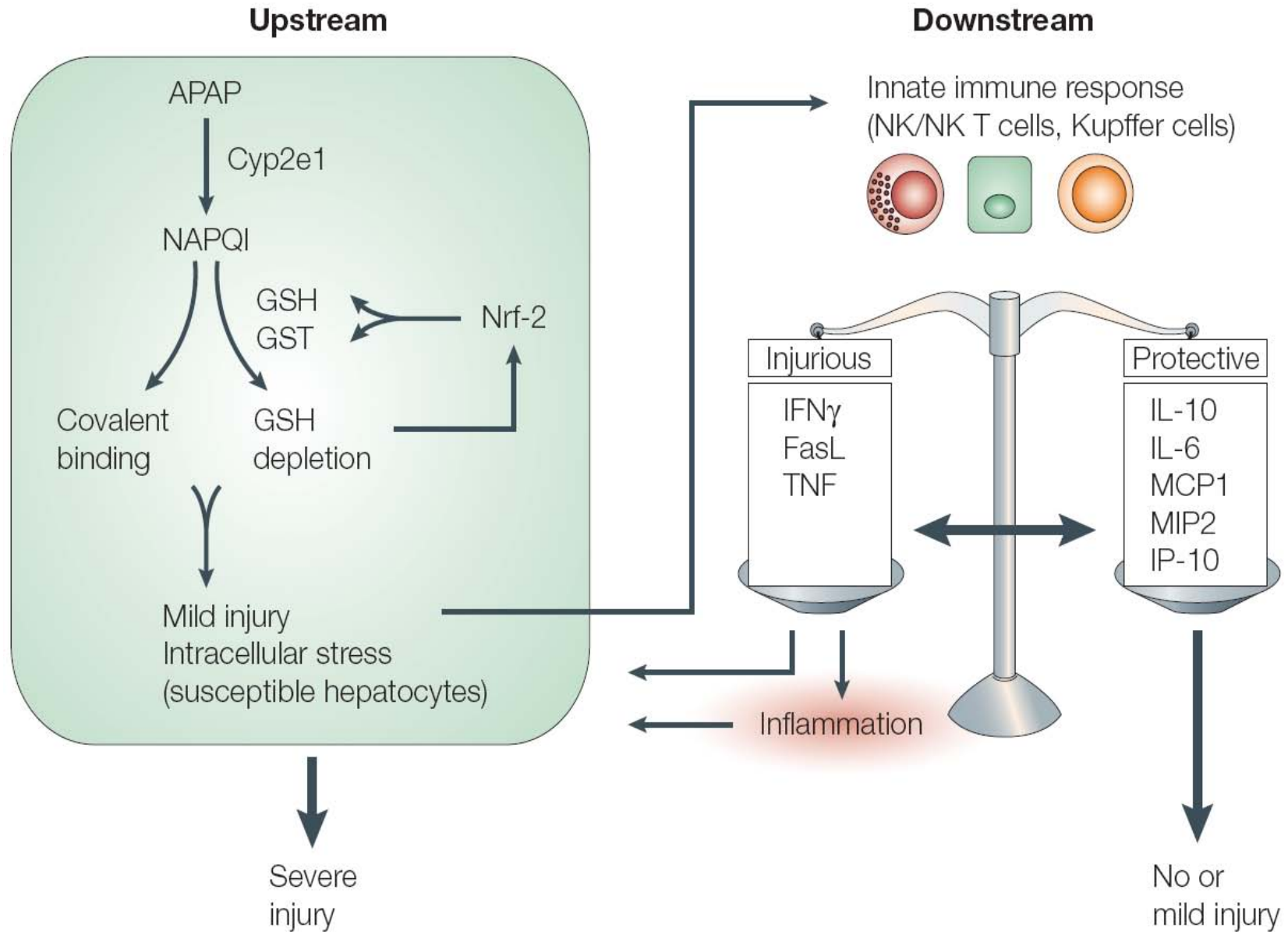
Profiles of susceptibility to toxicant stress
U19-ES011391 (Rusyn – PI, Project #4)

Toxicogenomic Consortium Standardization Experiment #3 (Phase 2):
Toxicogenetic Analysis of Susceptibility to Acetaminophen-Induced Liver Injury

Time-response: 4, 24 and 72 hrs
Dose: APAP 300 mg/kg by gavage

Panel of 36 inbred strains

ENDPOINTS
- **Histopathology (liver, kidney)**
- **Clinical chemistry (ALT, AST, BUN)**
- **Other toxicity endpoints (survival, GSH, CYPs protein levels, etc.)**
- **Gene expression profiles**
- **Genotypes** (160K SNPs to whole genome sequences)

Strain selection

**Dose-response: APAP 30 – 1,200 mg/kg by gavage**

| Selected strains (Sensitive) | Selected strains (Resistant) |

ENDPOINTS
- **Histopathology (liver, kidney)**
- **Clinical chemistry (ALT, AST, BUN)**
- **Other toxicity endpoints (survival, GSH, CYPs proteins, etc.)**
- **Gene expression profiles**
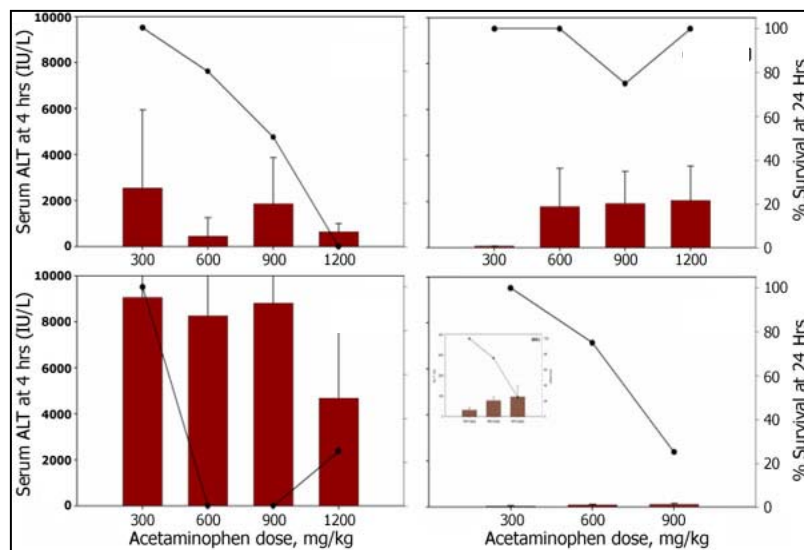- **Genotypes** (160K SNPs to whole genome sequences)

Profiling Liver Toxicity to APAP in a Genetically-Diverse Population
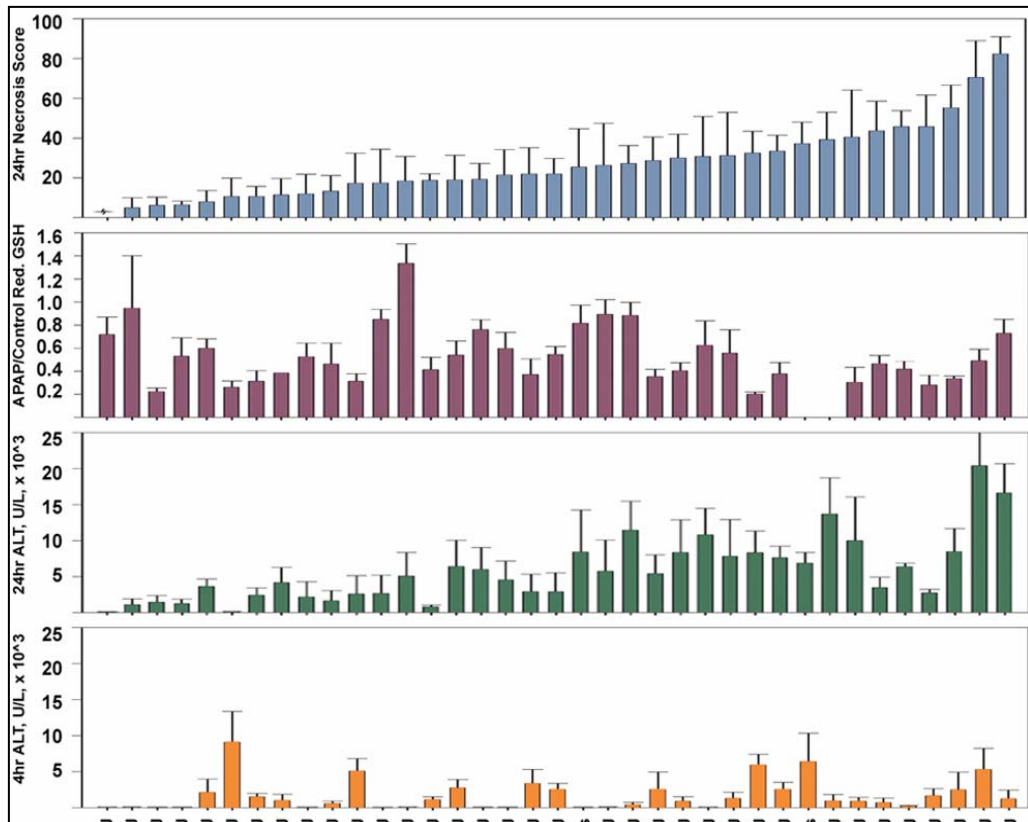
Multi-strain profiling of APAP-induced liver injury:
% liver necrosis (24h), reduced GSH (4h), ALT (24h), ALT (4h)
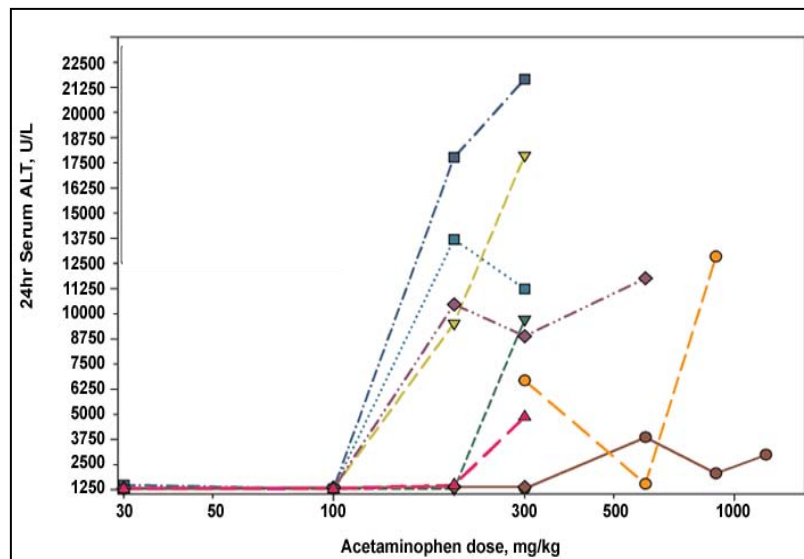
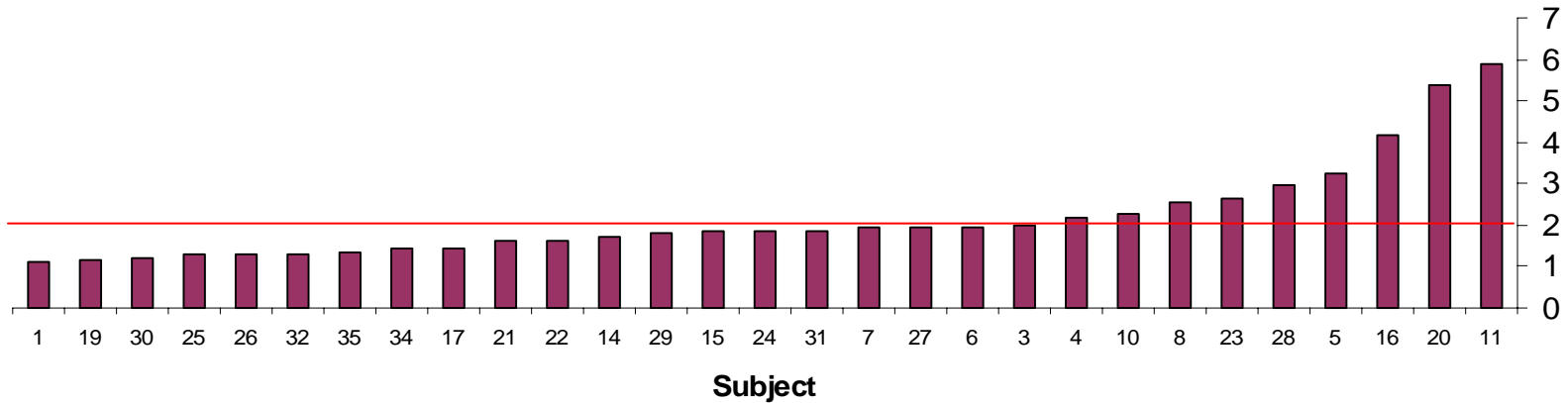Dose response to liver injury: ALT (24 h)

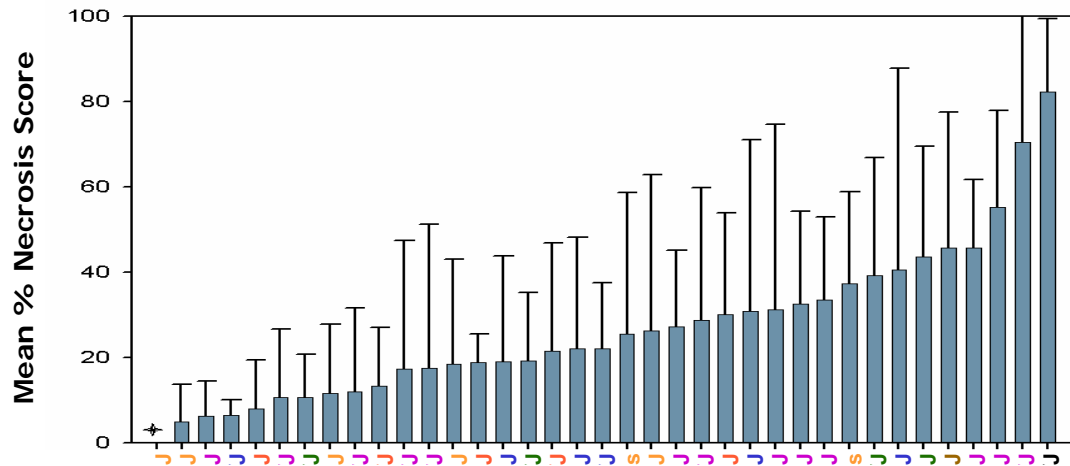Dose response to liver injury (4 h) vs survival (24 h)

Acetaminophen-Induced Liver Injury: Species Comparison

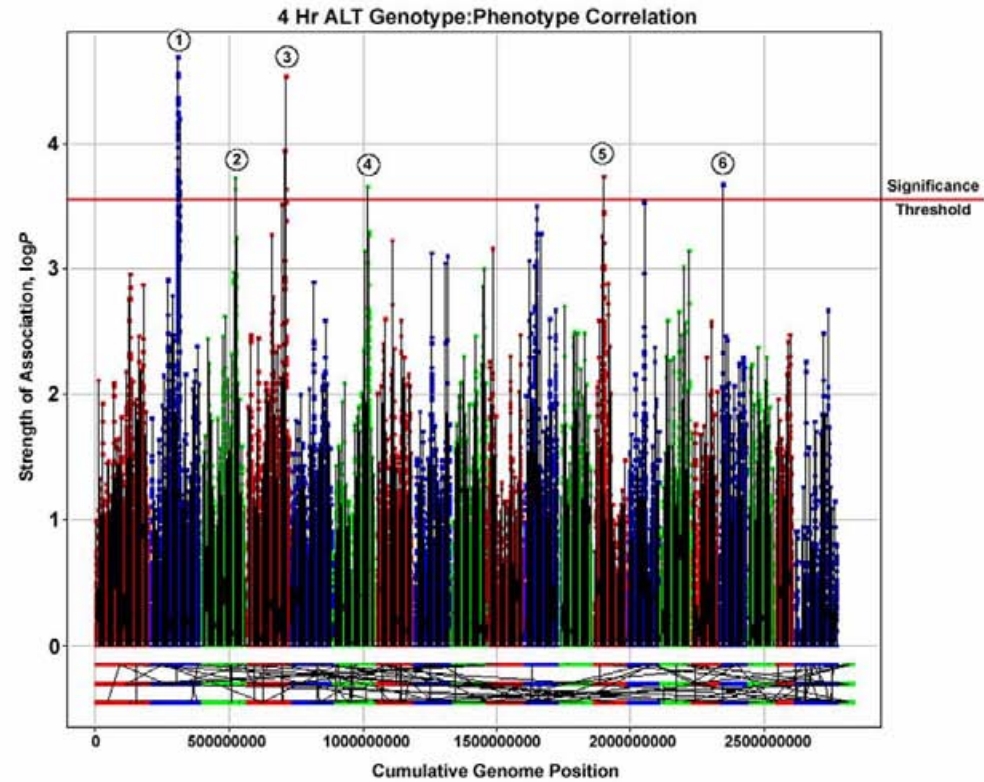## Human Subject Rank (Treated): Fold Change in ALT from Baseline

Data from clinical studies by Drs. Paul Watkins and Mark Russo at UNC Hospitals

Mouse Strain Rank (Treated): Liver Necrosis
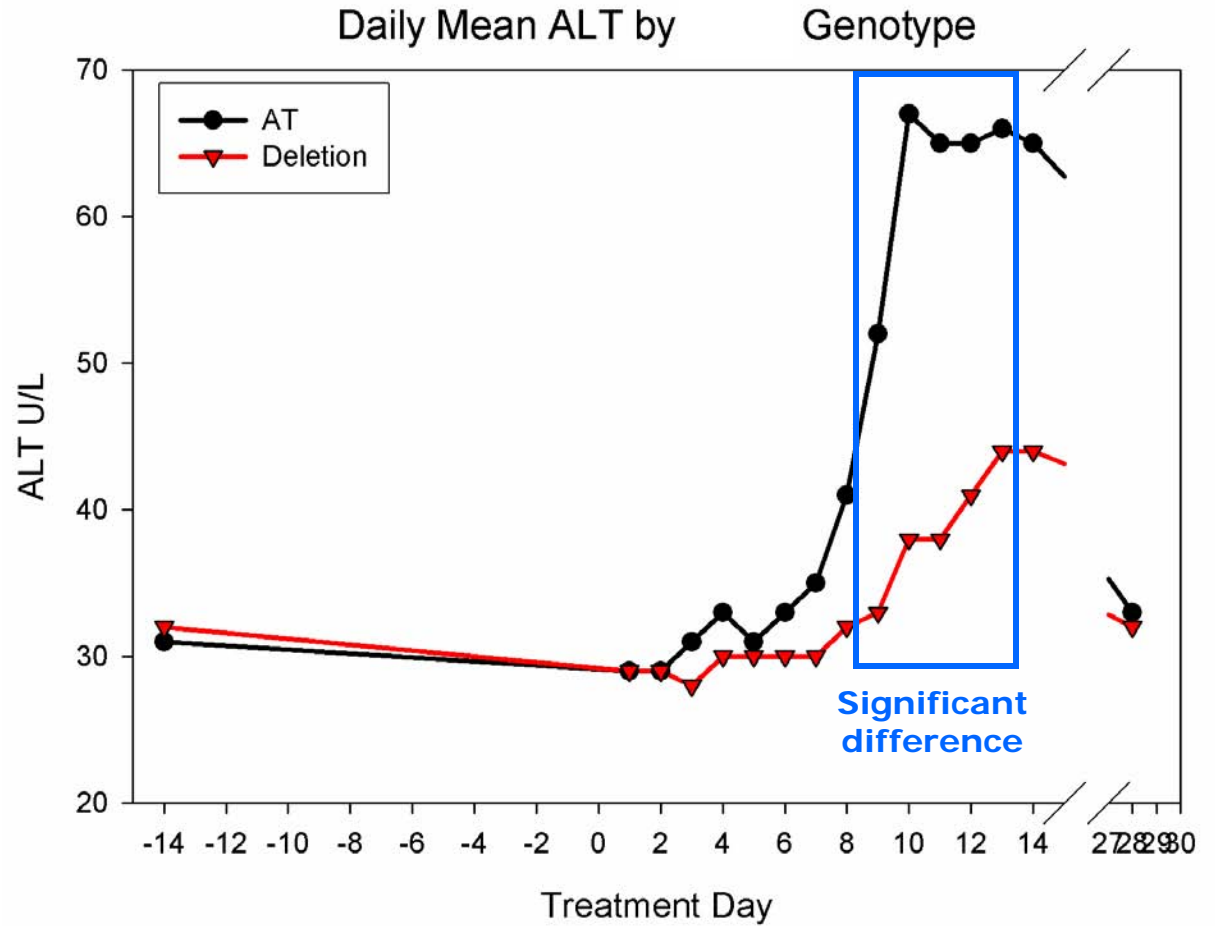
Haplotype-Associated Mapping

QTL mapping procedure: McClurg et al. BMC Bioinformatics (2006)

Haplotype-Associated Mapping: Mouse-to-Human discovery

Day	p value	AT	----
-14	0.8614	31	32
1	0.8655	29	29
2	0.9565	29	29
3	0.5907	31	28
4	0.5456	33	30
5	0.4501	31	30
6	0.4614	33	30
7	0.314	35	30
8	0.07857	41	32
9	0.01059	52	33
10	0.01691	67	38
11	0.007893	65	38
12	0.02055	65	41
13	0.02537	66	44
14	0.06552	65	44
28	0.8636	33	32

Daily Mean ALT by Genotype

ALT U/L vs Treatment Day

AT
Deletion

Significant difference

Biostatistics Issues:

- Data analysis procedures in concert with Project 1, including principal component analyses, distance-weighted discrimination, SAFE, etc.
- Specific data mining approaches also proposed, such as subspace clustering (SNPs vs. phenotypes, gene expression), that fall outside of typical statistical framework

Computational and Bioinformatics Issues:

- Software technology – federated systems and architectures
- Execution platforms – workstations, grid computing, supercomputing
- Data access and management – data mining, formats and data interchange, common abstractions/metadata issues