

Drug toxicity signatures for acetaminophen using the PharmGenix panel of rats

Steven H. Nye Ph.D.¹, Darin L. Evans¹, Jordan F. Baye¹, Heather J. Vernon¹, Martin J. Hessner Ph.D.², Xujing Wang, Ph.D.², Richard J. Roman Ph.D.^{1,2}, Howard J. Jacob Ph.D.^{1,2}
¹PhysioGenix, Inc., 10437 Innovation Drive, Milwaukee, WI, USA, ²Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI, USA

Abstract

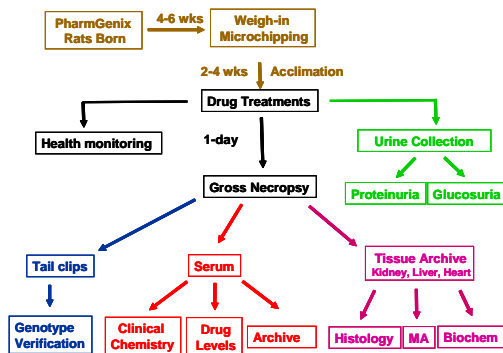
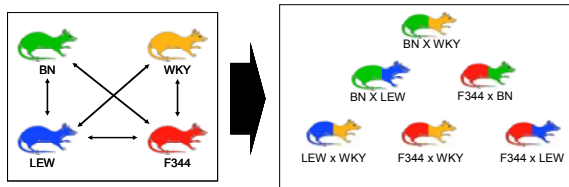
The ability of toxicogenomics to predict drug toxicity is unmet for reasons including the kind of animal model being studied. PhysioGenix has combinatorially bred 4 inbred rat strains to produce 6 F1 hybrids, called the PharmGenix panel. This strategy maximizes genetic diversity, enriches the chance to detect toxicity over inbred and outbred strains, and provides an ideal platform for creating drug toxicity signatures using microarray. Serum and tissue samples were collected from PharmGenix and F344 rats 24 hours after a single treatment with the hepatotoxin acetaminophen (1000mg/kg). Liver damage was assessed by ALT and found to be 10 to 20-fold more elevated in F344 and two sensitive PharmGenix strains (F344xBN, F344xLEW) than the resistant hybrid (F344xWKY). Histopathology revealed extensive hemorrhage and necrosis, but only in the sensitive strains. Microarray analysis of centrilobular liver tissues detected 300 differentially expressed genes for the F344 rat and 502 for the 3 PharmGenix rats (all F344 hybrids). Having both sensitive and resistant strains with a common genome background enabled us to categorize genes/ESTs for exposure (38), toxicity (47) and resistance (97). Insight into the mechanism of acetaminophen toxicity was gained by detecting genes in the glutathione elimination pathway. Upregulation of genes ($P < 0.05$) for 5 key enzymes was detected including glutathione-S-transferase $\alpha 2$ (2-4x), glutathione synthase (4-12x), glutamate cysteine ligase (4-7x), glutathione reductase (2-4x) and glucose-6-P-dehydrogenase (9-18x). We conclude that using a drug vs. vehicle comparison in a single strain cannot distinguish between gene expression differences caused by the drug exposure, toxicity, secondary or strain-specific responses. By including multiple sensitive and resistant strains with a common genome background, we distinguished likely exposure genes/ESTs from those due to toxicity.

Concepts

1. For preclinical drug studies, the use of the PharmGenix panel offers researchers genetic diversity along with the added power of reproducibility.

- Use of a single inbred strain enables reproducibility, but is genetically equivalent to testing one person.
 - Outbred strains have uncontrolled and limited genetic diversity.
2. Integrating microarray data with phenotypic data from PharmGenix drug studies enables complex gene expression signatures to be simplified.
- Use of sensitive and resistant strains helps to distinguish the different causes of gene expression responses following administration of a drug.
 - More accurate signatures and meaningful biomarkers may be detected due to a significant reduction in the individual animal and strain-specific "noise".

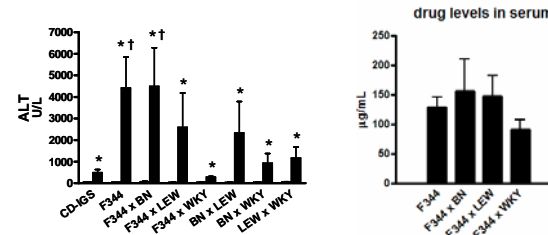
Breeding the PharmGenix panel and workflow



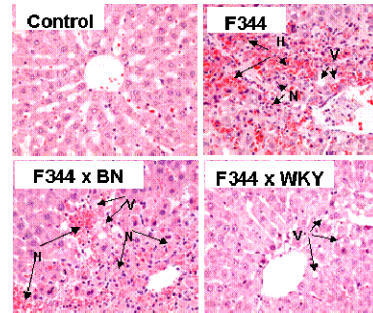
Study design and results

Rats were randomly assigned to vehicle (4-6) or drug (6-8) treatment groups. Animals weighed between 250-450g at the time of acetaminophen (1000 mg/kg dissolved in 50% aqueous propylene glycol) or vehicle (50% aqueous propylene glycol) treatment. Rats were administered a single IP dose and the study terminated after 24 hours. CD-1GS and F344 were included as industry standard comparisons. For alanine aminotransferase (ALT) measurements, all drug-treated strains were significantly different from vehicle controls. * $P < 0.05$ for drug vs. vehicle. † $P < 0.05$ vs. CD-1GS.

A. Strain differences found in clinical chemistry but not drug levels



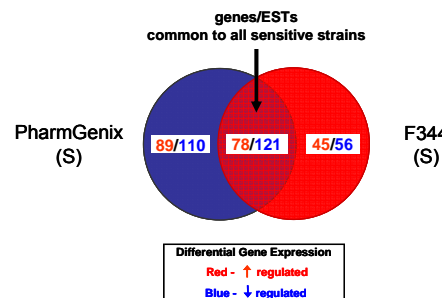
B. Liver damage correlates with elevated ALT



Representative histological comparisons of the left lateral lobe of the liver from a vehicle for F344 (control), and after acetaminophen for F344, F344xBN and F344xWKY. Profound damage due to drug is evidenced by hemorrhage (H), necrosis (N), and vacuolization (V) as seen in the liver of F344 rats. The damage to the liver is less severe in the F344xBN strain and F344xLEW (not shown), but very subtle in the F344xWKY strain.

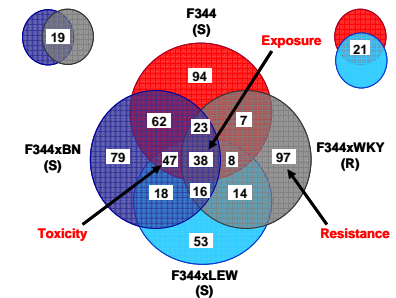
C. Microarray comparison of the most sensitive strains

Differentially expressed genes were detected in the left lateral lobe of animals showing a toxic response following treatment with acetaminophen. Sensitive (S) PharmGenix animals (F344xBN, F344xLEW) were selected based on clinical chemistry, histopathology, and having a genome background common to the F344. RNA from 4 drug treated animals was hybridized with a pool of vehicle RNA (4 individuals) to 18,000 element cDNA microarrays. Duplicate arrays with a flip-dye design resulted in 16 measurements for each gene/EST per strain. Genes/ESTs were filtered based on criteria that they are differentially expressed ($P < 0.05$) in 75% of the individuals for each strain.



D. Subclassification of gene expression responses

Drug toxicity signatures were classified into genes/ESTs differentially expressed due to either exposure, toxicity, resistance or strain-specific reasons by overlapping the responses from multiple strains that are either very sensitive (F344, F344xBN, F344xLEW) or much less affected (F344xWKY) to acetaminophen. "S" refers to sensitive; "R" to less affected/resistant.



E. Subclasses for the genes most responsive to acetaminophen

SubClass	Gene Symbol	Differential Gene Expression ¹				Function
		S F344xBN	S F344xLEW	S F344	R F344xWKY	
Toxicity	Big2	3.11	3.92	3.52	ND	extracellular signaling (anti-proliferative)
	Pa1f	2.76	1.84	3.42	ND	inflammation
	Cp2g21	2.56	3.61	3.38	ND	cell signaling
	Mt3	2.49	2.15	2.47	ND	oxidative stress
	Tdag	2.28	3.01	3.42	ND	cell death
	Myd116	2.03	2.76	2.88	ND	myeloid differentiation
Resistance	Fac12	-2.32	-2.43	-2.40	ND	lipid biosynthesis, FA metabolism
	Sult1c2	-2.47	-1.96	-2.17	ND	drug detoxification
	Alb1ra1	ND	ND	ND	2.30	fatty acid synthesis
	G6pc	ND	ND	ND	-1.63	gluconeogenesis
	Hsf1	ND	ND	ND	-2.12	stress response regulator
	Slc10a1	ND	ND	ND	-2.14	bile acid transport mediator
	Insig1	ND	ND	ND	-2.23	insulin-related growth response
	Pctk1	ND	ND	ND	-2.25	cell cycle regulation
	Oat	ND	ND	ND	-2.43	urea cycle
	Cd74	ND	ND	ND	-2.49	cell proliferation/survival
Sc4mol	ND	ND	ND	-2.58	fatty acid synthesis	
Exposure	G6pdx	3.27	2.73	2.95	3.38	glucose turnover
	Myc	2.65	3.69	3.24	1.41	transcription regulator
	Ifrd1	2.39	2.99	3.30	1.43	cell signaling
	Igf1a	-2.51	-2.56	-2.43	-1.50	insulin-like growth factor control
	Glut	-2.60	-2.18	-2.32	-2.45	glutamine metabolism

¹Differentially expressed genes are the log₂ values of the ratios comparing drug to vehicle responses
 ND refers to "Not Detected"

F. Pathway Identification

Real-Time PCR validation for genes detected in the glutathione elimination pathway

Gene Name	Differential Gene Expression ¹			
	S F344xBN	S F344xLEW	S F344	R F344xWKY
Glucose-6-phosphate dehydrogenase	18.10 ± 1.30	9.30 ± 0.21	12.25 ± 7.11	9.08 ± 3.75
Glutathione synthetase	12.19 ± 0.88	4.80 ± 0.11	4.88 ± 2.83	5.11 ± 2.11
Glutathione reductase	3.84 ± 0.28	2.35 ± 0.05	2.31 ± 1.34	2.40 ± 0.99
Glutamate-cysteine ligase (gamma-glutamylcysteine synthetase)	5.03 ± 0.36	7.46 ± 0.17	4.76 ± 2.77	7.30 ± 3.01
Glutathione-S-transferase, alpha type2	3.59 ± 0.26	2.80 ± 0.06	2.58 ± 1.50	2.47 ± 1.02

¹ Fold expression changes measured in 4 acetaminophen individuals per strain as compared to 4 control individuals

Conclusions

- By integrating microarray data with phenotypic data collected from multiple PharmGenix and F344 rats, we prioritized 47 differentially expressed genes/ESTs for acetaminophen toxicity from over 800 that we originally detected, enabling a complex gene expression signature to be greatly simplified.
- Finding genetically related PharmGenix strains with different phenotypic responses aids in subclassification of gene/EST responses.
- The similar, but different, drug signatures discovered in the F344 and the F344 PharmGenix hybrids point to the role that the heterogenous genome background and the different allelic combinations may play in drug toxicity.