Compensatory Transcriptome Analyses in Homozygous KOMP Knockout Mice

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Introduction

A powerful tool for understanding cellular and systems biology, and to dissect the role of specific genes, is to manipulate gene expression (e.g., knockdowns, knockouts, over-expression) or manipulate protein function (e.g. site directed mutagenesis, dominant negatives) and to evaluate compensatory transcriptome changes in cell culture or whole animals. For the KOMP Phenotyping Project (www.kompphenotype.org), we are using transcriptome analyses in homozygous (HOM) mutant mice to predict gene function for genes with little or no functional annotation. In this report, we validated our transcriptome assays and pathway analyses methods by comparing liver transcriptome changes in obese lepr$^{db/db}$ (db/db) mutant mice with previously published transcriptome analyses of other mouse models of fatty liver (steatosis). We then describe the transcriptome analyses from two Riken clone mutants with no published functional annotation: 130002K09Rik and 1700029I15Rik.

Methods

- For each mutant strain, we used 3-4 HOM mutant male mice, ~50 days-old, and compared the expression profiles against age-matched wildtype or heterozygous isogenic controls;
- Expression profiling was completed on individual total RNA preps (RNeasy, Qiagen) from each tissue/animal using the Illumina Beadarray Ref6 chips at the UC Davis Genome Center;
- Criterion for significant differences between the HOM and paired control was > 2-fold. For pathway analyses, we used proprietary software from Seralogix (Bayesian Statistical Approach; www.seralogix.com) and IPA from Ingenuity (www.ingenuity.com). Pathways with a score of more than +3 induction or less than -3 suppression for Seralogix were compared to pathways with p-value less than ~0.05 from the IPA analysis;
- Transcriptome analyses were completed in liver for db/db; liver, kidney and lung for the 130002K09Rik; and liver, kidney, lung and spleen for the 1700029I15Rik mutants. However, for the purposes of this presentation only the liver data are presented;
- Liver transcriptome changes in db/db were compared with liver transcriptome changes in a mouse model of hepatic steatosis (Kang et al., Physiological Genomics, 2011).

Results

- In db/db liver, 85 genes were up- & 67 down-regulated (Top genes affected shown in **Table 1**). 13 pathways were significantly affected by both the Seralogix and IPA analysis (**Table 2**). Many of the affected pathways are expected based upon the known physiology of hepatic steatosis, including 5 pathways of lipid metabolism, 3 pathways of amino acid metabolism, and 2 pathways of carbohydrate metabolism. Comparison with the pathways affected in another mouse model of hepatic steatosis (ROR)

Table 1. Top Genes Affected in db/db Liver

Top genes	Fold up	Top genes	Fold down
APOA4	14.72	CYP7B1	-11.80
CYP27A1	11.25	EGFR	-8.42
CIDEC	6.99	NADP	-6.16
LY6D	6.82	C8B	-5.05
LCN2	6.57	IGFBPS	-5.03
WFDC2	4.66	SOCS2	-4.35
GSTA1	4.33	GSTP1	-3.67
G0S2	4.12	SERPINA12	-3.36
CBR3	3.86	CIB3	-3.35
LGALS1	3.76	CISH	-3.40

color: hit 1 pathway color: hit 3 or more pathways

Table 2. Top Pathways Affected in db/db Liver

db/db-LIVER	IPA(p-value)	Seralogix	KEGG pathway #	Category
Metabolism of Xenobiotics by Cytochrome P450	1.90E-07	3.72	mmu00980	Xenobiotics Biodegradation & Metabolsim
Histidine Metabolism	2.41E-04	4.52	mmu00340	Amino Acid Metabolism
Arachidonic Acid Metabolism	6.00E-04	3.17	mmu00590	Lipid Metabolism
Bile Acid Biosynthesis	6.92E-04	4.11	mmu00120	Lipid Metabolism
Glycerolipid Metabolism	3.71E-03	3.16	mmu00561	Lipid Metabolism
C21-Steroid Horm Metabolism	2.12E-02	-4.70	mmu00140	Lipid Metabolism
JAK/Stat Signaling	2.27E-02	-3.42	mmu04630	Signal Transduction
Tryptophan Metabolism	8.47E-02	3.48	mmu00380	Amino Acid Metabolism
Butanoate Metabolism	1.37E-01	4.09	mmu00650	Carbohydrate Metabolism
Lysine Degradation	1.37E-01	3.03	mmu00310	Amino Acid Metabolism
Linoleic Acid Metabolism	1.62E-01	-4.23	mmu00591	Lipid Metabolism
Pyruvate Metabolism	1.91E-01	3.28	mmu00620	Carbohydrate Metabolism
Bladder Cancer Signaling	2.27E-01	-4.13	mmu05219	Cancers

color: hit by 1 of top 10 genes; color: hit by 2 of the top 10 genes; color: hit by 3+ of top 10 genes

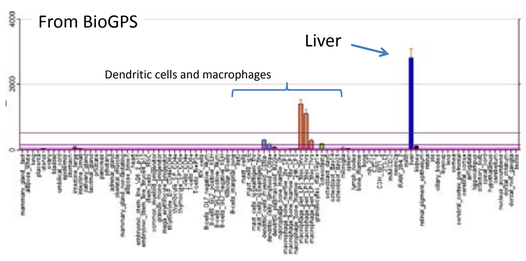
Table 3. Pathways Affected in db/db & ROR Liver

Ingenuity Canonical Pathways	db/db (p-value)	ROR (p-value)
LPS/IL-1 Mediated Inhibition of RXR Function	1.08E-08	1.44E-10
PXR/RXR Activation	2.02E-08	5.33E-07
Aryl Hydrocarbon Receptor Signaling	6.41E-08	3.03E-05
Metab of Xenobiotics by Cytochrome P450	2.36E-07	1.98E-02
Xenobiotic Metabolism Signaling	6.29E-07	4.09E-09
Histidine Metabolism	3.44E-04	1.14E-04
Bile Acid Biosynthesis	7.27E-04	3.73E-06
Androgen and Estrogen Metabolism	5.26E-03	8.24E-08
Glycerolipid Metabolism	8.55E-03	3.97E-09
Estrogen-Dependent Breast Cancer Signaling	3.22E-02	3.14E-04
Lysine Degradation	1.07E-01	5.08E-10
LXR/RXR Activation	1.26E-01	1.26E-03
Tryptophan Metabolism	1.49E-01	7.01E-10
Butanoate Metabolism	1.91E-01	4.12E-10

showed a high degree of overlap (**Table 3**);

- Expression of 1300002K09Rik is high in liver and low in all other tissues based upon published tissue surveys and LacZ reporter expression (**Fig 1**). In 1300002K09Rik liver, 107 genes were up- and 8 down-regulated (top genes affected shown in **Table 4**). The 18 pathways affected (**Table 6**) include 5 belonging to carbohydrate metabolism, 5 to amino acid metabolism (including tryptophan metabolism; **Fig 2**), 2 pathways belonging to metabolism of other amino acids and xenobiotics biodegradation & metabolism. Very few pathways were hit in kidney by

Fig 1. Tissue Expression of 1300002K09Rik



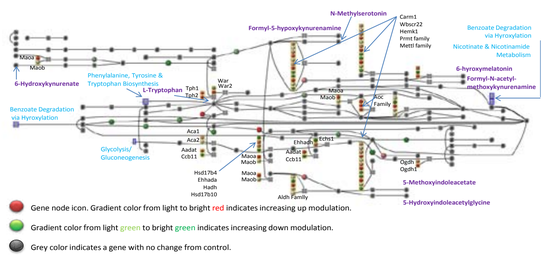
Histochemical staining in liver from HET 1300002K09Rik KO mice using a LacZ reporter driven by the native gene promoter; only the liver stained specifically for LacZ in this mutant

Table 4. Top Genes Affected in 130002K09Rik Liver

Top gene	Fold up	Top gene	Fold down
GCLC	6.19	BHMT	-3.12
HSPE1	5.50	PECI	-2.83
HSPA5	5.42	GADD45G	-2.74
GSTA1	5.30	PAIP2	-2.74
ID2	4.82	SLC38A4	-2.73
KMO	4.04	SPON2	-2.62
ALDH1A1	3.91	PECR	-2.38
TDO2	3.66	FGF21	-2.37
MT-ND5	3.55	GSTA2	-2.29
NAMPT	3.44	MT-CO2	-2.25

color: hit 1 pathway; color: hit 3 or more pathways

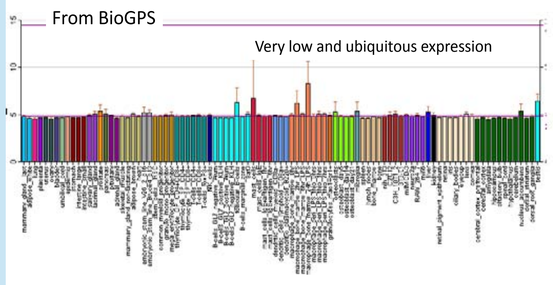
Fig 2. Tryptophan Metabolism in 130002K09Rik Liver



both Seralogix and IPA analysis (data not shown);

- Gene expression of 1700029I15Rik is low in all tissues tested (**Fig 3**). 89 genes were up- and 69 down-regulated in this mutant liver (top genes affected shown in Table 5). There were 13 pathways affected in this mutant (see **Table 6**). 3 of 13 pathways belong to lipid metabolism and another 3 to amino acid metabolism, 2 to Metabolism of Cofactors & Vitamins, 1 each to Energy Metabolism, Metabolism of Other Amino Acids, Xenobiotics Biodegradation & Metabolism, Glycan Biosynthesis & Metabolism and Cancers.
- There was very little overlap of the pathways affected in the liver by these three mutants (**Table 6**); suggesting that the liver expression profile is a sensitive phenotype that can distinguish among mutants.

Fig 3. Tissue Expression of 1700029I15Rik



There was no LacZ staining detected in tissues from HET 1700029I15Rik KO mice

Table 5. Top Genes Affected in 1700029I15Rik Liver

Gene	Fold up	Gene	Fold down
A1BG	23.37	ELOVL3	-8.57
FMO3	12.69	GSTP1A	-6.91
CYP17A1	11.12	SERPINA12	-6.91
NT5E	3.99	NADP	-6.07
ACOT4	3.90	ALAS2	-5.03
SQLE	3.75	ERRFI1	-4.26
XIST	3.30	PLK3	-4.17
EGR1	3.18	MT-ND5	-4.16
FDPS	2.84	G6PC	-4.12
SERPINA6	2.72	CYP7B1	-3.63

color: hit 1 pathway; color: hit 2 pathways; color: hit >2 pathways

Table 6. Affected Liver Pathways in 3 Mutants

db/db-LIVER	IPA(p-value)	Seralogix
Bile Acid Biosynthesis	6.92E-04	4.11
Metab of Xenobiotics by P450	1.90E-07	3.72
Lysine Degradation	1.37E-01	3.03
Pyruvate Metabolism	1.91E-01	3.28
Tryptophan Metabolism	8.47E-02	3.48

db/db-LIVER Unique		
Arachidonic Acid Metab	6.00E-04	3.17
Bladder Cancer Signaling	2.27E-01	-4.13
Butanoate Metabolism	1.37E-01	4.09
C21-Steroid Horm Metab	2.12E-02	-4.70
Glycerolipid Metabolism	3.71E-03	3.16
Histidine Metabolism	2.41E-04	4.52
JAK/Stat Signaling	2.27E-02	-3.42

1300002K09Rik-LIVER	IPA (p-value)	Seralogix
Bile Acid Biosynthesis	1.09E-04	-3.22
Metab of Xenobiotics by P450	6.00E-04	-4.14
Lysine Degradation	1.42E-02	4.09
Pyruvate Metabolism	2.12E-04	3.49
Tryptophan Metabolism	1.39E-04	3.48
Glutathione Metabolism	7.65E-05	-3.92
Gly, Ser and Threo Metab	1.37E-01	-3.42
Nicotinate & Nicotinamide Metab	1.49E-01	4.20

1300002K09Rik-LIVER Unique		
Citrate Cycle	1.01E-05	3.14
Fatty Acid Metabolism	8.60E-05	-3.46
Glycolysis/Gluconeogenesis	6.60E-07	5.05
Inositol Metabolism	1.39E-05	-3.81
MODY Signaling	5.08E-01	3.30
Propanoate Metabolism	1.64E-04	3.68
Retinol Metabolism	1.14E-03	3.43
Tyrosine Metabolism	2.95E-01	3.04
Val, Leu and Isoleu Degrad	2.31E-04	-3.21

1700029I15Rik-LIVER	IPA(p-value)	Seralogix
Bile Acid Biosynthesis	6.60E-04	-3.07
Metab of Xenobiotics by P450	4.96E-03	4.72
Glutathione Metabolism	4.52E-04	3.77
Gly, Ser and Threo Metab	6.56E-05	3.09
Nicotinate and Nicotinamide Metab	4.68E-03	4.03

1700029I15Rik-LIVER Unique		
Androgen and Estrogen Metab	2.26E-07	5.26
Biosynthesis of Steroids	1.25E-07	3.44
Cysteine Metabolism	4.97E-02	3.80
Keratan Sulfate Biosynthesis	9.68E-03	4.55
Methionine Metabolism	1.17E-02	3.86
Prostate Cancer Signaling	1.62E-01	4.18
Sulfur Metabolism	9.76E-06	3.55

color: hit 1 pathway; color: hit 2 pathways; color: hit >2 pathways

Significance

Our transcriptome methodology was validated by comparing the db/db liver results with another published transcriptome dataset in a mouse model of steatosis. There was a remarkable overlap of the liver pathways affected despite a low overlap of the differentially expressed genes; suggesting that the pathway analysis is a sensitive and valid measure of the metabolic consequences of the mutations.

In the 1300002K09Rik mutant liver; the significantly altered pathways of amino acid metabolism and pathways using amino acids for biosynthesis suggest that the gene is involved in some aspect of protein or amino acid metabolism. Follow-up studies underway include serum chemistry and liver histopathology.

Although the overall expression for the 1700029I15Rik gene is quite low, the knockout resulted in significant perturbations of liver gene expression; however, no specific hypotheses have been generated from these data regarding gene function.

Collectively, these data illustrate the sensitivity and reliability of the compensatory transcriptome analysis to provide functional annotation for protein coding genes.

Acknowledgements

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