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Genetic Analysis of Tissue Glutathione Concentrations and Redox Balance

Yang Zhou¹, David E. Harrison², Kimberly Love-Myers³, Yi Chen³, Arthur Grider¹, Kathie Wickwire¹, John R. Burgess⁴, Mateusz A. Stochelski⁴, and Robert Pazdro^{1,2,*}

¹Department of Foods and Nutrition, University of Georgia, Athens, GA USA 30602

²The Jackson Laboratory, Bar Harbor, ME USA 04609

³Department of Statistics, University of Georgia, Athens, GA USA 30602

⁴Department of Nutrition Science, Purdue University, West Lafayette, IN USA 47907

Abstract

Glutathione redox balance — defined as the ratio GSH/GSSG — is a critical regulator of cellular redox state, and declines in this ratio are closely associated with oxidative stress and disease. However, little is known about the impact of genetic variation on this trait. Previous mouse studies suggest that tissue GSH/GSSG is regulated by genetic background and is therefore heritable. In this study, we measured glutathione concentrations and GSH/GSSG in liver and kidney of 30 genetically-diverse inbred mouse strains. Genetic background caused an approximately three-fold difference in hepatic and renal GSH/GSSG between the most disparate strains. Haplotype association mapping determined the loci associated with hepatic and renal glutathione phenotypes. We narrowed the number of significant loci by focusing on those located within protein-coding genes, which we now consider to be candidate genes for glutathione homeostasis. No candidate genes were associated with both hepatic and renal GSH/GSSG, suggesting that genetic regulation of GSH/GSSG occurs predominantly in a tissue-specific manner. This is the first quantitative trait loci study to examine the genetic regulation of glutathione concentrations and redox balance in mammals. We identified novel candidate genes that have the potential to redefine our knowledge of redox biochemistry, its regulation, and inform future therapeutic applications.

Keywords

glutathione; genetics; redox; mice

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*Corresponding author: Robert Pazdro, Ph.D., Department of Foods and Nutrition, University of Georgia, 305 Sanford Drive, Athens, GA USA 30602, Phone: (706) 542-7504, Fax: (706) 542-5059, rpazdro@uga.edu.

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INTRODUCTION

Glutathione is an essential cellular antioxidant. This tripeptide, which is composed of cysteine, glutamate, and glycine, exists predominantly in its reduced form, GSH. GSH is utilized for a broad range of functions, including peroxide clearance and xenobiotic metabolism (6). Utilization of GSH by antioxidant enzymes generates the oxidized, dimer form of glutathione: GSSG. GSSG must then be converted back to two molecules of GSH through endogenous recycling mechanisms. Glutathione redox balance — the ratio of GSH/GSSG — is a useful measure of cellular redox state. An increase in GSH/GSSG is indicative of augmented antioxidant capacity, while a decrease is suggestive of oxidative stress and diminished antioxidant defenses. Changes in GSH/GSSG are closely associated with cellular processes like proliferation and apoptosis (1) as well as progression of conditions like diabetes and liver disease (6). GSH/GSSG is therefore critically tied to the fate of the cell in health and disease.

Studying the role of genetic variation in regulation of GSH/GSSG is essential to better understand disease processes. Several studies have shown a relationship between inbred strain and tissue GSH/GSSG in mice. Rebrin, Forster, and Sohal demonstrated that brain and erythrocyte GSH/GSSG differ between the inbred strains C57BL/6J (B6) and DBA/2J (D2) (15, 16). In a panel of 14 inbred strains, Tsuchiya et al. provided evidence that a strain effect occurs for hepatic GSH/GSSG as well (20). These findings suggest natural genetic variation regulates GSH/GSSG, as with other oxidative stress-related phenotypes like lipid peroxidation (7) and resistance to stressors like hyperoxia (4), paraquat (9), and MPTP (5). Since these oxidative stress-related traits are heritable, finding the genes and alleles responsible for their regulation will define the genetic basis of susceptibility to oxidative stress and disease.

In this study, we measured GSH and GSSG concentrations in the liver and kidneys of mice from 30 inbred mouse strains representing three mouse subspecies. Natural genetic variation is associated with up to a three-fold difference in hepatic and renal GSH/GSSG among strains. Haplotype association mapping (HAM) identified loci and candidate genes that regulate hepatic and renal GSH/GSSG. Our findings support the hypothesis that variation in distinct genes regulates GSH/GSSG in different tissues.

MATERIALS AND METHODS

Mouse strains

Female 129S1/SvImJ (129; JAX[®] #002448), A/J (AJ; JAX[®] #000646), AKR/J (AKR; JAX[®] #000648), ALR/J (ALR; JAX[®] #003070), BALB/cByJ (BALB; JAX[®] #001026), BTBR T⁺ Itpr3^{tf}/J (BTBR; JAX[®] #002282), C3H/HeJ (C3H; JAX[®] #000659), C57BL/6J (B6; JAX[®] #000664), C57BL/10J (B10; JAX[®] #000665), C57BLKS/J (BLKS; JAX[®] #000662), C57L/J (C57L; JAX[®] #000668), CAST/EiJ (CAST; JAX[®] #000928), CBA/J (CBA; JAX[®] #000656), DBA/1J (D1; JAX[®] #000670), DBA/2J (D2; JAX[®] #000671), FVB/NJ (FVB; JAX[®] #001800), LP/J (LP; JAX[®] #000676), MRL/MpJ (MRL; JAX[®] #000486), NOD/ShiLtJ (NOD; JAX[®] #001976), NON/ShiLtJ (NON; JAX[®] #002423), NOR/LtJ (NOR; JAX[®] #002050), NZB/BINJ (NZB; JAX[®] #000684), NZO/HILtJ (NZO;

JAX[®] #002105), NZW/LacJ (NZW; JAX[®] #001058), PL/J (PL; JAX[®] #000680), PWK/PhJ (PWK; JAX[®] #003715), SJL/J (SJL; JAX[®] #000686), SM/J (SM; JAX[®] #000687), SWR/J (SWR; JAX[®] #000689), and WSB/EiJ (WSB; JAX[®] #001145) mice were bred and housed at The Jackson Laboratory, Bar Harbor, ME USA. Mice were housed in a 12-hour light/dark cycles and at 3–4 months of age, mice were humanely euthanized by cervical dislocation (ACUC approved procedure LAH93-26).

Assessment of GSH/GSSG

Liver and kidneys were removed and diced in ice-cold PBS, blotted on paper towel, and flash frozen. Tissues were homogenized on ice in PBS containing 10 mM DTPA (12). An equal volume of 10% perchloric acid containing 1 mM DTPA was added and samples were centrifuged at 16,000g for 7 minutes at 4°C. Acidified supernatants were transferred to another tube, flash frozen in liquid nitrogen, and stored at –80°C. GSH and GSSG were quantified by the enzymatic recycling method (14) on a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA USA). All reagents and glutathione standards were purchased from Sigma-Aldrich, St. Louis, MO USA. Total homogenate protein was quantified by Pierce BCA Protein Assay (Thermo Fisher Scientific, Rockford, IL, USA).

Statistical analysis

All analyses were conducted using SAS 9.3. Before performing any analysis on the data, the normality of hepatic and renal GSH/GSSG and median lifespan were checked visually in order to meet the assumptions of later statistical modeling. Hepatic GSH/GSSG ratios were normally distributed while renal ratios were skewed to the right. A Box-Cox analysis suggested that a natural log transformation was appropriate for renal ratios; hence, the variable used for all analyses was $\ln(\text{renal GSH/GSSG})$. The distribution of median lifespan was skewed to the left and hence a square transformation was performed on median lifespan.

We used ANOVA to determine the role of genetic background in the regulation of GSH/GSSG. The model used for hepatic GSH/GSSG was

$$\text{Hepatic } \frac{\text{GSH}}{\text{GSSG}} = \mu + \text{Strain}_i + \varepsilon_{ij}, i = 1, \dots, 30, j = 1, \dots, n_i$$

where μ is the overall mean of hepatic GSH/GSSG, Strain_i is the effect of the i^{th} strain, ε_{ij} is the error for mouse j from strain i and follows a normal distribution with mean zero and variance σ^2 , and n_i is the number of mice from strain i . The ANOVA table and analysis results are as follows:

Source	DF	Sum of Squares	Mean Square	F Value	P-value
Model	29	51840.78	1787.61	11.67	<.001
Error	167	25587.69	153.220		
Corrected Total	196	77428.47			

R-Square	Root MSE
0.670	12.378

Similarly, the ANOVA table and analysis results for ln(renal GSH/GSSG) are:

Source	DF	Sum of Squares	Mean Square	F Value	P-value
<i>Model</i>	29	17.30	0.60	11.30	<.001
<i>Error</i>	167	8.81	0.053		
<i>Corrected Total</i>	196	2444.65			

R-Square	Root MSE
0.662	0.230

The Pearson Correlation Coefficient between average hepatic GSH/GSSG and average ln(renal GSH/GSSG) was calculated by first averaging the hepatic and natural-log transformed GSH/GSSG measures within each strain, for a total of 30 pairs of measures (one per strain). The correlation was then calculated for the thirty pairs of measures.

We also performed an ANCOVA to test whether the relationship between hepatic and renal GSH/GSSG within strains is different across strains. The model is:

$$\ln\left(\frac{\text{renal GSH}}{\text{GSSG}}\right) = \mu + \text{Strain}_i + \alpha \times \left(\frac{\text{hepatic GSH}}{\text{GSSG}}\right) + \beta_i \times \left(\frac{\text{hepatic GSH}}{\text{GSSG}}\right) + \varepsilon_{ij}, i=1, \dots, 30, j=1, \dots, n_i$$

where μ is the overall mean of ln(renal GSH/GSSG), Strain_i is the effect of the i^{th} strain, α is the average coefficient for hepatic GSH/GSSG across strains, β_i is the change in the coefficient for hepatic GSH/GSSG with strain i , ε_{ij} is the error for mouse j from strain i and follows a normal distribution with mean zero and variance σ^2 , and n_i is the number of mice from strain i :

Source	DF	Sum of Squares	Mean Square	F Value	P-value
<i>Model</i>	59	20.19	0.34	7.91	<.001
<i>Error</i>	137	5.92	0.04		
<i>Corrected Total</i>	196	26.11			

R-Square	Root MSE
0.773	0.208

Source	DF	Type III SS	Mean Square	F Value	P-value
<i>LiverGlutathione</i>	1	0.73	0.73	16.85	<.001

Source	DF	Type III SS	Mean Square	F Value	P-value
<i>Strain</i>	29	3.02	0.10	2.41	<.001
<i>LiverGlutathi*Strain</i>	29	2.29	0.08	1.82	0.012

The Pearson Correlation Coefficients between average hepatic GSH/GSSG and (median lifespan)², and between average ln(renal GSH/GSSG) and (median lifespan)² were calculated by first averaging the hepatic and natural-log transformed GSH/GSSG measures within each strain for 25 strains. Then, the Pearson Correlation between average hepatic GSH/GSSG and (median lifespan)² was calculated by using 25 pairs of average and (median lifespan)². The correlation between ln(renal GSH/GSSG) and (median lifespan)² was calculated in the same way.

Haplotype Association Mapping

GSH and GSSG concentrations, and GSH/GSSG, were collected and the data transformed (log10 or square-root) to maintain normal distribution. Values were imported into EMMA and tested for associations with 132,000 SNPs on file for 29 of our 30 strains. ALR/LtJ was not an available option and was therefore excluded from EMMA analysis. SNPs with p values less than 10⁻⁵ were selected by the program to be significant. The Mouse Genome Informatics Database (<http://www.informatics.jax.org/>) was searched by the location of significant SNPs to identify the protein coding genes or known QTLs covering corresponding SNPs.

RESULTS

Genetic Background Regulates Glutathione Concentrations and Redox Balance

To analyze the relationship between genetic background and GSH/GSSG, a panel of 30 inbred mouse strains was constructed to reflect the genetic diversity in the mouse genome. Petkov et al. assembled a mouse phylogenetic tree wherein inbred mouse strains were separated into seven groups (Fig. 1) (13); individual groups share a general lineage as follows: Group 1 – Bagg albino derivatives; Group 2 – Swiss mice; Group 3 – Japanese and New Zealand inbred strains; Group 4 – C57/58 strains; Group 5 – Castle's mice; Group 6 – C.C. Little's DBA and related strains; and Group 7 – wild-derived strains (heterogeneous group). Each group is represented by at least three strains in our panel.

Liver is frequently utilized for the measurement of GSH/GSSG as hepatic glutathione concentrations are higher than those in other organs, likely due to the role of the liver in xenobiotic metabolism. We hypothesized that if genetic background has a significant influence on GSH/GSSG, this will most likely manifest in the liver. To test if our trends were specific to liver or were indicative of genes and alleles that regulate GSH/GSSG in the whole organism, we tested renal GSH/GSSG as well.

In a panel of 30 genetically-diverse inbred mouse strains, genetic background had a significant effect on both hepatic and log transformed renal GSH/GSSG ($p < 0.001$ for both). A three-fold difference was observed in hepatic GSH/GSSG between the strains with

the lowest (CAST; 33.01 ± 2.13) and highest (B10; 100.73 ± 7.38) phenotypes (Fig. 2). Interestingly, renal GSH/GSSG was also shown to have a similar three-fold difference between the two most disparate strains (22.83 ± 1.28 in AKR versus 65.27 ± 6.62 in NZO; Fig 3). These results provide definitive new data to guide the design of future genetic studies.

Strains that belong to group 3 on the mouse family tree are of special interest as we map the genes responsible for GSH/GSSG. NZO and NZW had very high renal and hepatic GSH/GSSG (Fig. 2 and Fig. 3). NZB had a high renal GSH/GSSG, while NON was shown to have high hepatic GSH/GSSG. Shared alleles in this group may lead to the inheritance of a predisposition for high tissue GSH/GSSG, a hypothesis that will be tested in future investigations.

Lifespan is an inherited trait that may be influenced by oxidative stress and antioxidant capacity. The lifespans for most of the inbred mouse strains in our study have been previously reported by Yuan et al. (23). We tested for correlations between median lifespans and GSH/GSSG; neither hepatic nor renal GSH/GSSG was associated with the square of median lifespan ($r^2 = 0.000$, $p = 0.999$ and $r^2 = 0.001$, $p = 0.898$ respectively). Glutathione redox balance of young mice apparently does not influence overall longevity.

Some Wild-derived Alleles May Negatively Influence GSH/GSSG

The inbred strains CAST, PWK, and WSB are subspecies that originate from mice captured in the wild. These mice increase the amount of genetic diversity in our study because most classical inbred strains originate from domesticated mice bred by mouse fanciers, which limited genetic diversity. Because wild-derived mouse strains were inbred without domestication, they retain many genetic differences from domesticated mice. The considerable genetic differences between classical inbred strains and wild-derived inbred strains are useful for the analysis of quantitative traits and follow-up mapping studies.

In this study, our three wild-derived strains represent three mouse subspecies: *Mus musculus musculus* (PWK/PhJ), *Mus musculus castaneus* (CAST/EiJ), and *Mus musculus domesticus* (WSB/EiJ). The inclusion of these strains demonstrates the influence of wild-derived alleles on GSH/GSSG. While tissue GSH/GSSG ratios in PWK were higher than many of the classical inbred strains, WSB and CAST had the two lowest hepatic GSH/GSSG ratios in the entire panel. These two strains also had very low renal GSH/GSSG (Fig. 2 and Fig. 3). Our data suggest that an association exists between some, but perhaps not all, wild-derived alleles and GSH/GSSG.

Hepatic and Renal GSH/GSSG are Correlated

We first tested the significance of the Pearson Correlation between average hepatic and renal GSH/GSSG across the averages of the 30 strains (note that renal measures were natural log transformed due to skewness). The two measures were significantly correlated ($r^2 = 0.148$, $p = 0.036$), so that 14.8% of variability in renal and hepatic GSH/GSSG is related. We then performed an analysis of covariance (ANCOVA) to test whether the relationship between hepatic and renal GSH/GSSG within strains is different across strains. A significant

interaction between strain and hepatic GSH/GSSG indicates that the strength of the relationship between hepatic and renal GSH/GSSG is different for each strain ($p = 0.012$; Fig. 4). This indicates that the variations in renal and kidney GSH/GSSG due to non-genetic sources and random differences are more strongly linked in some strains than in others.

Identification of Novel Candidate Genes in Regulation of Glutathione Redox Balance

Efficient Mixed-Model Association (EMMA) was used to assess the relationship between glutathione phenotypes and 132,000 SNPs (2, 10). SNPs with p-values less than 10^{-5} were selected as significant (Fig. 5 and Fig. 6). Significant SNPs were further narrowed by selecting those that are found within protein-coding genes (Mouse Genome Informatics Database, <http://www.informatics.jax.org>), which we considered candidate genes for glutathione regulation (Table 1 and Table 2). We also discovered significant overlap between the genes that regulate GSSG concentrations and GSH/GSSG in kidney (Table 2). This suggests that renal GSH concentrations are not regulated by the genes and alleles that regulate glutathione redox balance.

DISCUSSION

Glutathione concentrations and redox balance are regulated by genetic background. Previous studies in mice have shown a strain effect on glutathione-related phenotypes (15, 16, 20). However, these studies discovered the relationships during investigations into calorie restriction and alcohol-induced liver injury. We reasoned that glutathione concentrations and redox balance are quantitative traits, and like other quantitative traits, these phenotypes can be mapped. Our approach would provide essential information regarding individual susceptibility to disease, as well as highlight potential new therapeutic targets to prolong or improve tissue function in disease. Here, we present the first putative QTLs — and candidate genes — that regulate mammalian tissue glutathione concentrations and redox balance.

In this study, we found a three-fold difference in both liver and kidney GSH/GSSG among 30 inbred mouse strains. A weak correlation exists between liver and kidney GSH/GSSG. If liver and kidney were regulated exclusively by the same genes, the correlation should be more significant. To explain these findings, we propose a model to help address this correlation. Here we designate Ratio GSH/GSSG (liver) as RL and Ratio GSH/GSSG ratio (kidney) as RK . We then consider that there may be some genes expressed in both the liver and kidney. We classify these genes as G . Those only expressed in the liver and which regulate GSH/GSSG are classified as GL , and similarly those in the kidney are classified as GK . Non-genetic sources of variability are classified as NG , though these non-genetic sources of variability may be influenced by genetic background. Mathematically, we suppose the following two equations: $RL = f(G, GL, NG, NGL) + \varepsilon$, $RK = f(G, GK, NG, NGK) + \varepsilon$, (ε represents noise). The significant correlation between liver and kidney GSH/GSSG argues for the role of G genes expressed in both organs simultaneously. However, other genes, GL and GK , may regulate this phenotype in a tissue-specific manner, and could be partially responsible for the approximate 85% of variability that renal and hepatic GSH/GSSG do not share. These equations represent our hypotheses to explain the weak

correlation between liver and kidney GSH/GSSG. These hypotheses will be tested in future genetics studies utilizing outbred mouse populations.

Our HAM results revealed no protein-coding genes that regulate both liver and kidney GSH/GSSG, yet we still discovered a weak association between these phenotypes. To explain these findings, we predict that the sequences of G genes in our model, which are expressed both in liver and kidney, might be largely conserved and therefore contribute to the weak association between liver and kidney GSH/GSSG without being detected by HAM analysis. One possible example of this is glutamate-cysteine ligase catalytic subunit (GCLC), which is responsible for glutathione biosynthesis. We searched for SNPs within this gene among the genomes of 25 inbred strains included in our study (informatics.jax.org). We found no nonsynonymous coding SNPs. Instead, we discovered several synonymous coding SNPs and the large number of remaining SNPs were located within introns. GCLC is therefore less likely to be listed as a candidate gene by our methods since non-synonymous coding SNPs are not present to cause an amino acid sequence alteration. The role of variation in genes like GCLC will be tested in future studies.

We also found evidence that some of the novel genes uncovered by HAM analysis may directly impact glutathione redox balance. Among them, RAR-related orphan receptor alpha (Rora) was associated with hepatic GSH concentration, and a previous study demonstrated that Rora controls expression of the rate-limiting enzyme for GSH biosynthesis as a repressor (21). In another example, we found Brip1 to be associated with renal GSH. Brip1 is essential to DNA repair responses to stressors like ochratoxin A (8) that also deplete GSH (18). Our next study will determine whether candidate genes like Rora and Brip1 have real associations with GSH in liver and kidney, respectively.

HAM is a powerful approach that has been used to identify candidate genes involved in the regulation of a wide array of phenotypes in mice, including red blood cell count (RBC) and high density lipoprotein cholesterol (HDL) (3) and albuminuria (19). However, this approach is not without limitations, as it has also been proposed that this method may lack the power to find QTLs for some phenotypes (11). HAM also has a high rate of false positives, often requiring experimental crosses to validate loci (3). Our future studies will generate outcrossed mice derived from strains with high tissue GSH/GSSG and strains with low GSH/GSSG. These studies will verify the validity of glutathione-associated genes and test if additional genes and alleles regulate glutathione phenotypes.

Glutathione redox balance — GSH/GSSG — is a regulator of a multitude of cellular processes and is associated with the development of many diseases. To our knowledge, this study is the first to map the genes that regulate glutathione concentrations and redox balance in mammalian tissues. Our study focuses on variation within the nuclear genome that is associated with glutathione homeostasis, which complements previous efforts that revealed different alleles within the mitochondrial genome can influence glutathione redox state in mice (17). Genetic regulation of GSH/GSSG in mice has significant clinical importance. A recently reported study conducted in human subjects provides data suggesting that red blood cell GSH/GSSG may be heritable in humans as well (22), but genetic effects in humans have not been tested in liver and kidney. Findings that support a relationship between genetics

and oxidative stress-related phenotypes like GSH/GSSG create remarkable promise for disease treatments. The next stages of this research — the validation of genes and alleles that regulate GSH/GSSG — will contribute to the development of the field of personalized medicine, as well as highlight potential therapeutic targets to ameliorate oxidative stress in clinical disease.

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LIST OF ABBREVIATIONS

129	129S1/SvImJ
AJ	A/J
AKR	AKR/J
ALR	ALR/J
ANCOVA	Analysis of covariance
B6	C57BL/6J
B10	C57BL/10J
BALB	BALB/cByJ
BLKS	C57BLKS/J
BTBR	BTBR T ⁺ Itpr ^{3^{tf}} /J
C3H	C3H/HeJ
C57L	C57L/J
CAST	CAST/EiJ
CBA	CBA/J
D1	DBA/1J
D2	DBA/2J
DTPA	diethylenetriaminepentaacetic acid
FVB	FVB/NJ
GSH	reduced glutathione
GSSG	oxidized glutathione
HAM	Haplotype association mapping
LP	LP/J
MRL	MRL/MpJ

NOD	NOD/ShiLtJ
NON	NON/ShiLtJ
NOR	NOR/LtJ
NZB	NZB/BINJ
NZO	NZO/HILtJ
NZW	NZW/LacJ
PL	PL/J
PWK	PWK/PhJ
QTL	quantitative trait loci
SJL	SJL/J
SM	SM/J
SWR	SWR/J
WSB	WSB/EiJ

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HIGHLIGHTS

- The relationship between genetic variation and redox state is not well-defined.
- We measured GSH/GSSG in liver and kidney of 30 diverse inbred mouse strains.
- Genetic background is associated with a three-fold range in tissue GSH/GSSG.
- We performed first QTL mapping of glutathione homeostasis in mammals.
- Novel glutathione homeostasis candidate genes were discovered.

Figure 1. Genetic relatedness of inbred mouse strains

The 30 inbred strains employed in this study were separated into their genetic groups as proposed by Petkov, et al (13). Each group is represented by at least three strains in our panel.

Figure 2. Liver GSH concentrations, GSSG, concentrations, and GSH/GSSG ratio in 30 inbred mouse strains

The livers of female mice (n = 6–10) representing 30 inbred strains were analyzed for GSH and GSSG concentrations, which were normalized to total protein and calculated as a ratio. Data are presented as mean $\pm$ S.E.

Figure 3. Kidney GSH concentrations, GSSG, concentrations, and GSH/GSSG ratio in 30 inbred mouse strains

The kidneys of female mice ($n = 6-10$) representing 30 inbred strains were analyzed for GSH and GSSG concentrations, which were normalized to total protein and calculated as a ratio. Data are presented as mean $\pm$ S.E

Figure 4. Relationship between liver and kidney GSH/GSSG ratio

A) Plot of liver versus log kidney GSH/GSSG for 30 inbred mouse strains. B) Correlation between liver and log kidney GSH/GSSG for each inbred strain separately. The relationship between liver and kidney GSH/GSSG depends on genetic background.

Figure 5. HAM genome scan for liver GSH and GSSG concentrations, as well as GSH/GSSG ratio

The labeling on the x-axis notes the chromosomes in the mouse genome and the y-axis represents HAM score, which is $-\log p$ -value. Peaks with cross shape indicates significant SNPs.

Figure 6. HAM genome scan for kidney GSH and GSSG concentrations, as well as GSH/GSSG ratio

The labeling on the x-axis notes the chromosomes in the mouse genome and the y-axis represents HAM score, which is $-\log p$ -value. Peaks with cross shape indicates significant SNPs.

Table 1

HAM peaks and protein-coding genes associated with liver glutathione phenotypes

Phenotype	Chromosome	Locus (bp)	Number of SNPs	Protein-coding Genes in Interval
GSH	3	137227778–137559816	2	Emcn, Gm4861, Gm567
GSH	8	14761415	1	Dlgap2
GSH	8	56109297	1	Glra3
GSH	8	110469117	1	Hydin
GSH	8	111471007	1	Wdr59
GSH	9	68901235	1	Rora
GSH	14	117019508–117381589	9	Gpc6
GSSG	4	130724603	1	Pum1
GSSG	14	62215307–62285021	3	Dleu7
GSSG	16	32783263	1	Muc20
GSH/GSSG	2	160594981	1	Gm14221
GSH/GSSG	4	99947162	1	Pgm2
GSH/GSSG	4	100256660–100031636	2	Ror1
GSH/GSSG	4	118045081–118097145	3	St3gal3
GSH/GSSG	14	117572316	1	Gpc6

Table 2

HAM peaks and protein-coding genes associated with kidney glutathione phenotypes

Phenotype	Chromosome	Locus (bp)	Number of SNPs	Protein-coding Genes in Interval
GSH	1	20598463	1	Pkhd1
GSH	1	81080496	1	Nyap2
GSH	1	138111815	1	Ptpcr
GSH	5	75003877	1	Chic2
GSH	5	123504936	1	Lrrc43
GSH	5	144959470	1	Smurf1
GSH	11	82722506	1	Cct6b
GSH	11	82765715	1	Zfp830
GSH	11	86178368–86193392	3	Brip1
GSH	11	93177374	1	Car10
GSH	13	37903516	1	Rreb1
GSH	13	43624914	1	Rnf182
GSH	13	44730456–44798929	3	Jarid2
GSH	14	70791410	1	Adams16
GSH	14	70629808	1	Dmtn
GSH	15	27663667	1	Fam105a
GSH	15	27746793	1	Trio
GSH	18	14645957	1	Ss18
GSH	18	14883202–14887645	2	Taf4b
GSH	18	16605612	1	Cdh2
GSSG	6	134119453	1	Etv6
GSSG	8	11244537	1	Col4a1
GSSG	8	15953204–16860830	16	Csmd1
GSSG	8	47982037–47988542	2	Wwc2
GSSG	8	48244906	1	Tenm3
GSSG	8	54793602	1	Wdr17
GSSG	8	55416311	1	Gm392
GSSG	12	73752989	1	Prkch
GSSG	19	21397909	1	Gda
GSSG	19	22148587–22457228	2	Trpm3
GSH/GSSG	8	11206425–11244537	2	Col4a1
GSH/GSSG	8	15953204–16860830	19	Csmd1
GSH/GSSG	8	47982037	1	Wwc2
GSH/GSSG	8	48244906	1	Tenm3
GSH/GSSG	18	12293017	1	Ankrd29
GSH/GSSG	19	21397909	1	Gda

Phenotype	Chromosome	Locus (bp)	Number of SNPs	Protein-coding Genes in Interval
GSH/GSSG	19	22148587–22457228	2	Trpm3