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Original Contribution

Genetic analysis of tissue glutathione concentrations and redox balance

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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 threefold 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 locus 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 and its regulation and inform future therapeutic applications.

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, whereas a decrease is suggestive of oxidative stress and diminished antioxidant defenses. Changes in GSH/GSSG are closely associated with cellular processes such as proliferation and apoptosis [1] as well as progression of conditions such as 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 the 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/6 J and DBA/2 J [15,16]. In a panel of 14 inbred strains, Tsuchiya et al. [20] provided evidence that a strain effect occurs for hepatic GSH/GSSG as well. These findings suggest that natural genetic variation regulates GSH/GSSG, as with other oxidative stress-related phenotypes such as lipid peroxidation [7] and resistance to stressors such as hyperoxia [4], paraquat [9], and MPTP [5]. Because 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.

Abbreviations: 129, 129S1/SvImJ; A, A/J; AKR, AKR/J; ALR, ALR/J; B6, C57BL/6 J; B10, C57BL/10 J; BALB, BALB/cByJ; BLKS, C57BLKS/J; BTBR, BTBR T^{+} $Itpr3^{tf}$/J; C3H, C3H/HeJ; C57L, C57L/J; CAST, CAST/EiJ; CBA, CBA/J; D1, DBA/1 J; D2, DBA/2 J; 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 locus; SJL, SJL/J; SM, SM/J; SWR, SWR/J; WSB, WSB/EiJ

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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 threefold 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 No. 002448), A/J (A; JAX No. 000646), AKR/J (AKR; JAX No. 000648), ALR/J (ALR; JAX No. 003070), BALB/cByJ (BALB; JAX No. 001026), BTBR T^{+} $Itpr3^{tf}$/J (BTBR; JAX No. 002282), C3H/HeJ (C3H; JAX No. 000659), C57BL/6 J (B6; JAX No. 000664), C57BL/10 J (B10; JAX No. 000665), C57BLKS/J (BLKS; JAX No. 000662), C57L/J (C57L; JAX No. 000668), CAST/EiJ (CAST; JAX No. 000928), CBA/J (CBA; JAX No. 000656), DBA/1 J (D1; JAX No. 000670), DBA/2 J (D2; JAX No. 000671), FVB/NJ (FVB; JAX No. 001800), LP/J (LP; JAX No. 000676), MRL/MpJ (MRL; JAX No. 000486), NOD/ShiLtJ (NOD; JAX No. 001976), NON/ShiLtJ (NON; JAX No. 002423), NOR/LtJ (NOR; JAX No. 002050), NZB/BINJ (NZB; JAX No. 000684), NZO/HILtJ (NZO; JAX No. 002105), NZW/LacJ (NZW; JAX No. 001058), PL/J (PL; JAX No. 000680), PWK/PhJ (PWK; JAX No. 003715), SJL/J (SJL; JAX No. 000686), SM/J (SM; JAX No. 000687), SWR/J (SWR; JAX No. 000689), and WSB/EiJ (WSB; JAX No. 001145) mice were bred and housed at The Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed on a 12-h light/dark cycle and at 3–4 months of age 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 phosphate-buffered saline (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 16,000*g* for 7 min 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 any analysis on the data was performed, the normality of hepatic and renal GSH/GSSG and median life span were checked visually to meet the assumptions of later statistical modeling. Hepatic GSH/GSSG ratios were normally distributed, whereas 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(renal GSH/GSSG). The distribution of median life span was skewed to the left and hence a square transformation was performed on median life span.

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

$$\text{hepatic GSH/GSSG} = \mu + \text{strain}_i + \varepsilon_{ij};\ i = 1, \ldots, 30;\ j = 1, \ldots, n_i;$$

Table 1
Results for hepatic GSH/GSSG.

Source	*DF*	Sum of squares	Mean square	*F* value	*p* value
Model	29	51,840.78	1787.61	11.67	< 0.001
Error	167	25,587.69	153.220		
Corrected total	196	77,428.47			

Table 2
Results for ln(renal GSH/GSSG).

Source	*DF*	Sum of squares	Mean square	*F* value	*p* value
Model	29	17.30	0.60	11.30	< 0.001
Error	167	8.81	0.053		
Corrected total	196	2444.65			

Table 3
Results for relationship between hepatic and renal GSH/GSSG within strains.

Source	*DF*	Sum of squares	Mean square	*F* value	*p* value
Model	59	20.19	0.34	7.91	< 0.001
Error	137	5.92	0.04		
Corrected total	196	26.11			
		Type III SS			
Liver glutathione	1	0.73	0.73	16.85	< 0.001
Strain	29	3.02	0.10	2.41	< 0.001
Liver glutathione × strain	29	2.29	0.08	1.82	0.012

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 0 and variance σ^2, and n_i is the number of mice from strain *i*. The ANOVA table and analysis results are shown in Table 1 ($R^2 = 0.670$ and root MSE = 12.378).

Similarly, the ANOVA table and analysis results for ln(renal GSH/GSSG) are shown in Table 2 ($R^2 = 0.662$ and root MSE = 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 30 pairs of measures.

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

$$\ln(\text{renal GSH/GSSG}) = \mu + \text{strain}_i + \alpha \times (\text{hepatic GSH/GSSG}) + \beta_i \times (\text{hepatic GSH/GSSG}) + \varepsilon_{ij};\ i = 1, \ldots, 30; j = 1, \ldots, n_i;$$

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 0 and variance σ^2, and n_i is the number of mice from strain *i* (Table 3; $R^2 = 0.773$ and root MSE = 0.208).

The Pearson correlation coefficients between average hepatic GSH/GSSG and (median life span)^2, and between average ln(renal GSH/GSSG) and (median life span)^2, were calculated by first averaging the hepatic and natural-log transformed GSH/GSSG measures within each strain for 25 strains. Then, the Pearson