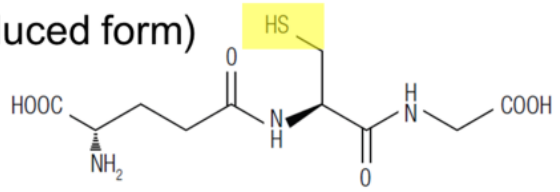
Genetic Control of the Glutathione Redox System

Rob Pazdro, Ph.D.
Assistant Professor
Department of Foods and Nutrition
University of Georgia

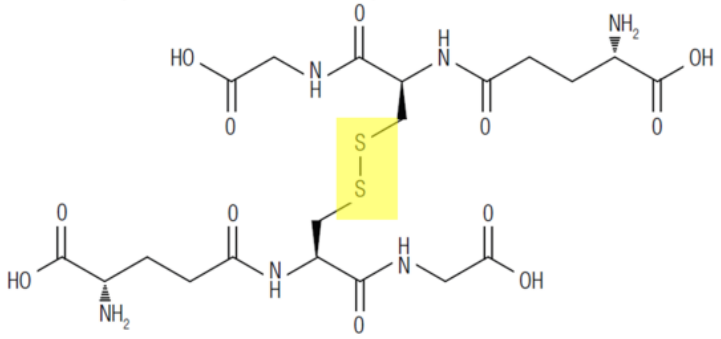
Glutathione

- Endogenous tripeptide antioxidant
- Coordinates diverse stress response functions
- Ubiquitous, essential for the survival of all eukaryotic cells
- Regulates cellular processes and signaling

GSH (reduced form)



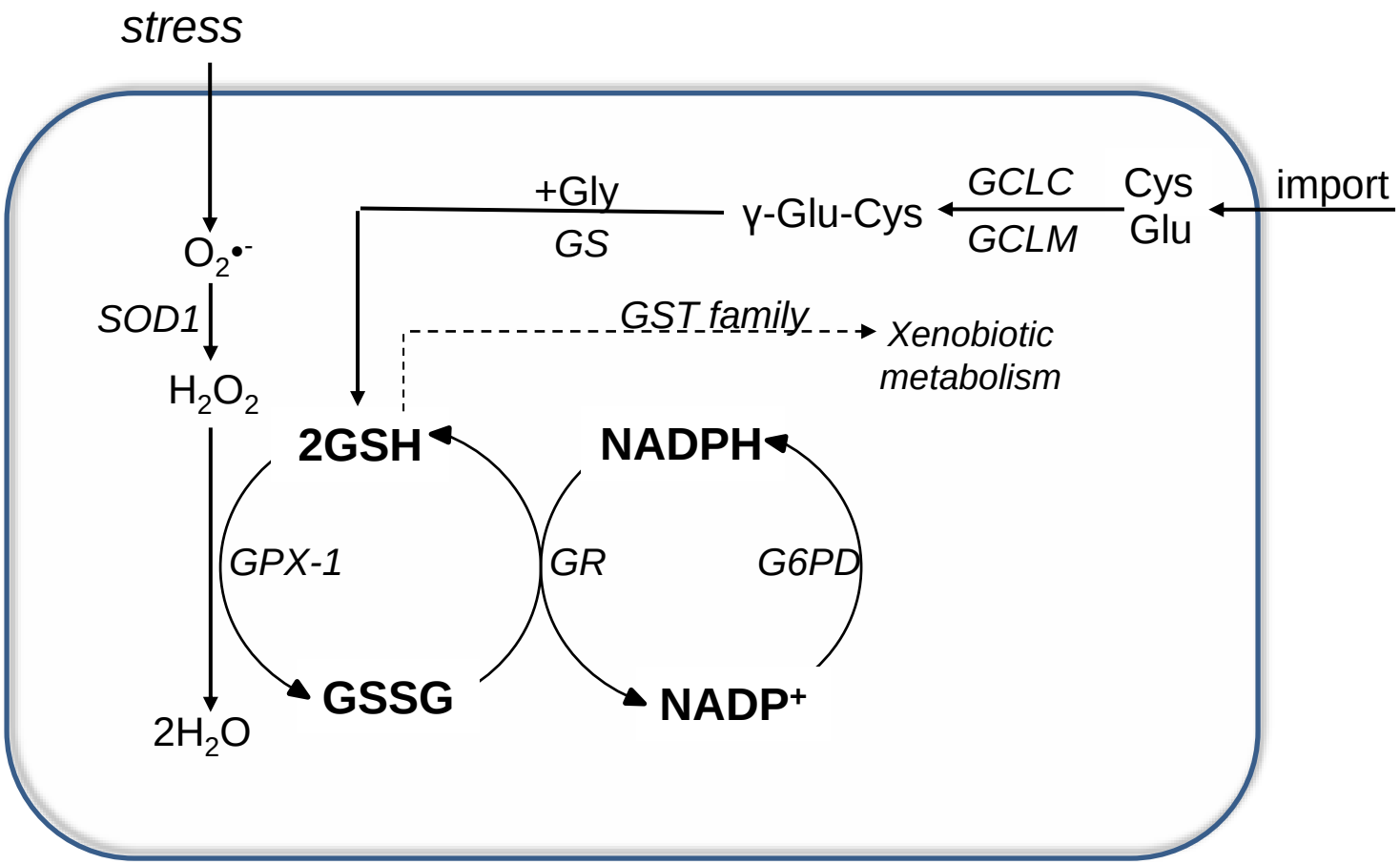
GSSG (oxidized form)



GSH is the cornerstone of an endogenous stress response system.

It must be controlled at a genetic level, right?

Basic GSH System



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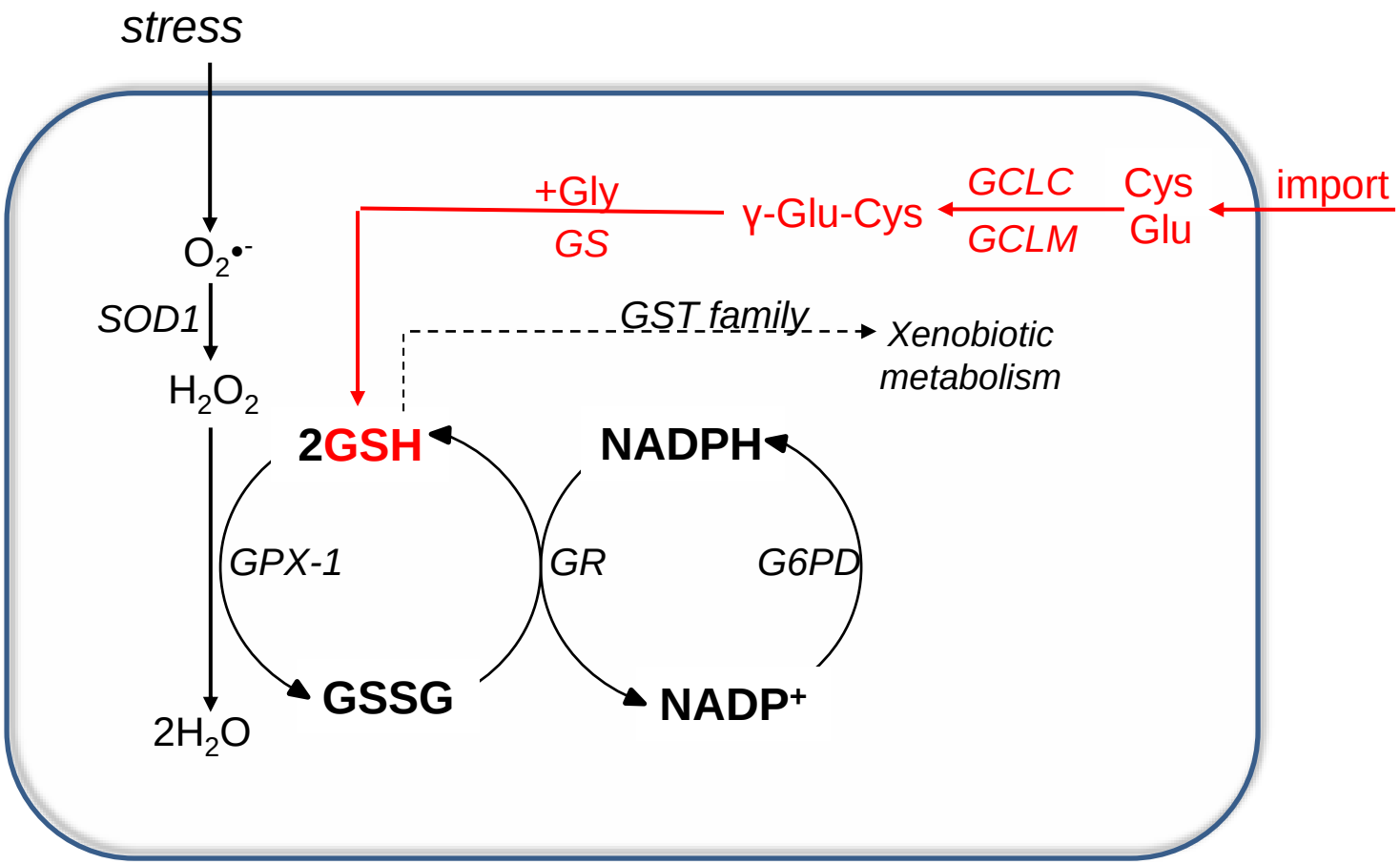

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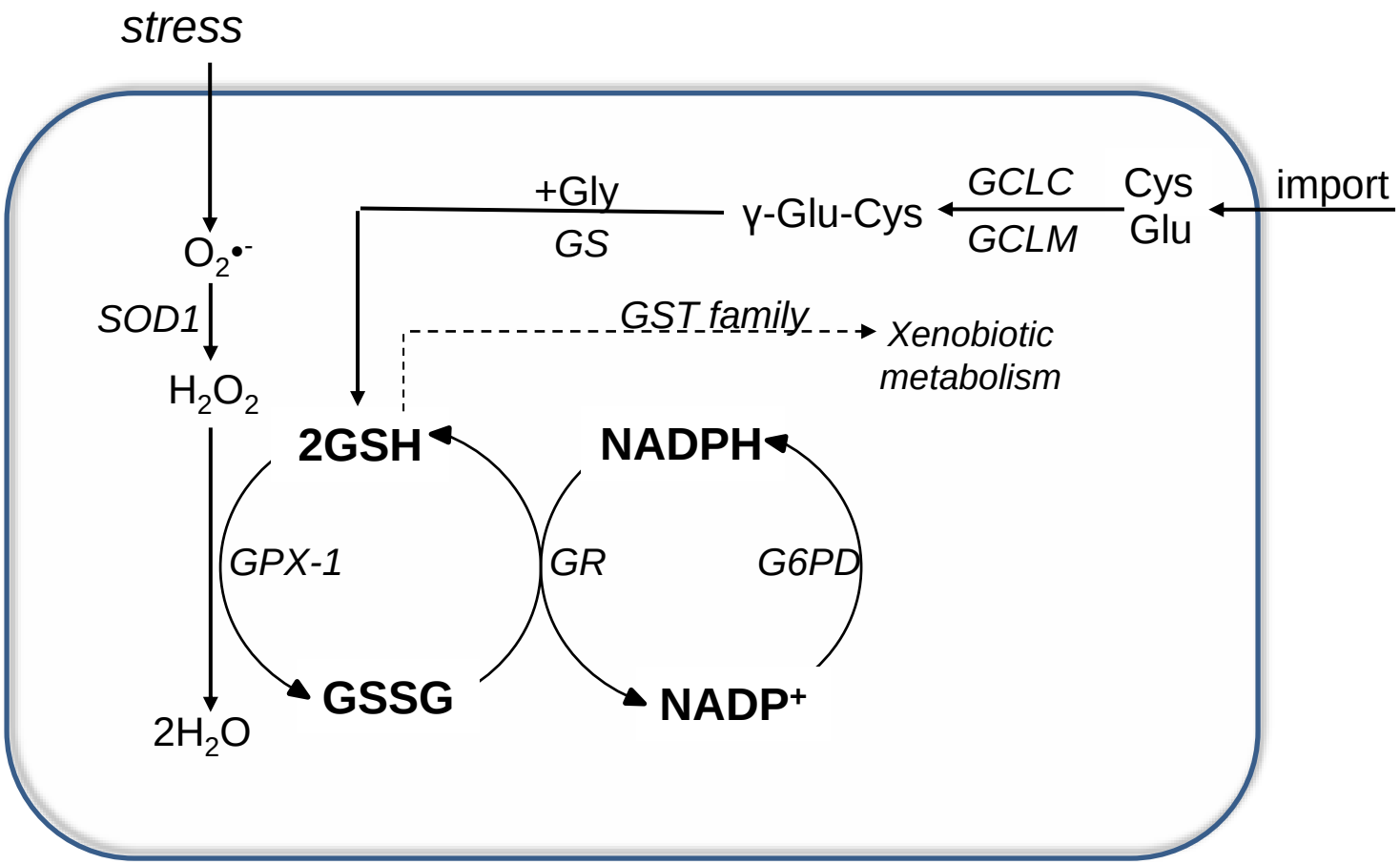

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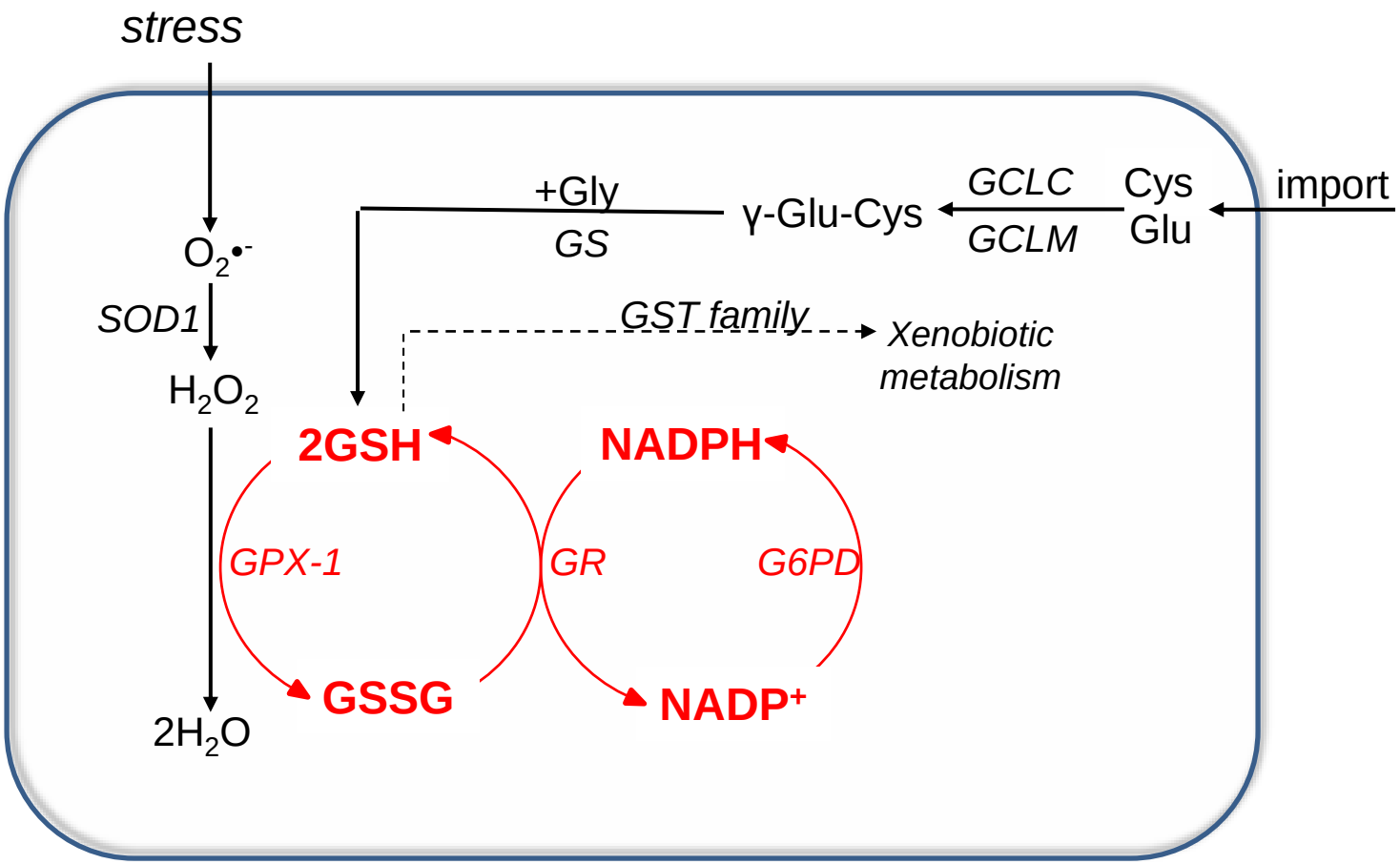

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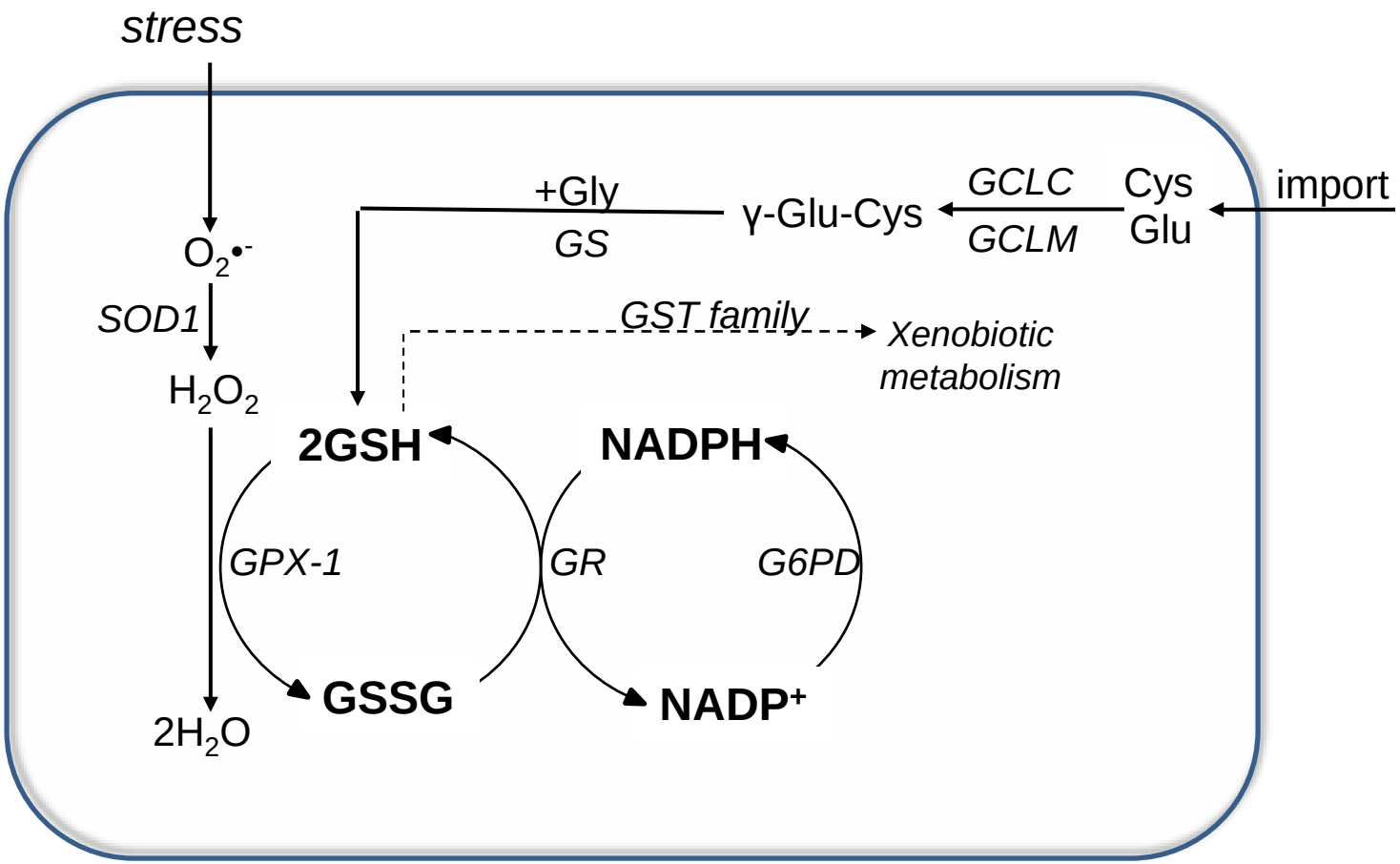

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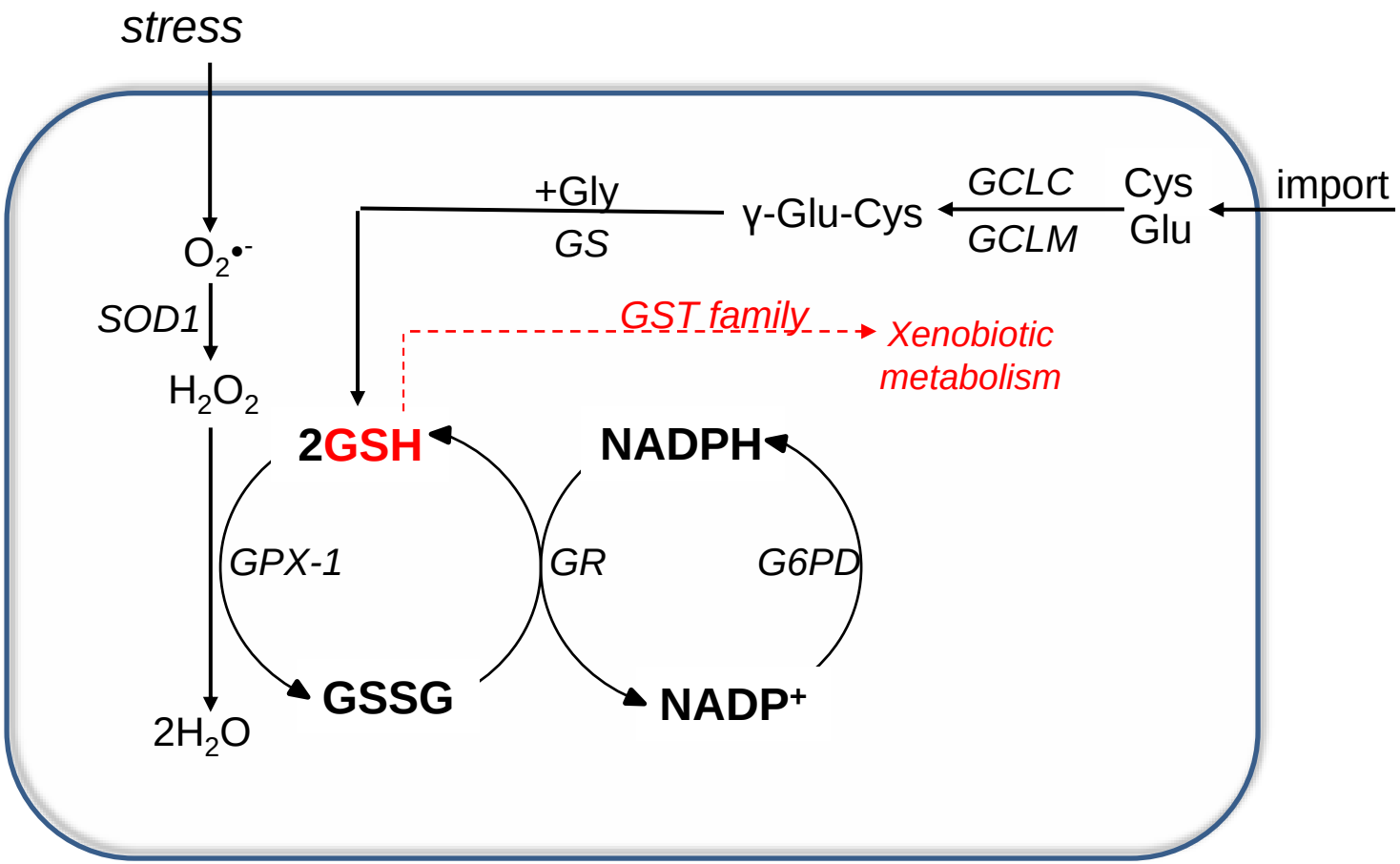
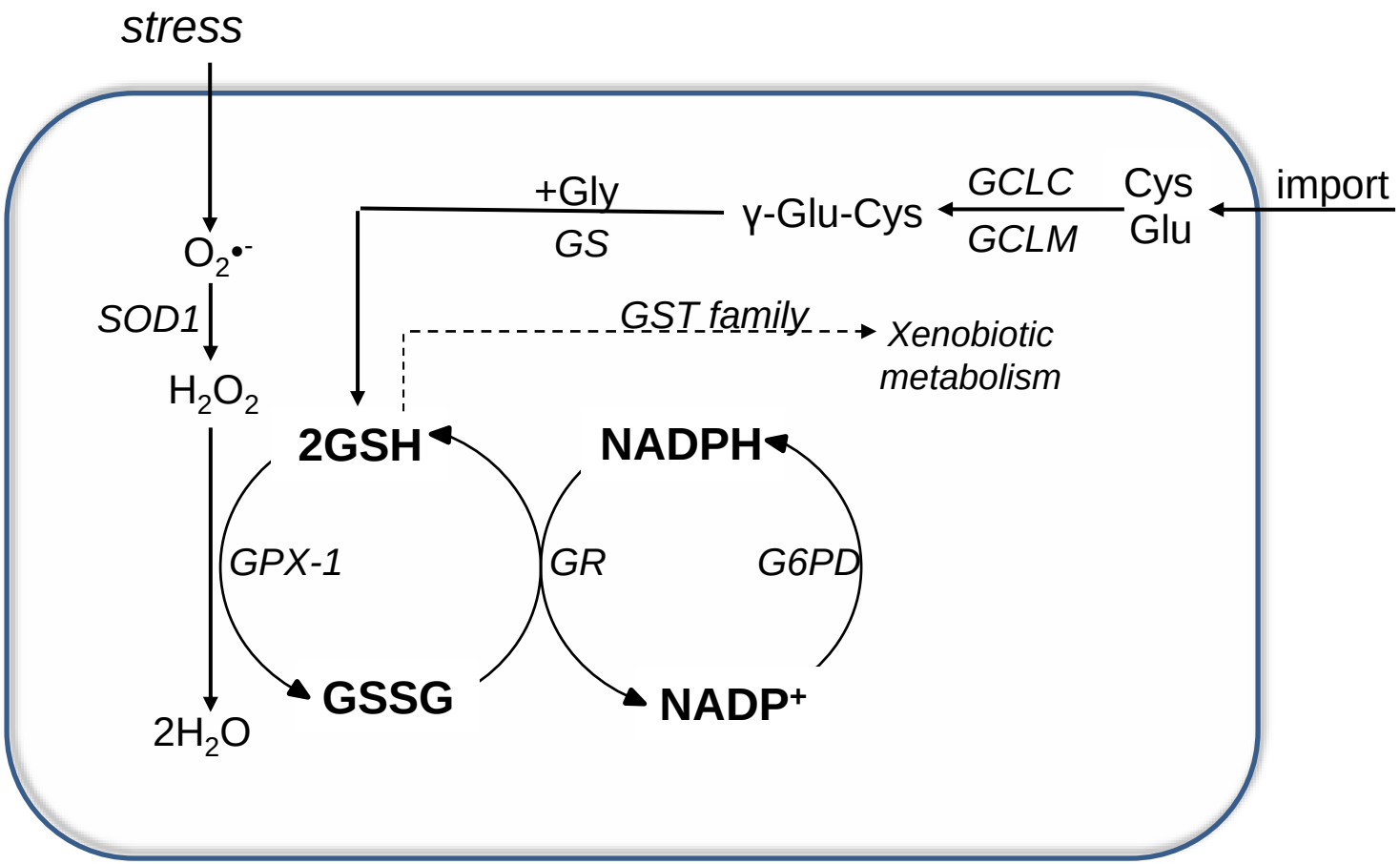
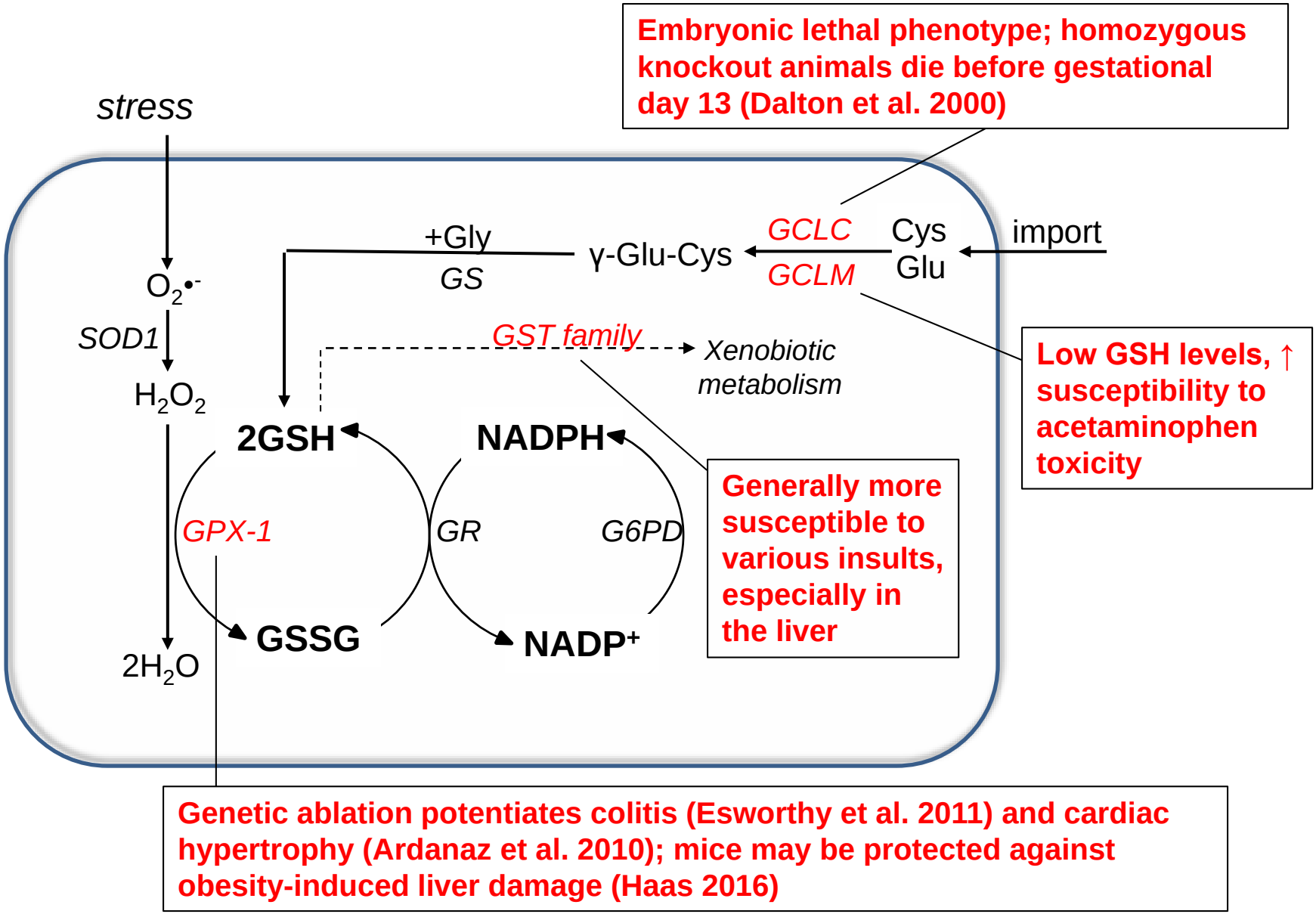


Current Knowledge of GSH Genetics



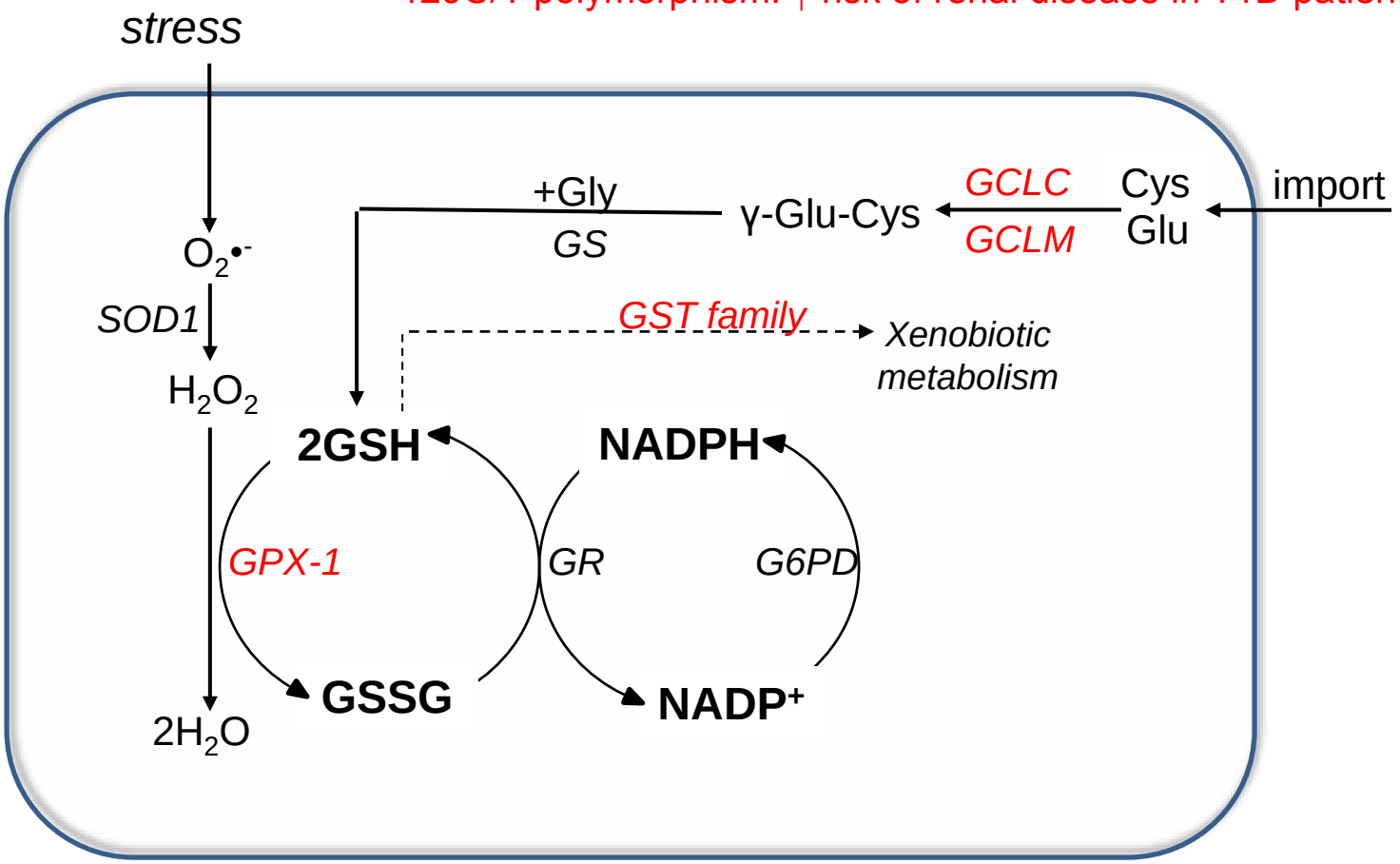
Model Organisms: Genetic Mutants



Candidate Gene Studies in Humans

GCLC

- GAG-repeats in 5'-UTR: ↓ blood GSH levels (Nichenametla et al. 2008)
- Same repeats: ↑ risk of lung cancer (Nichenametla et al. 2013)
- -129C/T polymorphism: ↑ risk of renal disease in T1D patients (Viera 2011)



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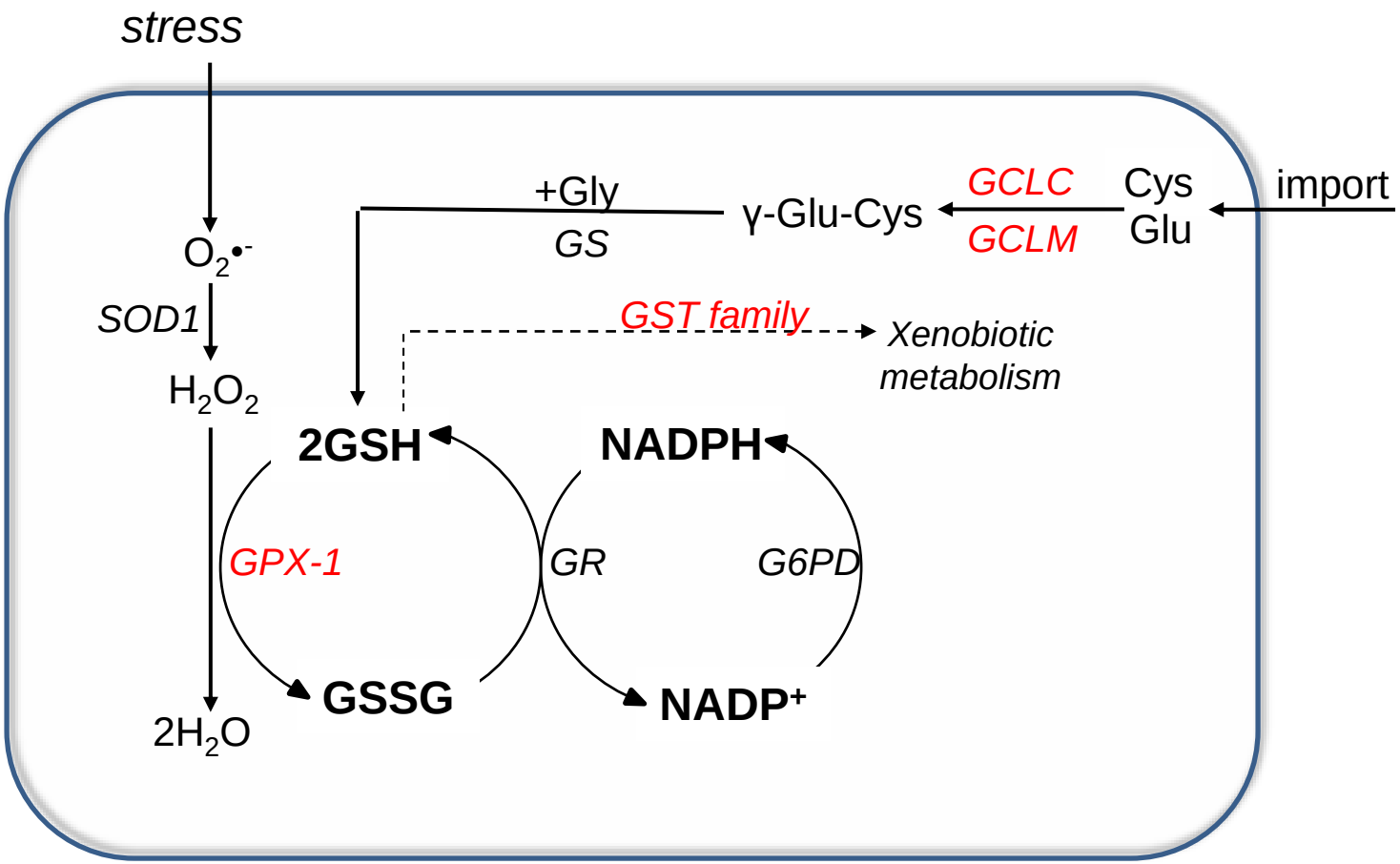


GPX-1

- Pro198Leu polymorphism: ↑ risk diabetic neuropathy (Buraczynska et al. 2016)
- Same polymorphism: no effect on CHD risk (Souiden et al. 2016)
- Same polymorphism: C allele (Pro) more common in panic disorder (Cengiz 2015)

Critical Knowledge Gaps

Many polymorphisms have been shown to affect enzyme activity, and for some, GSH levels.

However, systems genetics approaches have never been applied to GSH.

- Are these the most relevant polymorphisms?
- In such a vital system, might there be additional, non-canonical genes to consider?

Overarching Hypothesis

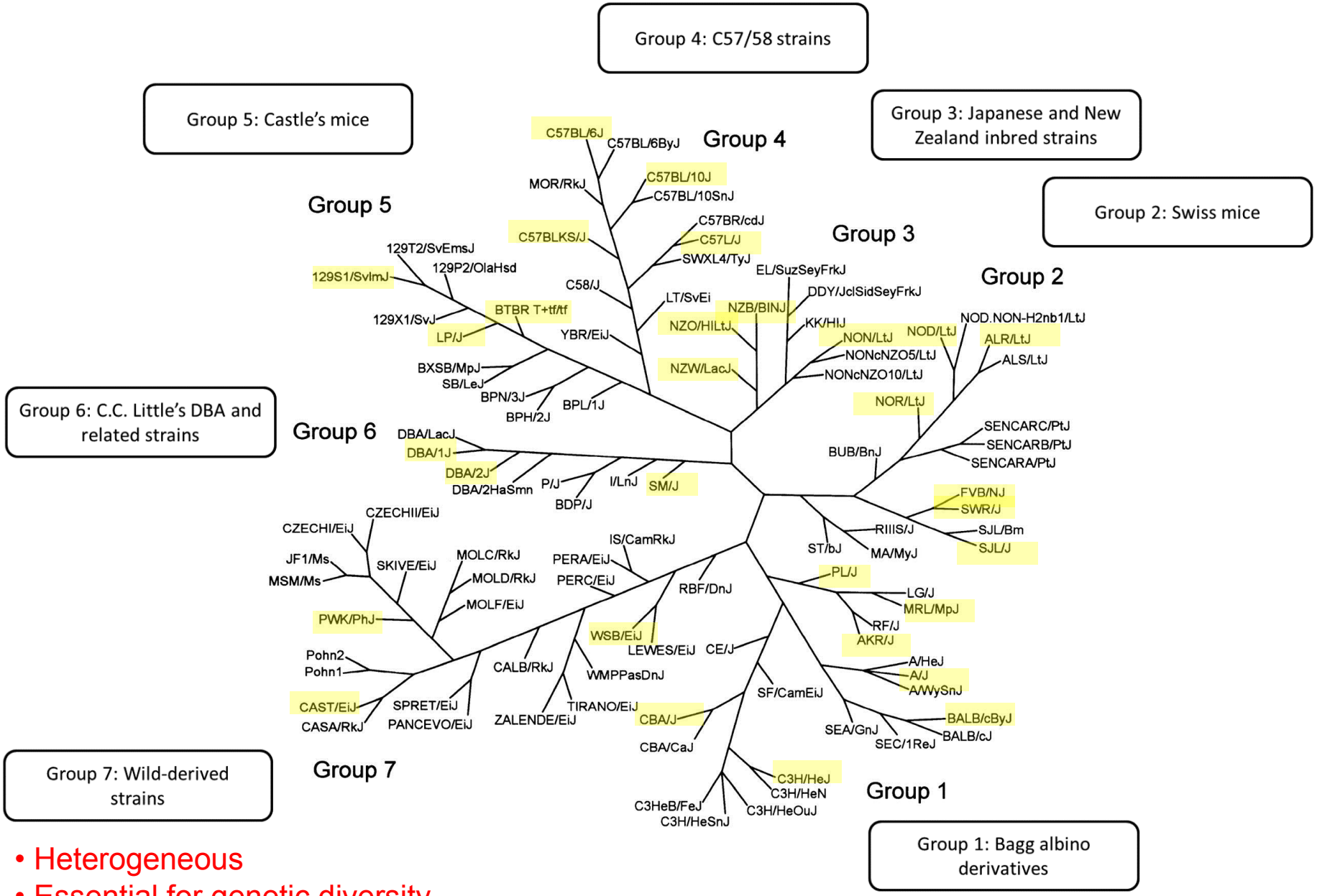
Genetic control of the GSH system:

1) Includes canonical genes, such as GCLC, GCLM, GPX-1, and the GST family

2) Extends to non-canonical genes that also affect GSH

Pilot Project in GSH Genetics

- Developed project at The Jackson Laboratory
- Project funded by NIGMS, completed at UGA
- Goal: Define role of genetic background in regulating tissue GSH levels and GSH/GSSG
- Design
  - Analyze tissue GSH levels and GSH/GSSG in liver and kidney
  - Mice 3-4 months of age
  - 30 genetically-diverse inbred mouse strains

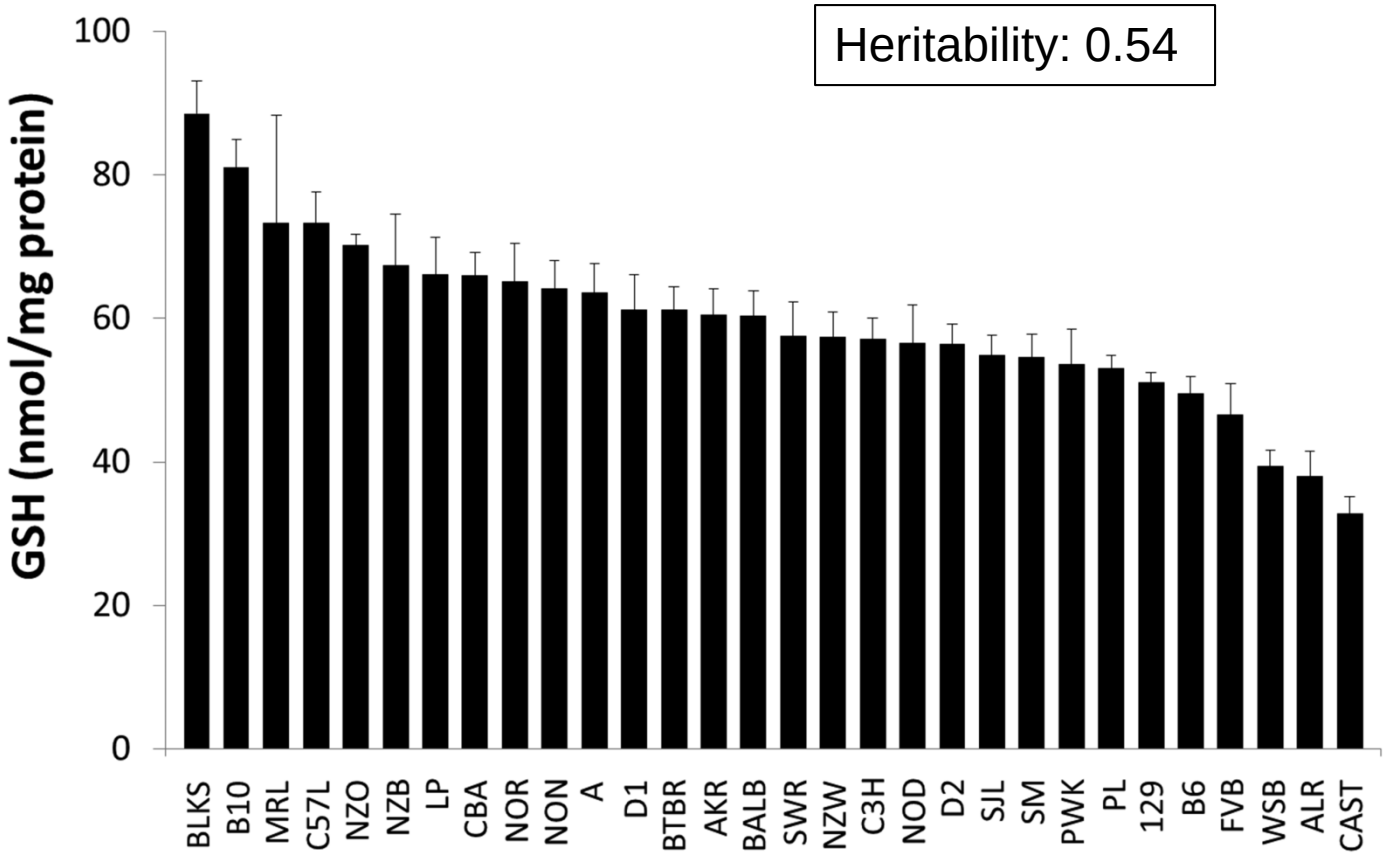


- Heterogeneous
- Essential for genetic diversity

Petkov PM, et al. Genome Res. 2004; 14: 1806-11.

Liver GSH Phenotypes in 30 Inbred Mouse Strains

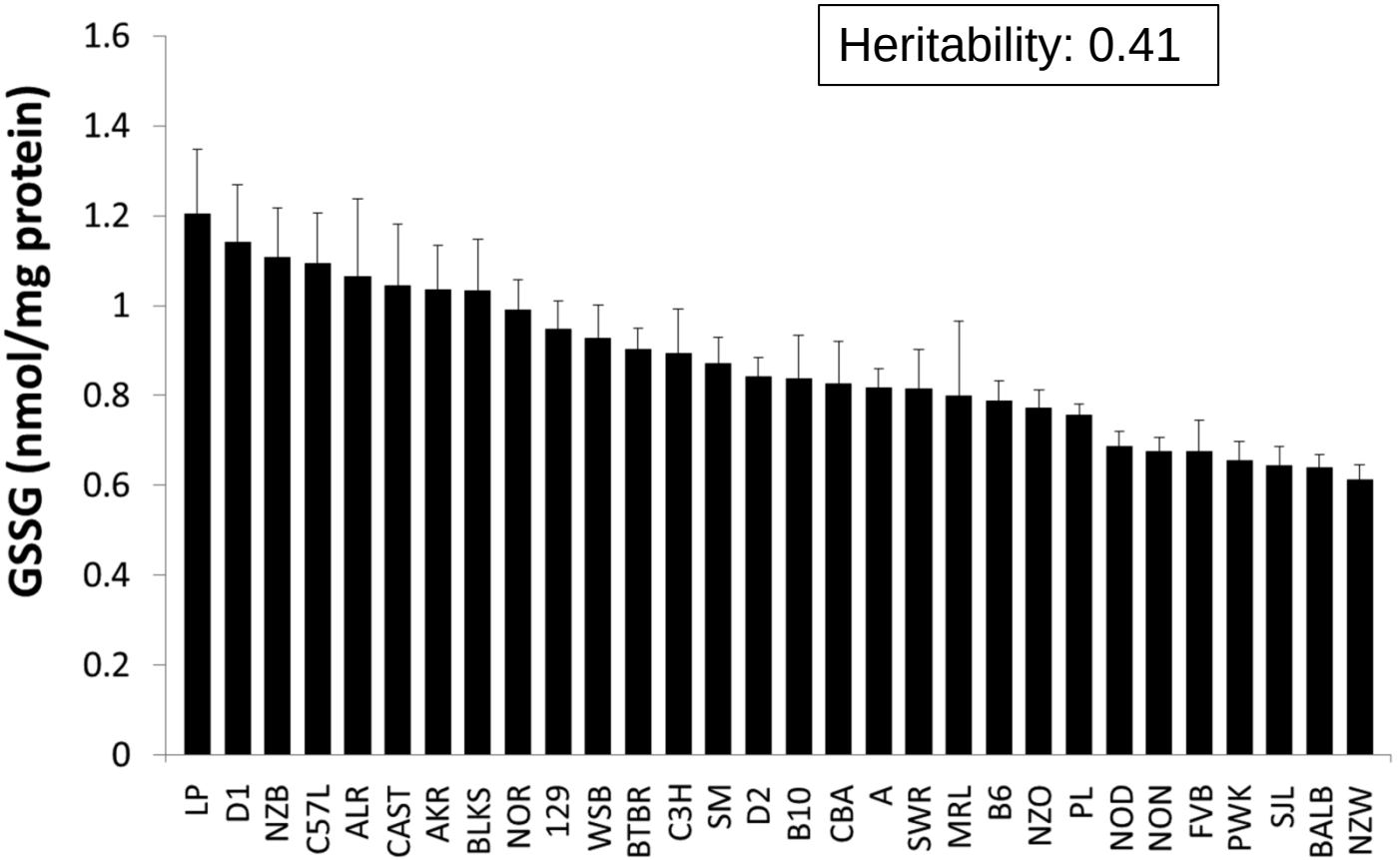
Liver GSH Phenotypes



N = 6-10 per strain. Data presented as mean ± S.E.

Zhou, et al. Free Radic Biol Med, 2014.

Liver GSH Phenotypes



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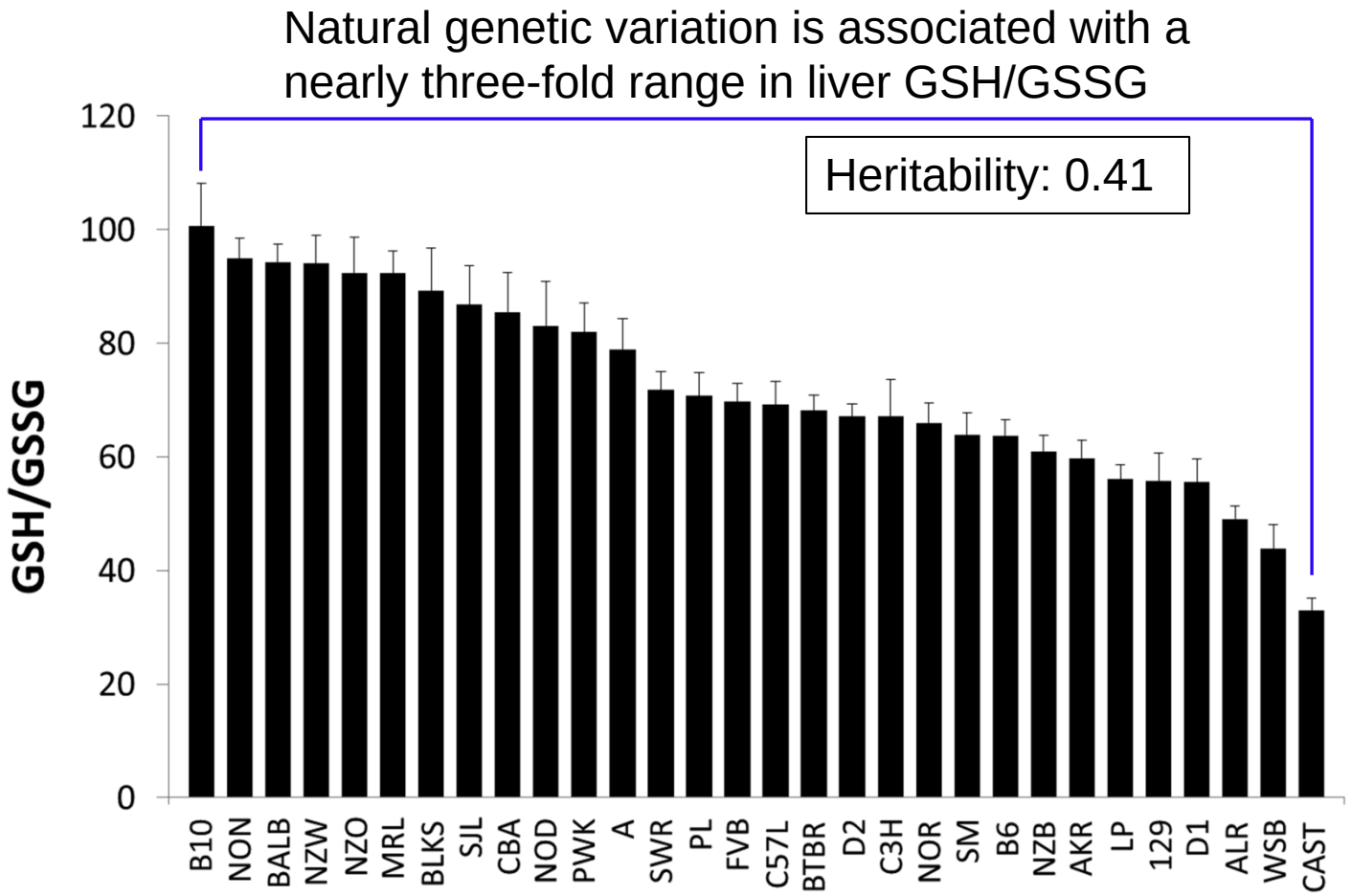

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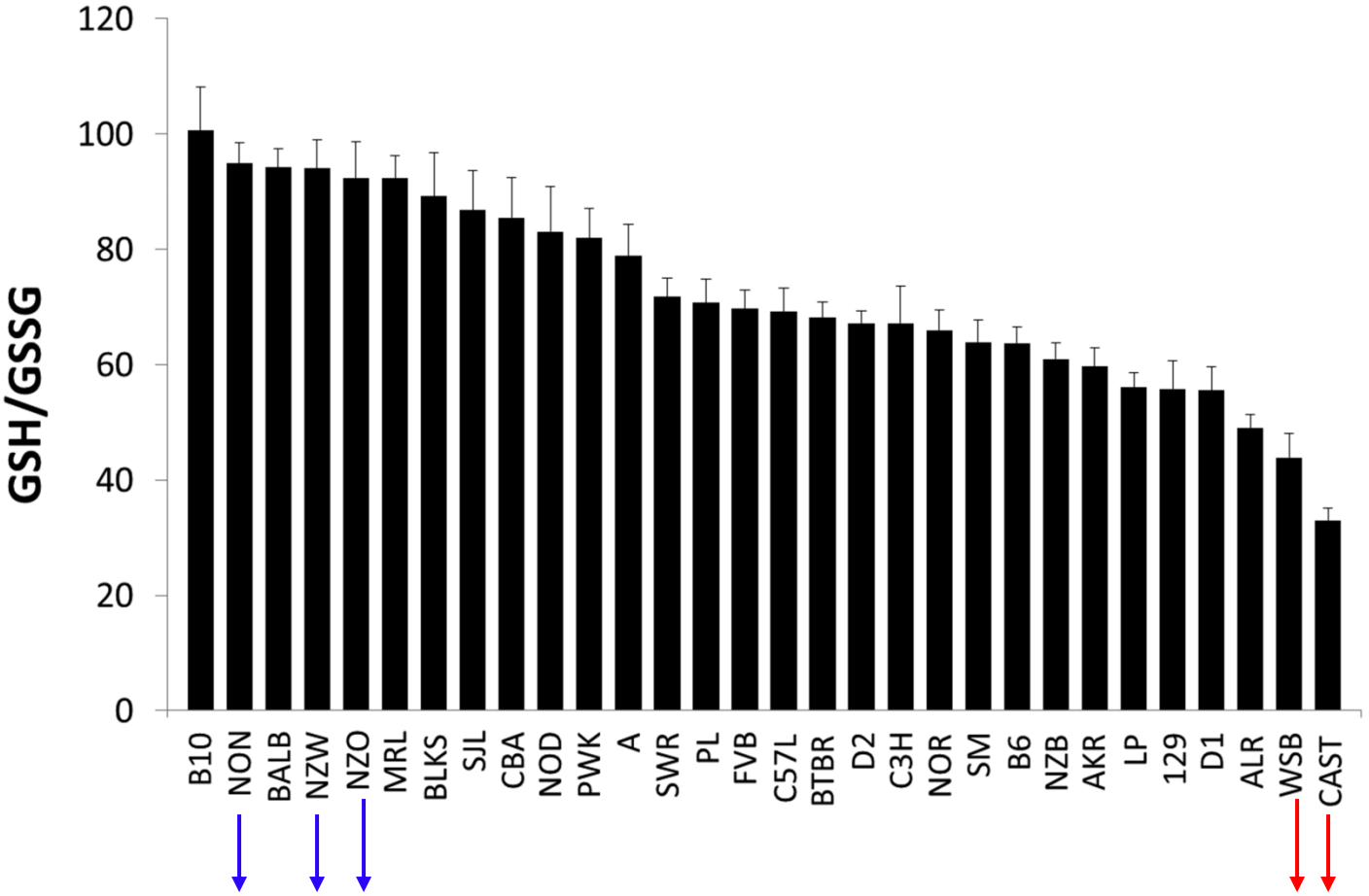
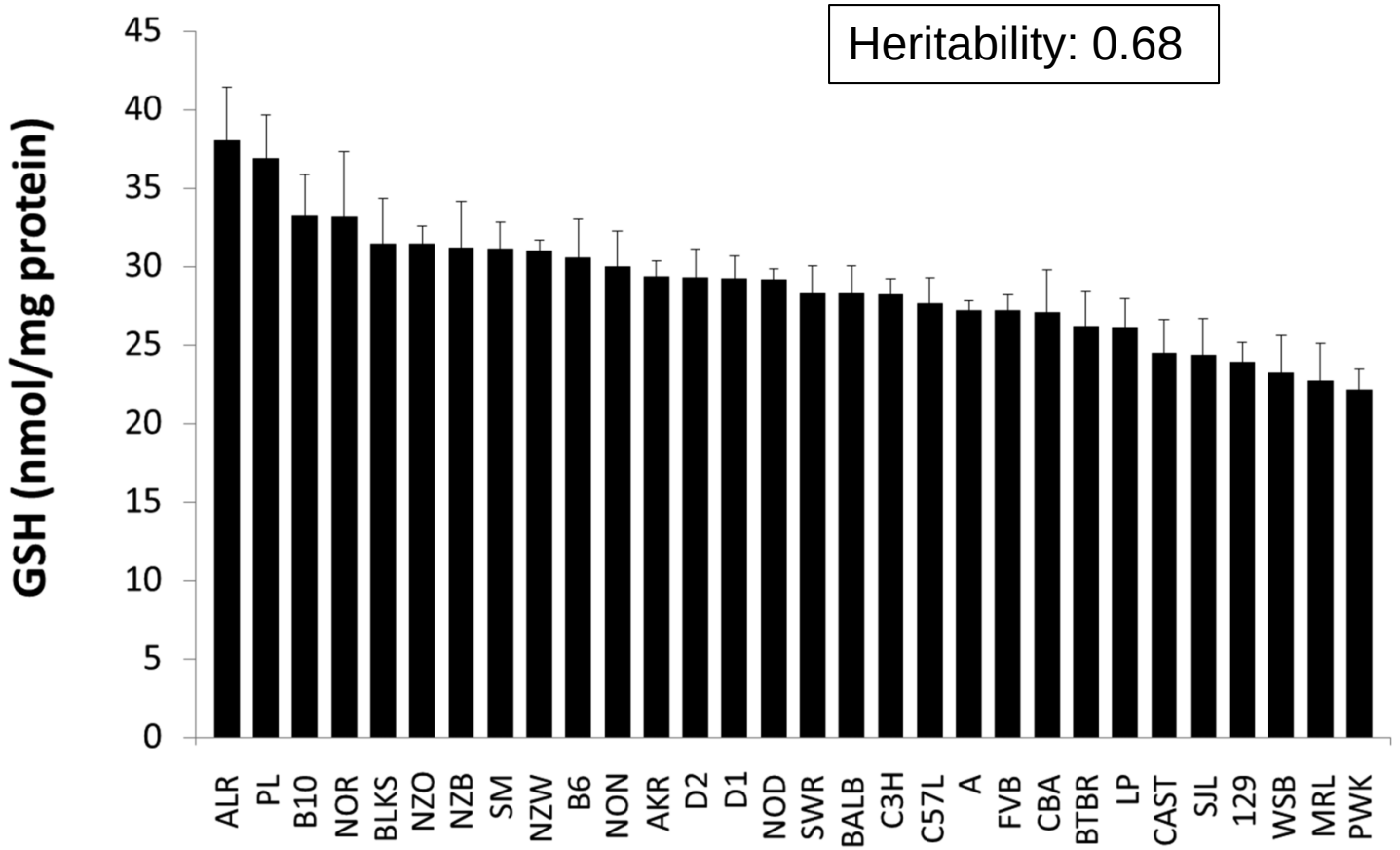


Hypothesis: Shared alleles in this group may contribute to high GSH/GSSG

Hypothesis: Some wild-derived alleles may negatively influence GSH/GSSG

Kidney GSH Phenotypes in 30 Inbred Mouse Strains

Kidney GSH Phenotypes



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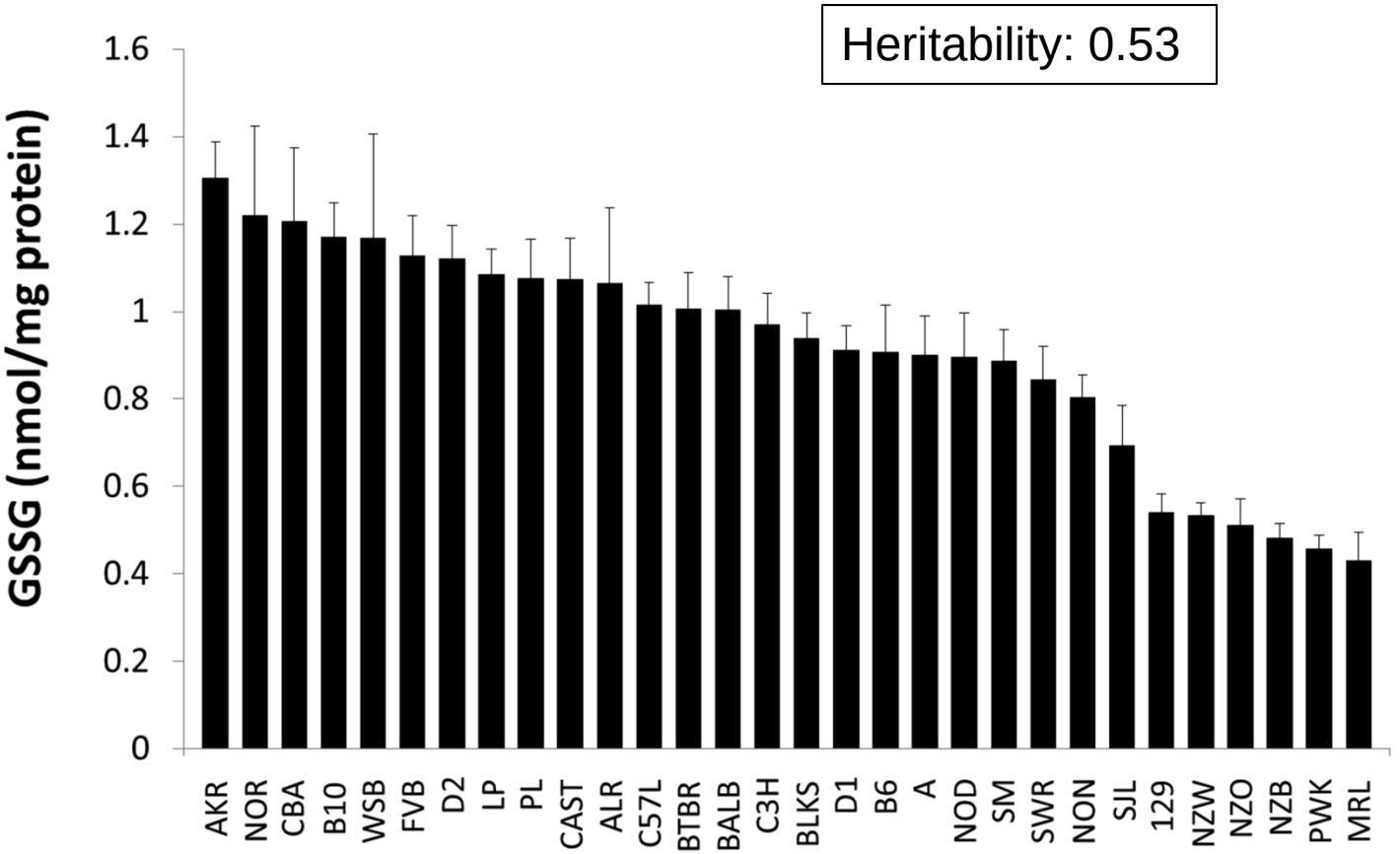
Kidney GSH Phenotypes



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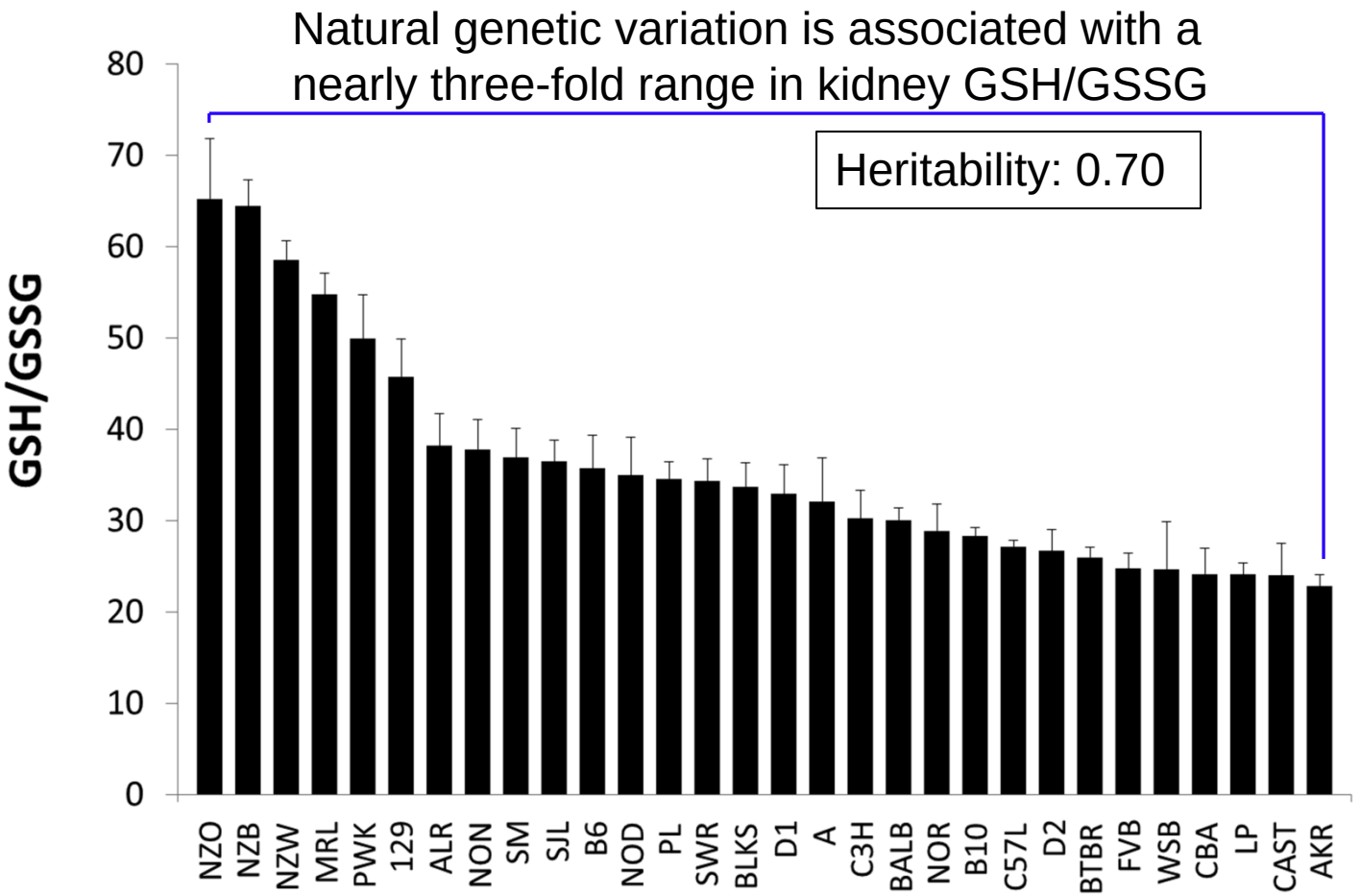

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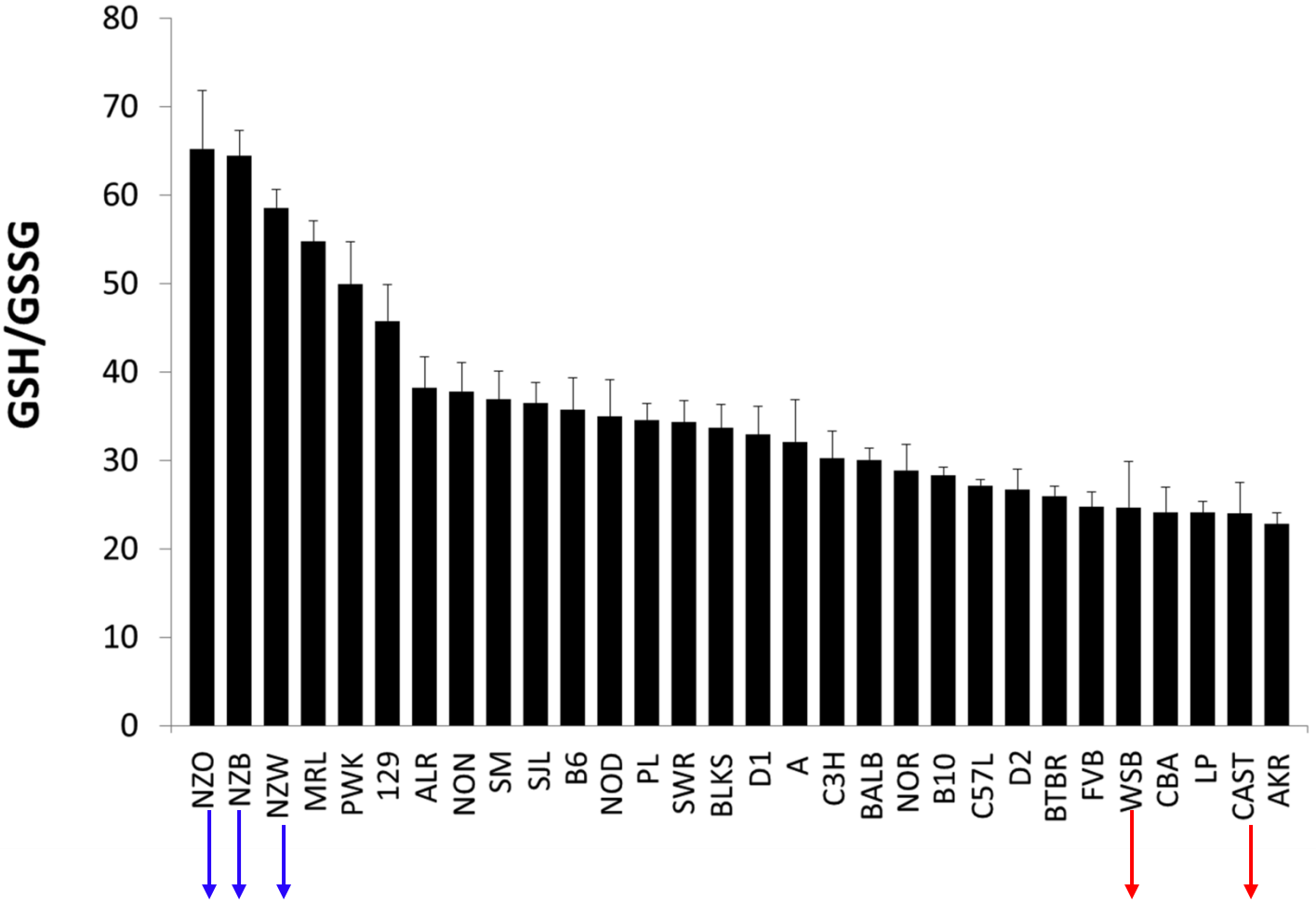
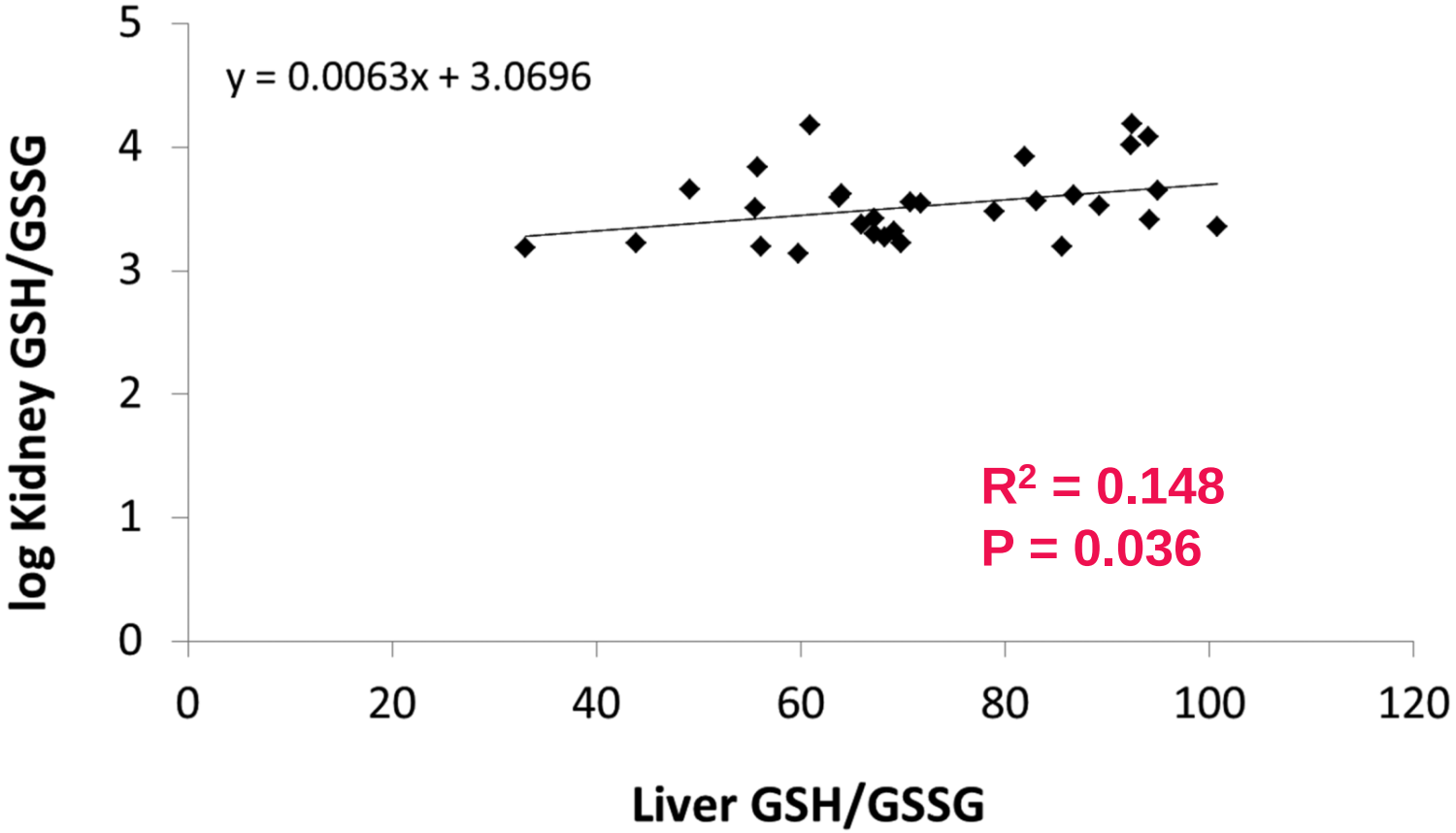


Hypothesis: Shared alleles in this group may contribute to high GSH/GSSG

CAST and WSB are not associated with high GSH/GSSG

Does a correlation exist between hepatic and renal GSH/GSSG?

Liver versus Kidney GSH/GSSG



GSH/GSSG in Liver and Kidney

Correlation

- Statistically significant, but relatively weak
- Does not support model that all of the same genes and alleles regulate GSH/GSSG across multiple tissues

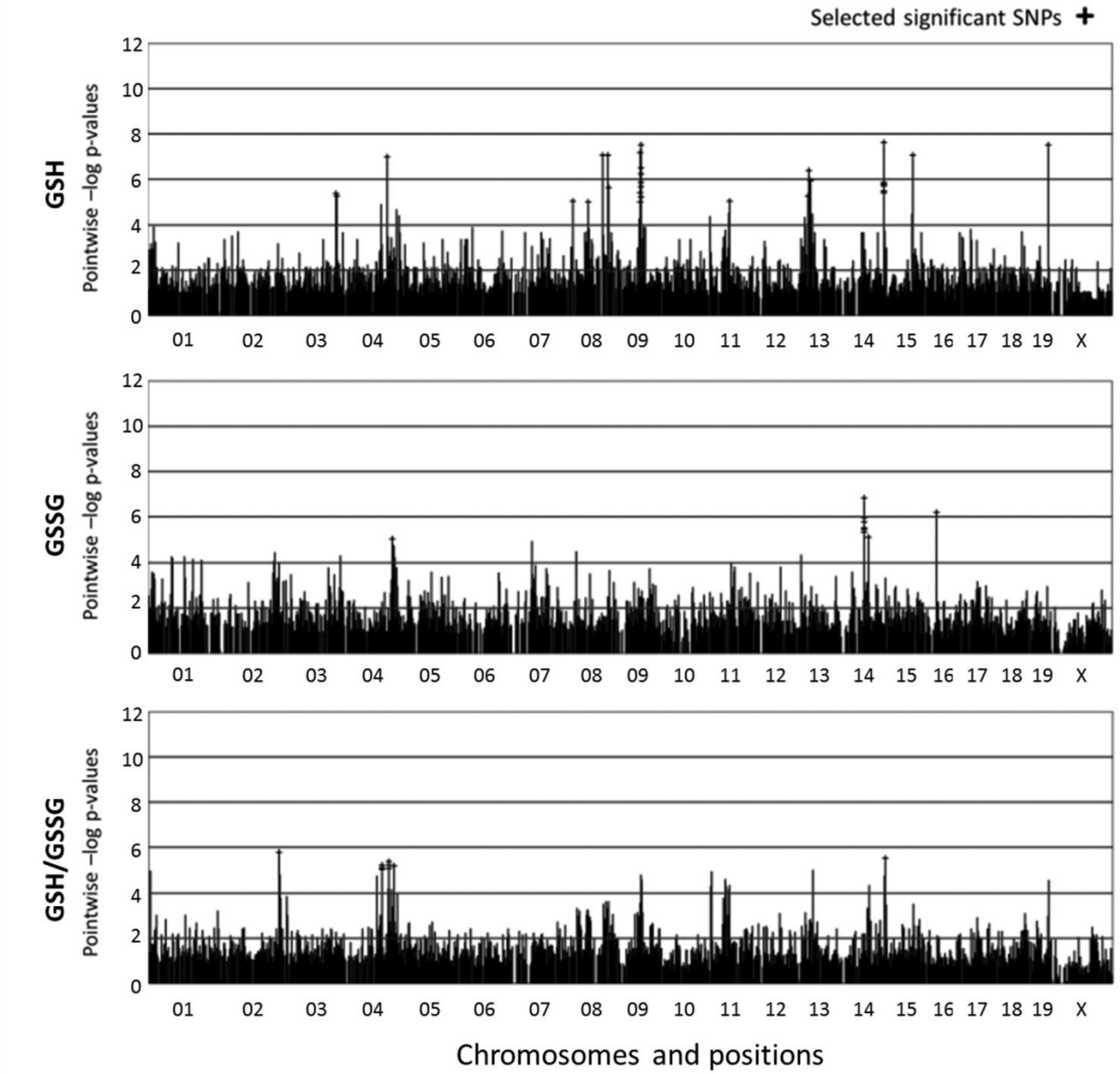
We hypothesize that GSH/GSSG is regulated by:

1. Genes expressed in multiple tissues simultaneously
   - e.g., GCL
   - These genes contribute to the correlation
2. Tissue-specific genes
   - These genes contribute most to glutathione homeostasis
3. Non-genetic sources of variability

Testing our Hypothesis

- *In silico* mapping
- Efficient Mixed-Model Association (EMMA)
- Performed on strain survey data
- 132,000 imputed SNPs from 29 inbred strains
- SNPs with p-values less than 10^{-5} were selected as significant

Mapping Hepatic GSH Phenotypes



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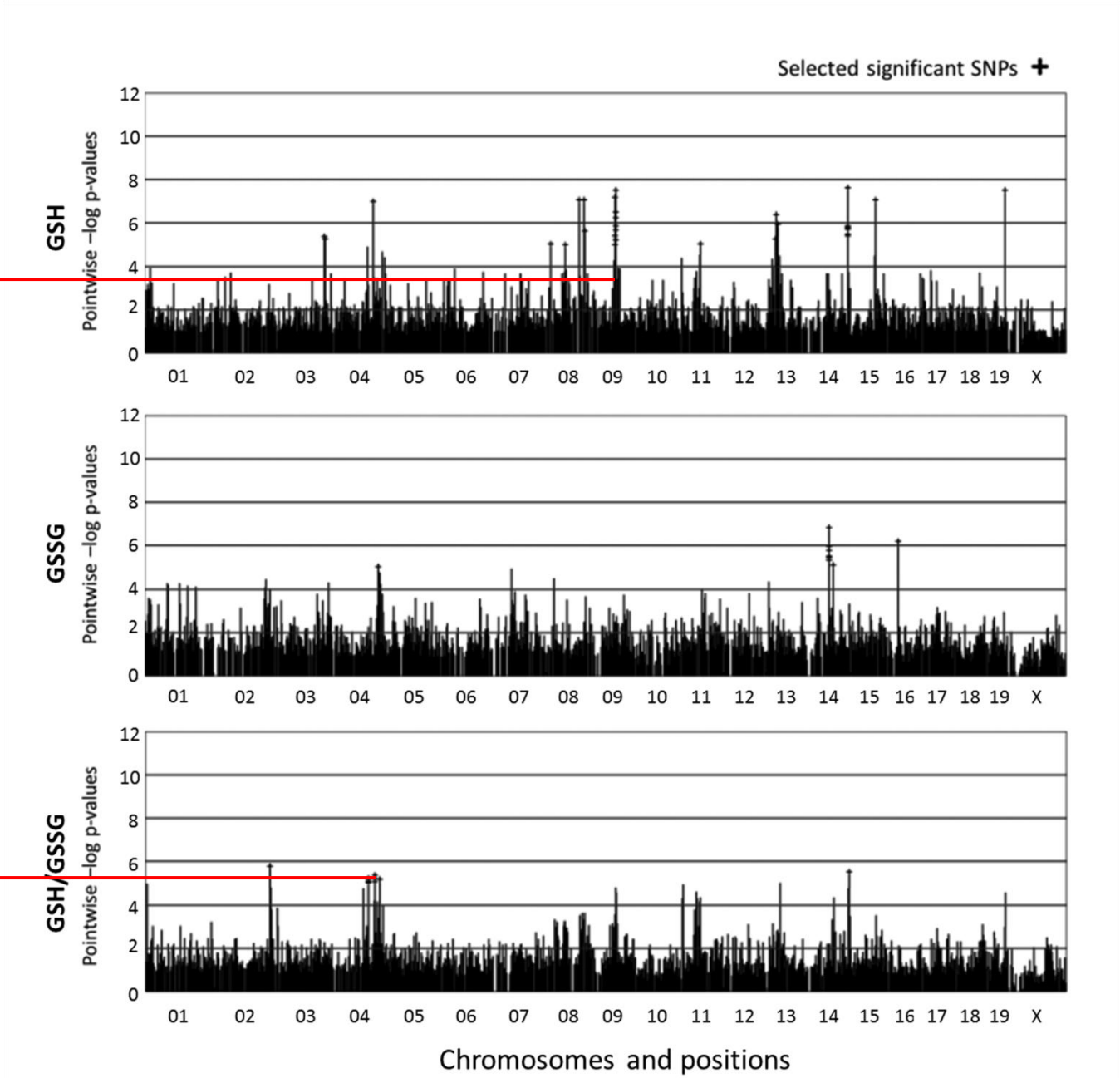


Zhou, et al. Free Radic Biol Med, 2014.

Hepatic GSH Candidate Genes

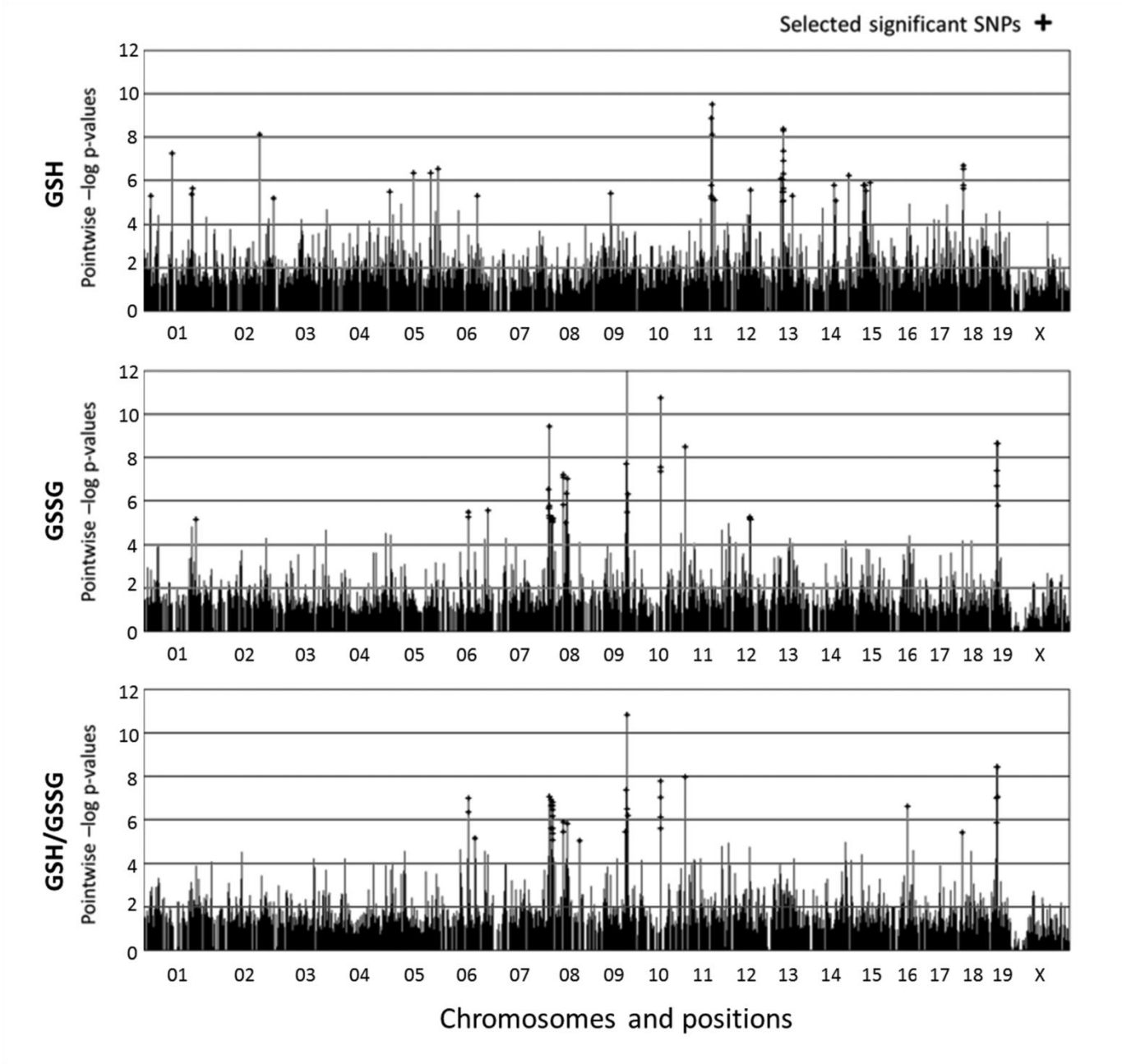
Rora

- RAR-related orphan receptor alpha
- Represses expression of GCL, the rate-limiting enzyme for GSH synthesis (*Urata, et al. Free Radical Biol Med (1999); 27: 838.)*

Pgm2

- Phosphoglucomutase 2
- Converts glucose-1-phosphate to glucose-6-phosphate (G6P)
- G6P then utilized by G6P dehydrogenase (G6PD) to generate NADPH
- NADPH used for glutathione recycling. G6PD is essential for GSH/GSSG

Mapping Renal GSH Phenotypes



Zhou, et al. Free Radic Biol Med, 2014.

Renal GSH Candidate Genes

GSH		GSSG	GSH/GSSG
Pkhd1	Rreb1	Col4a1	Col4a1
Nyap2	Rnf182	Csmd1	Csmd1
Ptprc	Jarid2	Wwc2	Wwc2
Chic2	Adamts16	Tenm3	Tenm3
Lrrc43	Dmtn	Gda	Gda
Smurf1	Fam105a	Trpm3	Trpm3
Cct6b	Trio	Etv6	Ankrd29
Zfp830	Ss18	Wdr17	
Brip1	Taf4b	Gm392	
Car10	Cdh2	Prkch	

- Significant overlap in candidate genes for GSSG levels and GSH/GSSG
- Results decouple GSH levels and GSH/GSSG
- *Hypothesis: GSH levels and GSH/GSSG are regulated by distinct genes and alleles.*

Conclusions

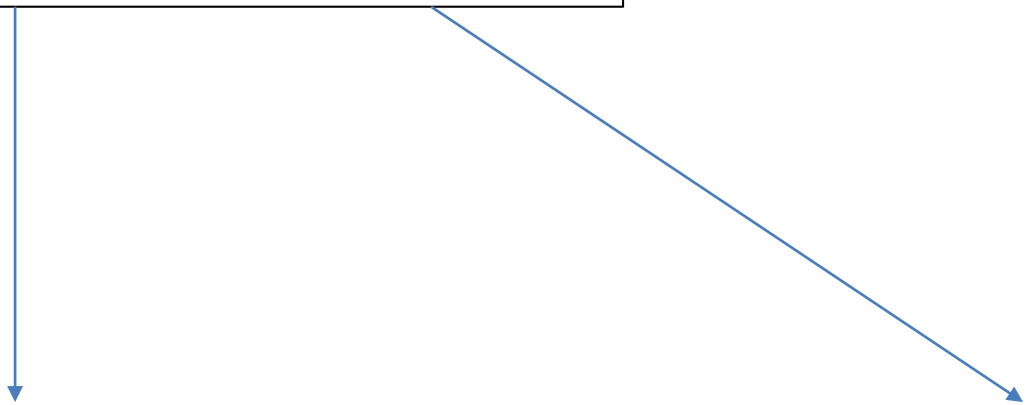
1. GSH levels and GSH/GSSG are heritable traits that can be mapped using forward genetics approaches

2. Heritability estimates were similar to those from human erythrocytes (van ‘t Erve 2013)
    - *GSH levels*: 0.57
    - *GSSG levels*: 0.51
    - *GSH/GSSG*: 0.70

3. Genetic control of GSH appears to be mostly tissue-specific

Moving Forward

GSH levels and GSH/GSSG are heritable traits



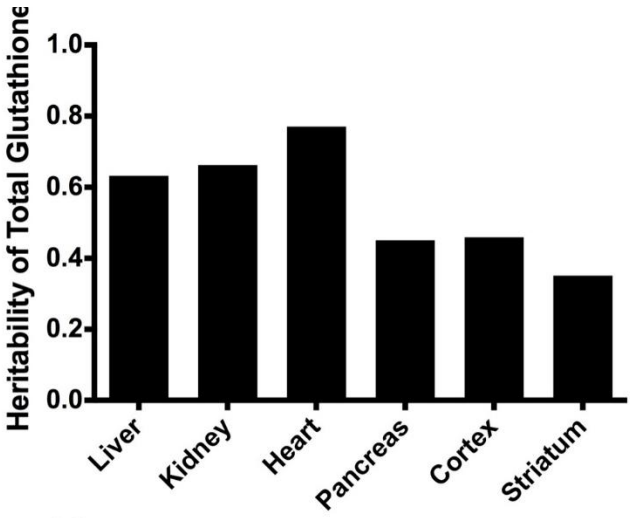
Goal 1: Use systems approaches to define the genetic architecture of the GSH system. Critical because of power limitations with *in silico* approaches.

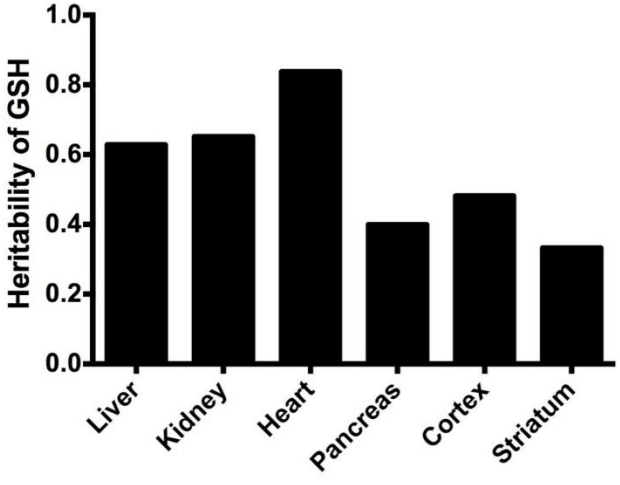
Goal 2: Understand the effects of aging on GSH heritability, and whether age-specific genes regulate the GSH system

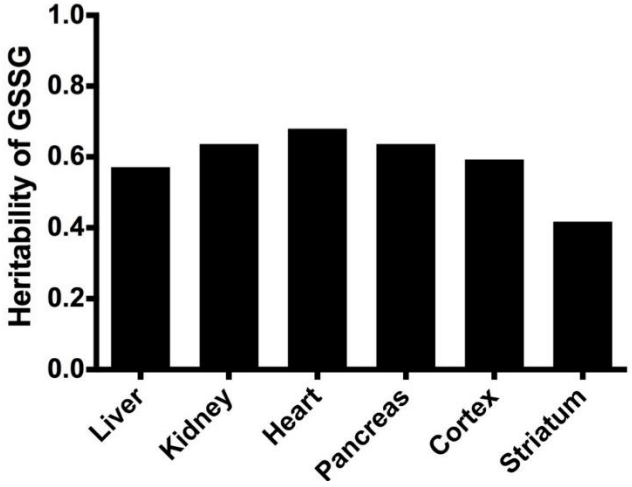
Effects of Aging on GSH Heritability

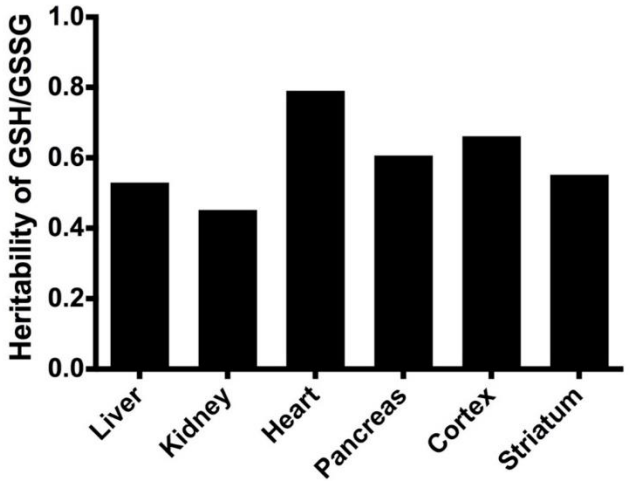
- Advanced age is associated with a decrease in GSH levels and GSH/GSSG
- Does randomness increase with old age? Or, are these phenotypes equally heritable later in life?
- Strain survey of 20 inbred mouse strains
- Same strains as before, without the short-lived strains

Effects of Aging on GSH Heritability









Moving Forward

GSH levels and GSH/GSSG are heritable traits

Goal 3: Determine whether genetic background governs the effects of diet on GSH

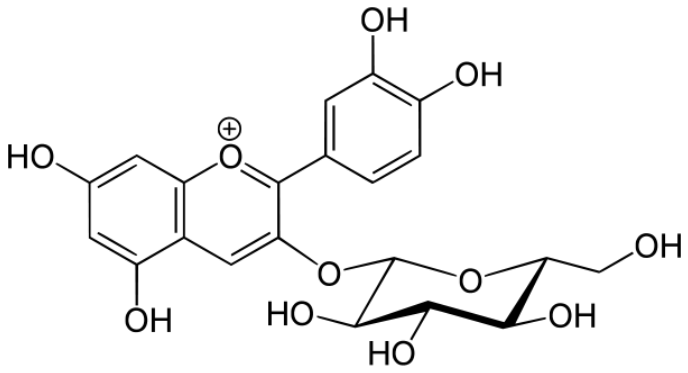
Goal 1: Use systems approaches to dissect the genetic architecture of the GSH system. Critical because of power limitations with *in silico* approaches.

Goal 2: Understand the effects of aging on GSH heritability, and whether age-specific genes regulate the GSH system

Does genetic background determine the effects of diet on GSH?

Background

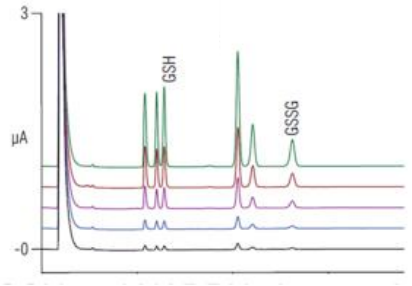
- Foods such as blueberries, blackberries, and purple corn are rich in anthocyanins, a class of flavonoid glycosides
- These compounds are antioxidants, and have been shown to increase tissue levels of GSH
- We predict that genetic background may determine the effects of anthocyanins on GSH
- Cyanidin-3-O-β-glucoside (C3G)
    - 100 mg/kg diet
    - Shown to increase GSH



Design: 6 week intervention

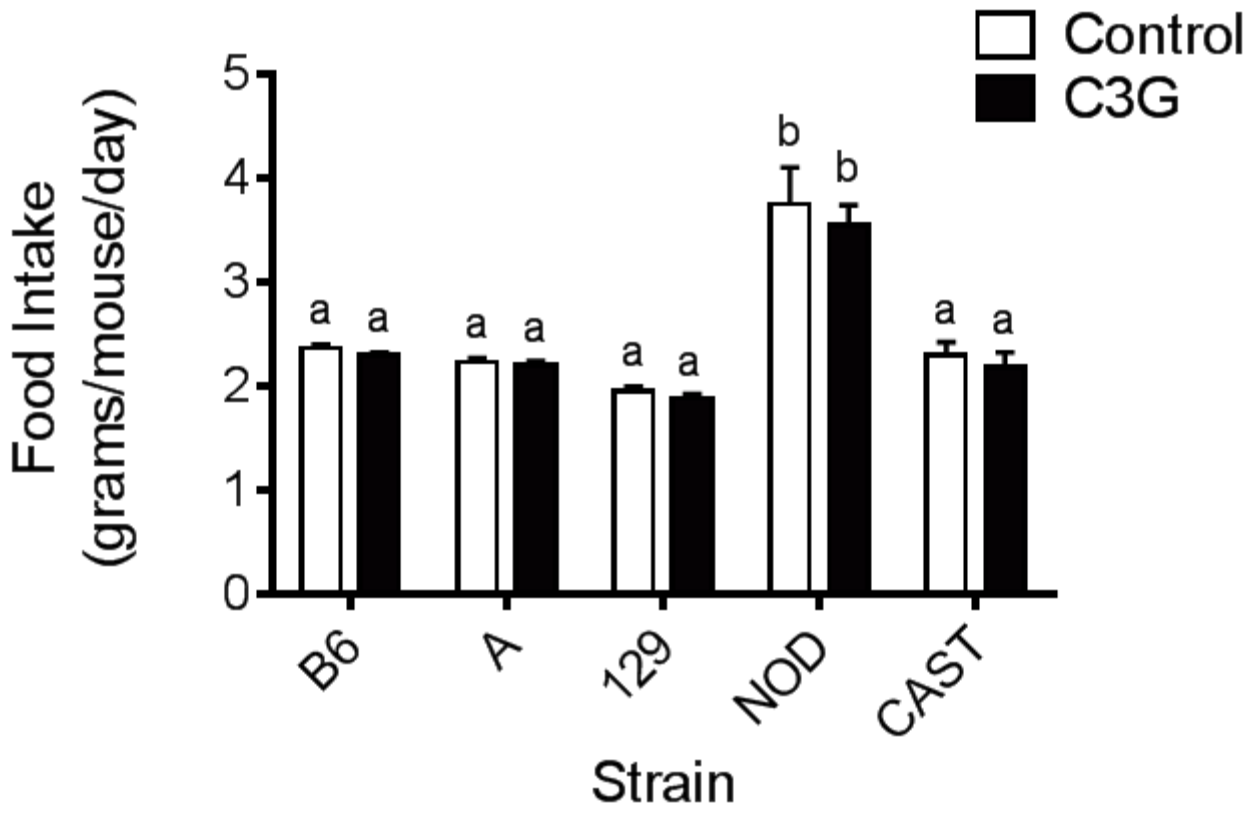
	 B6	 A	 129	 NOD	 CAST
Control Diet	N=5F	N=5F	N=5F	N=5F	N=5F
C3G Diet 100 mg/kg	N=5F	N=5F	N=5F	N=5F	N=5F

Tissue Panel



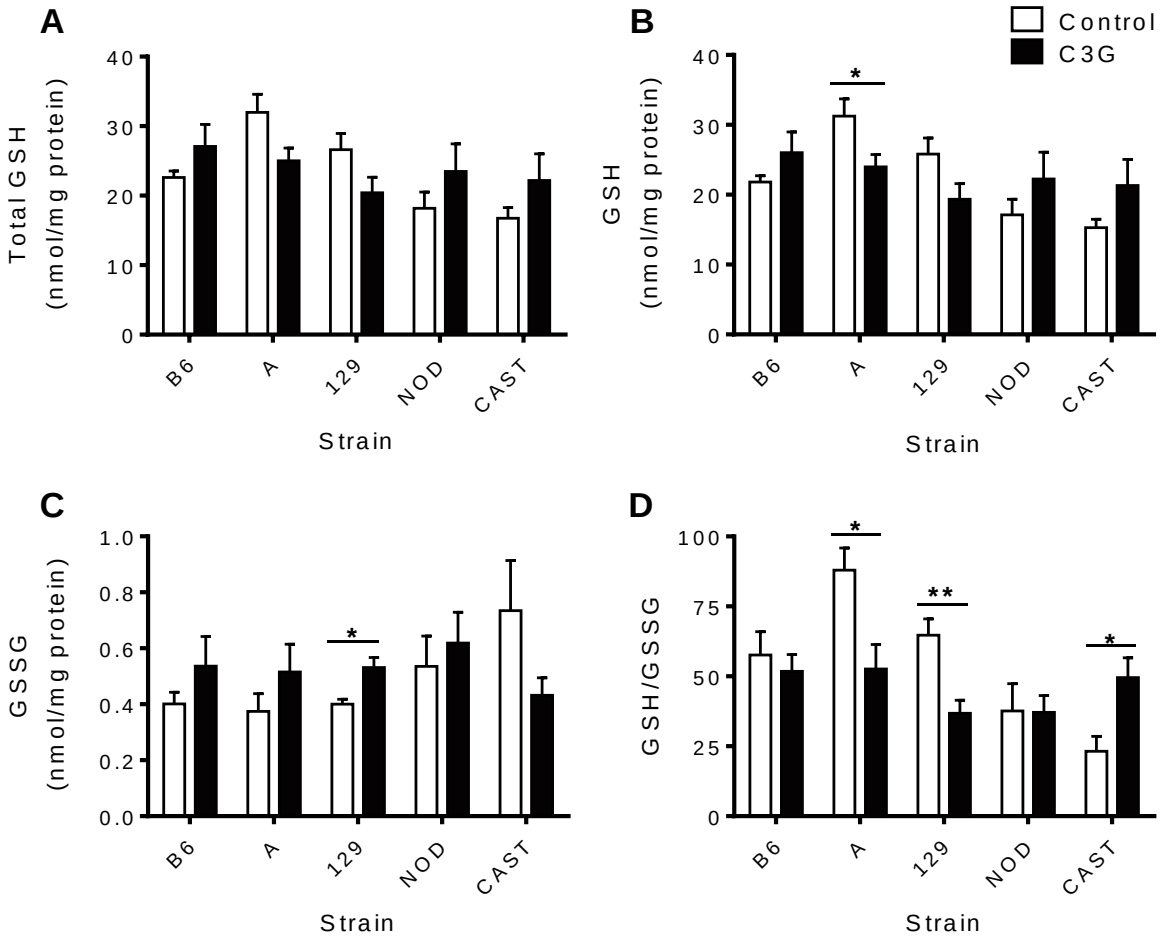
GSH Phenotyping

Food Intake



Norris, et al. Redox Biol, 2016.

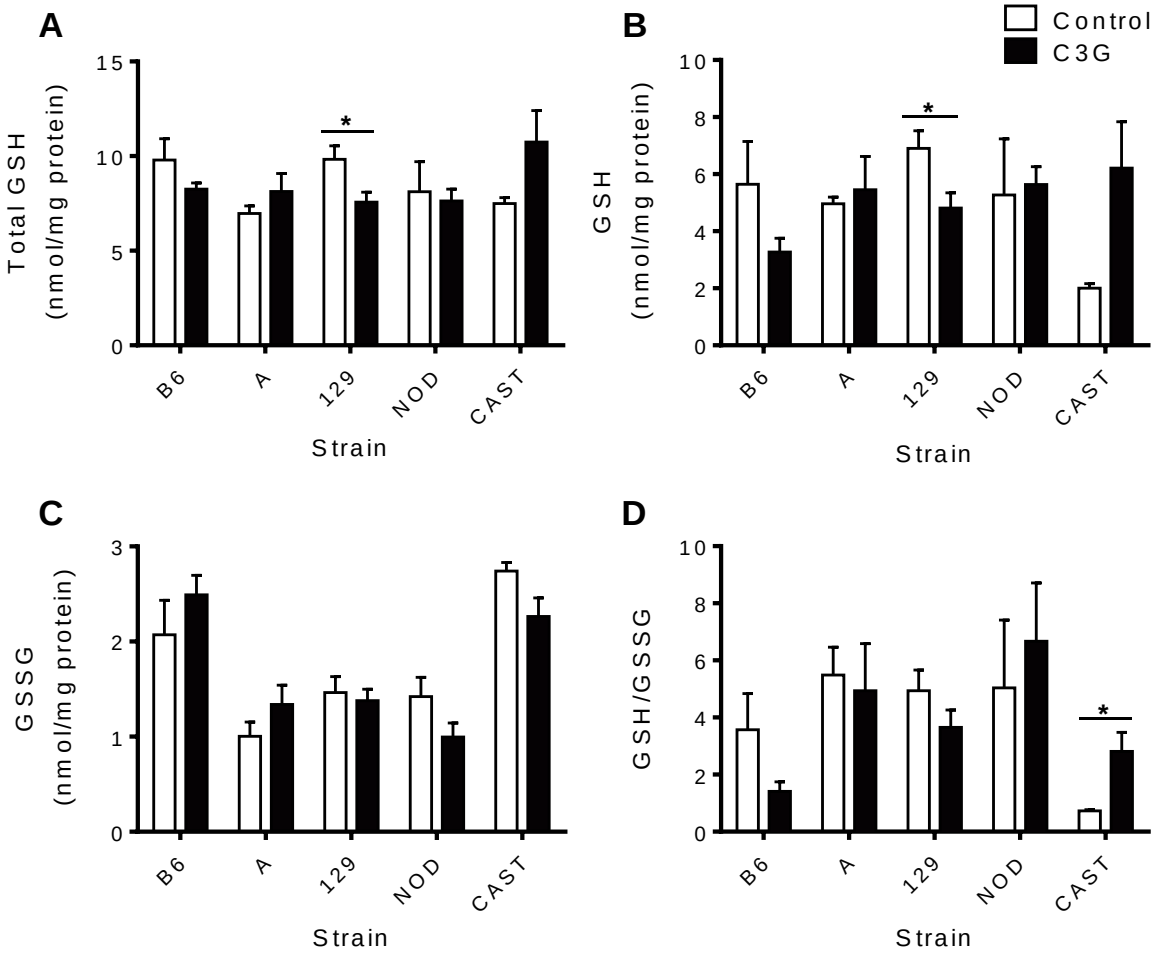
GSH: Liver



*$P < 0.05$; **$P < 0.01$

Norris, et al. Redox Biol, 2016.

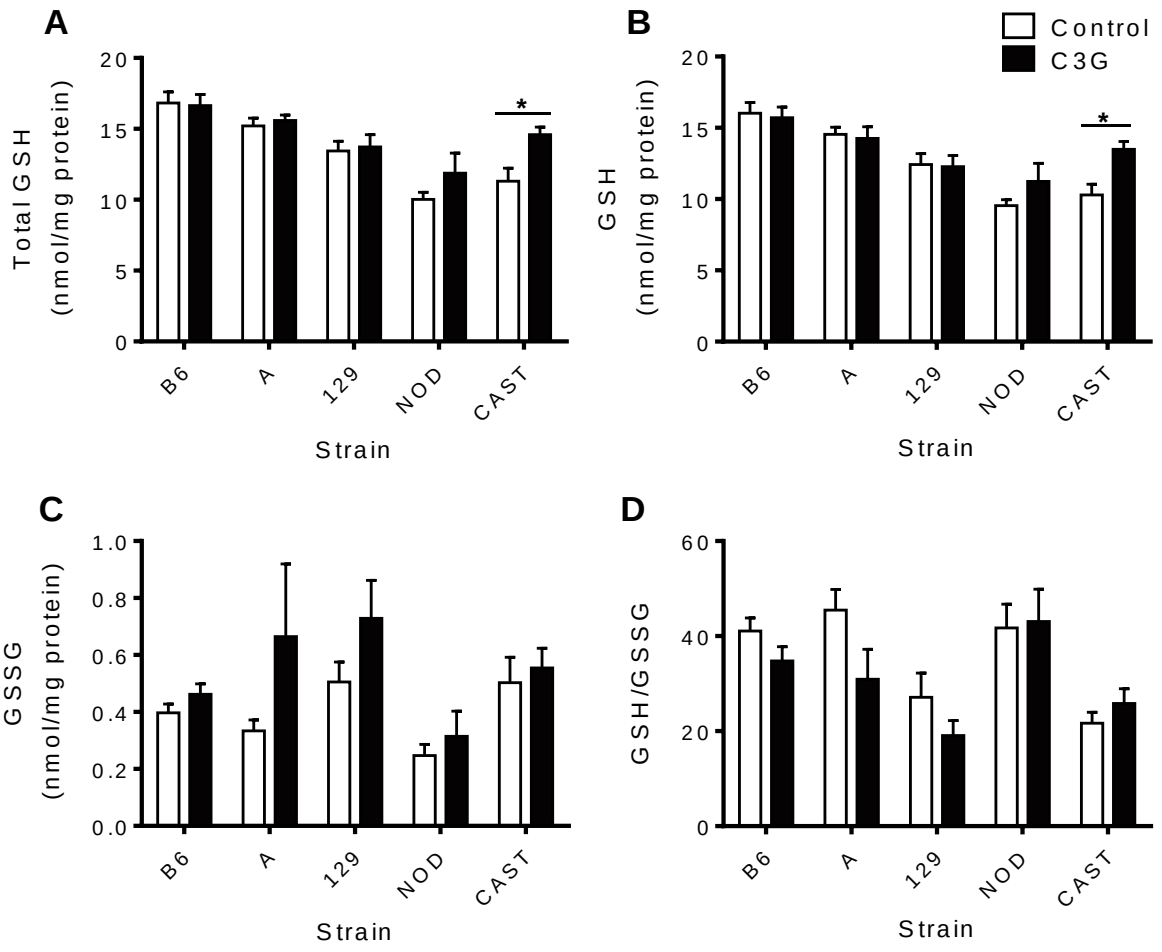
GSH: Heart



*$P < 0.05$

Norris, et al. Redox Biol, 2016.

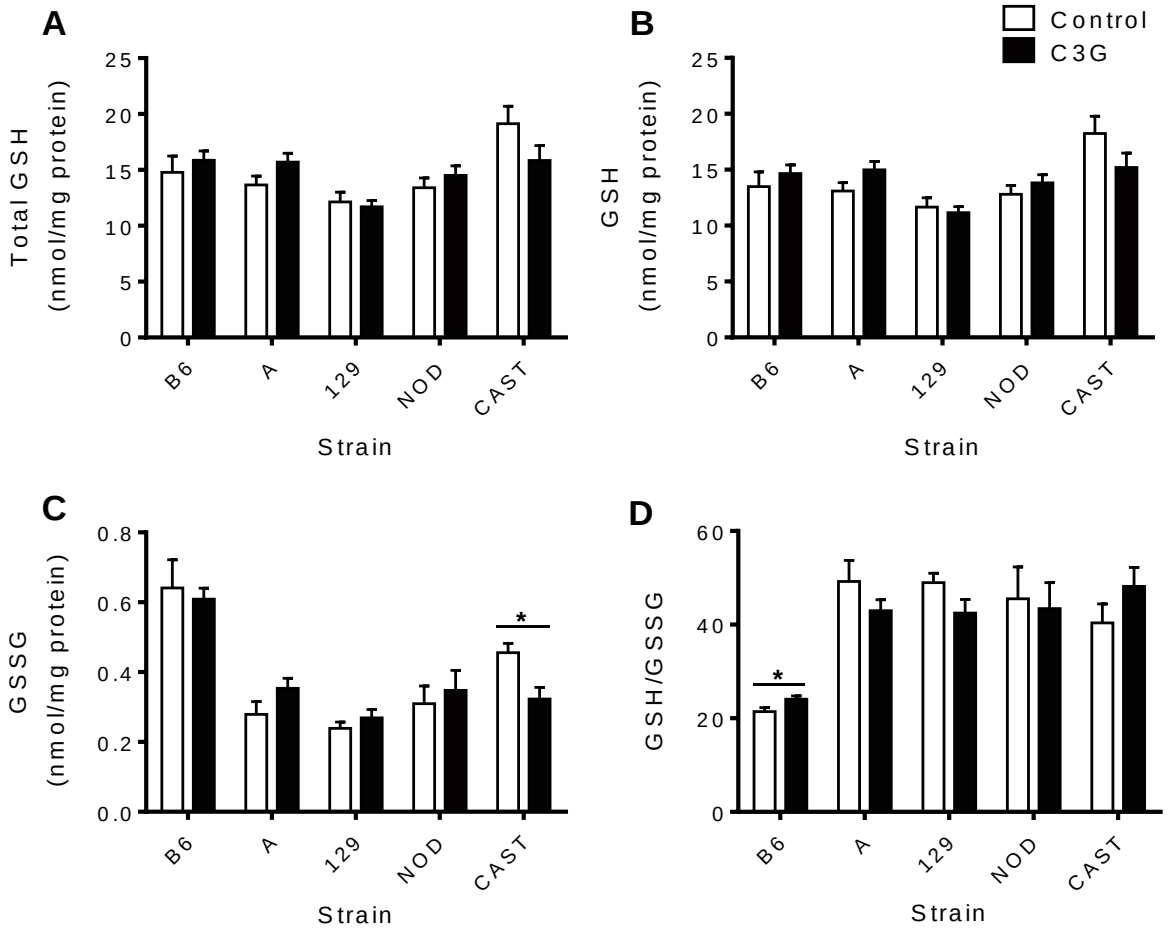
GSH: Kidney



*$P < 0.05$

Norris, et al. Redox Biol, 2016.

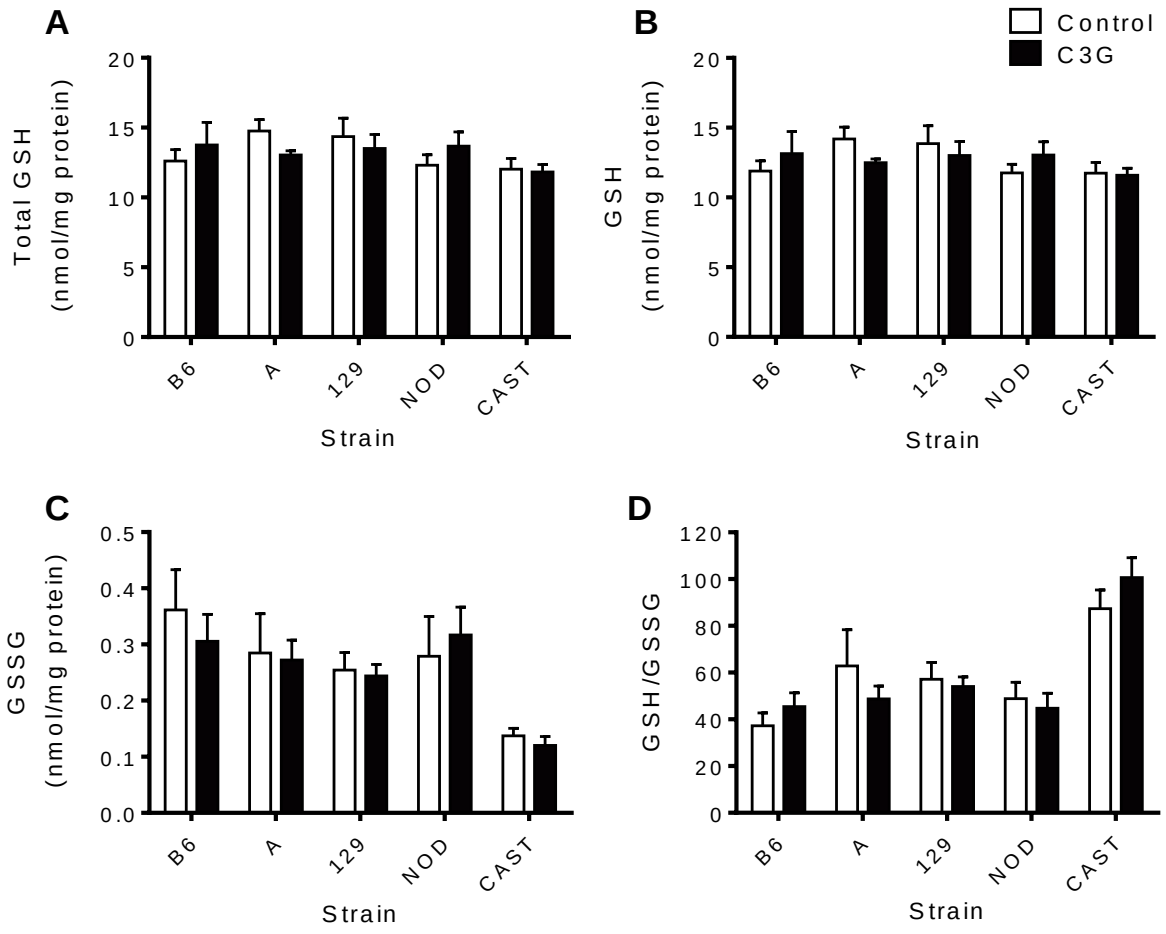
GSH: Pancreas



*$P < 0.05$

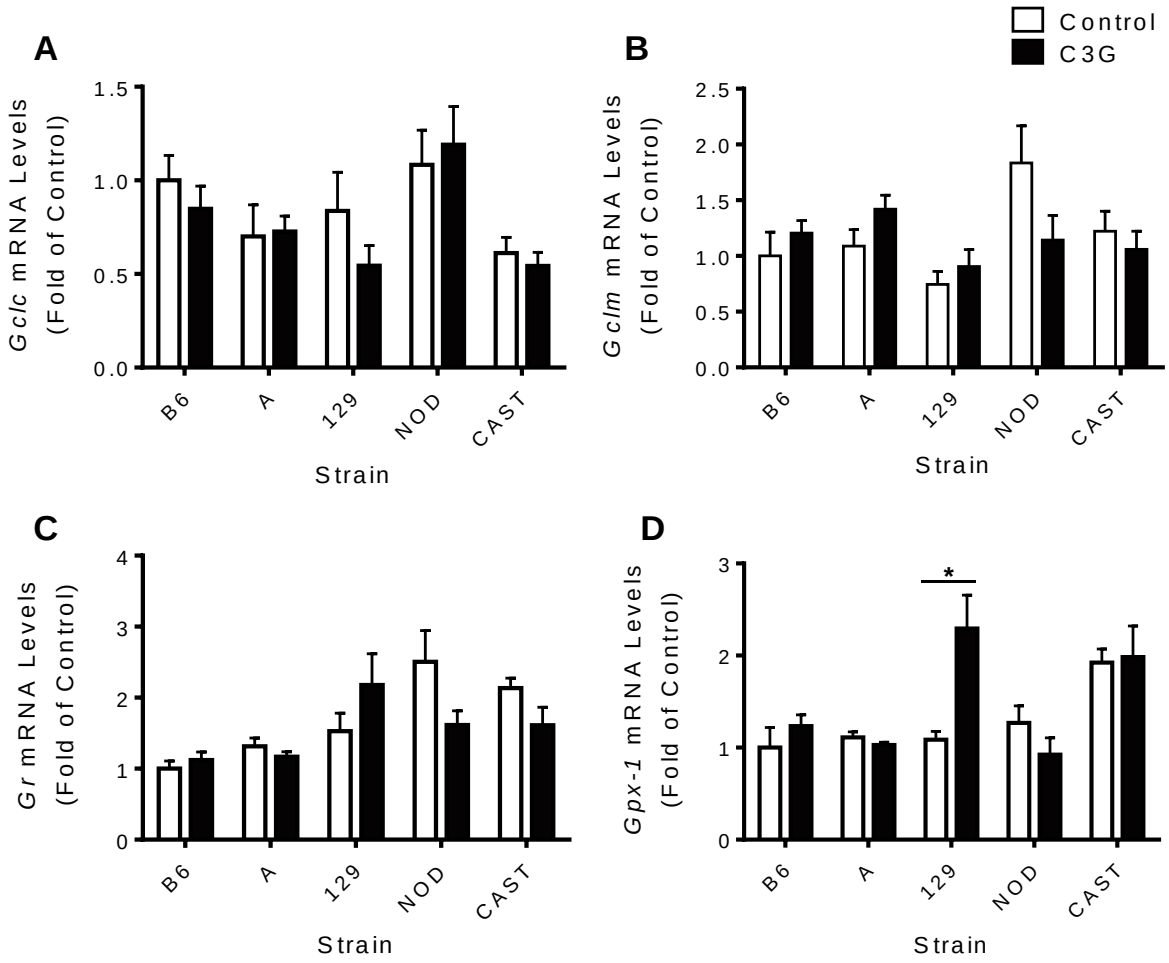
Norris, et al. Redox Biol, 2016.

GSH: Whole Brain



Norris, et al. Redox Biol, 2016.

Expression of GSH Enzymes: Liver



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Norris, et al. Redox Biol, 2016.

Conclusions

- The effects of anthocyanins are most prominent in liver, least prominent in brain

- Results contradicted previous reports that C3G induces threefold increase in GSH. Perhaps due to lack of wild-type controls in previous study.

- Genetic background governs the effects of dietary anthocyanins on the GSH system. A/J and 129S1/SvImJ showed that C3G *depletes* GSH.

- Genetic effects may reconcile findings from various epidemiological and clinical studies. Next step: dissect the genetic basis of C3G effects.

Future Directions

- We can use systems genetics to improve our understanding of an essential biochemical system

- We predict that the approach will:
    - Improve candidate gene studies by directing us to the most impactful and relevant (i.e. tissue-specific) genes
    - Provide critical insight into the roles of age and diet in regulation of the GSH system
    - Highlight potential therapeutic targets to slow the rate of tissue degeneration under various stress conditions

Acknowledgements

Lab Members

- Master's students: Yang Zhou and Katie Norris
- Undergrad: Claire Yakaitis, Erica Coe, Yanfang Xiao, Elizabeth Harris

The Jackson Laboratory

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University of Georgia

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- Statistics: Kim Love and Yi Chen
- Gaylen Edwards

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Questions