The genetic architecture underlying inter-individual variation in the ER stress transcriptional response

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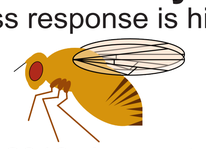
1. Introduction

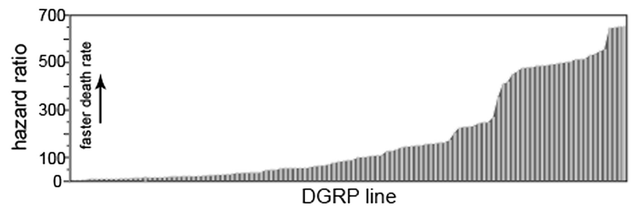
The phenotypic expression of a disease can vary greatly between individuals. This phenotypic variation can manifest as differences in age-of-onset, severity, and other disease associated phenotypes. The genetic background among patients can be a major contributor to differences in disease outcomes observed in the clinic. In order to understand how genetic variation among individuals contributes to disease outcomes, laboratory studies need to incorporate inter-individual genetic variation into study designs. I am applying this strategy to study how natural genetic variation impacts the endoplasmic reticulum (ER) stress response, a basic cellular pathway. The ER is a large eukaryotic organelle involved in protein folding. ER stress is the result of the accumulation of misfolded proteins in the lumen of the ER. The cell responds to ER stress with the unfolded protein response (UPR), which includes a large transcriptional response involving thousands of genes. If left unresolved, this can lead to cell death and disease. ER stress is an important component to many diseases, such as neurodegeneration and diabetes. Changes in the ER stress response can impact disease outcomes. I hypothesize that inter-individual genetic variation in the ER stress response pathway is an important contributor to variable disease phenotypic expression. I will study how genetic variation impacts the transcriptional response to ER stress and how variable ER stress genes function to produce differing responses.

2. Genetic diversity influences the ER stress response

The ER stress response is highly variably across different genetic backgrounds.

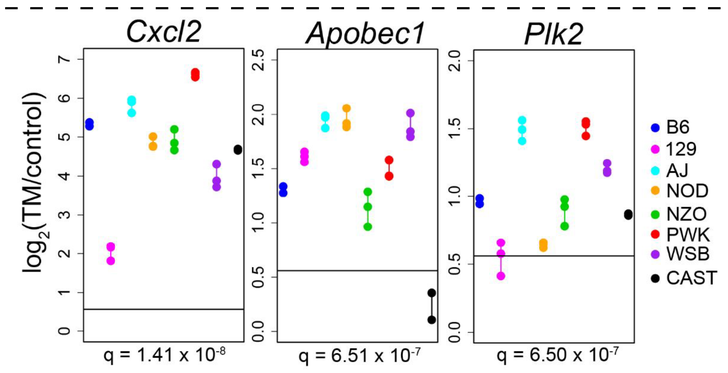


Chow et al., 2013 demonstrated that different genetic backgrounds among *Drosophila* have highly variable survivability under ER stress conditions.



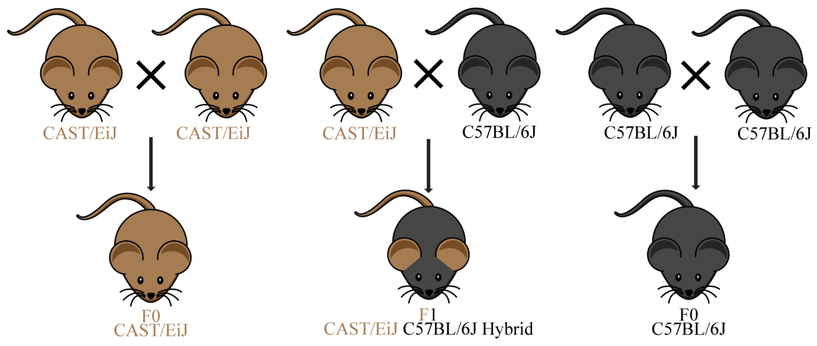
MEFs

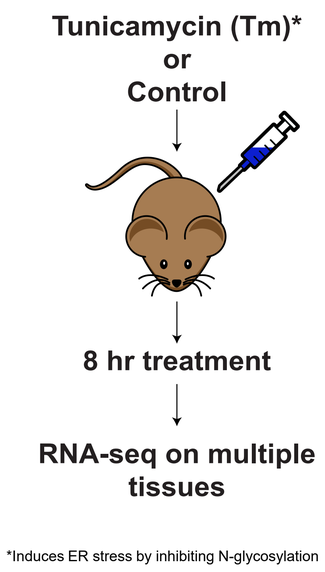
Chow et al., 2015 found that certain genes had highly variable expression, in response to ER stress, across different genetic backgrounds.



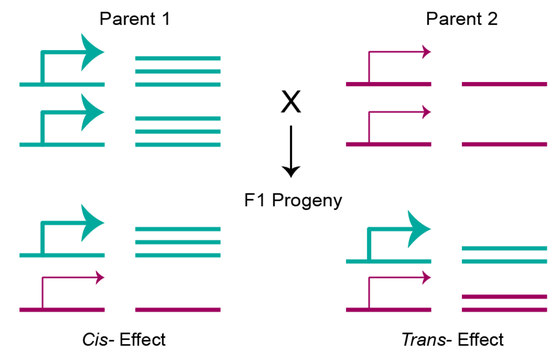
3. Experimental design

Previous studies investigating the role of genetic background on ER stress variability was done in cultured fibroblasts providing no tissue-specific data. I am performing an *in vivo* study to better understand how the *cis*- and *trans*- regulatory landscape drives variation in the ER stress response in a physiological tissue-specific manner. I will use two mouse strains, C57BL/6J (B6) and CAST/EiJ (CAST), which are very genetically divergent and cross these two strains to produce F1 hybrid mice. In each mouse, I will induce ER stress and then perform RNA-seq on multiple tissues.





*Induces ER stress by inhibiting N-glycosylation

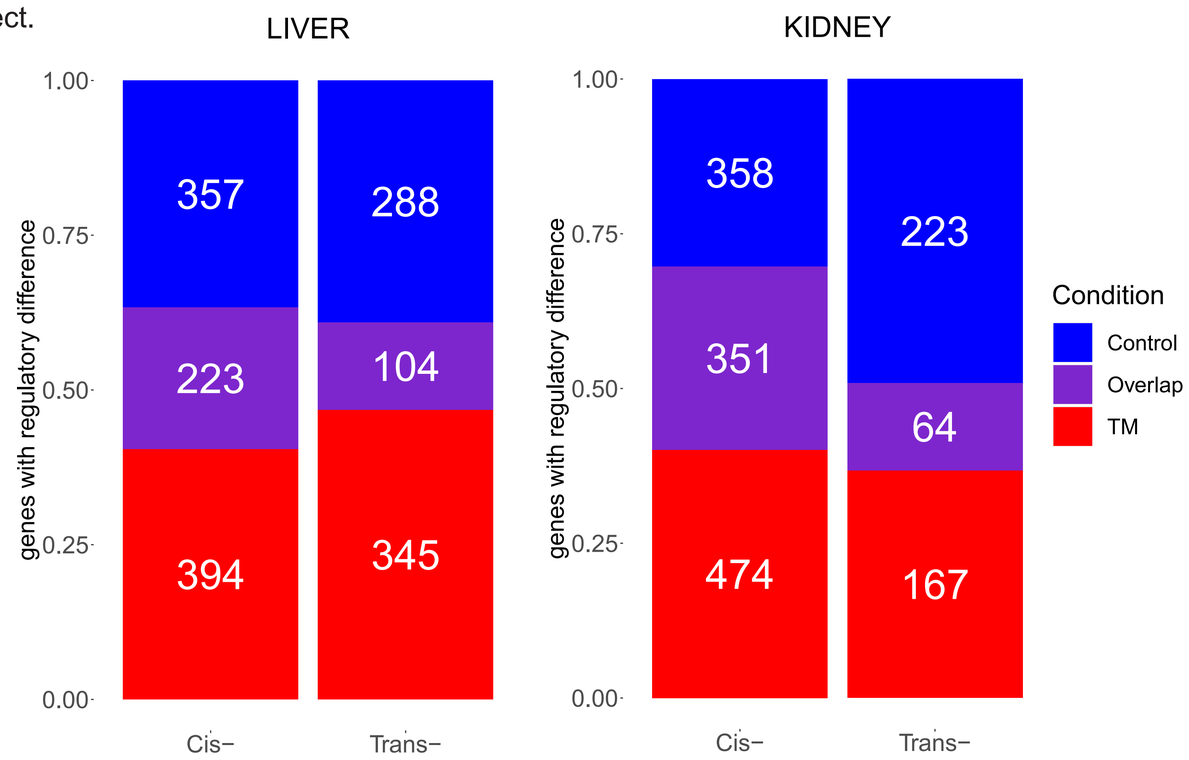


Differentiating Between *Cis*- and *Trans*- Regulatory Effects

Diagram depicting how alleles will be tracked through RNA-seq analysis. This will allow us to compare relative allelic expression in the parental CAST, parental B6, as well as the F1s. By doing this we can determine if a gene is being regulated through *cis*- or *trans*- mechanisms.

4. Variation in ER stress-responsive genes across strains

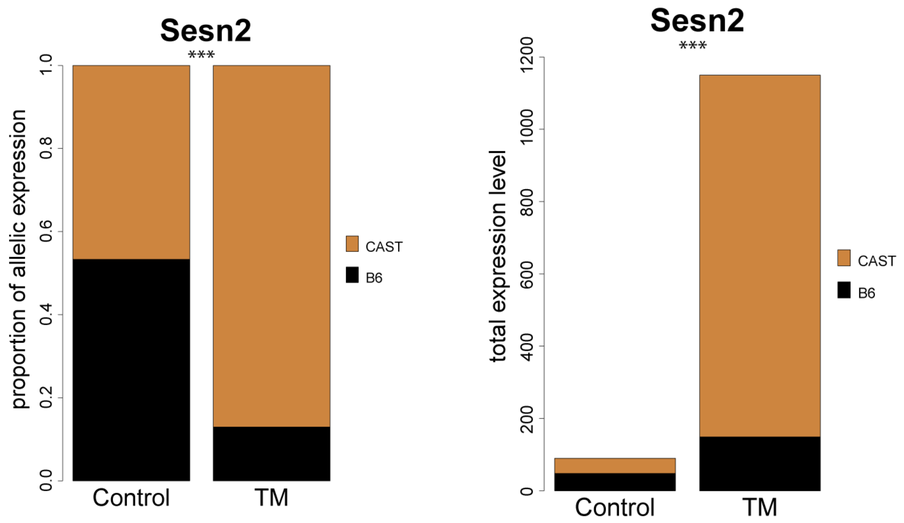
For each strain, RNA-seq data was processed through Deseq2 to determine $\log_2$(Fold Change). Significance was selected by setting a $q < 0.05$ and a fold change (FC) > 1.5 cutoff. Allele specific expression (ASE) was performed on B6, CAST, and F1 ER stress-responsive genes. ASE was used to determine whether a gene displayed a *cis*- or *trans*- regulatory effect.



Presence of ER stress reveals cryptic regulatory variation. ER stress responsive genes in the F1 hybrid mouse that were determined to display a cis- or trans- effect. These regulatory effects were seen in either stress conditions, control conditions, or both. In both liver and kidney, the majority of genes that showed a regulatory difference between strains depends on the presence or absence of ER stress.

Tissue	B6 v CAST	B6 v CAST v F1	Enrichment
Liver	126/2,240 (5.7%)	80/2,240 (3.6%)	Immunity, Inflammation
Kidney	321/2,744 (12%)	270/2,744 (9.8%)	Metabolism, AA transport

Significantly variable ER stress-responsive genes across strains. We performed an ANOVA test to test for genes that are ER stress responsive and are highly dependent on genetic background. We performed this on genes in liver and kidney, both B6 vs. CAST and B6 vs. CAST vs. F1. We found that genes that are highly variable are involved in a variety of processes, such as inflammation and metabolism. This implicates that perhaps the variability in the ER stress response is from peripheral processes, not canonical ER stress genes.



ER stress influences RNA transcripts levels & ASE. Sesn2, in the F1 hybrid mouse liver, displays ER stress responsiveness in both total RNA transcript levels and ASE. This demonstrates how ER stress and *cis*- regulatory variants can impact expression in an environmental and allele specific manner. Similar pattern seen in kidney.

5. Conclusions -

This work will address what is driving the variability of the ER stress response seen in the population. We will approach this question at three different levels. First, the genome wide level. Our *cis*- and *trans*- studies will provide us with a better understanding the the large transcriptional response to the ER stress response genome wide. Second, the gene level. Our functional studies in *Drosophila* will provide us with an idea as to how our candidate modifier gene is functioning in the variable ER stress response. Third, the SNP level. Future studies using a large outbred mouse population (Diversity Outbred Mice) and high resolution eQTL analysis will be critical for uncovering causative SNPs of the variability seen in the ER stress response. These different approaches and variety of tools will provide us with a more holistic approach to understanding how these modifier genes could potentially be used as a therapeutic target in the treatment of ER stress driven diseases.

1. C. Y. Chow, X. Wang, D. Riccardi, M.F. Wolfner, A.G. Clark, PLoS genetics 11, 2015
2. C. Y. Chow, M. F. Wolfner, A. G. Clark, Proc Natl Acad Sci U S A 110, 9013 (2013)

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