# Modeling of systems genetics reveals genetic buffering mechanisms involved in HDL homeostasis


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## Abstract

**Most common human diseases are genetically complex and may involve gene-gene and gene-environment interactions. Mouse models provide a powerful tool to identify genes involved in complex interactions. Stressful environmental factors such as a high-fat, high-cholesterol diet can induce specific regulatory responses that vary in different strain backgrounds. Modeling the effects of the epistatic interactions on genomewide mRNA expression profiles and a clinic trait (HDL) in a mouse intercross population (C57BL6/J x DBA/2J), we are able to identify candidate regulatory and target genes involved in the genetic buffering mechanisms that play an important role in the homeostasis of HDL. The genetic system is characterized by multiple epistatic interactions involving gene expression traits, and balanced allelic effects in different physiological pathways. Epistatic interactions affect HDL phenotype through specific transcripts that help to bridge the gap between genotypes and phenotypes. Understanding of the genes involved in this buffering system may provide new therapeutic targets for drug discovery.**

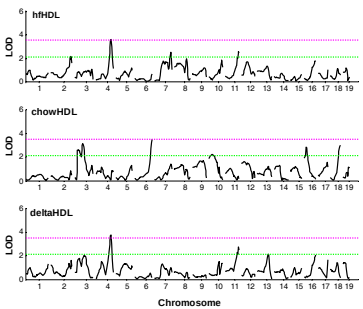

## Introduction

**Epistasis** is the masking or unmasking of the effects of allelic substitution at one locus by the allelic states at a second locus (Bateson, 1909)

**The BXD intercross**

- 111 female mice fed a chow diet until 12 months of age, then switched to an atherogenic (HF) diet for another 4 months (Schadt, et al. 2003);
- Lipids measured at 12 and 16 months of age, respectively (Colinayo et al. 2003);
- Profiling of 23536 transcripts on liver tissue at 16 months of age;
- Difference in HDL in B6 females fed on chow versus HF diets (54±2 vs 55±8 mg/dL) is quite small;
- Difference in HDL among the BXD intercross population on the two diets (60±1 vs 53±2 mg/dL) is also small;
- Public data (http://www.diabetesgenome.org/thirdpartydata/);
- Our analysis focuses on HDL levels measured at 16 months.

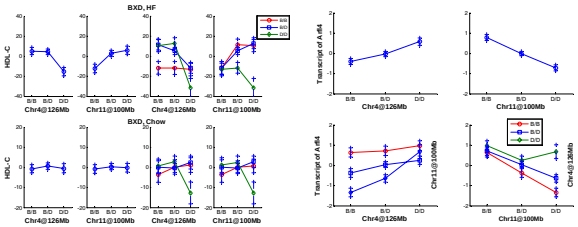
## Figure 1. Genome scans of HDL QTL




**Genome scans for HDL measured at 12 months (chowHDL), at 16 months (hfHDL), and the change in HDL are shown (left). Significant (p<0.05) and suggestive (p<0.63) genomewide thresholds are indicated by dashed lines. QTLs for hfHDL are noted on Chr 4, 11, 7 and 2.**

## Figure 2. Genetic buffering

**We detected a significant epistatic interaction that impacts hfHDL but has no significant effect on chowHDL (left panel). The same interaction is also found for transcript abundance of Arfl4 (right panel), but in opposite directions. HDL measurements are centered at the population means (60 and 53 mg/dL for chowHDL and hfHDL, respectively). In both interactions, homozygosity of B6 alleles at Q11 has a buffering effect on Q4.**



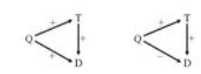

## Methods

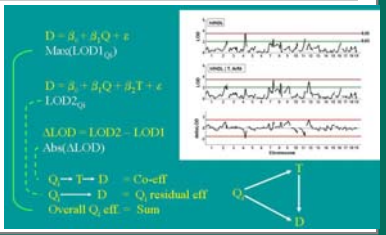
**Structural equation modeling (SEM)** is a multivariate technique used to infer causal relationships from correlational structure of multiple variables measured on a population. We use SEM to systematically screen for local eQTLs that colocalize with QTL (Q) for a clinic trait (D). Changes in LOD scores in conditional genomewide QTL scans, when conditioning on a covariate (T), indicate potential causal interactions.

**Screening algorithm:**

1. **Scan genome for D-QTLs to identify target regions**
2. **Select eQTLs with LOD scores above 3.5 that colocalize within the target regions**
3. **Run conditional scans LOD(D|T) and select transcripts with a ΔLOD above 1.5**

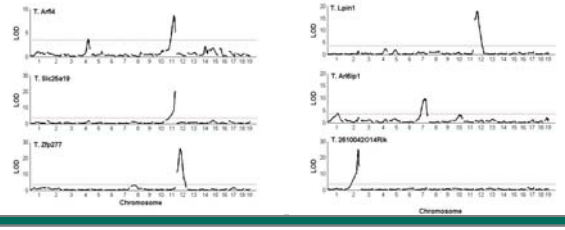
**Feedforward loop:**



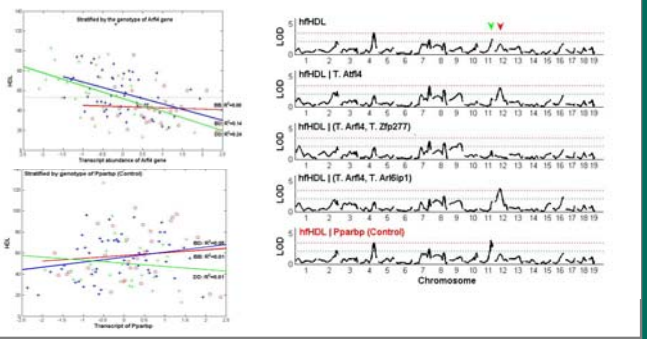

## Figure 3. Scans for eQTLs

**We detected several eQTLs that pass the filters.**



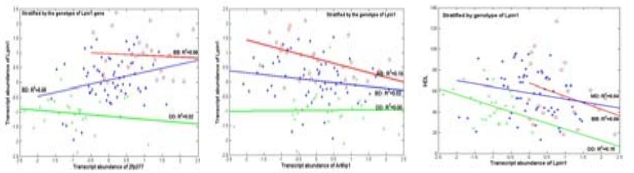

## Figure 4. Interactions between transcripts

**We identified that homozygosity BB of Arfl4 gene has a buffering effect on hfHDL, while a closely linked gene Pparbp, also has a strong local QTL on Chr 11, but has no effect on hfHDL. Conditional scans indicate complex interactions between thanscripts of Arfl4, Zfp277, and Arl6ip1 genes in their effects on HDL.**



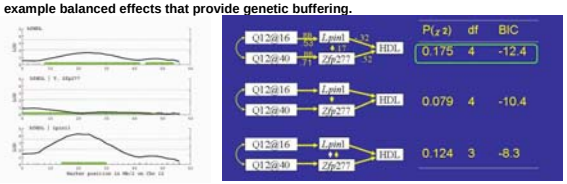

## Figure 5. Allele-specific coupling of transcripts

**Allele-specific coupling of transcripts includes several scenarios. One of them is the transcription factor-target gene interaction where transcript abundance of the two partners may not be correlated due to post-transcriptional and post-translational modifications of the regulatory gene. But allele-specific effects are clear. In the second scenario of transcript-transcript relationships, transcripts are preferentially correlated for at least one specific genotype. These allele-specific effects on gene targets and/or downstream genes have significant influences on HDL.**



## Figure 6. Example: Building a two-transcript model

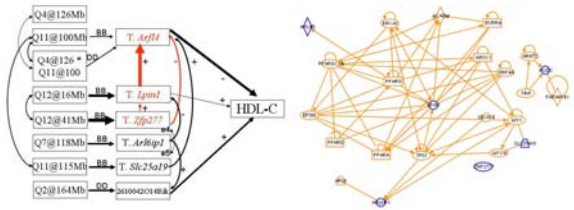
**In order to illustrate the SEM, we consider two candidate genes on Chr 12: Zfp277 a transcript with a downward change pattern and Lpin1 a transcript with an upward change pattern. The best fitting SEM indicates that Zfp277 transcript up-regulates Lpin1. Lpin1 down-regulates HDL through an unknown pathway. The residual effect from Zfp277 to HDL is large but in an opposite direction. This is an example balanced effects that provide genetic buffering.**




## Figure 7. System-level modeling

**We fit a model (left) with genetic variation at QTLs, transcription abundances of corresponding candidate genes identified, and hfHDL, using SEM (Li et al. 2006). This model points to a third mechanism in the homeostasis of HDL; that is, balance of multiple pathways with opposite directions. There is a residue for each transcript and HDL in the model, and e4 and e5 covary. This model provides a plausible mechanism for HDL regulation that can be tested experimentally.**

**Interestingly, four out of the selected candidate genes are documented in the gene network from Ingenuity Database (right). The four genes are involved in lipid metabolism that is connected to cell cycle and apoptosis pathways.**




## Conclusions

- **High-fat, high-cholesterol diet can induce specific regulatory response that impacts multiple gene targets involved in an interacting system;**
- **Specific gene targets have genetic buffering effects for the regulatory response and for robustness of the system;**
- **Balanced effect of multiple physiological pathways is the direct cause of HDL homeostasis;**
- **Epistatic interactions affecting HDL are through specific transcripts.**

## References


Schadt EE, AJ Lusis, et al. (2003). Genetics of gene expression surveyed in maize, mouse and man. Nature 422(6929): 297-302.
Colinayo VV, AJ Lusis, et al. (2003). "Genetic loci for diet-induced atherosclerotic lesions and plasma lipids in mice." Mamm Genome 14(7): 464-71.
Li R, GA Churchill, et al. (2006). Structural model analysis of multiple quantitative traits.PLoS Genet 2(7): e114.



**We thank Drs. E Schadt, A.J. Lusis, and T. A. Drake for generating the publicly available BXD data.**