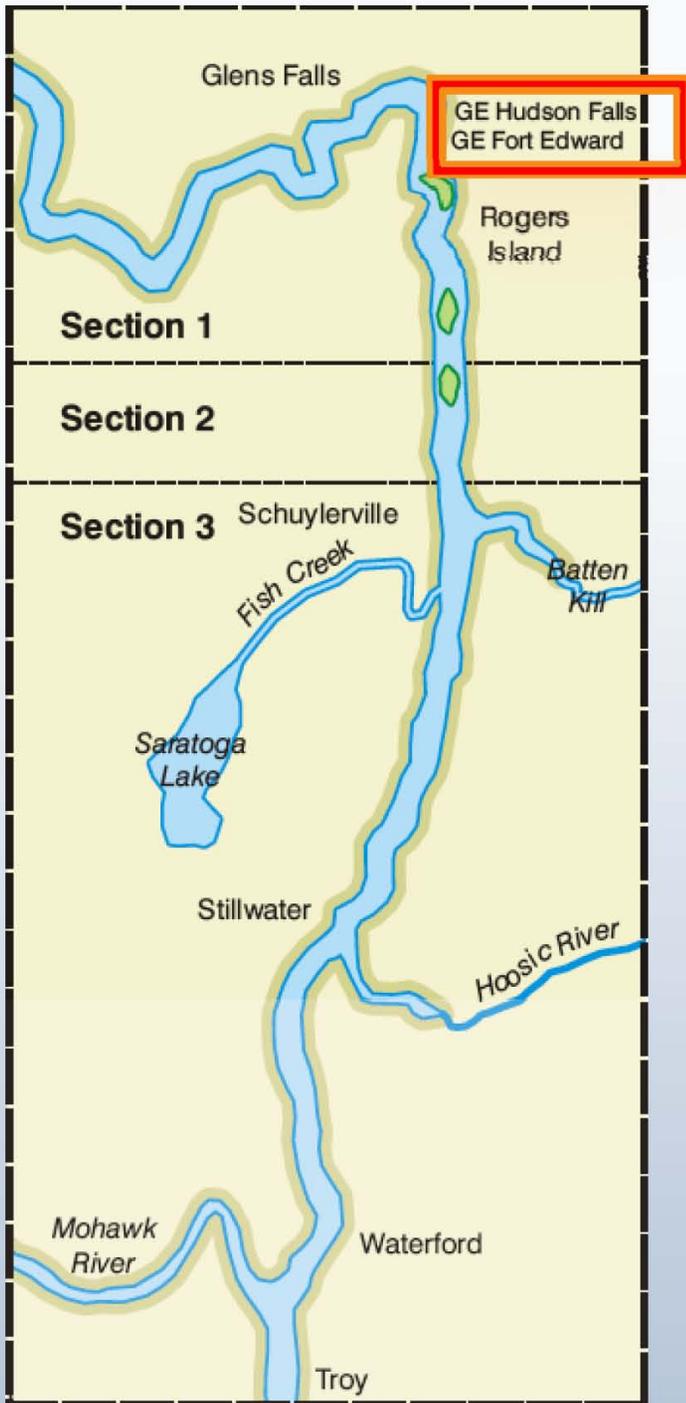


Differential Expression of Liver Regulatory Genes with Embryonic PCB Exposure

32nd Annual SETAC North America Meeting
Molecular Toxicology, Toxicogenomics, and Biomarkers
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Meredith Bohannon
University of Maryland

The opinions expressed in this presentation are those of the authors; the research does not represent the official position of the funding agency.



Background

- General Electric plants on Hudson River (HR)
- PCBs deposited into HR from 1940s until 1977 (PCBs banned) – 209K – 1.3M lbs dumped (EPA estimate)
- Superfund site



Background

- PCBs entered river as mixtures (Aroclors)
- Our study – mixture based on congener profile found in spotted sandpiper
- Interested in sublethal endpoints – gene expression
 - Looking for affected pathways
 - Looking for novel biomarkers
 - Whole-genome approach – Microarray
- Model animal – Japanese quail
- Liver as organ of interest

Japanese Quail

- Not as sensitive as chickens to toxic effects
 - LD50s (Head et al., 2008)
 - AhR Sequence (Head et al., 2008)
- Good model of endocrine system
- Conserved homology with chickens

Study Design and Methods

- Egg Collection, Injection, and Incubation
 - Incubated for 3 days prior to injection
- Tissue Collection
 - Livers collected at hatch
- RNA Extraction

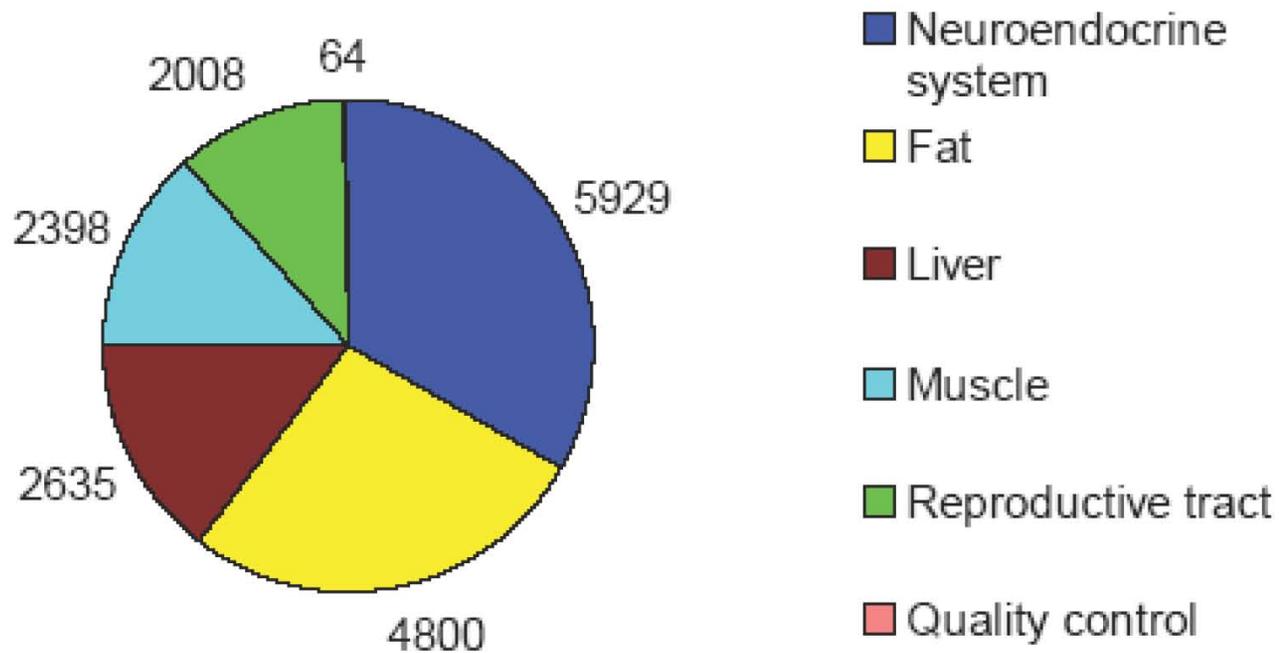
PCB Concentration (ng PCB/g)	TEQs (ng/g)
0	0
6000	0.70

Study Design and Methods Cont'd

- Microarray Validation
 - Five species: Quail, American Kestrel, Tree Swallow, Domestic Turkey, and Domestic Duck
- Microarray Experiment
 - Quail females
 - N= 7 (Vehicle), 5 (Low), 2 (Medium), and 2 (High)
 - Statistics
 - Genes BLASTed against chicken genome
 - Cluster and Pathway Analyses
- qPCR
 - Males (n= 6 [untreated], 4, 2, 3, and 7)
 - Genes of interest from microarray experiment

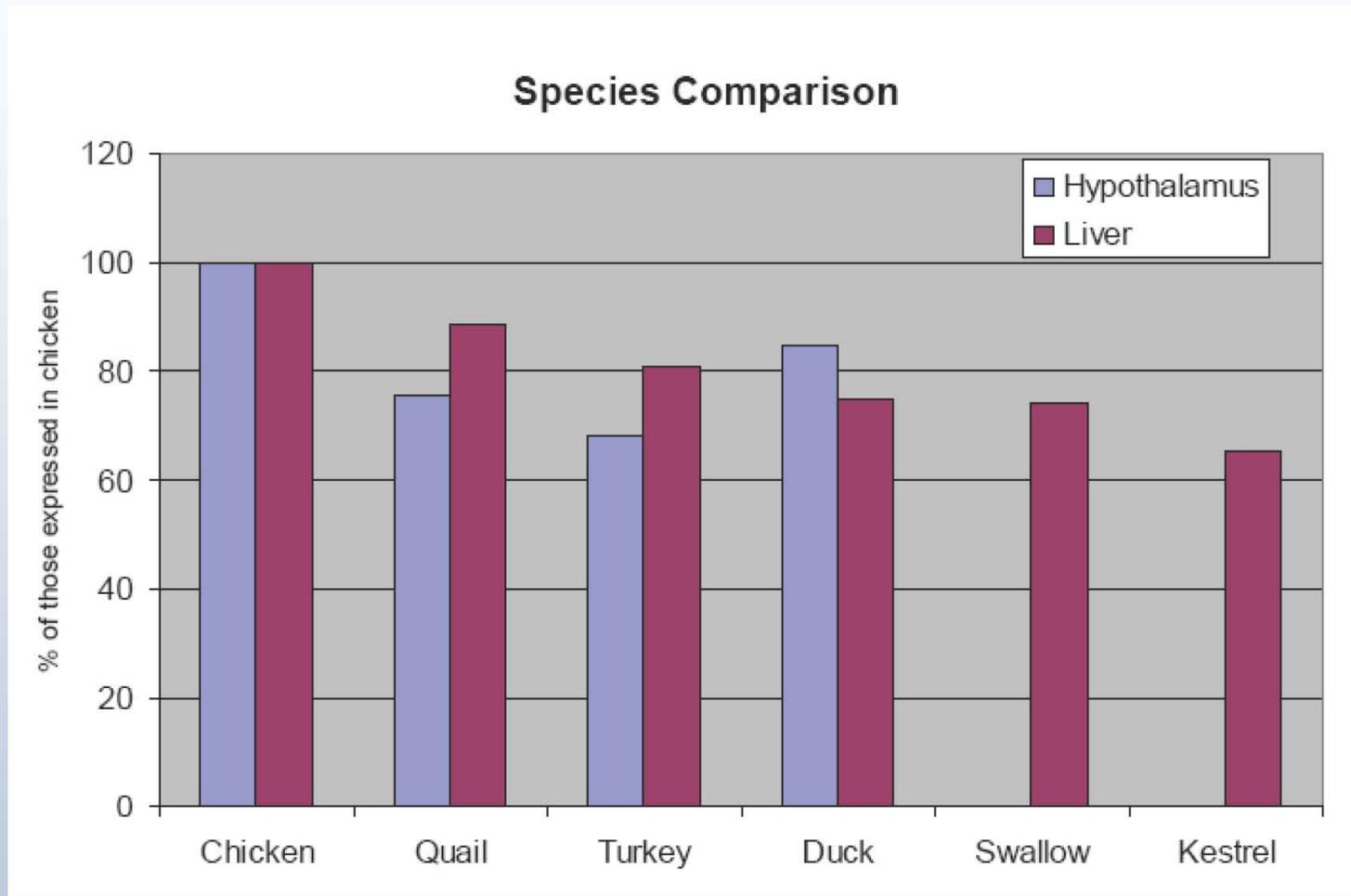
Results - Microarray Validation

Del-Mar 14K Chicken Integrated System Microarray



Data Generated by Tom E. Porter Lab,
University of Maryland

Results - Microarray Validation

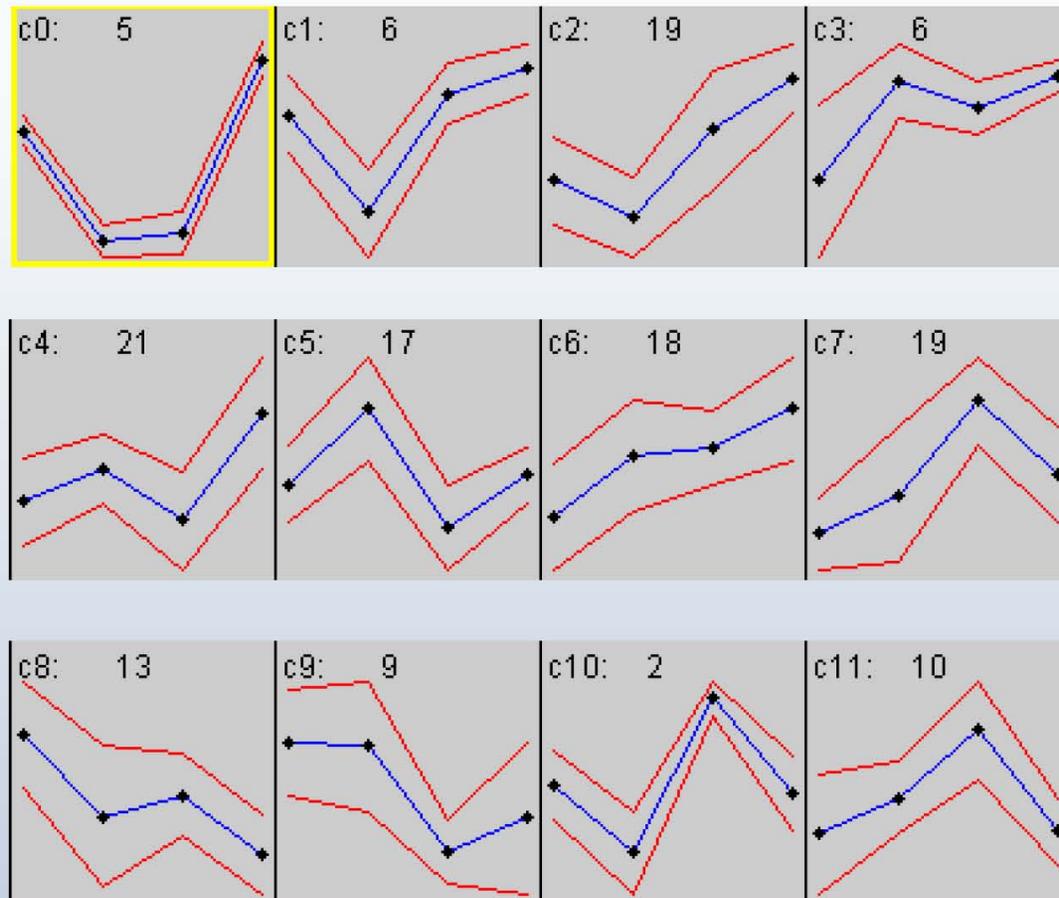


Data Generated by Tom E. Porter Lab,
University of Maryland

Results – Microarray Analysis

- SAS analysis of microarray data
 - One-way ANOVA for each spot
 - 285 spots on array were statistically significant
 - 159 spots had ≥ 8 samples represented – submitted to cluster analysis
- Cluster analysis (GeneCluster)
 - Finding similar expression profiles by allowing genes to cluster into self-organizing maps (SOMs)

Results – Cluster Analysis

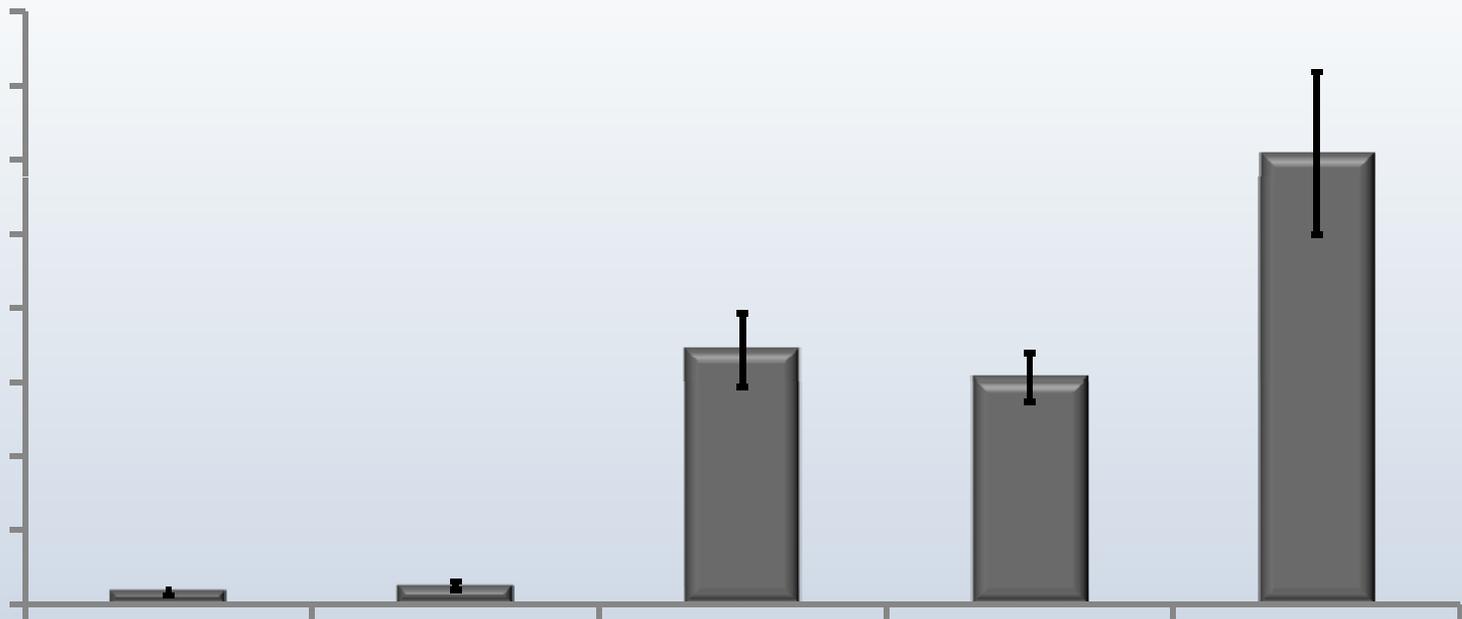


Results – Pathway Analysis

- Biorag (www.biorag.org)
- 124 spots submitted
- 40 spots (i.e. genes) retained
- Many pathways returned with only a few genes in them, but several genes fell into a number of pathways, so...

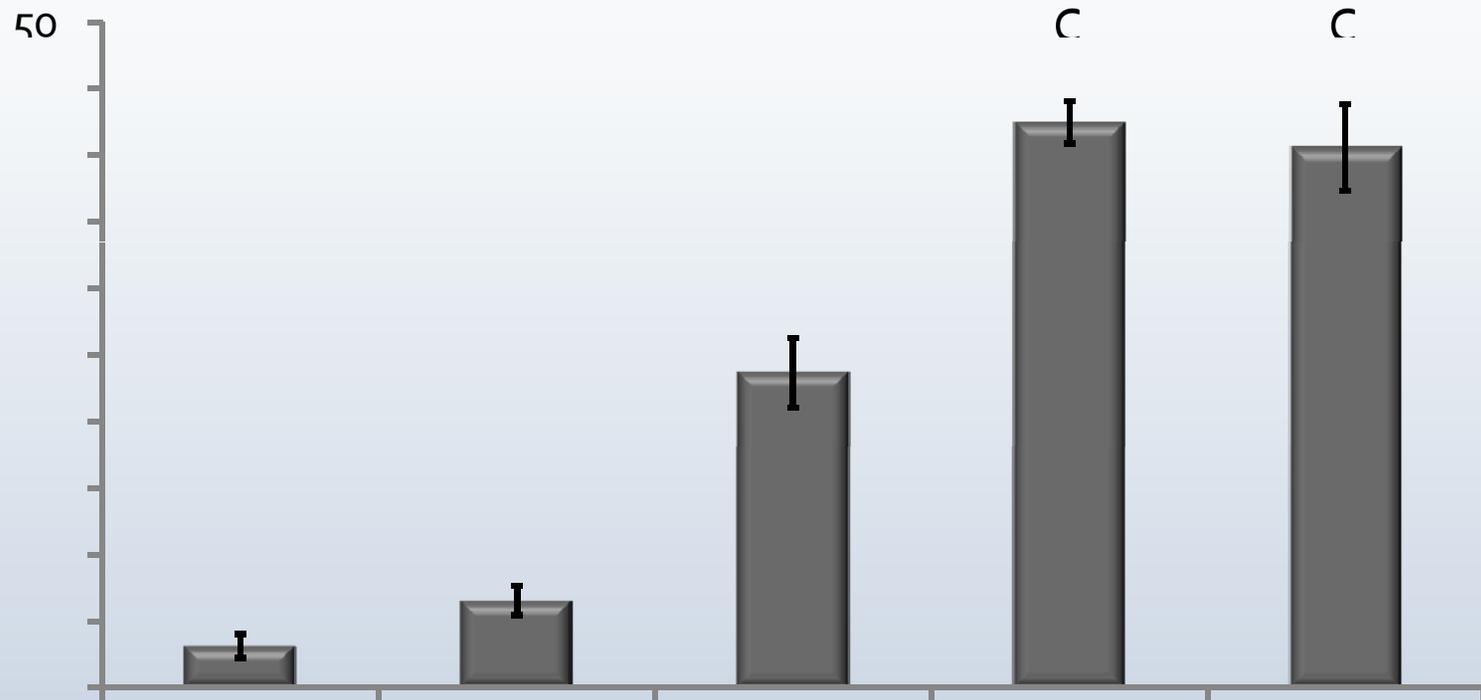
Results – q PCR

Cytochrome P₄₅₀ 1A5



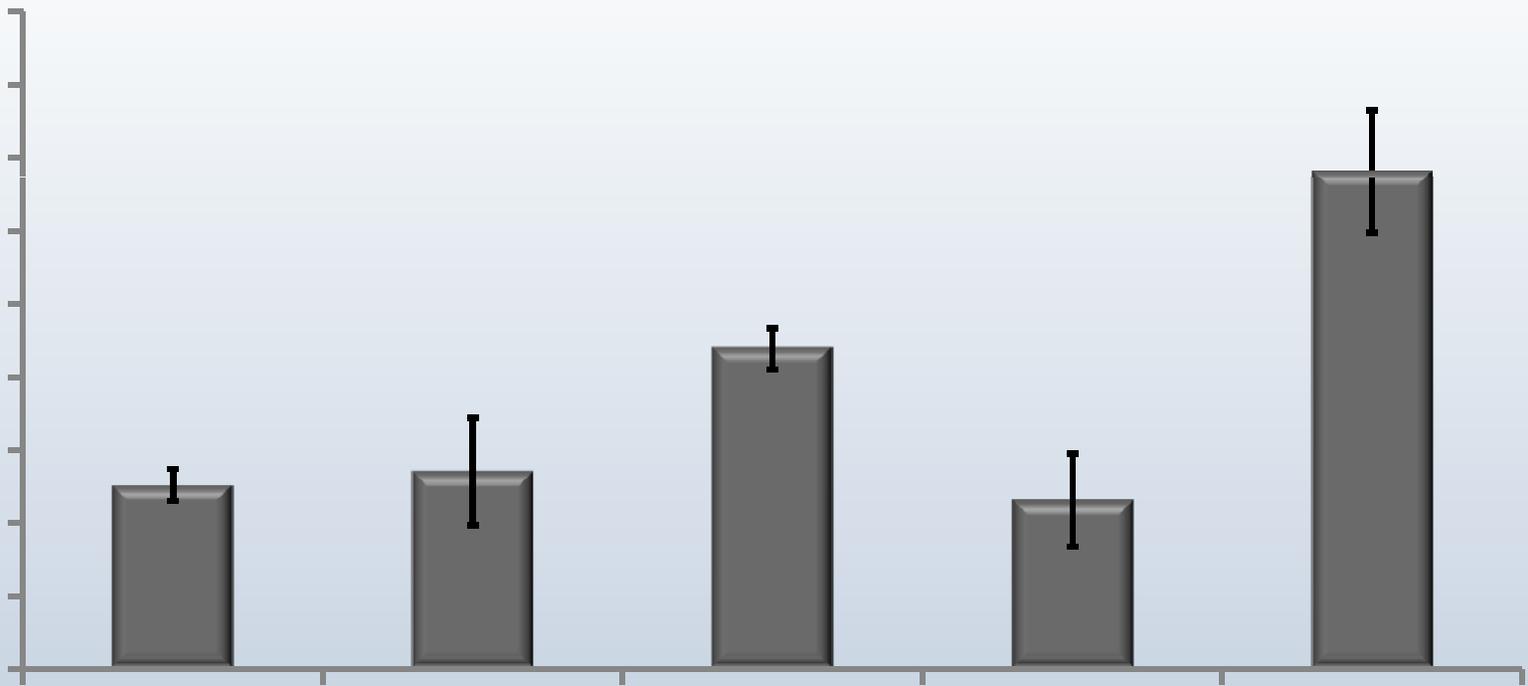
Results - qPCR

Cytochrome B5



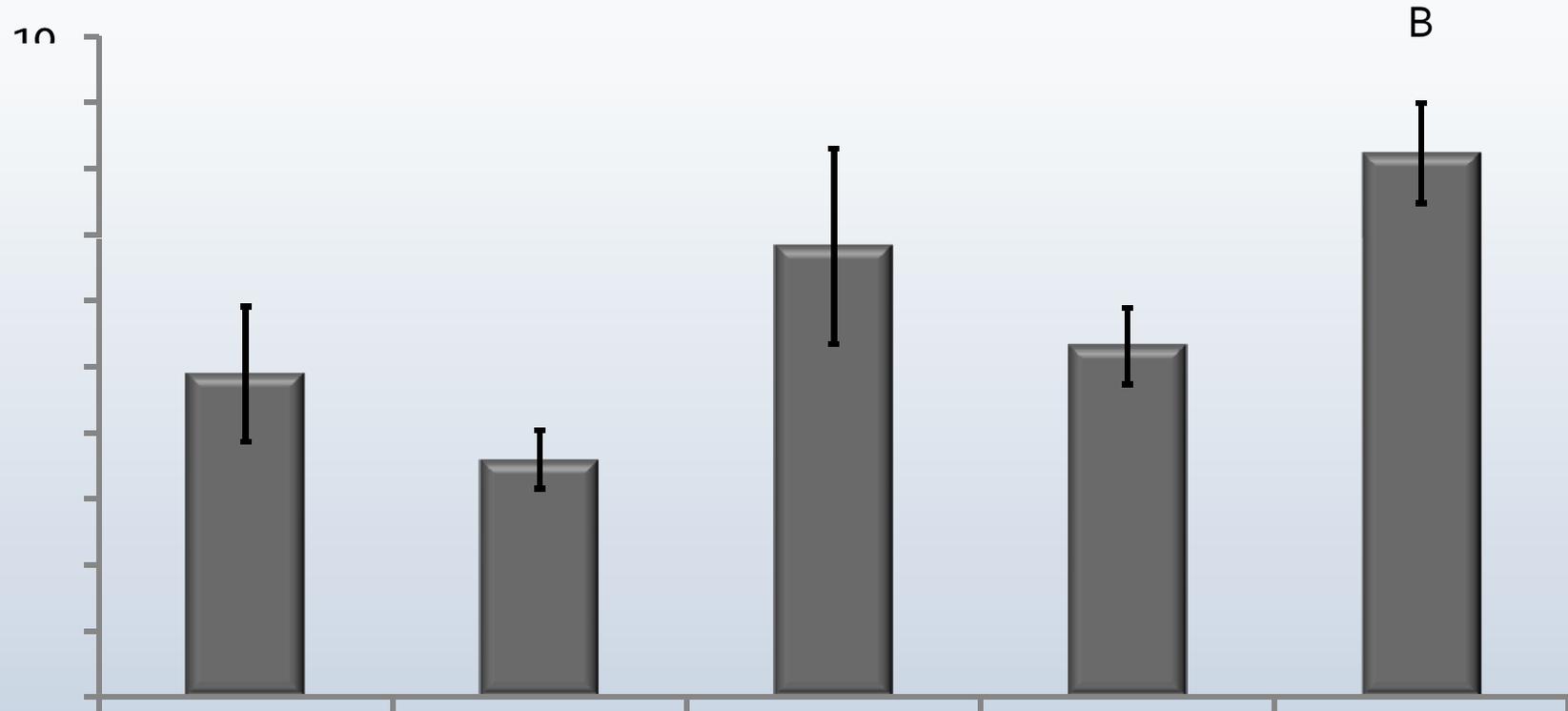
Results - qPCR

Glutathione S Transferase



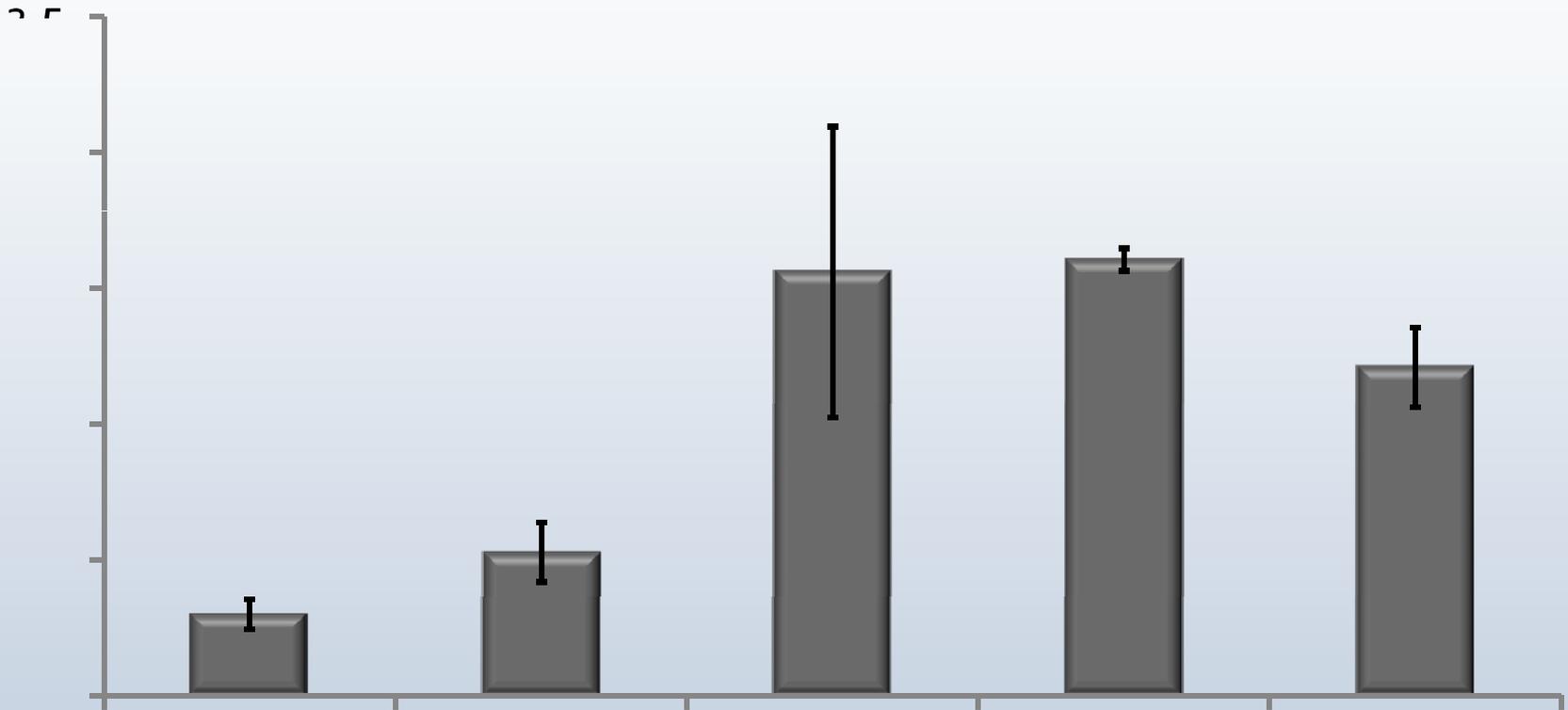
Results - qPCR

GAPDH



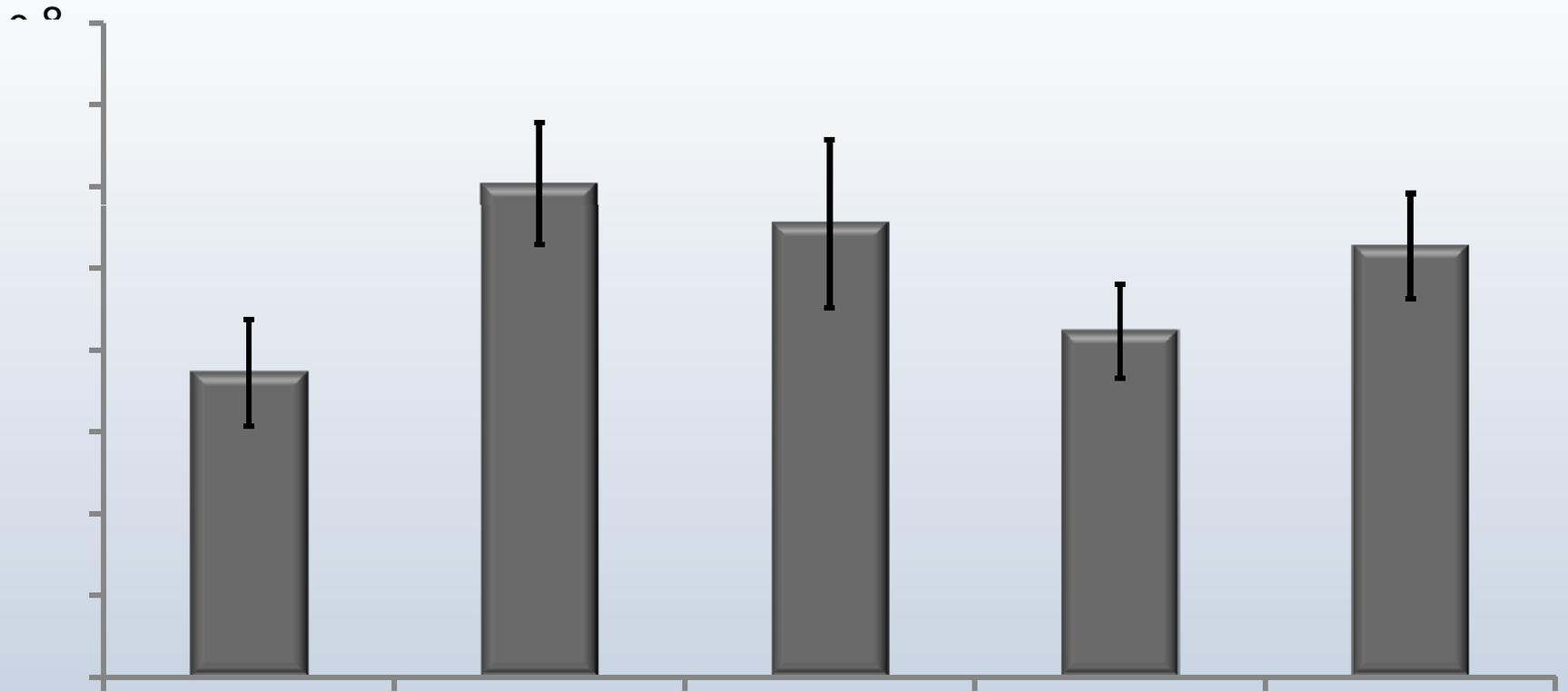
Results - qPCR

Fructose Bisphosphate Aldolase B



Results - qPCR

KDELR2



Conclusions and Future Directions

- Genes can be detected by microarray and qPCR technologies
- Sex Difference?
- Many affected pathways
 - Can explore MOA and find new biomarkers
- Further studies:
 - Steroid metabolism and clearance
 - Other CYP₄₅₀ genes
 - Endocrine disruption
 - Energy balance

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